International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.8, pp 196-205, 2015

Formulation and Evaluation of Multiparticulate Gel Beads containing Tinidazole for Stomach Specific Delivery

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Abstract: The purpose of this research was to prepare multiparticulate system for stomach specific delivery of Tinidazole towards the effective eradication of *Helicobacter pylori*, a major causative agent for peptic ulcer. Different formulations containing peppermint oil entrapped in optimized sodium alginate and pectin polymers were prepared by emulsion gelation technique. The purpose of addition of volatile oil (peppermint oil) was to provide flavour as well as cool sensation after intake to the patient. This also tends to make Tinidazole beads with excellent floating ability (more than 12 hrs) hence ensured gastroretentive property in fasted state. The prepared beads were evaluated for particle size, density, percentage yield, biodegradation, entrapment efficiency, buoyancy of beads and in-vitro drug release kinetics. The results clearly indicated that the percentage of oil plays an important role in controlling the floating of the beads. Scanning electron microscopy (SEM) revealed that the beads were almost spherical in shape with numerous pores on their surface. SEM of the cross section of the swelled beads demonstrated small oil globules confirming traces of oil entrapment throughout the inner portion of the beads. The drug release kinetics was best fitted with the near zero order following fickian diffusion model. The results provide evidence that formulated gel beads may be used to incorporate other antimicrobials which may be effective when administered locally in stomach to cure *Helicobacter pylori* infection.
Keywords: Multiparticulate system, Tinidazole, Peppermint oil, Gastroretentive, *Helicobacter pylori.*

Introduction

Multi-particulate drug delivery systems are meant for oral dosage forms consisting of a multiplicity of small discrete units, which exhibit some desired characteristics. In these systems, the dosage of the drug substances is divided on a plurality of subunit, typically consisting of thousands of spherical particles with diameter of 0.05-2.00mm[1]. Thus multiparticulate dosage forms are pharmaceutical formulations in which the active substance is present as a number of small independent subunits. To deliver the recommended total dose, these subunits are filled into a sachet, capsule and encapsulated or compressed into a tablet[2]. Multiparticulates provide many advantages over single-unit systems because of their small size. Multiparticulates are less dependent on gastric emptyting, resulting in less inter and intra-subject variability in gastrointestinal transit time. They are also better distributed and less likely to cause local irritation[3]. They have potential benefits such as increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying[4].

Helicobactor pylori(*H.* pylori) is a small, spiral, microaerophilic, gram-negative bacteria with a 4-6 bulbous tipped sheathed flagella at one end, which helps it to penetrate the gastric mucosa and colonize on the

gastric antrum[5,6]. Although *H.* pyloriare highly sensitive to most antibiotics, its eradication from patients is very difficult, even with the current best therapies[7, 8]. Conventional tablets or capsules are, in general, used for eradication therapy, but these preparations do not remain in the stomach for the long time. Therefore, it is difficult to reach minimum effective concentrations in the gastric mucus where *H.* pyloricolonize. If *H. Pylori* is not eradicated from stomach its infection can lead to problems such as ulcers developing in stomach or duodenum. *H. pylori* infection is also associated with stomach cancer and an inflammation inside your stomach known as "gastritis".

To overcome the problems in *H.* pylori treatment, a novel drug delivery system that will localize the antibiotic at the site of infection to achieve bactericidal concentration is highly desirable. Floating drug delivery systems have a bulk density lower than gastric fluids and thus remain buoyant in the stomach for a prolonged period of time, without affecting the gastric emptying rate. While the system is floating on the gastric contents, the drug is released slowly at a desired rate from the system and remaining part of the residual system is slowly emptied from the stomach and enhanced gastroretentive time of the drug[9, 10, 11]. The extended retention of the drug can maintain a higher antibiotic concentration in the gastric region where *H.* pyloriexists and thereby improve the therapeutic efficacy.

