DESIGN CHARCTERIZATION AND OPTIMIZATION OF COLON SPECIFIC TINIDAZOLE MICROSPHERES

Kothapally D. L.*, Sridhar Rao and Aleti P.

*Research Scholar, JJT University, Jhunjhunu, Rajasthan, India.
Department of Pharmaceutics, HOD Chaitanya College of Pharmacy Education and Research, Hanamkonda, Telangana.

*Corresponding Author: Kothapally D. L.

Research Scholar, JJT University, Jhunjhunu, Rajasthan, India.

ABSTRACT

The objective of the present study was to formulate and optimize colon targeted tinidazole microspheres. To achieve these objective nine formulations of microspheres were prepared by emulsion solvent evaporation method using Eudragit and Ethyl cellulose polymer. A 3^2 factorial design was employed in formulating the microspheres with concentration of surfactant (X_1) and stirring speed (X_2) as independent variables. Percent drug release was considered as dependent variable. The effect of drug-polymer concentration, surfactant concentration, cross-linking agent and stirring speed were evaluated with respect to entrapment efficiency, particle size, surface characteristics, micromeritic properties, DSC study and in vitro drug release studies. The particle size and entrapment efficiency were found to be varied by changing various formulation parameters like surfactant concentration and stirring speed etc. IR study confirmed the drug-polymer compatibility and scanning electron microscopy indicates that the microspheres have the rough and porous surface due to arising as a trace of solvent evaporation during the process.

KEYWORD: Eudragit, DSC, Microsphere.

INTRODUCTION

Oral drug delivery still is the preferred route of administration for drug products. The evolution of oral drug delivery technology may be described by a three-stage course to reach its current level. With every step forward in drug delivery technology, scientists strive to gain more control over the pharmacokinetics of the drug substance with the goal to increase the therapeutic benefit-risk ratio or to improve bioavailability.[1,2]

Delivery of drug substances to the ileocolonic region may be an essential element of successful drug treatment (improved efficacy or reduced systemic toxicity) in topical treatment of the colon. Release of mesalazine and corticosteroids in the ileocolonic region has proved to be a successful approach for the treatment of ulcerative colitis. Extension of this approach to other anti-inflammatory and immunosuppressive drug substances (e.g. 6-thioguanine, tacrolimus, ciclosporin A) is envisaged as a promise. Moreover, topical treatment of other colon pathologies also appears to be rational from a clinical pharmacological point of view, such as Crohn's disease (budesonide, infliximab), colon cancer (sulindac)[3,4] luminal amoebiasis (antibiotics), diarrhea (prebiotics) an inflammatory bowel disease (probiotics). The common denominator of these therapies is that a high intraluminal concentration of drug substance in the ascending colon is related to a beneficial outcome of drug treatment. Another reason for investigating oral ileocolonic drug delivery may be found in food science to support weight management and the treatment of obesitas. Consumer research has highlighted the need to better control hunger when on a diet to enhance and sustain compliance in maximizing weight loss success. According to recent market research in the United States, the majority (53%) of respondents claimto cheat on a diet because they are hungry Microspheres have played a major role in the development of controlled and or sustained release drug delivery systems. Microspheres have been of particular interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled drug release.[5] There are several publications based on drug-containing microspheres using the Eudragit series of polymers as the encapsulating materials. The Eudragits are a family of polymers based on acrylic and methacrylic acids suitable for use in orally administered drug delivery systems. These polymers are available in various grades possessing a range of physicochemical properties.[8,9]

The objective of the investigation is to design and develop colon targeted drug delivery system of tinidazole

microspheres by using Eudragit L 100 and Ethyl cellulose as a pH sensitive polymer. by directly targeting the drug to colon.

MATERIALS AND METHODS
Materials
Tinidazole was a gift sample from Dr.Reddy Lab Hyderabad, Eudragit L100 and Ethyl cellulose was procured from MSN Lab Hyderabad., all the solvents are purchased from Evonik India Pvt. Ltd.

