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FORMULATION AND EVALUATION OF ETHAMBUTOL MICROSPHERES

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ABSTRACT

Emulsion cross linking method can be successfully employed to fabricate Ethambutol microspheres than Ionotropic gelation method. The technique provides characteristic advantage over conventional microsphere method, which involves an "all-aqueous" system, avoids residual solvents in microspheres. Other methods utilize larger volume of polymer, uneasy in dropping through syringe, air pollution, toxicity and difficult to remove traces during filtration. FT-IR spectra of the physical mixture revealed that the drug is compatible with the polymers and copolymer used. Micromeritic studies revealed that the mean particle size of the prepared microspheres was in the size range 528µm to 644µm. Increase in the polymer concentration led to increase in % Yield, % Drug entrapment efficiency, Particle size, % swelling and % Mucoadhesion. Floating microspheres of drug with HPMC and Ethyl cellulose were buoyant while those with Eudragit S 100 showed greater buoyancy. *Invitro* drug release decreased with increase in the polymer and copolymer concentration.

KEYWORDS: Ethambutol, Ionotropic gelation technique, Floating Microspheres, Eudragit S100, In vitro drug release studies.

1. INTRODUCTION

One of the most feasible approaches for achieving a prolonged and predictable drug delivery in the GI tract is to control the gastric residence time by using gastro-retentive dosage forms (GRDFs). It remains in the gastric region for several hours and hence prolongs the gastric residence time of drug. It has several advantages over immediate release dosage form including the minimization of fluctuations in drug concentration in plasma and at the site of

action over prolonged periods of time, resulting in optimized therapeutic efficiencies and reduce the side effect, reduction of total dose administered and reduction of administration frequency leading to improved patient compliances.[1,2] Gastric emptying of dosage forms is an extremely variable process and ability to prolong and control the emptying time is a valuable asset for dosage forms, which reside in the stomach for a longer period of time than conventional dosage forms.[1] Floating microspheres are gastro-retentive drug delivery systems based on non-effervescent approach. Hollow microspheres are in strict sense, spherical empty particles without core. These microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers. Floating microspheres have emerged as an efficient means of enhancing the bioavailability[3,4] Ethambutol is a potent first line anti tubercular drug, rapidly absorbed from GIT and. Peak plasma concentration is achieved within 2-4 hours. Ethambutol HCL has the half life of 3-4 hours.[5] the objective of present study is to formulate and evaluate Ethambutol HCL microspheres for oral sustained drug delivery. Here comparison is made between methods of preparation of floating microspheres.

2. MATERIALS AND METHODS

2.1 MATERIALS

Ethambutol was obtained as a gift sample from Chandra Labs, Hyd. HPMC, Eudragit S-100 and Carbopol 940 were purchased from S D fine-chem. Limited, Mumbai. All other chemicals were used as analytical grade.

2.2 METHODOLOGY

Preparation of floating microspheres of ethambutol

Floating microspheres were prepared by the solvent evaporation method. Various concentration of polymer in suitable solvents were mixed well with the Ethambutol with different ratios of polymer as shown in Table and this pasty, mass was introduced into 50ml of aqueous saline phase containing 0.04 % (20 mg) polyvinyl alcohol (PVA) and 10% (5 ml) ethanol. The system is stirred using propeller at 300 rpm at room temperature for 2-3 hr. The drug loaded floating microspheres formed were filtered, washed and dried in a hot air oven at 60˚C[52].

Formulation design

Table 1: Formulation of Ethambutol Floating Microspheres.

Ingredients(mg/Dose)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ethambutol	200	200	200	200	200	200	200	200	200
HPMC	200	400	600	-	-	-	-	-	-
Eudragit S100	-	-	-	200	400	600	-	-	-
Ethyl cellulose	-	-	-	-	-	-	200	400	600
$NaHCO_3$	100	150	200	100	150	200	100	150	200
Water (ml)	q.s	q.s	q.s	-	-	-	-	-	-
Dichloromethane:Ethanol (2:1) (ml)	-	-	-	q.s	q.s	q.s	-	-	-
Ethanol (ml)	-	-	-	-	-	-	q.s	q.s	q.s

q.s – Quantity sufficient

Drug-Excipient Compatibility study (FTIR)

The IR absorption spectra of the pure drug and with different excipients were taken in the range of 4000-400 cm^{-1} using KBr disc method, 1-2 mg of the substance to be examined was triturated with 300-400 mg, specified quantity, of finely powered and dried potassium bromide. These quantities are usually sufficient to give a disc of 10-15mm diameter and pellet of suitable intensity by a hydraulic press.