Based on the above mentioned concept, to enhance the efficacy, an attempt has been made to develop oil entrapped floating alginate and pectin beads for stomach specific delivery of Tinidazole for treatment of *H.* pyloriinfection[12]**.**

Materials and Methods

Materials

Tinidazole was procured as gift sample from Nacto Pharmaceuticals Ltd, Hyderabad, India. Sodium alginate and pectin were purchased from Sigma Aldrich Chemicals (Mumbai, India). Peppermint oil and calcium chloride were purchased from Merck, Mumbai, India. All chemicals and reagents used were of analytical grade. Deionized water was used throughout the study.

Preparation of Tinidazole Floating Beads by Ionotropically Emulsion Gelation Method[13]

Tinidazole loaded peppermint oil entrapped multi-unit gel beads were prepared by emulsion gelation method. In this method, different polymer ratios of sodium alginate and pectin (1:1), (2.5:1.5) & (1.5:2.5) were taken in 25ml distilled water to prepare polymers solutions followed by addition of Tinidazole (1gm) in the polymers solutions. Peppermint oil (5, 10, 15%v/v) was added drop wise to the aqueous slurry with stirring by high mixer homogenizer fitted with stirring shaft with blade type impeller.

The stirring was continued for 1 hour to get a stable o/w type emulsion. Finally this emulsion was dropped through 20G needle in to 3%w/v calcium chloride solution to get spherical beads. These beads were kept in contact with calcium chloride solution for 30 minutes and then removed from calcium chloride solution by straining, washed, air dried and stored in desiccators for further studies. The formulation variables are given in **table 1**.

Table-1: Formulation Composition

Formulation code	Tinidazole (%w/v)	Sodium alginate (%w/v)	Pectin (%w/v)	Oil (ml)	Calcium chloride (%w/v)
SP_1	1	1	1	5	3
SP_2	1	1	1	10	3
SP_3	1	1	1	15	3
SP_4	1	2.5	1.5	5	3
SP_5	1	2.5	1.5	10	3
SP_6	1	2.5	1.5	15	3
SP_7	1	1.5	2.5	5	3
SP_8	1	1.5	2.5	10	3
SP_9	1	1.5	2.5	15	3

Evaluation of the Floating Beads

Particle Size Analysis[14]

The sample of prepared microbeads was randomly selected and their size was determined using an optical microscope with the help of eye piece and stage micrometer. In all measurements at least 50 beads in five different fields were examined. Each experiment was carried out in triplicate.

Scanning Electron Microscopy (SEM) [15]

Morphological examination of the surface and internal structure of the dried Tinidazole beads was carried out using a scanning electron microscope equipped with secondary electron detector at an accelerating voltage of 10 kV. The samples were coated with gold to a thickness of about 30 nm in a vacuum evaporator. The internal structure of beads was examined by cutting them with a steel blade.

UV-Visible Spectroscopy [16]

Tinidazole solutions were prepared in fasted state gastric fluid (pH1.2) and then diluted suitably. The UV spectrums of thesolutions were taken on UV Spectrophotometer. The solutions exhibited UV maxima at 278nm in fasted state gastric fluid.

Fourier Transforms Infrared Radiation Measurement (FTIR) [17]

Drug polymer interactions were studied by FT-IR spectroscopy. Tinidazole, polymers and physical mixtures of samples were weighed and mixed properly with Potassium bromide to a uniform mixture. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure. The IR spectrum of the pellet from 500-4000cm^{-1} was recorded taking air as the reference and compared to study any interference.

Flow Property [18]

The flow characteristics are measured by angle of repose. Angle of repose was determined by using funnel method; the accurately weighed beads were taken in funnel. The height of funnel was adjusted in such a way that the tip of funnel just touches the apex of heap of blends. The blends were allowed to flow through funnel freely on to surface. The diameter of powder cone was measured; angle of repose was calculated by using following equation.