Method
Preliminary studies for surfactant level selection
Span 60 was used as surfactant in the microsphere formulation, at various concentration Span 60 was added and evaluated for EE% and DR%, from data obtained below it was confirmed that when the surfactant concentration is 1.8 ml EE% is high.

Table 1: Level of selection of span60.

Batch code	Span 60(ml)	Drug(g.m)	Polymer Ratio Eudragit:EC	EE%	DR%
A1	1.8	0.9	1:2	56.01±0.03	87.11±0.43
A2	1.8	0.9	1:2	61.33±0.11	89.02 ±0.72
andA3	1.1	0.9	1:2	42.01±0.33	72.66±0.17
A4	0.4	0.9	1:2	40.31±0.22	69.23±0.66

Preliminary studies for RPM level selection
At various rpm the formulation of microsphere trial formulation was prepared and evaluated for EE% and DR%, The trial results are given below in table noXXXX.

Table no. 2: Level of selection of RPM.

Batch code	RPM	Drug(g.m)	Polymer Ratio Eudragit:EC	EE%	DR%
A5	2000	0.9	1:2	64.31±0.83	77.11±0.43
A6	2000	0.9	1:2	63.23±0.01	80.02 ±0.12
A7	1500	0.9	1:2	52.01±0.23	77.66±0.27
A8	1000	0.9	1:2	50.31±0.12	70.23±0.26

Preparation of Tinidazole microspheres
Tinidazole microspheres were prepared by emulsification solvent evaporation method. Accurately weighed EL 100 and EC in 1:2 ratios were dissolved in ethanol and acetone to form a homogenous polymer solution. Tinidazole was added into the polymer solution and mixed thoroughly. Plasticizer (dibutyl phthalate 50% w/v) was added to above solution. The above organic phase was slowly poured at 30^0C into liquid paraffin (15 mL) containing span 60 of different concentrations with stirring speed at different rpm to form a smooth emulsion. Thereafter, it was allowed to attain room temperature and stirring was continued until residual acetone and ethanol evaporated and smooth walled, rigid and discrete microspheres were formed. The microspheres were collected by decantation and the product was washed with petroleum ether (40^0 -60^0C), three times and dried at room temperature for 3 h. The microspheres were then stored in a desiccators over fused calcium chloride for further use. Nine batches were performed with optimization.[10,11]

Table no. 3: Experimental Variable in 3^2 Factorial Design.

Coded Value		Actual value	
	X_1 (%)		X_2(rpm)
-1	0.4		1000
0	1.1		1500
+1	1.8		2000

Independent Variables

X_1=surfactant concentration

X_2=RPM

Dependent Variables

Y_1= % of drug release

Y_2= Entrapment efficienc

Table no. 4: 3^2 Factorial Design for Tinidazole microsphere.

Formulation code	X_1	X_2
F1	+1	-1
F2	+1	0
F3	+1	+1
F4	0	-1
F5	0	0
F6	0	+1
F7	-1	-1
F8	-1	0
F9	-1	+1

Table no. 5: Fomulation chart of Tinidazole Microsphere.

Formulation code	Drug(gm) EL:EC	Polymer concentration	Surfactant	RPM
F1	0.9	1:2	1.8	2000
F2	0.9	1:2	1.8	1500
F3	0.9	1:2	1.8	1000
F4	0.9	1:2	1.1	2000
F5	0.9	1:2	1.1	1500
F6	0.9	1:2	1.1	1000
F7	0.9	1:2	0.4	1000
F8	0.9	1:2	0.4	1500
F9	0.9	1:2	0.4	2000

Characterization of Tinidazole microspheres
Drug-polymer interaction (FTIR) study
FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan).