Characterization of microspheres

Scanning electron microscopy (SEM)[10]

The morphology of the microspheres was studied using scanning electron microscopy (SEM).The samples for SEM were prepared by lightly sprinkling on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with gold film under reduced pressure. The stub containing the coated samples was placed in the scanning electron microscope (Hitachi S3400N) chamber. The samples were then randomly scanned, and photomicrographs were taken at the acceleration voltage of 5 kV. Microphotographs were taken on different magnification and higher magnification was used for surface morphology.

Drug content[11]

20 microspheres of each formulation were weighed and powdered. The quantity of powder equivalent to 100 mg of Ethambutol was transferred in to a 100 ml volumetric flask and the volume adjusted to 100ml with 0.1N HCl. Further 1ml of the above solution was diluted to 100 ml with 0.1N HCl and the absorbance of the resulting solution was observed at 268 nm.

In vitro Buoyancy studies[12]

The in vitro buoyancy was determined by floating lag time, and total floating time. (As per the method described by Rosa et al) The microspheress were placed in a 100ml beaker containing 0.1N HCl. The time required for the microspheres to rise to the surface and float was determined as floating lag time (FLT) and the duration of the time the microspheres constantly floats on the dissolution medium was noted as the Total Floating Time respectively (TFT).

$$\% \text{ Buoyancy} = Q_f / (Q_f + Q_s) \text{ X } 100$$

Where Q_f and Q_s are the weight of the floating and settled microspheres respectively.

Swelling Index Studies[13]

The swelling behavior of a dosage unit was measured by studying its weight gain. The swelling index of microspheres was determined by placing the microspheress in the basket of dissolution apparatus using dissolution medium as 0.1N HCl at 37±0.5°C. After 0.5, 1, 2, 3, 4, 5, and 6h, each dissolution basket containing microspheres was withdrawn, blotted with tissue paper to remove the excess water and weighed on the analytical balance (Schimdzu, AX 120). The experiment was performed in triplicate for each time point. Swelling index was calculated by using the following formula

$$\text{Swelling index} = \frac{\text{(Wet weight of microspheres – Dry weight of microspheres)}}{\text{Dry weight of microspheres.}}$$

Drug Loading and Drug Entrapment[14]

Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl (pH-1.2) repeatedly. The extract was transferred to a 100ml volumetric flask and the volume was made up using 0.1N HCl (pH-1.2). The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV 1700, Shimadzu, Japan) at 268 nm against appropriate blank. The amount of drug loaded and entrapped in the microspheres was calculated by the following formulas:

$$\textbf{Drug loading} = \frac{\textbf{weight of drug in microspheres} \times \textbf{100}}{\textbf{microspheres sample weight}}$$

$$\text{(Drug entrapment efficiency (\%)} = \frac{\text{Amount of drug actually present} \times 100}{\text{Theoretical drug load expected}}$$

Determination of percentage yield[15]

The dried microspheres were weighed and percentage yield of the prepared microspheres was calculated by using the following formula.

$$\text{Percentage yield} = \frac{\text{Practical yield (mg)} \times 100}{\text{Theoretical yield}}$$

In-vitro Release Study[16]

The drug release study was performed for microsphere containing quantity equivalent to Ethambutol dose by using USP dissolution apparatus Type I in 900 ml of 0.1N HCl dissolution media (pH-1.2) at 100 rpm and 37^0C temperature. 10 ml of sample was withdrawn at predetermined time interval for 12 hours and same volume of fresh medium was replaced to maintained sink condition. Withdrawn samples were assayed spectrophotometrically at 268 nm. Drug release was also performed for pure drug.

Release Kinetics[17]

To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to, Zero order, First order, Higuchi matrix, Peppas and Hixson Crowell model. In this by comparing the r-values obtained, the best-fit model was selected.

