



## FORMULATION AND EVALUATION OF TINIDAZOLE MICROSPHERES

Ravi Jayant\*, Mohammad Rashid, Sokindra Kumar, Arun Kumar

1. R. V. Northland Institute, Gr Noida, India

ARTICLE INFO	ABSTRACT
<p><b>Published on:</b> 15-03-2016 <b>ISSN:</b> 0975-8216</p>	<p>The microspheres of Tinidazole in six batches was prepared using Hydroxy propyl methyl cellulose (K4M) and ethyl cellulose in different drug and polymer ratio taking into account non-aqueous solvent evaporation method. The formulation of different batches was subjected to various physicochemical studies such as yield value, particle size determination, buoyancy percentage, drug entrapment efficiency and in vitro drug release determination. In -vitro release study of each formulation was carried out on dissolution apparatus using 1.2 pH HCl buffer and simulated gastric fluid. Various results were inferred such as percentage yield value (78.8% to 92.14%) the particle size (244<math>\mu</math>m to 294.8<math>\mu</math>m), drug entrapment efficiency (32.9% to 60.6%) and buoyancy percentages (52.5% to 70%). The best drug release profiles were seen with formulation A<sub>2</sub> at the ratio of drug: ethyl cellulose of 1:2.5.</p>
<p><b>Keywords:</b> Microspheres; Tinidazole; ethyl cellulose; HPMC.</p>	
<p><b>Corresponding author:</b> Ravi Jayant R. V. Northland Institute, Gr Noida, India</p>	

### INTRODUCTION

The present era comprises of devouring challenges in the field of public health care and unrelenting researches have laid down several queries towards the design of various formulations capably possessing highest possible pharmacodynamic as well

as the pharmacokinetic properties to render the particular formulation appreciably significant acceptability to the patient care systems prevailing all across the globe with narrower spectrum of toxicities. Such queries encompass problems of variable intensities and hence invite the attention of

manufacturing chemists to devise new techniques which may exert control over the rate of drug delivery, sustain the duration of therapeutic activity and /or target the delivery of drug to a tissue or particular organ.

Microspheres are the formulation that can achieve maximum bioavailability with the desired properties of novel drug delivery systems.

## MATERIALS AND METHOD

**Materials:** All the chemicals used in this work were procured from industry of repute. The drug Diclofenac sodium was procured by the institute itself.

**Methods:**

**Preparation of Standard calibration curve in pH 1.2 Hydrochloric acid buffer:**

**(a) Stock-A (1000 µg/ml):**

150 mg of drug equivalent to 100 mg Tinidazole was accurately weighed and transferred into a 100 ml volumetric flask. The drug was then dissolved in 10 ml methanol and diluted up to the mark with pH 1.2 HCl buffer solution.

**(b) Stock-B (100µg/ml):**

From solution-A 10ml was pipetted out and diluted to 100ml using pH 1.2 HCl buffer solution. Different aliquots containing 5,

10, 15, 20, 25µg/ml of Tinidazole was prepared using Stock B.<sup>50</sup>

**Estimation of  $\lambda_{max}$ :**

A sample solution of (100 µg/ml) stock B was scanned at range of 200-400 nm to access the  $\lambda_{max}$  value for Tinidazole which was reproduced and confirmed by obtaining the overlain U V spectra of the drug with different concentrations i.e 5, 10, 15, 20, 25µg/ml.

The standard calibration curve was obtained with the samples of same concentrations as opted in the process.

## DRUG POLYMER COMPATIBILITY STUDY:

**FTIR analysis:**

The drug-polymer compatibility was studied by FTIR spectrophotometer. The mixture of drug and potassium bromide was ground into a fine powder using mortar Pestle and then compressed into a KBr discs in a hydraulic press at a pressure of 75 Kg/cm<sup>2</sup>. Each KBr disc was scanned 45 times at a resolution of 2 cm<sup>-1</sup>. The characteristic peaks were recorded.

