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FORMULATION AND EVALUATION OF SPIRAMYCIN LOADED FLOATING MICROSPHERES

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Abstract
Floating microspheres of spiramycin were prepared by novel oil-in-water emulsion solvent evaporation technique, using various biodegradable polymers such as HPMC E-15 and ethyl cellulose in order to retain drug in body for longer period of time. Spiramycin is insoluble in water and has short half life of 1.5 h. It requires frequent dosing before meals due to short half life and thereby imposing side effects. The drug requires a novel gastroretentive drug delivery system which can provide an extended period of time in stomach and improve oral bioavailability. Floating microspheres were characterized for floating ability, compatibility study, particle size and shape, drug content, in vitro drug release, entrapment efficiency. Due to their low density, these multi particulate drug delivery systems showed good floating ability and remained in gastric environment for more than 12 h. HPMC based microspheres showed its buoyancy for more than 12 h, required for sustained therapeutic activity in comparison to Ethyl cellulose based microspheres. Major advantages of the system include ease of preparation, good floating ability, high encapsulation efficiency and sustained drug release over several hours. From this study it was concluded that formulation of floating microspheres of spiramycin offers prolonged gastric residence time and continuous release of the medication over an extended period of time thus oral bioavailability of the drug and subsequent efficacy is improved.

Keywords: Floating microspheres; Spiramycin; HPMC E-15; Ethyl cellulose;

INTRODUCTION
Oral route drug administration is by far the most preferable route for taking medications. However, their short circulating half life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. Rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamics profile is to release the drug in a controlled manner and site specific manner. Microspheres are small spherical particles, with diameters 1 µm to 1000 µm. They are spherical free flowing particles consisting of proteins or synthetic polymers which are biodegradable in nature. There are two types of microspheres; microcapsules and micromatrices, which are described as, Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall, and micromatrices in which entrapped substance is dispersed throughout the matrix. Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Microsphere play an important role to improve bioavailability of conventional drugs and minimizing side effects. Ideal characteristics of microspheres: [5,6].

- The ability to incorporate reasonably high concentrations of the drug.
- Stability of the preparation after synthesis with a clinically acceptable shelf life.
- Controlled particle size and dispersability in aqueous vehicles for injection.
- Release of active reagent with a good control over a wide time scale.
- Biocompatibility with a controllable biodegradability.
- Susceptibility to chemical modification.

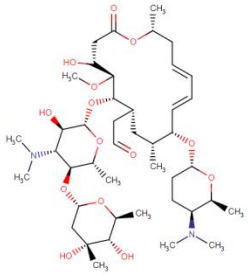
Advantages of microspheres: [6]

- Particle size reduction for enhancing solubility of the poorly soluble drug.
- Provide constant and prolonged therapeutic effect.
- Provide constant drug concentration in blood there by increasing patent compliance,
- Decrease dose and toxicity.
- Protect the drug from enzymatic and photolytic cleavage hence found to be best for drug delivery of protein.
- Reduce the dosing frequency and thereby improve the patient compliance
- Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
- Microsphere morphology allows a controllable variability in degradation and drug release.
- Convert liquid to solid form & to mask the bitter taste.
- Protects the GIT from irritant effects of the drug.
- Biodegradable microspheres have the advantage over large polymer implants in that they do not require surgical procedures for implantation and removal.
- Controlled release delivery biodegradable microspheres are used to control drug release rates thereby decreasing toxic side effects, and eliminating the inconvenience of repeated injections.

MATERIAL AND METHODS

Drug Profile

Name : Spiramycin
Molecular Formula : $C_{43}H_{74}N_2O_{14}$
Chemical structure :



Drug spiramycin and other Excipients

Drug spiramycin with working standard was obtained as a gift sample from SUN Pharma Dewas. Ethanol and coconut oil were purchased from S.K. Traders Indore. HPMC E-15, ethyl cellulose, dichloromethane, tweene 80, light liquid paraffin, petroleum ether were obtained from store of our institute. All the chemicals were made by Renkem, Mumbai.

Preformulation Studies

Before formulation and characterization of floating microspheres, preformulation studies were carried out in term of test for identification (Physical appearance, Melting point and IR Spectra), solubility profile and qualitative estimation of drug.