Zero Order Kinetics

Drug dissolution from Pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation

$Q_t = Q_o + K_o t$

Where,

Q_t = Amount of drug dissolved in time t,

Q_o = Initial amount of drug in the solution and

K_o = Zero order release constant.

First Order Kinetics

To study the first order release rate kinetics the release rate data were fitted to the following

equation.

$\log Q_t = \log Q_o + K_1 t / 2.303$

Where,

Q_t = Amount of drug released in time t,

Q_o = Initial amount of drug in the solution and

K_1 = First order release constant.

Higuchi Model

Higuchi developed several theoretical models to study the release of water soluble and low-soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The higuchi equation is

$Q_t = K_H \text{ x } t_{1/2}$

Where,

Q_t = Amount of drug released in time t and,

K_H = Higuchi dissolution constant.

Peppas Release Model

To study this model the release rate data is fitted to the following equation

$M_t / M_\infty = K. t^n$

Where,

M_t / M_∞ = Fraction of drug release,

K= Release constant,

t = Drug release time and

n = Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form.

Stability Studies

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light, and enables recommended storage conditions.

ICH guidelines the length of study and storage conditions:

Accelerated testing - 40°C/75% RH for 6 months.

The accelerated stability study of the best formulations was carried out as per the ICH guidelines.

3. RESULTS AND DISCUSSION

Preformulation parameters

Drug-Excipient Compatibility study (FTIR)

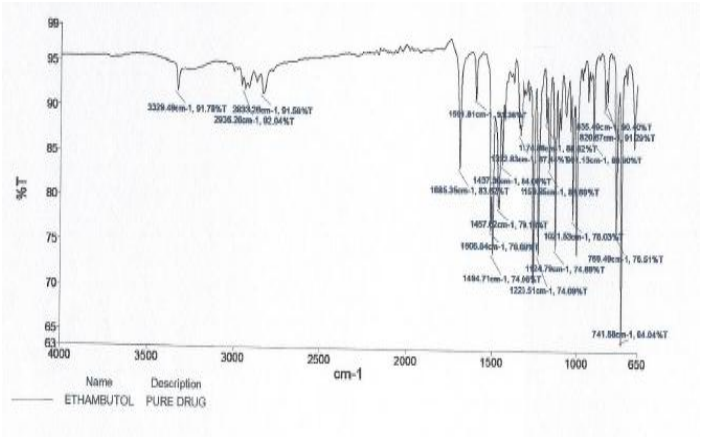

Fig. 1: FTIR Spectra of Ethambutol pure drug.

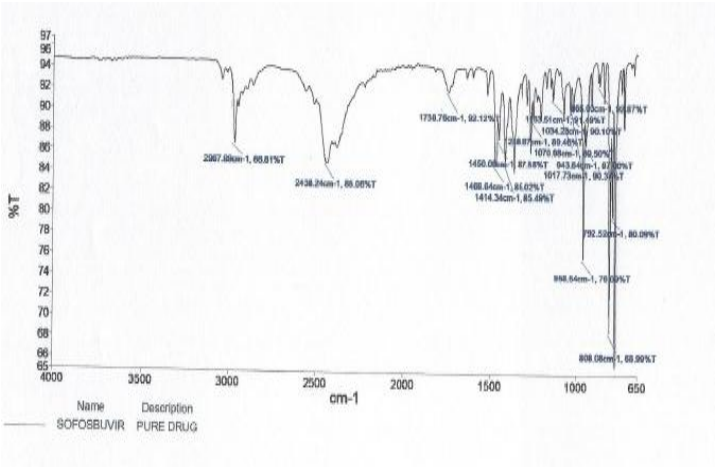

Fig. 2: FTIR Spectra of Ethambutol optimized formulation.

Evaluation and characterisation of microspheres

Percentage yield

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug- polymer solution, adhesion of polymer solution to the magnetic bead and microspheres lost during the washing process.

Drug entrapment efficiency

Percentage Drug entrapment efficiency of Ethambutol arranged from 56 to 92% for microspheres. The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency.

Table 2: Percentage yield and percentage drug entrapment efficiency of the prepared microspheres.