## FORMULATION DESIGN:

The formulation was divided into six batches prepared with different ratio of suitably chosen polymers as depicted in the table below:

Table No.1: Formulation design of Microspheres:

Sr.no	Ingredients	A1	A2	A3	B1	B2	B3
1	Drug	1	1	1	1	1	1
2	Ethylcellulose(gm)	2	2.5	3	-----	-----	-----
3	HPMCK4M (gm)	-----	-----	-----	2	2.5	3
4	Ethanol (ml)	25	25	25	25	25	25
5	DCM (ml)	25	25	25	25	25	25
6	Tween-80 (ml)	0.18	0.18	0.18	0.18	0.18	0.18
7	Liquid paraffin (ml)	60	60	60	60	60	60
8	RPM	1200	1200	1200	1200	1200	1200

#### PREPARATION OF TINIDAZOLE FLOATING MICROSPHERES:

Microspheres containing Tinidazole as a core material were prepared by a non-aqueous solvent evaporation method. Drug and polymer were dispersed in the solvent (dichloromethane and ethanol in ratio 1:1). The slurry was slowly introduced into 30 ml of light liquid paraffin containing Tween 80 (0.01% w/v) as emulsifier with continuous stirring at 1200 rpm using a propeller type mechanical stirrer at room temperature. The solution was stirred for 2 hrs for complete evaporation of solvent and filtered. The microspheres thus obtained were washed repeatedly and dried at room temperature until free flowing particles were obtained.<sup>23</sup>

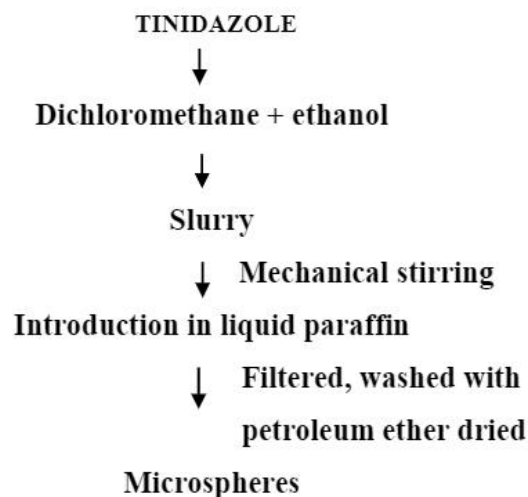


Fig no.1: Schematic representation:

#### EVALUATION OF MICROSPHERES:

##### Percentage yield (% yield):

The percentage yield was determined on the basis of method as reported by **Amitava et.al.**<sup>53</sup> The yield was calculated as the weight of the microspheres recovered from each batch divided by total weight of drug and polymer used in the preparation of the particular batch.



$$\% \text{ Yield} = \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \times 100$$

#### Particle size analysis:

The analysis of particle size was carried out using a photomicroscope fitted with micrometric tools. The particle size distribution was determined and the average diameter was calculated for each batch of microspheres.

#### Bulk density:

The principle involved in such determination was derived from the text reference. The Bulk density was calculated by manual tapping method introducing microspheres in 10 ml graduated cylinder. The ratio of weight of particles to that of its volume gave the bulk density as mentioned below:

$$\text{B.D} = \frac{\text{wt.of microspheres}}{\text{vol.of microspheres}}$$

#### Buoyancy percentage:

The experiment to determine this parameter was performed as reported by **Anand et. al.** The microspheres (0.3 g) were spread over the surface of USP (TDT 06L) dissolution apparatus (Type II) filled with 900 ml of 1.2pH HCl buffer containing 0.01% of Tween 80. The medium was agitated with a paddle rotating at 100 rpm for 12 h. The floating and the settled portions of microspheres were recovered, dried and weighed separately. Buoyancy percentage

was calculated as the ratio of the mass of particles that remained floating and the total mass of the recovered microspheres.

#### Drug Entrapment Studies:

The practical drug content was determined by UV analysis and entrapment efficiency was calculated.

#### Surface Morphology:

The morphology and surface characteristics of microspheres were studied by Scanning electron microscopy (Quanta FEI 200F). The dried microspheres were coated with gold foil (100 Å) under an argon atmosphere in a gold coating unit and micrographs were obtained at both higher and lower resolutions.

#### In-Vitro Release Studies:

In vitro drug release studies were carried out for all batches by using USP (TDT 06L) type I dissolution test apparatus. The sample of Microspheres equivalent to 100 mg of the pure Tinidazole was used for the study. 5 ml sample were withdrawn, diluted suitably and analyzed for the drug content spectrophotometrically at  $\lambda_{\text{max}}$  318 nm using dissolution media (pH 1.2 HCl Buffer and SGF) as blank.

#### Stability Study:

The stability study of drug loaded microspheres was carried out for a period of

90 days at  $40 \pm 2^\circ\text{C}$  temperature and relative humidity of  $75 \pm 5\%$  using stability chamber. Sample was collected after 90 days and evaluated for drug loading.