Identification of Drug

Physical appearance

Through visual inspection the physical appearance analysis of spiramycin pure drug was carried out.

Melting Point

Melting point determination of the drug sample was done by open capillary method using melting point apparatus. Drug was taken in glass capillary tube whose one end was sealed by means of flame. The capillary tube was placed in a melting point apparatus attached to a thermometer to measure the melting point. The sample holder was heated gradually and the temperature at which drug melts was recorded. Melting point of a drug sample is a first indication of purity of sample.

FTIR Analysis

A spectra of FTIR helps in identification of different functional groups of a molecule. It helps in identification of a compound based on the functional groups present in it. The FTIR spectrum of spiramycin was recorded on Bruker spectrophotometer using KBr pellet technique and reported in wave number (cm^{-1}). The scanning range was 450-4000 cm^{-1} and the resolution was 2 cm^{-1}. The FTIR spectra of drug with polymer were compared with standard FTIR spectrum of the pure drug.

Solubility Profile

In this process, the solubility of spiramycin in different solvents such as ethanol, dichloromethane and water etc. Should be checked.

100 mg of Spiramycin was accurately weighed and transferred into 10ml volumetric flask and diluted

with water to get a solution concentration of 1000 µg/ml as a stock solution A.
1 ml of stock solution of A was again diluted to 100 ml with water to get a solution concentration of 100 µg/ml as a stock solution B.
Then further dilution were made with different solvents to get the solution in ranging from 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml.
Again 1.5 ml of stock solution B was diluted to 10 ml with different solvents such as ethanol, dichloro methane and water to get a solution of 15 µg/ml concentration.
Again 2.0 ml of stock solution B was diluted to 10 ml with different solvents such as ethanol, dichloro methane and water to get a solution of 20 µg/ml concentration.
Again 2.5 ml of stock solution B was diluted to 10 ml with different solvents such as ethanol, dichloro methane and water to get a solution of 25 µg/ml concentration.

Partition Coefficient
10 mg of spiramycin was accurately weighed and transferred into a separating funnel in which 10 ml distilled water and 10 ml octanol occurred.
Now, shake the separating funnel for 10 minutes and stand over this mixture for 24 hours. Then filter this mixture and separate the oil phase and water phase and prepared a solution concentration of 10 µg/ml, 15 µg/ml and 20 µg/ml.

Preparation of Floating Microspheres with HPMC E-15 polymer
Floating microspheres were prepared by solvent evaporation method. Accurately weighed drug and HPMCE15 were dissolved in ethanol and dichloromethane (1:1) to form a homogenous polymer solution. This solution is poured dropwise with niddle in light liquid paraffin and coconut oil (1:1) containing few drops of tween 80. This is maintained at 30-40°C subsequently stirred at agitation speed of 1000 rpm for 4 hours to allow the volatile liquid to evaporate. The microspheres formed were filtered, washed with petroleum ether and dried in vacume. The microspheres were then stored in a desiccator over fused calcium chloride.

Table 1: Formulation design for Spiramycin + HPMC E15 floating microspheres

S. No.	Ingredients	Quantity
1	Spiramycin	250 mg
2	HPMC E-15	250 mg
3	Ethanol	20 ml
4	Dichloromethane	20 ml
5	Light Liquid Paraffin	50 ml
6	Coconut Oil	50 ml
7	Tweene 80	0.3 ml

Preparation of Floating microspheres with Ethyl cellulose Polymer
Floating microspheres were prepared by solvent evaporation method. Accurately weighed drug and Ethyl Cellulose were dissolved in ethanol and dichloromethane (1:1) to form a homogenous polymer solution. This solution is poured drop wise with niddle in light liquid paraffin and coconut oil (1:1) containing few drops of tween 80. This is maintained at 30-40°C subsequently stirred at agitation speed of 1000 rpm for 4 hours to allow the volatile liquid to evaporate. The microspheres formed were filtered, washed with petroleum ether and dried in vacuum. The microspheres were then stored in a desiccators over fused calcium chloride.