$\tan \theta = h/r$

Determination of Density [19, 20]

The tapped density was measured in a 10 ml graduated measuring cylinder. Tapped density is the volume of powder determined by tapping by using a measuring cylinder containing weighed amount of sample. The cylinder containing known amount of microspheres was tapped for about 1 minute on a tapped density apparatus until it gives constant volume.

Tapped Density = Mass of microspheres/Volume of microspheres after tapping

Determination of Beads Buoyancy [21]

The floating ability was determined using USP dissolution tester apparatus II (Paddle method).Fifty beads were introduced in the vessels and the paddles were rotated at 50 rpm in 900 ml fasted state gastric fluid pH 1.2, maintained at 37±0.5 °C. The floating ability of the beads was measured by visual observation and the percent of floating beads was the average of three determinations. The preparation was considered to have buoyancy only when all beads floated on the test solution immediately or within a lag time which did not exceed 2 min.

Biodegradability Studies of Gel Beads [15]

The biodegradability studies were carried out using USP rotating basket apparatus. Amounts ofbeads (200 mg) were introduced into the baskets which were rotated at 50 rpm in 900 ml of 1.2 pH buffer solution maintained at 37 ± 0.5 °C.

Yield of Production[22]

The yields of production of micro beads 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 micro beads and percent production yields were calculated as per the formula mentioned below.

Percentage yield = Practical yield/ Theoretical yield ×100

Estimation of Drug Content and Percentage Entrapment Efficiency [23, 24]

An accurately weighed sample of beads(100mg) was crushed in a mortar; the crushed materials were dissolved in 100ml of fasted state gastric fluid pH 1.2. The sample was analysed by UV-Visible spectrophotometer at λ max 278nm against buffer pH 1.2. The drug entrapment efficiency percent can be calculated using the following equation.

%EE = Actual drug content/ theoretical drug content x 100

In Vitro Drug Release Study [25, 26]

In vitro dissolution studies were performed for all the formulations using USP Dissolution test apparatus I (basket type). An accurately weighed sample of beads (250mg) containing Tinidazole drug was dropped into 900ml of fasted state gastric fluid pH 1.2 maintained at temperature of (37±0.5º) and stirred at a speed of 50 rpm. At predetermined time intervals 5ml aliquot of the sample was withdrawn and the volume was replaced with an equivalent amount of plain dissolution medium. The collected samples werefiltered using Whattman's filter paper andanalyzed at λ max 278nm using a UV-visible spectrophotometer against buffer pH 1.2 as blank.

Results And Discussion

Particle Size Characterization

The mean particle size of the 9 formulations was between 1.12±0.06 and 1.91±0.08, revealed in **table 2**. When the beads were dried the higher volatile oil evaporated quickly leading to the uneven morphological characteristics and also alters the decrease in particle size. By visual inspection; the dried beads were of spherical geometry.

Table-2: Representing Particle Size, Flow Property, Tapped Density of Formulations

Formulation code	Particle size (mm)	Angle of repose	Free flowing	Tapped density (g/ml)
SP_1	1.91±0.08	17.20º	Yes	0.53
SP_2	1.16±0.08	12.63°	Yes	0.50
SP_3	1.57±0.08	14.30°	Yes	0.48
SP_4	1.39±0.07	13.75°	Yes	0.45
SP_5	1.13±0.07	14.40°	Yes	0.40
SP_6	1.22±0.07	15.12°	Yes	0.33
SP_7	1.55±0.06	15.10°	Yes	0.37
SP_8	1.12±0.06	10.20º	Yes	0.39
SP_9	1.68±0.06	15.60º	Yes	0.41

Scanning Electron Microscopy (SEM)

Before drying the cross section images of swelled Tinidazole beads were taken and small pocket structures **(Fig.2)**were found on the interior part of the beads showing the presence of oil droplets in the polymer matrix.SEM study of Tinidazole beads showed spherical morphology with rough or porous surface having corrugations where small pores or minute channels distributed throughout the surface **(Fig.3)**and **(Fig.4).**