## RESULTS AND DISCUSSION

### Spectrophotometric scan of Tinidazole.

The stock Solution ( $100 \mu\text{g/ml}$ ) of Tinidazole was prepared using 1.2 pH HCl buffer and scanned between 200-400nm. The scan concluded  $\lambda_{\text{max}}$  of 318 nm for 1.2 pH HCl buffer.

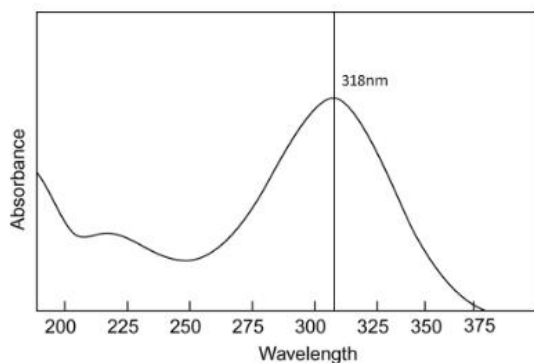


Fig no. 2: Spectrophotometric scan of Tinidazole.

### Validation of $\lambda_{\text{max}}$ :

The samples containing different concentration of the drug as depicted in table No: 6, were run and overlain spectra describing the reproducibility of the  $\lambda_{\text{max}}$  (earlier scanned) was obtained that confirmed and validated the process.

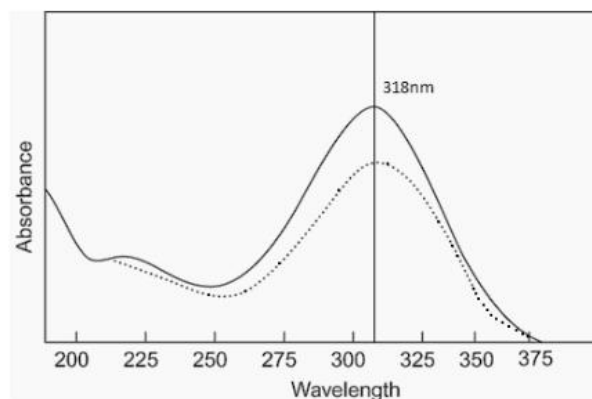


Fig no. 3: Spectrophotometric overlain scan of Tinidazole

### Preparation of calibration curve in 1.2 pH HCl buffer:

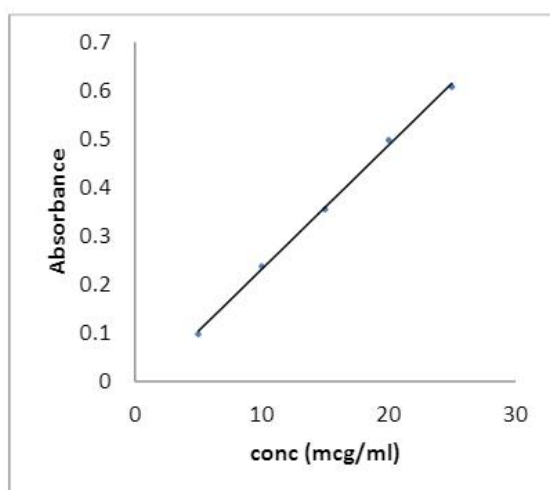
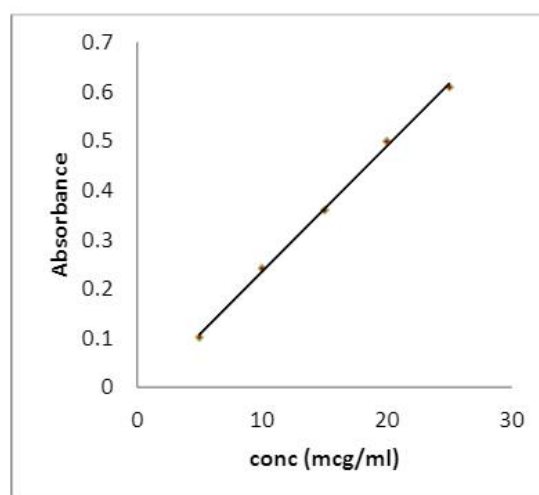
Various samples with different concentrations were loaded on the UV spectrophotometer and respective absorbances were obtained at the  $\lambda_{\text{max}}$  318 nm. A graph was plotted (Conc. Vs Absorbance) which resulted a straight line concluding that the drug followed Beer's Lambert's Law at the concentration range of 5-25  $\mu\text{g/ml}$ .