Table 2: Formulation design for Spiramycin + Ethyl Cellulose floating microspheres

S. No.	Ingredients	Quantity
1	Spiramycin	250 mg
2	Ethyl Cellulose	250 mg
3	Ethanol	20 ml
4	Dichloromethane	20 ml
5	Light Liquid Paraffin	50 ml
6	Coconut Oil	50 ml
7	Tweene 80	0.3 ml

Particle Size
Particle size and shape of the microspheres was determined by optical microscopy. The freshly prepared microspheres were examined on an optical microscope and the size of microspheres was measured by precalibrated ocular micrometer and stage micrometer. About 100 particles of each formulation were observed and measured.

***In-vitro* release Studies**
The in-vitro drug release studies of formulations were carried out in 0.1 N HCl (pH 1.2) for 2 h and in pH 6.8 buffer for 10 h. The drug release rate from

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floating microspheres was determined using basket type eight station dissolution test apparatus (Electrolab). A weighed amount of floating microspheres equivalent to 100 mg drug was kept in 0.1 N HCl (1.2 pH) maintained at 37± 0.5 ºC at a rotation speed of 100 r/min. Sink condition was maintained during the study. 5 ml sample was withdrawn at 60 min time interval, the initial volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawal, passed through 5 mm membrane filter and analyzed spectrophotometrically at 210 nm. The same process was repeated using pH 6.8 as dissolution medium, for both HPMC-E15 and Ethyl cellulose formulations.

RESULTS

Pre formulation Studies

Preformulation studies for the selected drug Spiramycin includes test for identification (examination of physical appearance, melting point determination and IR spectroscopy), solubility studies.

Identification of drug

Physical Appearance

Physical appearance od Spiramycin drug was white solid powder.

Melting Point

Melting point of spiramycin was found to be 151°C

FTIR Analysis

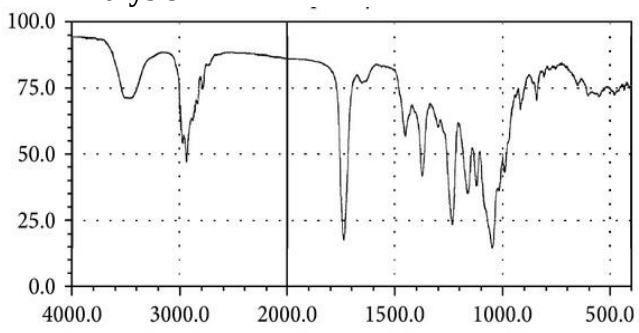

Figure 1: FTIR of Pure Spiramycin

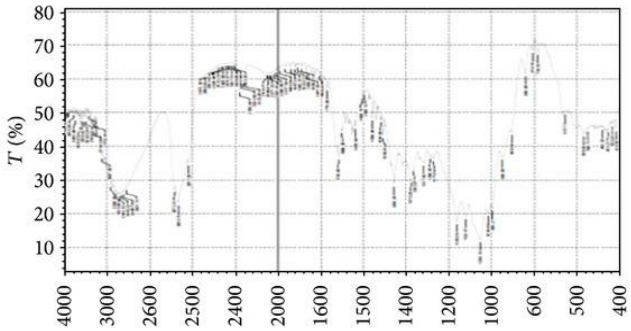

Fig. 2: FTIR of Pure Spiramycin + HPMC E-15

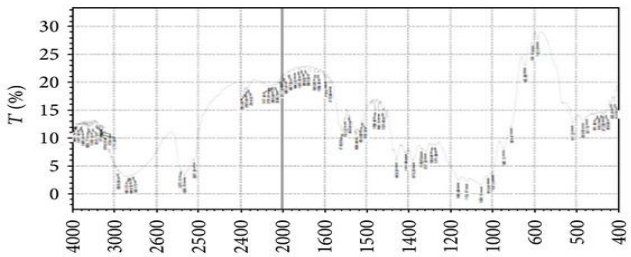

Fig. 3: FTIR of Pure Spiramycin + Ethyl Cellulose

Solubility Study

Solubility analysis was done to select a suitable solvent system to dissolve the drug and also to test its solubility in the dissolution medium which was to be used. Drug solubility study was performed by taking an excess quantity of drug in different solvents like water, ethanol, methanol, acetone and buffers. Spiramycin was found to be Insoluble in water; Very soluble in acetonitrile and methanol; Almost completely (>99.5) in ethanol. mg/mL (20 °C).