Particle size
The particle size of the microbeads was evaluated using an optical microscope fitted with a calibrated eyepiece micrometer under a magnification of 40X. The particle diameters of about 50 microbeads was measured randomly and the average particle size was determined using the Edmondson's equation:

$$D_{mean} = \frac{\Sigma \, nd}{\Sigma \, n}$$

Where, n - stands for the number of counted microbeads, and d - mean size range.

% Drug content
Accurately weighed 100 mg microbeads were taken in a mortar pestle, finely crushed and then small quantity of water is added. It was then kept overnight for complete solubilization of pectin and drug release from it. After suitable dilutions in methanol, absorbance was measured in uv-vis spectrophotometer and accordingly drug content is calculated. The study was repeated three times.[12,13]

Entrapment efficiency
Microspheres containing equivalent to 10 mg of drug was allowed to equilibrate in 100 mL of phosphate buffer pH 7.4 for 24 h. The solution was filtered using Whatman filter paper (44). The resulting solution was analyzed using a UV spectrophotometric method at 318nmin the presence of a blank prepared from microspheres containing all materials except the drug.

$$\% \text{ Drug entrapment} = \frac{\text{calculated drug concentration}}{\text{theoretical drug concentration}} \times 100$$

Differential scanning colorimetry (DSC)
DSC studies were performed using a DSC METTLERSwitzerland with thermal analyzer. Accurately weighed samples (about 5 mg) were placed in a sealed aluminum pan, before heating under nitrogen flow (20 mL/min) at a scanning rate of 20 _C per min from 40 to 300 _C. An empty aluminum pan was used as reference. DSC thermograms of pure substances, their physical mixtures and drug-loaded microparticles were recorded.

Surface morphology (SEM)
Scanning electron microscopy has been used to determine the surface morphology and texture. SEM studies were carried out by using JEOL Model JSM-6390LV scanning microscope.

Micromeritic properties of microspheres
The flow properties of microspheres were investigated by determining the angle of repose, bulk density, tapped density, Carr's and Hausner's ratio. The angle of repose was determined by the fixed-based funnel method. Bulk and tapped densities were measured in 10 mL of a graduated cylinder. The cylinder was tapped from a height of 2 inches until a constant volume was obtained. The volume occupied by the sample after tapping was recorded and bulk density, tapped density, Carr's index and Hausner's ratio was calculated.

In vitro drug release study
In vitro release study of microspheres was performed in pH progression medium at $37^0C \pm 0.5^0C$. The drug dissolution test of microspheres was performed by the

paddle method (USP dissolution apparatus Type II, Electrolab Limited, India).

Microspheres equivalent to 100 mg were weighed accurately and put in muslin cloth and tied this to paddle over the surface of 900 mL of dissolution medium. The content was rotated at 100 rpm. The pH of the dissolution medium was kept 1.2 for 2 h using 0.1 N HCl. After 2 h, the pH of the dissolution medium was adjusted to 7.4 with 0.1 N NaOH and maintainedup to 8 h. The samples were withdrawn from the dissolution medium at various time intervals using a pipette. The rate of drug release was analyzed using UV spectrophotometer (JASCO, Ahmadabad, India).[14]

Kinetic treatment of dissolution data

There are number of kinetic models, which described the overall release of drug from the dosage forms. One of the approach to investigate the kinetics of drug release from controlled release formulation is by using model dependent methods. Model dependent methods are based on different mathematical functions, which describe the dissolution profile. Once a suitable function has been selected, the dissolution profiles are evaluated depending on the derived model parameters. Following models are evaluated.[15]

Zero order Kinetics

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$Q_0 — Q_t = K_0 t$

Rearrangement of equation (9) yields

Where, Q_t is the amount of drug dissolved in time *t*, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as cumulative amount of drug released *versus* time.

First order Kinetics

This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order kinetics can be expressed by the equation:

$\log C = \log C_0 – K_E t/2.303$

Where C_0 is the initial concentration of drug, K is the first order rate constant, and t is the time. The data obtained are plotted as log cumulative percentage of drug remaining *vs*. time which would yield a straight line with a slope of - K/2.303.