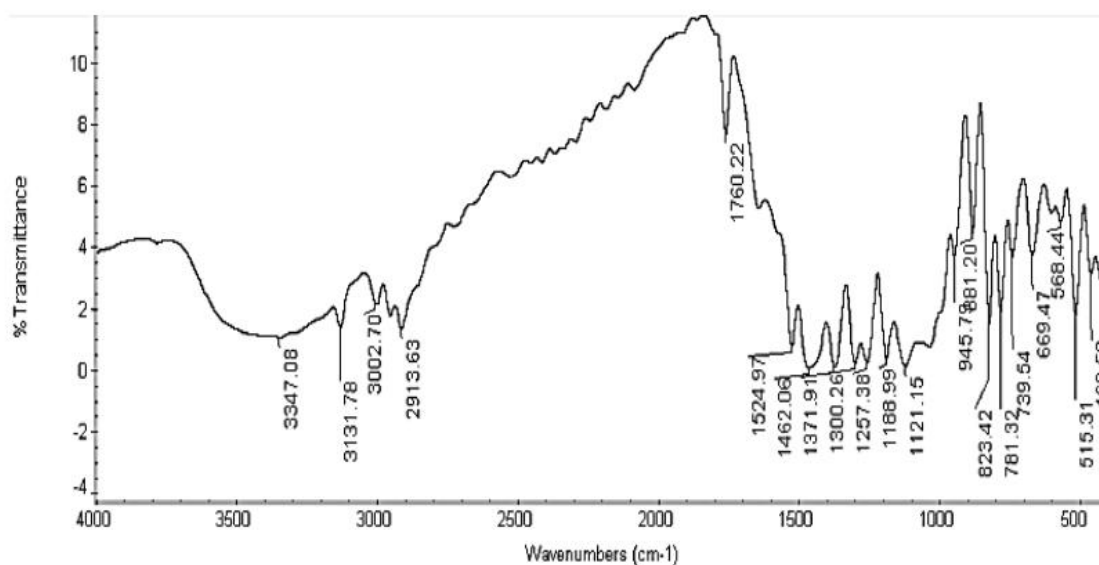
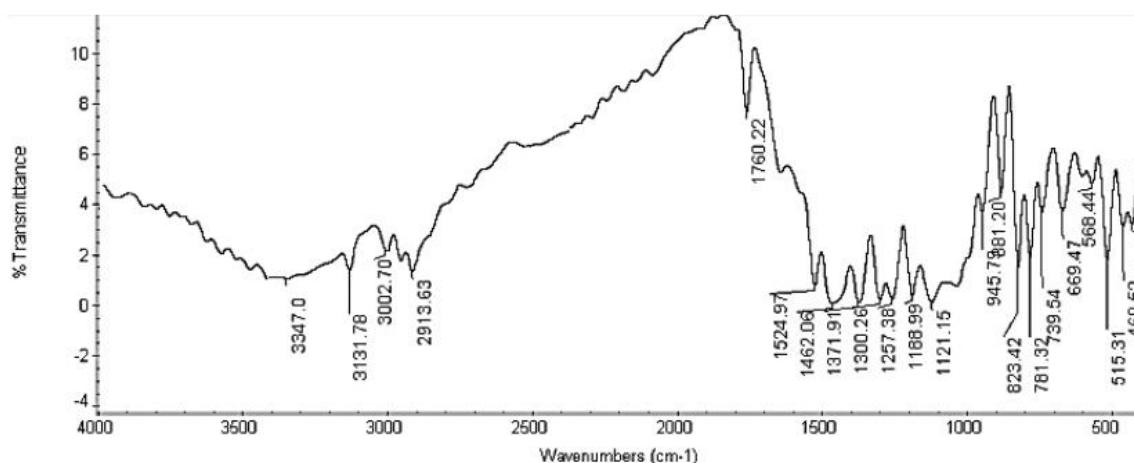
The regression analysis was carried out on these experimental data and Y &  $r^2$  values were calculated.

The obtained values for  $Y = 0.024x$  for  $r^2 = 0.995$  in 1.2 pH HCl buffer and  $Y = 0.024x$  for  $r^2 = 0.997$  in simulated gastric fluid were recorded.