S. No.	Formulation code	% Yield	% Buoyancy	% Drug entrapment efficiency	%Swelling Index
1	**F1**	80	63	62.66	33.32
2	**F2**	83.33	67	72	35.66
3	**F3**	85	75	89	30.91
4	**F4**	86	79	56	32.33
5	**F5**	87.22	89	92	38.11
6	**F6**	80	85	72	38.18
7	**F7**	88	70	80	36.55
8	**F8**	82	76	82	37.32
9	**F9**	80	84	67	35.66

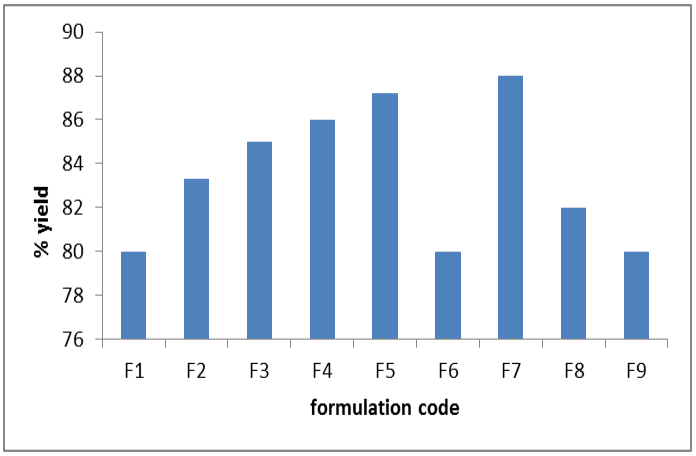

Fig. 3: Graph for % yield vs Formulation code.

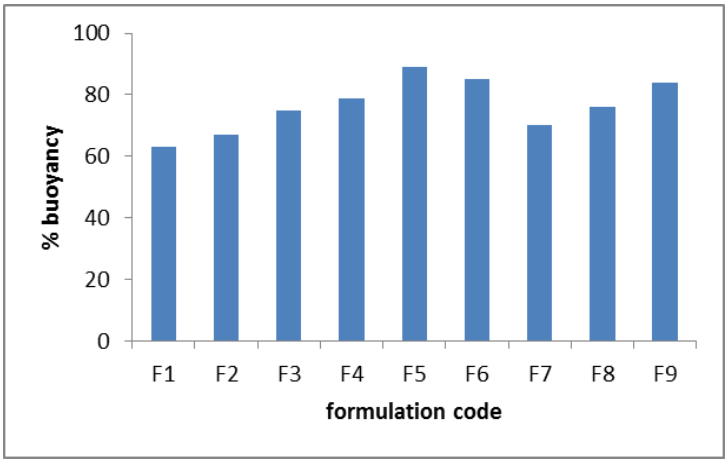

Fig. 4: Graph for % Buoyancy vs Formulation code.

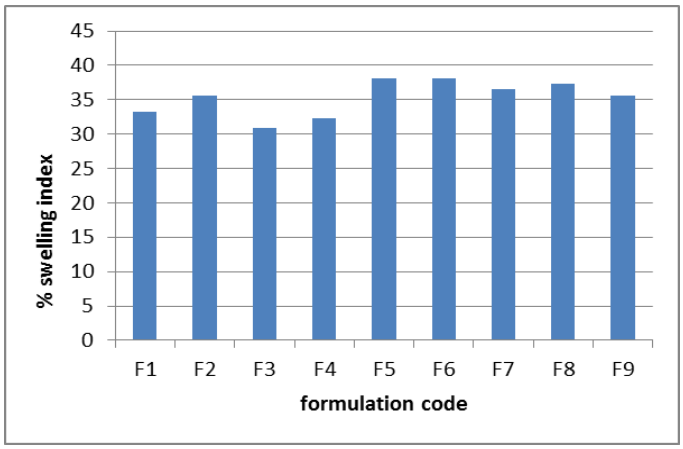

Fig. 5: Graph for % swelling index vs Formulation code.