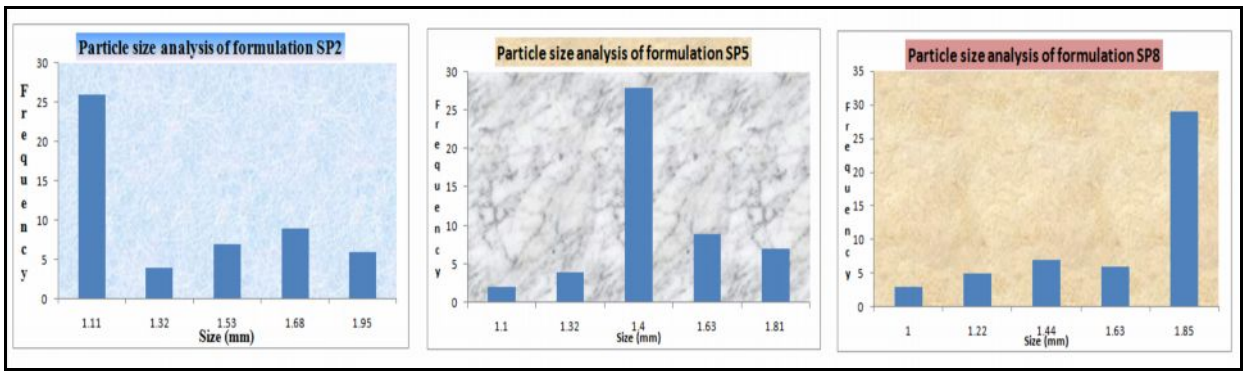

Fig.1: Histogram Representing Particle Size

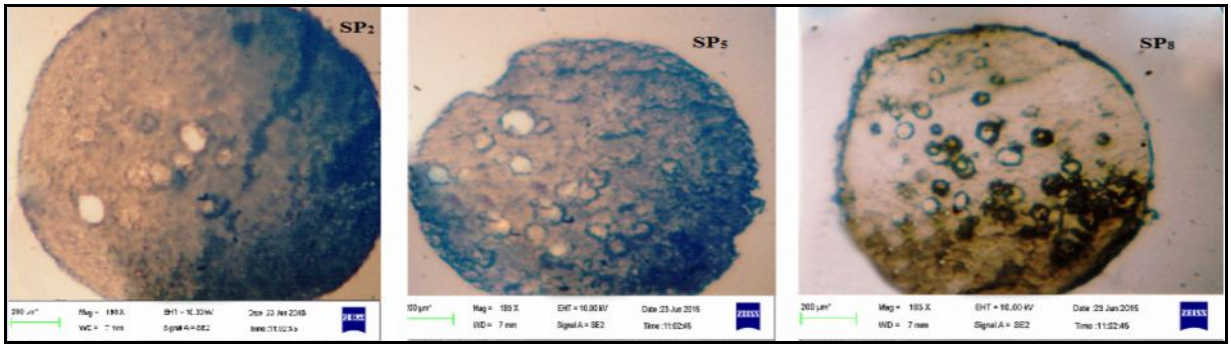

Fig.2: Sem Images of Tinidazole Microbeads (Cross Sectional Structure Before Drying)