**Table No. 2: Concentration Vs Absorbance data Tinidazole in 1.2 pH HCl buffer.**

Sr. No.	Concentration (mcg/ml)	Absorbance (mean $\pm$ SD)	Absorbance (mean $\pm$ SD)
1	0	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
2	5	0.098 $\pm$ 0.01	0.101 $\pm$ 0.01
3	10	0.238 $\pm$ 0.01	0.241 $\pm$ 0.01
4	15	0.355 $\pm$ 0.03	0.359 $\pm$ 0.00
5	20	0.498 $\pm$ 0.02	0.499 $\pm$ 0.00
6	25	0.608 $\pm$ 0.02	0.609 $\pm$ 0.02

**Preparation of Standard calibration curve of Tinidazole in 1.2 pH HCl buffer:****Fig no. 4: Standard calibration curve of Tinidazole in 1.2 pH HCl buffer.****Fig no. 5: Standard calibration curve of Tinidazole in simulated gastric fluid:**

**COMPATIBILITY STUDIES:****FTIR spectra of Tinidazole pure drug:****Fig no. 6 : FTIR spectra of Tinidazole pure drug.****Figno. 7: FTIR spectra of formulation blend.**

The IR absorption spectrum of Tinidazole was obtained using KBR pellet technique and peaks obtained were compared with the reference drug.

Similarly IR spectra of formulation blends containing ethyl cellulose and HPMC K4M were obtained. The compatibility was studied with the spectra

produced with drug + polymer combination comparing individual spectrum of drug.

**EVALUATION PARAMETERS:**

The analysis was performed for all six batches and the results as shown in table below:



Table no.3: Particle size analysis of batch A<sub>1</sub> to B<sub>3</sub>

Formulation Codes	Mean Particle Size (µm)	Bulk density (mg/ml)	Buoyancy percentage (%)	% drug content	Theoretical Yield (g)	Practical Yield (g)	Percentage Yield (%)
A <sub>1</sub>	244±16	0.833	62.5	41.7	6	5.31	88.5
A <sub>2</sub>	269±8	0.769	70	60.6	7	6.45	92.14
A <sub>3</sub>	292±24	0.757	65	56.6	8	6.31	78.8
B <sub>1</sub>	266±17	0.750	52.5	32.9	6	5.450	90.8
B <sub>2</sub>	272±6	0.769	57.5	42.7	7	6.29	89.8
B <sub>3</sub>	294.8±9	0.833	61.5	57.2	8	6.35	79.37

**Surface morphology:**

The surface morphology of microspheres belonging to significant batches i.e.

A<sub>2</sub>&B<sub>3</sub> was examined by scanning electron microscopy.

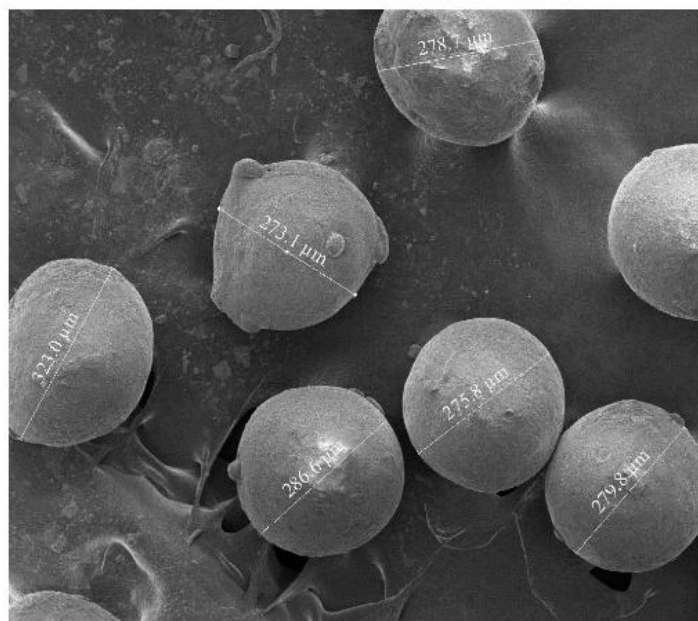
**SEM image of formulation**

Fig no.8: Scanning Electron Microscopy of formulation



**IN-VITRO DISSOLUTION STUDIES:****Table no. 4: Comparative release kinetic data of batch A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub> in 1.2 pH HCl buffer**