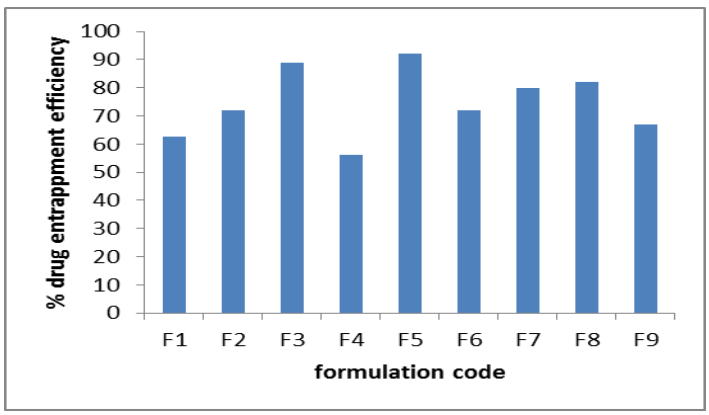

Fig. 6: Graph for % drug entrapment efficiency vs Formulation code.

Particle size analysis

The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size. Microspheres containing sodium alginate along with carbopol 934

as copolymer had a size range of 540µm to 644µm, microspheres containing sodium alginate along with carbopol 971 as copolymer exhibited a size range between 540µm to 644µm and microspheres containing sodium alginate along with HPMC K 4 M as copolymer had a size range of 588µm to 626µm. The particle size data is presented in Tables 5.4 to 5.15 and displayed in Figure 5.10 to 5.12. The effect of drug to polymer ratio on particle size is displayed in Figure 5.13. The particle size as well as % drug entrapment efficiency of the microspheres increased with increase in the polymer concentration.

Table 3: Average particle size of Ethambutol microspheres.

S.No	Batches	Mean Particle Size(µm)
1	F_1	540 µm
2	F_2	602 µm
3	F_3	644 µm
4	F4	612 µm
5	F5	528 µm
6	F6	624 µm
7	F7	588 µm
8	F8	598 µm
9	F9	626 µm

IN-VITRO Drug release studies

Dissolution studies of all the formulations were carried out using dissolution apparatus USP type I. The dissolution studies were conducted by using dissolution media, pH 1.2. The results of the in-vitro dissolution studies of formulations F_1 to F_9 are shown in table no.25 The plots of Cumulative percentage drug release Vs Time. Figure shows the comparison of % CDR for formulations F_1 to F_3, figure for formulations F4 to F6 and figure for formulations F7 to F9.

Table 4: Percentage cummulative drug release for all formulations.

TIME(hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1	23	18	16	28.4	16.25	14	25.3	23	11.30
2	32	27.2	24	40.3	21.3	20	37.2	38	19.6
3	41.5	36	31	49.7	28.6	26	44.3	45	25.4
4	57.6	45	42	55.3	30.4	28	52.4	50	28.2
5	68.2	53	49	62.4	38.2	38	57.8	54	36.3
6	79.7	67	54	68.3	44.3	42	65.2	63	40.4
7	86.4	72	58.7	76.9	51.6	48	70.8	69	46.8
8	-	84	70.4	83.2	57.2	54	79.2	78	59.3
10	-	-	-	86.9	78.3	63	85.2	83	62.4
12	-	-	-	-	86.2	76	-	-	71.2

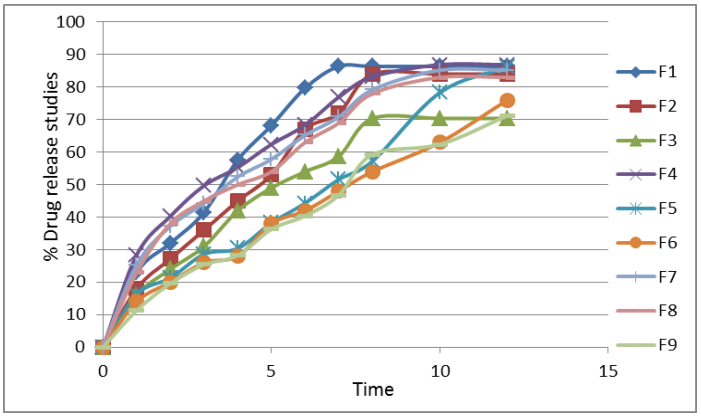

Fig. 7: Drug release studies of all formulations.