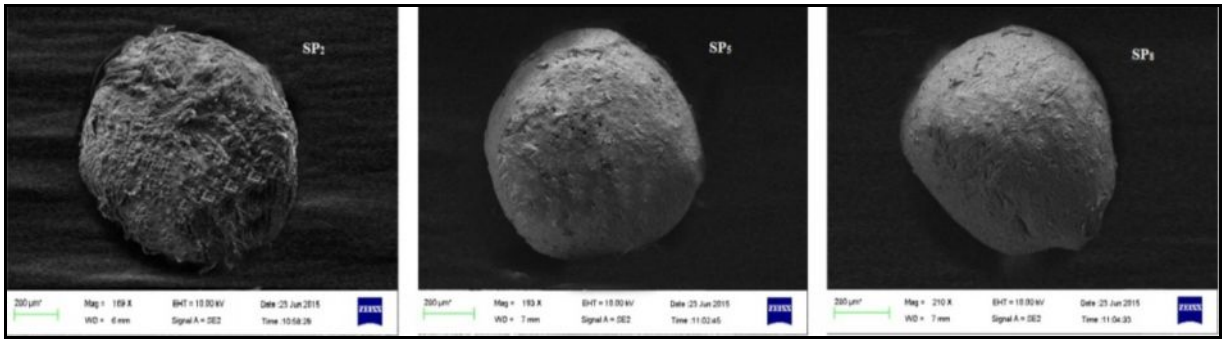

Fig.3: Sem Images of Tinidazole Microbeads (External Structure)

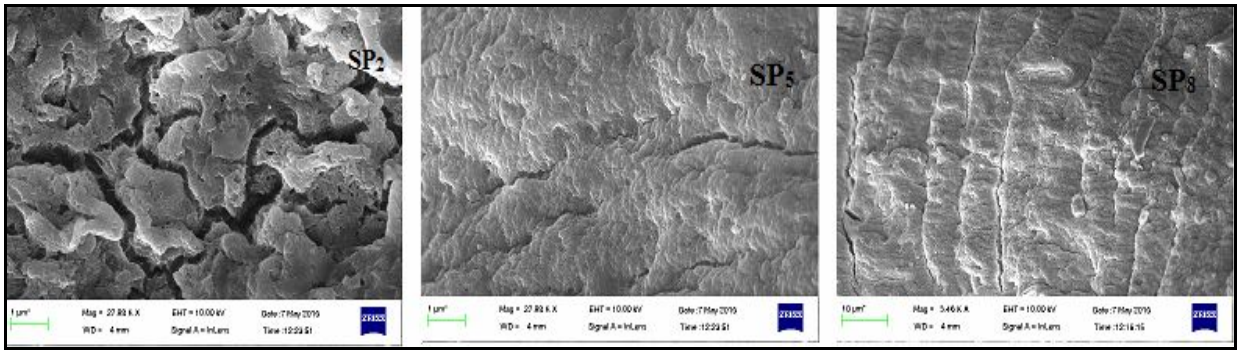

Fig.4: Sem Images of Tinidazole Microbeads (Enlarged External Structure)