SR NO	Time (minutes)	Time(t) <sup>1/2</sup>	% Drug Release					
			A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	60	7.7460	25.7349	21.6867	15.9036	7.3735	8.3855	6.9398
3	120	10.9545	28.6551	24.1687	19.8249	12.4419	13.1659	11.8631
4	180	13.4164	31.8390	25.7618	24.3112	17.5078	25.4604	18.0855
5	240	15.4919	33.5775	28.7997	27.3523	33.5616	31.2572	21.4177
6	300	17.3205	36.6156	30.1043	30.1027	45.8686	35.0227	25.9033
7	360	18.9737	38.9322	31.9852	33.4310	51.2316	41.9666	31.1131
8	420	20.4939	40.6697	36.7584	36.7600	56.0087	47.4683	38.2032
9	480	21.9089	43.1295	41.3902	41.3902	62.9537	52.2455	43.5605
10	540	23.2379	46.3129	43.1303	46.0218	67.5880	60.2026	50.7953
11	600	24.4949	51.2321	46.4575	50.7981	71.0630	67.4403	56.1528
12	660	25.6905	62.8039	51.2323	55.5745	76.9945	75.8339	59.9178
13	720	26.8328	67.2986	54.7075	60.4954	83.3626	78.0119	64.2593

**Table no. 5: Comparative release kinetic data of batch A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub> in simulated gastric fluid**

SR NO	Time (minutes)	Time(t) <sup>1/2</sup>	% Drug Release					
			A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	60	7.7460	28.1928	24.1446	18.3614	9.8313	10.8434	9.3976
3	120	10.9545	31.1157	26.6292	22.2855	14.9025	15.6265	14.3237
4	180	13.4164	34.2996	28.2223	26.7717	19.9684	27.9210	20.5460
5	240	15.4919	36.0381	31.2602	29.8129	36.0222	33.7178	23.8782
6	300	17.3205	39.0761	32.5648	32.5632	48.3292	37.4832	28.3639
7	360	18.9737	41.3928	34.4458	35.8916	53.6922	44.4271	33.5737
8	420	20.4939	43.1303	39.2190	39.2206	58.4692	49.9288	40.6638
9	480	21.9089	45.5900	43.8508	43.8508	65.4143	54.7060	46.0210
10	540	23.2379	48.7735	45.5908	48.4824	70.0485	62.6631	53.2559
11	600	24.4949	53.6927	48.9181	53.2586	73.5235	69.9009	58.6133
12	660	25.6905	65.2644	53.6929	58.0350	79.4551	78.2945	62.3783
13	720	26.8328	69.7592	57.1680	62.9560	85.8231	80.4724	66.7198

## IN-VITRO COMPARATIVE STUDY OF FINALIZED FORMULATION A<sub>2</sub>, B<sub>3</sub> WITH SIMULATED GASTRIC FLUID:

Table no. 6: Comparative release kinetic data of batch A<sub>2</sub>, B<sub>3</sub> in simulated gastric fluid:

SR NO	Time (minutes)	Time(t) <sup>1/2</sup>	% Drug Release	
			A <sub>2</sub>	B <sub>3</sub>
1	0.00	0.00	0.00	0.00
2	60	7.7460	24.1446	9.3976
3	120	10.9545	26.6292	14.3237
4	180	13.4164	28.2223	20.5460
5	240	15.4919	31.2602	23.8782
6	300	17.3205	32.5648	28.3639
7	360	18.9737	34.4458	33.5737
8	420	20.4939	39.2190	40.6638
9	480	21.9089	43.8508	46.0210
10	540	23.2379	45.5908	53.2559
11	600	24.4949	48.9181	58.6133
12	660	25.6905	53.6929	62.3783
13	720	26.8328	57.1680	66.7198

### 4.6.2.1 Zero order, Higuchi plot and Korsmeyerpeppas model of formulation A<sub>2</sub>, B<sub>3</sub> in simulated gastric fluid

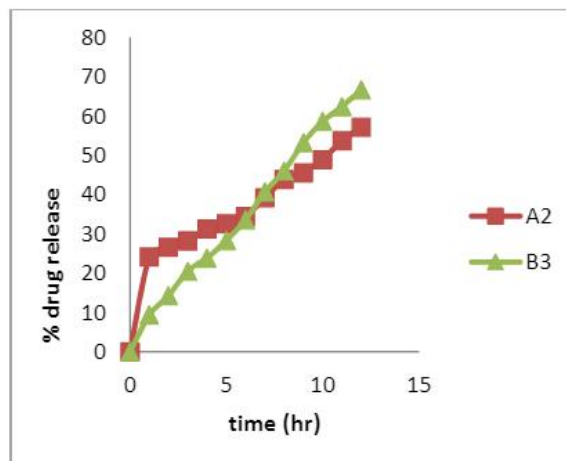


Fig no . 9: Comparative Kinetic Zero order release of batch A<sub>2</sub>, B<sub>3</sub> in simulated gastric fluid