Table 3: Solubility in Ethanol

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.034
2	15	0.098
3	20	0.168
4	25	0.242

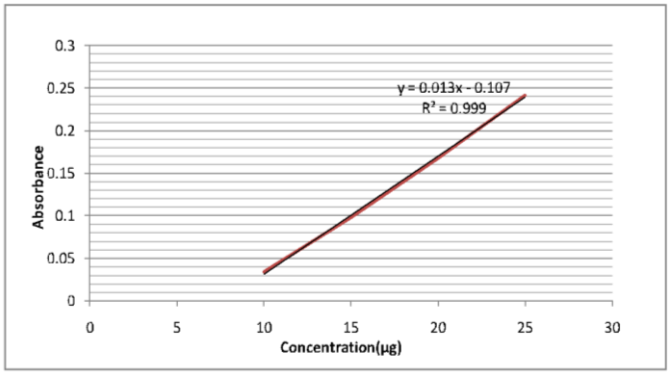

Fig. 4: Solubility in Ethanol

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Table 3: Solubility in Water

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.0021
2	15	0.0065
3	20	0.0095
4	25	0.0190

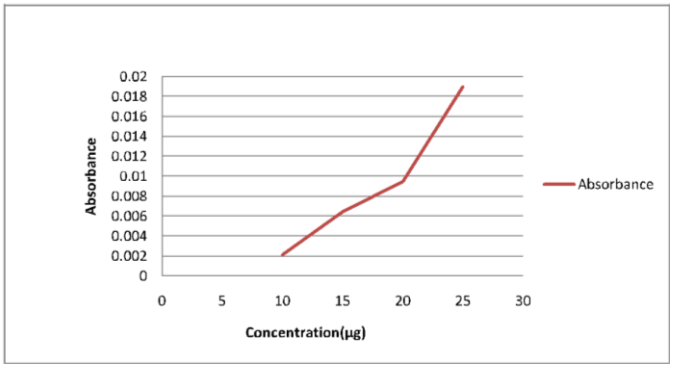

Fig. 5: Solubility in Water

Table 4: Solubility in Dichloromethane

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.076
2	15	0.170
3	20	0.260
4	25	0.350

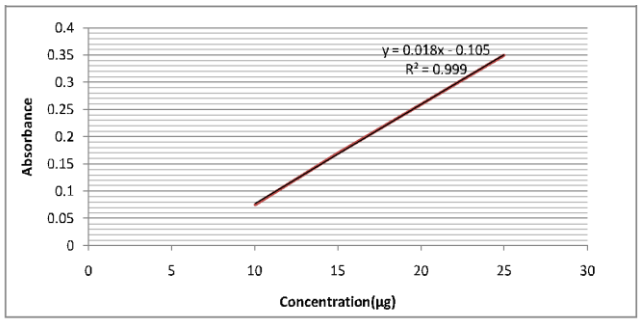

Fig. 6: Solubility in Dichloromethane

Table 5: Solubility in Different Solvents

S. No.	Solvent	Solubility
1	Ethanol	Soluble
2	Water	Slightly soluble / Insoluble
3	Dichloromethane	Soluble

Partition Coefficient

Table 6: Drug partition coefficient checked in Octanol

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.088
2	15	0.139
3	20	0.189

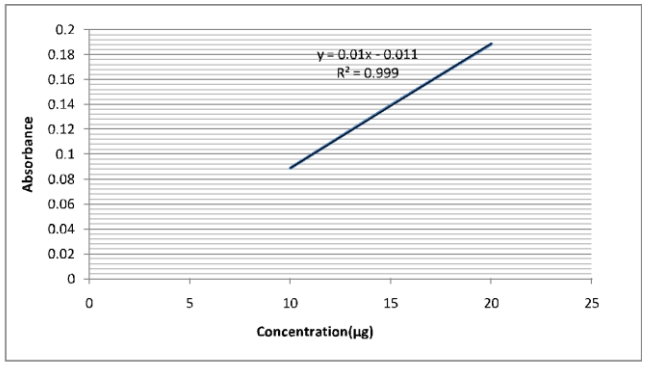

Fig. 7: Drug partition coefficient checked in Octanol

Table 7: Drug partition coefficient checked in Water

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.0044
2	15	0.0058
3	20	0.0071