Higuchi model

This model is based on the hypotheses that (i) initial drug concentration in the matrix is much higher than drug solubility; (ii) drug diffusion takes place only in one dimension (edge effect must be negligible); (iii) drug particles are much smaller than system thickness; (iv) matrix swelling and dissolution are negligible; (v) drug diffusivity is constant; and (vi) perfect sink conditions are always attained in the release environment. In a general way it is possible to simplify the Higuchi model as,

$f_t = Q = K_K \times t_{1/2}$

Where, K_H is the Higuchi dissolution constant. The data obtained were plotted as cumulative percentage drug release versus square root of time.

Hixson-crowell model

Hixson and Crowell (1931) recognized that the particles regular area is proportional to the cube root of its volume. They derived the equation:

$W_O — W_t = nt$

Where, W_0 is the initial amount of drug in the pharmaceutical dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form at time t and n (kappa) is a constant incorporating the surface volume relation. The equation describes the release from systems where there is a change in surface area and diameter of particles or tablets. Tostudy the release kinetics, data obtained from *in vitro* drug release studies were plotted as cube root of drug percentage remaining in matrix *versus* time.

Statistical design

Design-Expert software (Design Expert trial version 8.0.7.1; State-Ease Inc., Minneapolis, MN, USA) was used. A two-factor three-level full factorial design was used for systemic study of combination of polymers. Polynomial models including interaction and quadratic terms were generated for the entire response variables using multiple linear regression analysis (MLRA) approach. The general form of the MLRA model is represented in the equation.

Where Y is the dependent variable; b0 is the arithmetic average of all the quantitative outcomes of nine runs. b1, b2, b12 are the estimated coefficients computed from the observed experimental response values of Y and X1 and X2 are the coded levels of the independent variables. The interaction term (X1X2) shows how the response values change when two factors are simultaneously changed. Table 1 summarizes the translation of the coded levels to the experimental units used in the study and Table X summarizes the experiment runs used. In this study factorial design based on the response surface method was adopted to optimize effective factors for the release of the drug from the microspheres. Analysis of variance (ANOVA) and all statistical analysis were also performed using the software. Calculation of the effects was performed. The significant effects would constitute the model. The F-value was then calculated by comparing the treatment variance with the error variance. The multiple correlation co-efficient was calculated

which is a measure of the amount of variation about the mean, which is explained by the model. The main effects and interactions are plotted and results interpreted. All assumptions underlying the ANOVA are checked. For statistical purposes, the assumption is made that residuals are normally distributed and independent with constant variance.[16]

RESULTS AND DISCUSSION
Spectrometric estimation of Tinidazole
The Lamda max of drug was obtained by scanning 20µg/ml solution concentration in the range of 200-400nm using UV-Visible spectrometer and it was found that 317.9nm for phosphate buffer pH 6.8 and pH 7.4.

Preparation of standard calibration curve of Tinidazole
Tinidazole (10 mg) was dissolved in 0.1 N HCl and volume was made up to 100 mL in 100 mL volumetric flask. This solution (100 mcg/mL) was further diluted with 0.1 N HCl to obtain solution of 5 to 40 mcg/mL. Absorbance of each solution was measured at 228 nm using Shimadzu UV-1601 UV/Vis double beam spectrophotometer and 0.1 N HCl as reference standard. The standard curve was generated for the entire range from 5 to 40 mcg /mL. The results of standard curve preparation are shown in the Table 6,7 and Figure 1&2.

Preparation of standard stock solution
Stock solution of tinidazole was prepared in all four solvents (Methanol, 0.1N HCl, Phosphate buffer pH 6.8 and Phosphate buffer pH 7.4). 100 mg of drug was accurately weighed and transferred into 100 mL volumetric flask individually and the volume was made up to the mark with same solvent to obtain a concentration of 1000 µg/mL. From this, 1 mL solution was withdrawn and again diluted to 10 mL, with same media to achieve a final concentration of 100 µg/mL.