In-vitro drug release kinetics

For understanding the mechanism of drug release and release rate kinetics of the drug from dosage form, the in-vitro drug dissolution data obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix, and Krosmeyer-Peppas model. The coefficient of determination (R^2) was used as an indicator of the best fitting for each of the models considered. The kinetic data analysis of all the formulations reached higher coefficient of determination with the Zero order (R^2 = 0.985). From the coefficient of determination and release exponent values, it can be suggested that the mechanism of drug release follows Korsmeyer-Peppas model along with non-Fickian diffusion mechanism which leading to the conclusion that a release mechanism of drug followed combination of diffusion and spheres erosion.

Table 5: *In-vitro* drug release kinetics data for Formulation F5.

Zero order		First order		Higuchi's data		Korsmeyer-Peppas data	
Time (h)	% CDR	Time (h)	Log % CD Remaining	SQR Time	% CDR	Log Time	Log % CDR
1	16.25	1	1.922	1	16.25	0	1.21
2	21.3	2	1.895	1.414	21.3	0.301	1.328
3	28.6	3	1.853	1.732	28.6	0.477	1.456
4	30.4	4	1.842	2	30.4	0.602	1.482
5	38.2	5	1.790	2.236	38.2	0.698	1.582
6	44.3	6	1.745	2.449	44.3	0.778	1.646
7	51.6	7	1.684	2.645	51.6	0.845	1.712
8	57.2	8	1.631	2.828	57.2	0.903	1.752
10	78.3	10	1.336	3.162	78.3	1	1.893
12	86.2	12	1.139	3.464	86.2	1.079	1.935

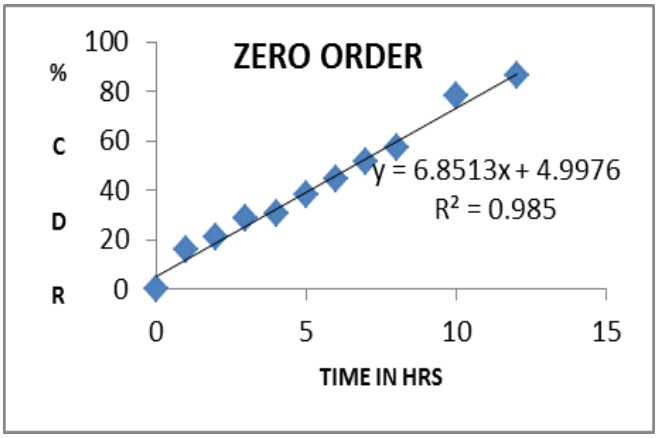

Fig. 8: Zero order kinetic graph for F5 batch.

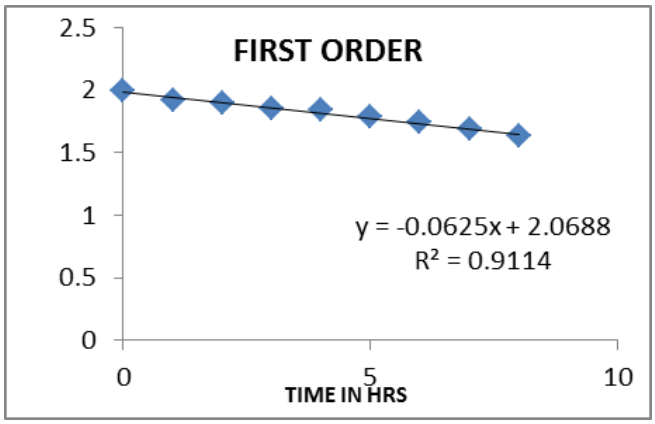

Fig. 9: First order kinetic graph for F5 batch.

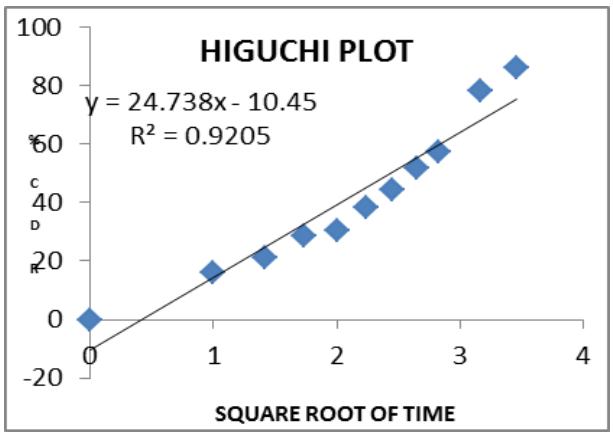

Fig. 10: Higuchis model kinetic graph for F5 batch.