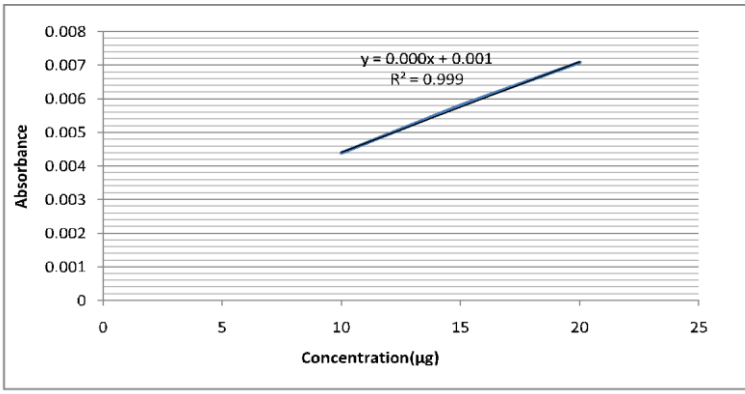

Fig. 8: Drug partition coefficient checked in Water

Characterization of Drug loaded Microspheres

Particle Size Measurement:

The average particle size range for formulations F1 and F2 was found to be 66 µm and 85 µm respectively. The results were showed in figure. The particle size of the microspheres increases with increase in polymer concentration respectively. This is because the viscosity of polymer solution increases with increasing polymer concentration resulting in enhanced interfacial tension, which in turn decreases the stirring efficiency, which results in increased particle size. Particle size was measured by using Malvern instrument and to get the result mentioned on graph.

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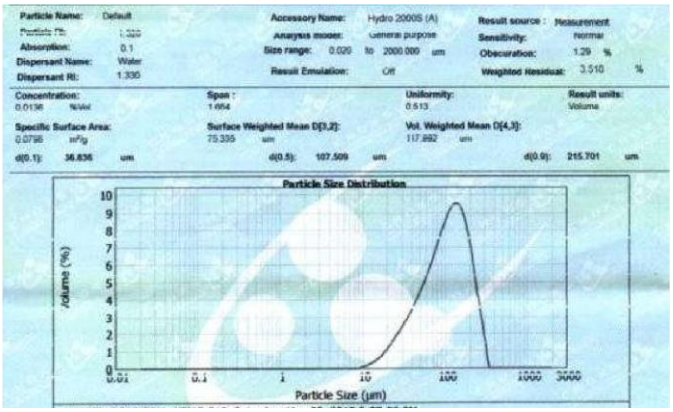

Fig. 9: Particle Size Analysis

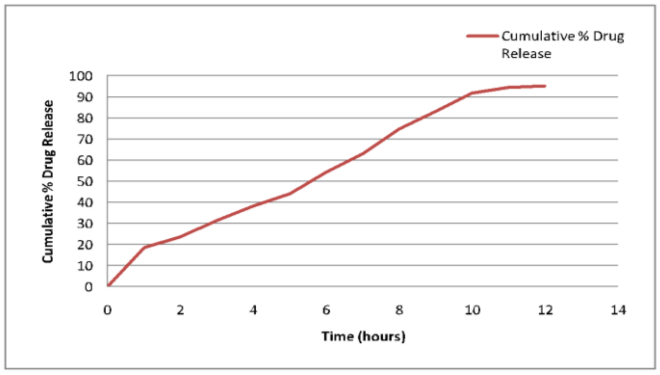

Fig. 10: Cumulative percentage Drug Release of Spiramycin + HPMC E-15 Formulation

In-vitro Drug Release Study

Table 8: Cumulative percentage Drug Release of Spiramycin + HPMC E-15 Formulation