Drug –polymer compatiblity results
The FTIR spectra of pure drug, Eudragit and tinidazole microspheres were shown in (Fig:3). It shows that no incompatibility reactions took place between drug and excipients.

DSC Study
DSC thermograph of tinidazole, Eudragit and tinidazole loaded Eudragit microspheres are shown in Fig5 The

pure drug tinidazole Fig. 5(a) gives rise to a sharp peak that corresponds to melting point at 126^0C, indicates its crystalline nature. The pure polymer Eudragit L 100 and Eu ragit S 100 exhibits a peak at 223^0C and 222^0C respectively, referring to the relaxation that follows the glass transition. peak of drug did not appear in the thermogram of prepared microspheres, it may indicate the drug was uniformly dispersed at the molecular level in the microspheres in Fig. 5.

SEM Study
The produced microspheres were spherical, non aggregated with rough and porous surface, as shown in scanning electron micrographs (Fig. 4). The surface of microspheres was rough due to arising as a trace of solvent evaporation during the process.

Micromeritic results
The value of angle of repose of formulation within the range of 17.43±0.13 to 29.13±0.22 indicating good flow properties for the microspheres. The bulk density values ranged between. 0.197±0.53 to0.127±0.43 The tapped density values ranged between 0.219±0.03 and 0.299±0.33 (gm/cm^3). The Carr's index values ranged between 28.63±0.03 and 28.63±0.03 which can described by Table 8.

In vitro drug release profile
The in vitro release study was carried out by buffer change method to mimic the GIT environment. Drug release for the initial 2 h i.e. in 0.1 N HCL, the drug release was found to be low in all cases. Then drug release is found 91.84% at the end of 8 h in pH 7.4 phosphate buffer, shown in Fig.6.

Release kinetics Results
Release kinetics was performed for the optimized batch. In vitro drug release of check point batch was best explained by zero order as the plot showed highest linearity. The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. Further, the mechanism of drug release fitted well with Hixon-crowell model, indicating sustain release mechanism. The plots and results of this study are shown in Figure 7.

Tables and Figures
Table 6: Calibration data of Tinidazole in pH 6.8 phosphate buffer.

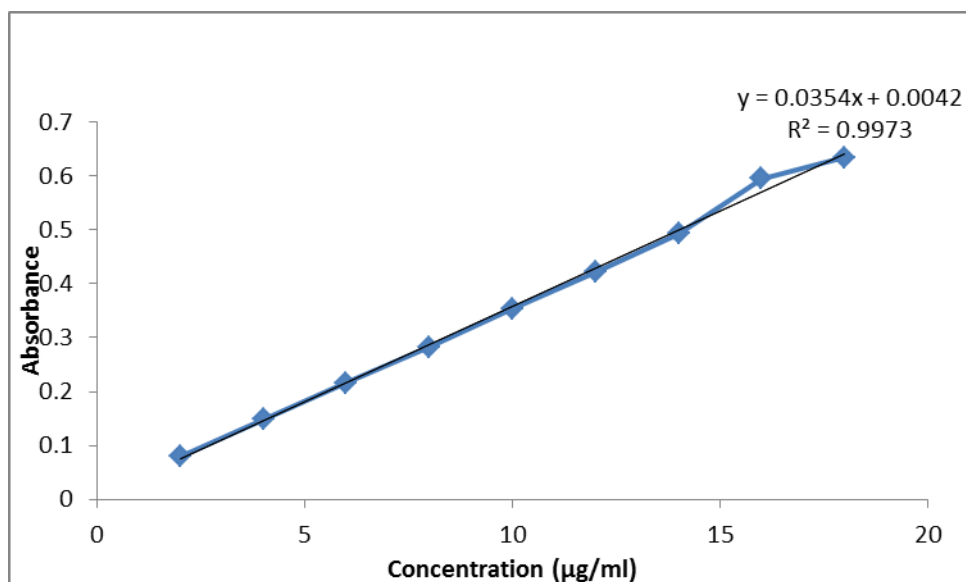| Concentration (µg/mL)	Absorbance			Mean absorbance[a]
	I	II	III	
2	0.082	0.079	0.08	0.080
4	0.149	0.148	0.147	0.148
6	0.217	0.216	0.217	0.216
8	0.284	0.282	0.283	0.283
10	0.356	0.354	0.354	0.354
12	0.424	0.421	0.422	0.422
14	0.494	0.492	0.493	0.493
16	0.596	0.594	0.595	0.595
18	0.636	0.634	0.634	0.634