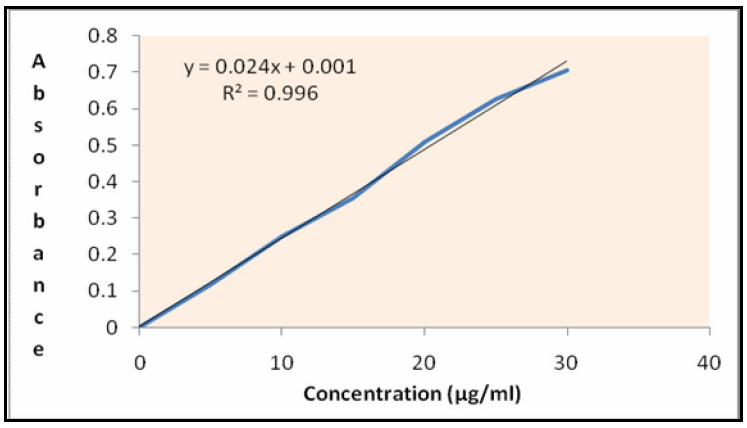

Fig. 5: Calibration Curve of Tinidazole At Ph 1.2

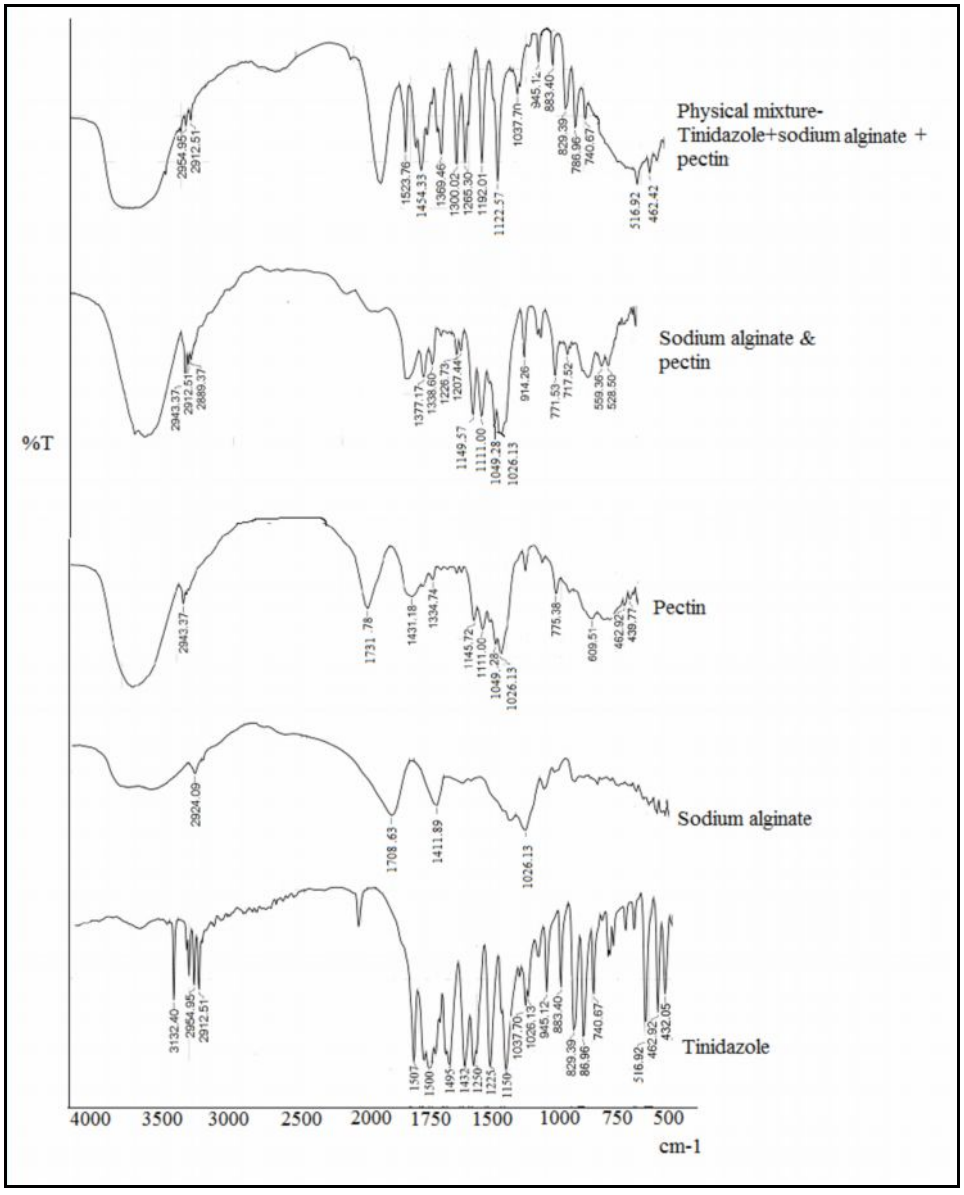

Fig.6: IR Spectrum of Drug, Polymers and Physical Mixture

UV-Visible Spectroscopy

The linear regression data obtained for the calibration curve showed a good linear relationship when a graph of concentration versus absorbance was plotted **(Fig.5)**.

Fourier Transforms Infrared Radiation Measurement (FTIR)

IR spectra of pure drug, sodium alginate, pectin and their combinations were done**(Fig. 6)**. It was found that, in IR spectra all peaks were found intact. Hence, no interaction between them and they are found to be compatible.

Flow Property

The flow properties of the formulations were investigated by measuring the angle of repose. All the formulations showed excellent flow. The comparative angles of repose were summarized in **table 2**.