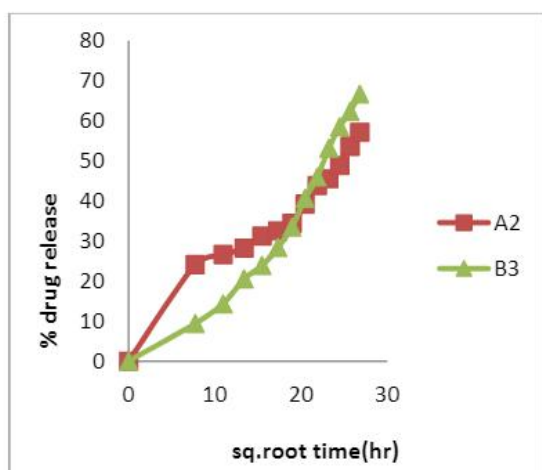


Fig no. 10: Comparative Higuchi plot of batch A<sub>2</sub>, B<sub>3</sub> in simulated gastric fluid

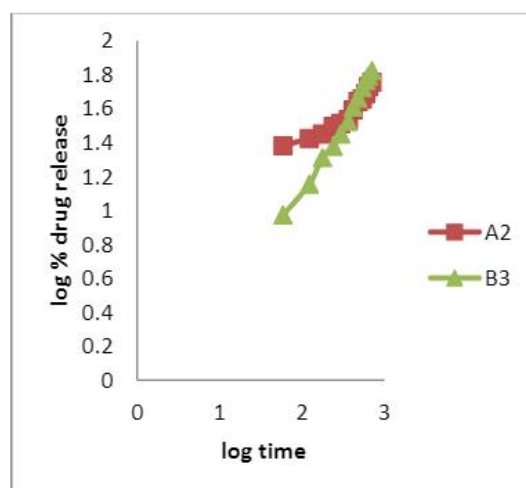


Fig no. 11: Comparative Korsmeyerpeppas model of batch A<sub>2</sub>, B<sub>3</sub> in simulated gastric fluid

### STABILITY DATA OF TINIDAZOLE MICROSPHERES:

The stability study was performed on the prepared formulations as per the ICH guidelines at accelerated condition.

( $40^{\circ}\pm 2^{\circ}\text{C}$ ,  $75\%\pm 5\%$  RH) and it showed that the formulations were stable with no physical change and also there was no significant reduction in drug content (table no: 33)

Table no. 7: Stability study of formulation A<sub>2</sub>, B<sub>3</sub> at  $45\pm 2^{\circ}\text{C}$ , at  $75\%\pm 5\%$  R.H:

Stability study	A <sub>2</sub>			B <sub>3</sub>		
	0 Day	30 days	90 days	0 day	30 days	90 days
Physical description	Buff yellow	No change	No change	Cream	No change	Light cream
Assay (% drug remaining)	100%	99.66%	99.07%	100%	99.68%	98.98%
Flow ability	good	good	good	good	good	good

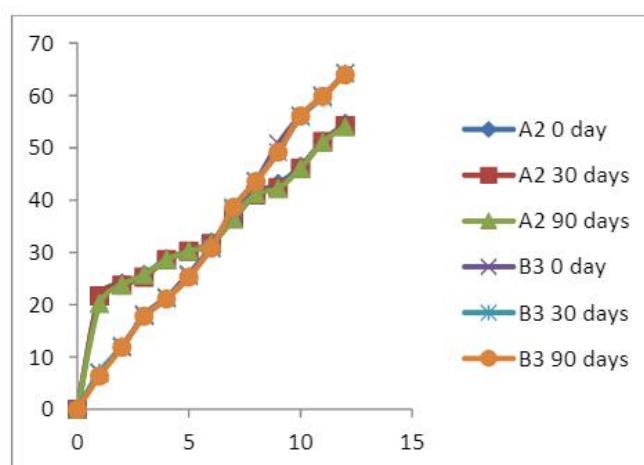


Fig no. 12: Comparative release profile of formulation A<sub>2</sub>, B<sub>3</sub> on stability



## CONCLUSION

The microspheres of Tinidazole were prepared with two polymers i.e. ethyl cellulose and HPMC K4M. The particle size determination by SEM techniques revealed that the mean particle diameter were in the range of 244  $\mu$ m -294  $\mu$ m. The mean particle size were in the order of  $A_1 < B_1 < A_2 < B_2 < A_3 < B_3$ . The morphological studies were conclusive to spherical shaped particles with smooth surface.