S. No.	Time in hours	Cumulative percentage Drug Release						
		F1	F2	F3	F4	F5	F6	Average
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1.00	18.64	18.56	18.40	18.65	18.74	18.62	18.60
3	2.00	23.72	23.84	23.45	23.78	23.64	23.57	23.67
4	3.00	31.35	31.89	31.48	31.25	31.89	31.78	31.61
5	4.00	38.58	38.65	38.59	38.48	38.95	38.69	38.66
6	5.00	43.45	43.22	43.49	43.19	43.57	43.28	43.37
7	6.00	52.65	52.99	52.67	52.38	52.47	52.34	52.58
8	7.00	64.58	64.59	64.87	64.25	64.65	64.18	64.52
9	8.00	74.65	73.99	74.68	74.26	74.69	74.52	74.47
10	9.00	82.65	82.36	82.19	82.47	82.56	82.31	82.42
11	10.00	92.64	92.35	92.48	92.35	92.38	92.54	92.46
12	11.00	94.36	94.62	94.76	94.32	94.21	93.26	94.26
13	12.00	95.26	95.48	95.64	95.32	95.66	95.23	95.43

Table 9: Cumulative percentage Drug Release of Spiramycin + Ethyl Cellulose Formulation

S. No.	Time in hours	Cumulative percentage Drug Release						
		F1	F2	F3	F4	F5	F6	Average
1	0	0	0	0	0	0	0	0.00
2	1	10.72	10.82	10.65	10.45	10.35	10.68	10.61
3	2	16.09	16.25	16.38	16.44	16.22	16.84	16.37
4	3	22.57	22.65	22.37	22.38	22.58	22.67	22.54
5	4	31.05	31.28	31.24	31.24	31.62	31.83	31.38
6	5	35.36	35.91	35.62	35.46	35.15	35.26	35.46
7	6	42.36	42.13	42.19	42.35	42.26	42.15	42.24
8	7	46.35	46.35	46.25	46.18	46.57	46.29	46.33
9	8	54.69	54.28	54.32	54.61	54.29	54.35	54.42
10	9	59.32	59.36	59.21	59.36	59.41	58.99	59.28
11	10	63.25	63.26	63.22	63.12	63.15	63.15	63.19
12	11	71.68	71.25	72.26	71.36	72.24	72.15	71.82
13	12	77.32	77.26	77.65	77.15	77.37	77.65	77.40

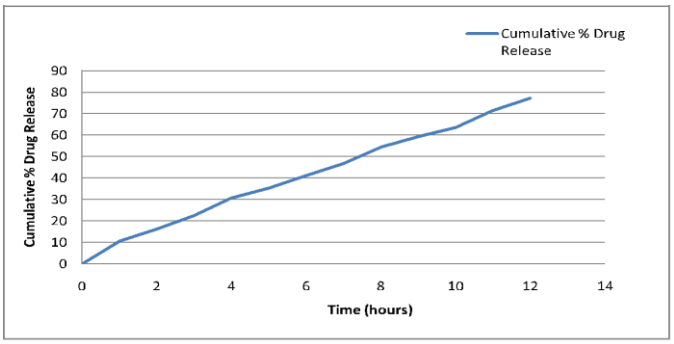

Fig. 11: Cumulative percentage Drug Release of Spiramycin + Ethyl Cellulose Formulation

CONCLUSION

Floating microspheres of spiramycin were prepared by novel oil-in-water emulsion solvent evaporation technique, using various biodegradable polymers such as HPMC E-15 and ethyl cellulose in order to retain drug in body for longer period of time. Spiramycin is insoluble in water and has short half life of 1.5 h. It requires frequent dosing before meals due to short half life and thereby imposing side effects. The drug requires a novel gastroretentive drug delivery system which can provide an extended period of time in stomach and improve oral bioavailability. Floating microspheres were characterized for floating ability, compatibility study, particle size and shape, drug content, in vitro drug release, entrapment efficiency. Due to their low density, these multi particulate drug delivery systems showed good floating ability and remained in gastric environment for more than 12 h. HPMC based microspheres showed its buoyancy for more than 12 h, required for sustained therapeutic activity in comparison to Ethyl cellulose based microspheres. Major advantages of the system include ease of preparation, good floating ability, high encapsulation efficiency and sustained drug release over several hours. From this study it was concluded that formulation of floating microspheres of spiramycin offers prolonged gastric residence time and continuous release of the medication over an extended period of time thus oral bioavailability of the drug and subsequent efficacy is improved.

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