Determination of Density

The tapped density of various formulations were found to be within the range of 0.33-0.53g/mlas shown in **table 2**, (i.e.) the results of all the formulations obtained were less than the density of fasted state gastric fluid pH 1.2 (1.004g/ml).

Determination of Beads Buoyancy

Table-3: Representing Buoyancy & Biodegradibility of the Formulations

Formulation code	Byouancy		Biodegradability
	pH 1.2	Water	
SP_1	>6hr	>6hr	<3hr
SP_2	>12hr	>12hr	<3hr
SP_3	>8hr	>8hr	<3hr
SP_4	>6hr	>6hr	<3hr
SP_5	>12hr	>12hr	<3hr
SP_6	>8hr	>8hr	<3hr
SP_7	>6hr	>6hr	<3hr
SP_8	>12hr	>12hr	<3hr
SP_9	>8hr	>8hr	<3hr

The buoyancy test of Tinidazole beads was performed to investigate the floatability of prepared beads. The data of floating properties of beads are shown in **table 3**. In alginate and pectin solution, peppermint oil gets emulsifies and undergoes fast gelation with the entrapment of oil in the polymer matrices resulting in number of oily pockets which may be the reason for beads buoyancy. Oil leakage has been observed in formulations prepared with 15% v/v of oil leading to their reduced floating time. The formulas containing 5% v/v oil had short floating time compared to formula containing 10% v/v peppermint oil.

Biodegradability Study of Gel Beads

Biodegradability studies revealed that the formulations were found to disintegrate and dissolve in intestinal pH within 3 hours **table3**. Formulations seemed to completely biodegrade in intestinal fluid and it is the pH of media, which is responsible for slow dissolution of the beads in intestinal fluid. This indicates that after gastric emptying the regular shaped beads, gradually become rough with an irregular surface and thereafter was degraded. Thus the beads proved to be suitable gastro retentive dosage form, as they have a rigid structure that resist biodegradation in gastric pH but exhibit complete biodegradation in buffer pH 8.

Percentage Yield

The percentage yield of all the formulations in a range of 61% to 85% shown in **table 4** was found to be satisfactory.

Table-4: Representing % Yield, Drug Content & %Entrapment Efficiency of the Formulations

Formulation code	Yield (%)	Drug content at pH 1.2 (mg)	Entrapment efficiency (%)
SP_1	61.2	0.69	65.36
SP_2	85.0	0.75	79.49
SP_3	69.8	0.71	84.56
SP_4	63.0	0.77	89.76
SP_5	84.1	0.89	91.18
SP_6	59.0	0.81	93.45
SP_7	63.9	0.88	90.10
SP_8	84.0	0.95	92.73
SP_9	66.8	0.89	96.88

Estimation of Drug Content and Percentage Entrapment Efficiency

Drug content of formulations at pH 1.2 were revealed in **table 4**. By comparing (SP1,SP4&SP7), (SP2,SP5&SP8), (SP3,SP6&SP9) it was found that formulas affected by using different peppermint oil percentage, as formulas used 15% v/v peppermint oil have lower percentage entrapment efficiency than those used 5 and 10% v/v. Since as the percentage of oil increases the barrier action of entrapped oil droplets protect the drug from diffusion to the surrounding medium during gelation process. Increased volume of oil occupied most of the volume of the bead and prevented the entrapment of sufficient amount of the drug.