The characteristic peaks of the pure drug were compared with that obtained with microspheres in different batches which remained nearly same. Conclusively Tinidazole was found to be compatible with the two polymers and other ingredients incorporated in microspherical formulations.

The other physicochemical parameters determined with the microspheres were bulk density (0.75-0.83g/ml), % yield (92.14%- 78.8%), buoyancy % in 1.2 pH HCl buffer (70.0%- 52.5%) and Drug entrapment efficiency (60.6 % - 32.9%). The in vitro drug release in 1.2 pH HCl buffer ranged from 83.3% -54.7% while in simulated gastric fluid it ranged from 85.8% - 57.17%.

Conclusively the % yield was maximum with  $A_2$  and minimum with  $A_3$  batch. The drug entrapment efficiency was found to be of the order of  $B_1 < A_1 < B_2 < A_3 < B_3 < A_2$  indicating the best results with microspheres of  $A_2$  batch.

The in-vitro release of formulation  $A_2$  in 1.2 pH HCl buffer and in simulated gastric fluid (SGF) were 54.7% and 57.17% respectively which showed sustained release over a period of 12 hrs.

All above data satisfactorily complied with the characteristics requirements of the formulation as microspheres.

The present worker tends to provide impetus for future researchers to design such novel drug delivery systems which can supersede conventional dosage forms with significant pharmacokinetic and pharmacodynamics properties.

## REFERENCES

1. Chawla G; Gupta P; Koradia V; And Bansal A. K; "Gastro retention: A Means to Address Regional Variability in Intestinal Drug Absorption" Pharmaceutical Technology, 2003, pp. 50-52.
2. Nayak AK; Maji R; Das B; "Gastro retentive drug delivery systems: a Review" Asian Journal of Pharmaceutical and Clinical Research, 2010, 3(1), pp. 3-8.
3. Garg R; Gupta GD; "Progress in Controlled Gastro retentive Delivery Systems" Tropical Journal of Pharmaceutical Research, 2008, 7 (3), pp. 1055-1066.
4. Hilton AK; Deasy PB; "In vitro and in vivo evaluation of an oral sustained release floating dosage form of

- amoxicillin trihydrate"Int. J. Pharm.,1992, 86, pp. 79-88.
5. Seth PR;Tossounian; "The hydrodynamicallybalanced system, a novel drug Deliverysystem for oral use"Drug Dev. Ind Pharm.,1984, 10, pp. 313-339.
6. Harrigan RM; "Drug delivery device for preventing contact of undissolved drug with the stomach lining"US Patent4, 1977,55, pp.178.
7. Whitehead L; Fell JT;"Collett JH. Development of agastroretentive dosage form"Eur. J. Pharm.Sci.,1996,4 (1), pp.182.
8. Kawashima Y;Niwa T; Takeuchi H; Hino T;Itoh Y;"Hollow microspheres forUse as a floating controlled drug delivery system in the stomach"J. Pharm. Sci.,1992, 81, pp.135-140.
9. Rubinstein A; Friend DR;"Specific delivery to the gastrointestinal tract" In DombAJ (Ed.) Polymeric site specific pharmacotherapy, Wiley, Chichester, 1994,pp. 282-283.
10. Klausner EA; Lavy E; Stepensky D; Friedman M;Hoffman A; "Novel Gastro retentive dosage form: evaluation of gastroretentivity and its effectOn riboflavin absorption in dogs"Pharm. Res.,2002, 19, pp. 1516-1523.
11. Moes AJ;"Gastroretentive Dosage forms"Crit. Rev.Ther. Drug Carrier Syst.,1993,10, pp. 143-195.
12. Arora S; Ali J;Ahuja A;Khar RKand Baboota S;"Floating DrugDelivery Systems: A Review"AAPS PharmSciTech.,2005, 6 (3), pp.72-90.
13. Benita S;"Method and industrial application of microencapsulation" Marcel Dekker, Inc., 73, pp.1-2.
14. Das MK; Rao KR; "Evaluation of zidovudine encapsulated ethyl celluloseMicrospheresprepared by water-in-oil-in-oil (w/o/o) double emulsion solvent diffusionTechnique"ActPolonaise.Pharmaceutical.Drug.Res.2006,63, pp. 141-148.