$$y = 0.0354x + 0.0042$$
$$R^2 = 0.9973$$

Figure 1: Standard curve of tinidazole in pH 6.8 phosphate buffer at 317.9 nm.

Table 7: Calibration data of Tinidazole in pH 7.4 phosphate buffer.

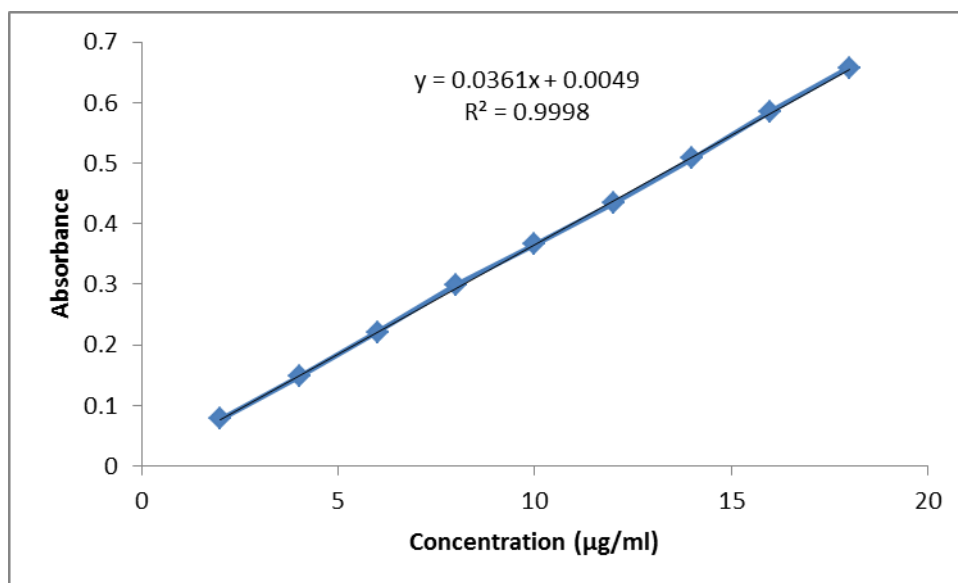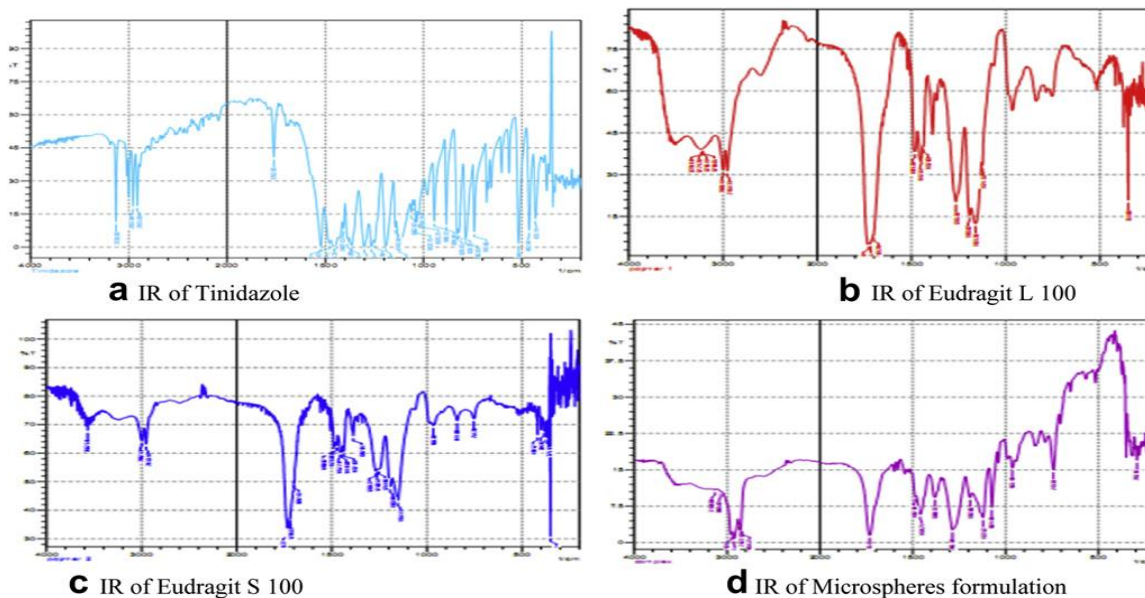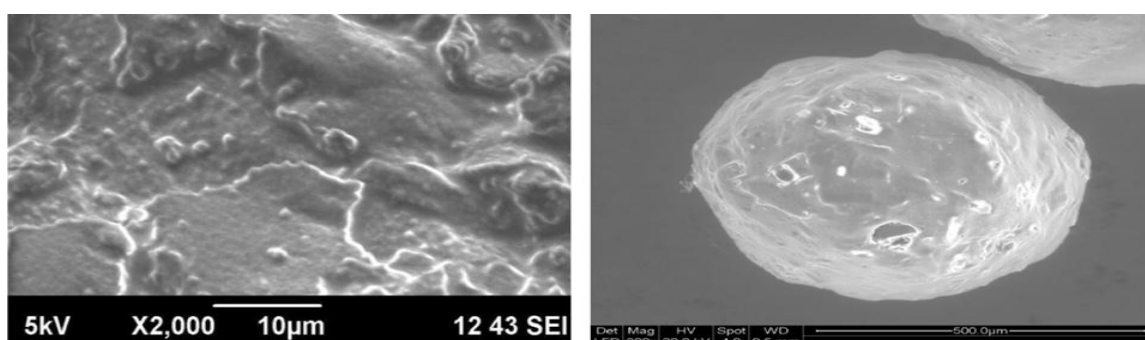| Concentration (µg/mL)	Absorbance			Mean absorbance[a]
	I	II	III	
2	0.079	0.073	0.082	0.078
4	0.144	0.147	0.153	0.148
6	0.221	0.220	0.221	0.221
8	0.289	0.293	0.315	0.299
10	0.360	0.372	0.366	0.366
12	0.427	0.433	0.443	0.434
14	0.500	0.506	0.519	0.508
16	0.580	0.580	0.595	0.585
18	0.651	0.654	0.665	0.657

$$y = 0.0361x + 0.0049$$
$$R^2 = 0.9998$$

Figure 2: Standard curve of tinidazole in pH 7.4 phosphate buffer at 316.7 nm.

Table 8: Micromeritic Properties of different batches of Tinidazole microsphere.