In Vitro Drug Release

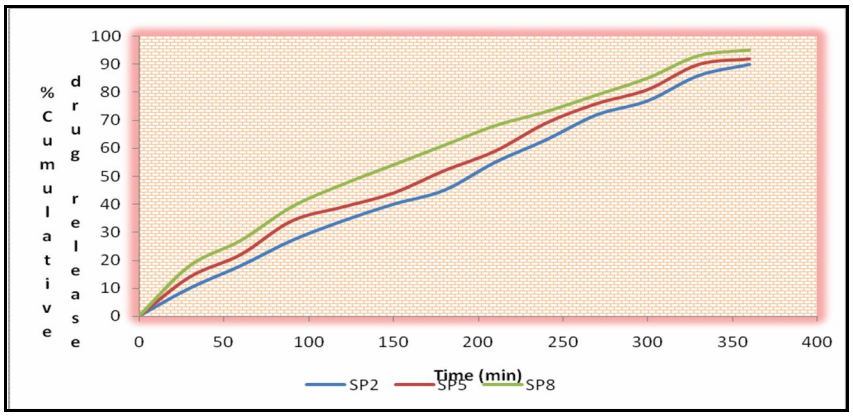

Fig.:7- In-Vitro Drug Release Kinetics For Sp-2, Sp-5 And Sp-8 Formulations of Tinidazole Microbeads At Ph 1.2

The results of in vitro dissolution studies of formulations at fasted state gastric pH were shown in **table 5**. Different batches of the formulations were able to release up (57 to 95%) drug after6hours under study**(Fig. 7)**. The drug release was found to be slower in formulation with higher oil concentration. The slow release of the drug from beads may be due to the formation of drug-oil dispersion system in oil pockets of the beads. In order to investigate the mechanism of drug release from the buoyant beads, the data were fitted to models representing zero order rate equation which describe the systems where the release rate is independent of the concentration of dissolved species. In vitro data were also fitted to Higuchi's square root of time model[27], which describes the release from systems where the solid drug is dispersed in an insoluble matrix, and the rate of drug release is related to the rate of drug diffusion. All the formulations followed zero order kinetics as evidenced by R^2 values as shown in **table 6**, which were higher when fitted to zero order kinetics which explains that the drug release rate is independent of its concentration. Whereas with evidence to b value the drug release by diffusion.

Table-5: Cumulative Release Profile of Formulations in Fasted State Gastric Fluid pH 1.2

TIME (Min)	Formulations (%Cumulative Drug Release)								
	SP_1	SP_2	SP_3	SP_4	SP_5	SP_6	SP_7	SP_8	SP_9
0	0	0	0	0	0	0	0	0	0
30	5	5	6	3	7	11	13	8	12
60	11	15	18	12	21	20	23	19	23
90	15	32	25	25	28	26	36	26	37
120	17	38	30	30	36	39	45	33	45
150	25	44	36	36	40	48	48	36	49
180	31	50	45	42	46	58	56	41	56
210	39	58	53	44	54	60	60	48	59
240	42	70	59	48	62	66	65	59	65
270	46	79	67	52	71	71	70	64	70
300	53	82	76	55	80	79	72	76	77
330	55	87	79	59	84	83	75	88	87
360	57	90	82	63	92	85	79	95	81

Table-6: Release Mechanisms of Tinidazole From Oil Entrapped Beads

Formulation Code	Fasted State pH 1.2			
	Zero order		Higuchi	
	R^2	b	R^2	b
SP2	0.951	0.28	0.839	3.798
SP5	0.981	0.258	0.8	3.5
SP8	0.966	0.23	0.817	3.12

Conclusion

A new gastroretentive multiparticulate hollow floating drug delivery system of oil entrapped sodium alginate and pectin beads was designed and prepared by emulsion gelation method and its morphology and release characteristics were studied. The beads were easy to prepare and their particle size increases with increase in the amount of the oil phase. The pore size of oil entrapped beads was affected by concentration of oil. The beads showed excellent sustaining properties as compared to conventional beads. Thus, oil entrapment technique novel approach towards the development of multiparticulate system for drug delivery.

Acknowledgement

I would like to express my special thanks of gratitude to my guide Shashi Kiran Misra as well as our HOD Nisha Sharma who gave me the golden opportunity to carry out the research work and I came to know about so many new things I am really thankful to them. I would also wish to thank the Management, Institute of Pharmacy, for providing facilities to carry out the research work.

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