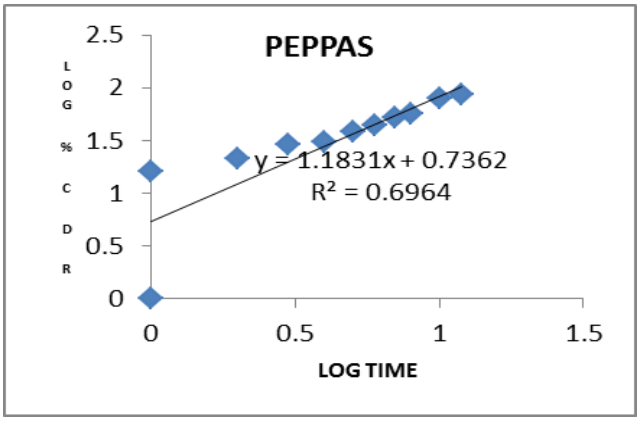

Fig. 11: Peppas model kinetic graph for F5 batch.

Table 6: R^2 values for release kinetics.

RELEASE KINEITCS				
	ZERO	**HIGUCHI**	**PEPPAS**	**FIRST**
	1	**2**	**3**	**4**
	Q Vs T	**Q Vs √T**	**Log C Vs Log T**	**Log % Remain Vs T**
Slope	**6.85**	**24.73**	**1.18**	**-0.06**
Intercept	**4.99**	**10.45**	**0.73**	**2.06**
Correlation	**0.99**	**0.95**	**0.83**	**-0.95**
R 2	**0.9850**	**0.9365**	**0.69**	**0.91**

Stability studies of ethambutol optimized formulation

The optimized formulation of Ethambutol (F5) were subjected to short-term stability testing by storing the microspheres at Accelerated temperature 25°C/60% RH.

Table 7: Stability studies of optimized formulation at Accelerated temperature.

Time	**Colour**	**Drug entrapment efficiency ± St.D. at accelerated Temperature**	**Cumulative % drug release ± St.D.**
First day	White	92.00± 0.91	86.20±0.55
30 days	White	91.72 ± 0.21	85.71±0.10
60 days	White	91. 01 ± 0.90	85.12±0.88
90 days	White	90. 66 ± 0.01	85.00±0.12

Results from stability studies indicate that the formulated microspheres are stable for a period of 3 months under accelerated temperature i.e., 40˚C temp and 70% RH. There were no remarkable changes were observed during the period of storage.

4. CONCLUSION

The present study has been a satisfactory attempt to formulate a floating Microspheres of Ethambutol with a view to control the release of the drug. From the experimental results it can be concluded that, FT-IR study shows no significant shifting of the peaks therefore it confirms the short term stability of the drug in the microspheres.

Biocompatible polymers like can be HPMC, Ethyl cellulose and Eudragit used to formulate a floating Microspheres. Good percentage drug entrapment and practical yields were obtained with the polymers. The flow properties of all formulations were within the acceptable range and therefore they could be easily filled into capsules. The floating microspheres of drug with HPMC and Ethyl cellulose were buoyant while those with Eudragit S 100 showed greater buoyancy. Cumulative percentage drug release significantly decreased with increase in polymer concentration. The overall curve fitting into various mathematical models was found to be on average. The formulations F 5 best fitted into zero order and shows non fickian diffusion mechanism. Formulated microspheres were stable and compatible at the room and accelerated temperature and humidity in storage for 90days. From the stability studies it was found that there was no significant change in the drug entrapment, release characteristics of the microspheres. Thus, the formulated floating microspheres seem to be a potential candidate as an oral gastroretentive controlled drug delivery system in prolonging the drug retention stomach and increasing the bioavailability of drug.

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