Batch	Angle of Repose	Bulk density(gm/cm³)	Tapped density(gm/cm³)	Carr;s Index	Hausner ratio
F1	28.33±0.03	0.187±0.13	0.289±0.13	33.63±0.33	1.13
F2	17.43±0.13	0.188±0.01	0.299±0.33	41.63±0.33	1.23
F3	26.13±0.23	0.197±0.23	0.219±0.03	46.63±0.33	1.33
F4	29.13±0.22	0.127±0.43	0.279±0.63	30.63±0.33	1.24
F5	18.33±0.63	0.187±0.07	0.269±0.22	33.68±0.36	1.22
F6	28.63±0.41	0.184±0.04	0.288±0.34	36.63±0.42	1.13
F7	22.33±0.73	0.177±0.66	0.287±0.06	32.23±0.37	1.63
F8	23.23±0.53	0.197±0.53	0.287±0.03	43.67±0.32	2.23
F9	21.33±0.03	0.187±0.33	0.282±0.05	28.63±0.03	1.03

Table 9: Characterization of Microsphere of different batches.

Formulation code	Entrapment efficiency (%)	Percentage of yield (%)	Average particle size(μm)
F1	51.18±0.03	**67.21**	**782.67**
F2	50.18±0.13	**63.17**	**794.11**
F3	48.11±0.13	**57.13**	**678.11**
F4	60.03±0.01	**70.11**	**734.21**
F5	68.76±0.03	**78.11**	**519.47**
F6	43.11±0.13	**63.17**	**812.37**
F7	42.65±0.07	**72.13**	**413.37**
F8	54.11±0.01	**61.17**	**512.37**
F9	51.11±0.11	**57.53**	**612.27**

a IR of Tinidazole

b IR of Eudragit L 100

c IR of Eudragit S 100

d IR of Microspheres formulation

Figure 3: FTIR RESULTS.

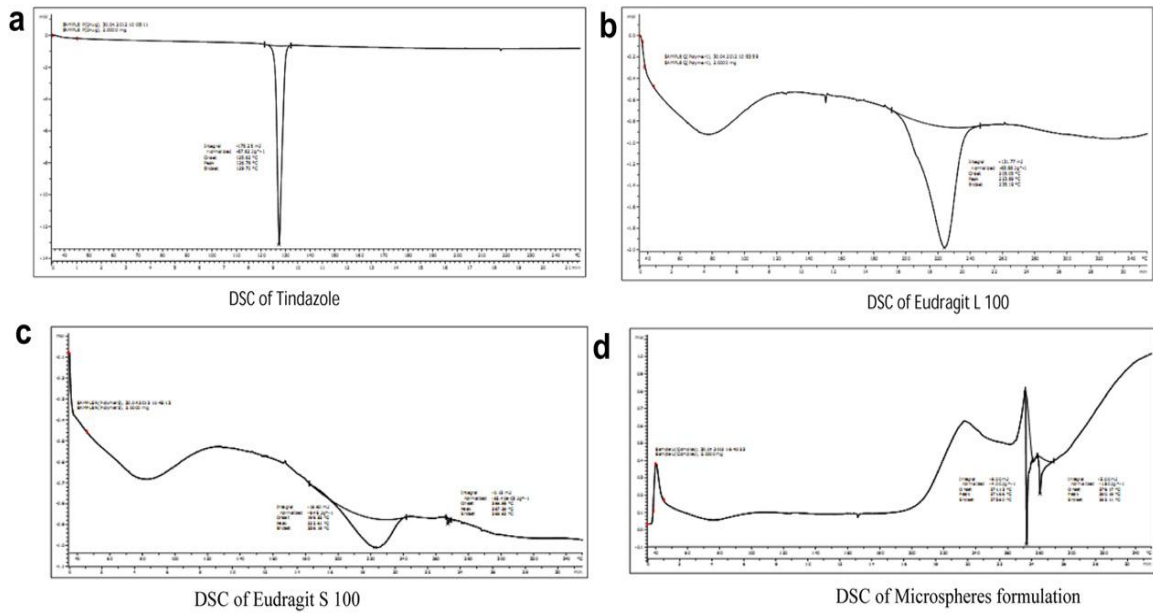**Figure 4: SEM RESULTS.**

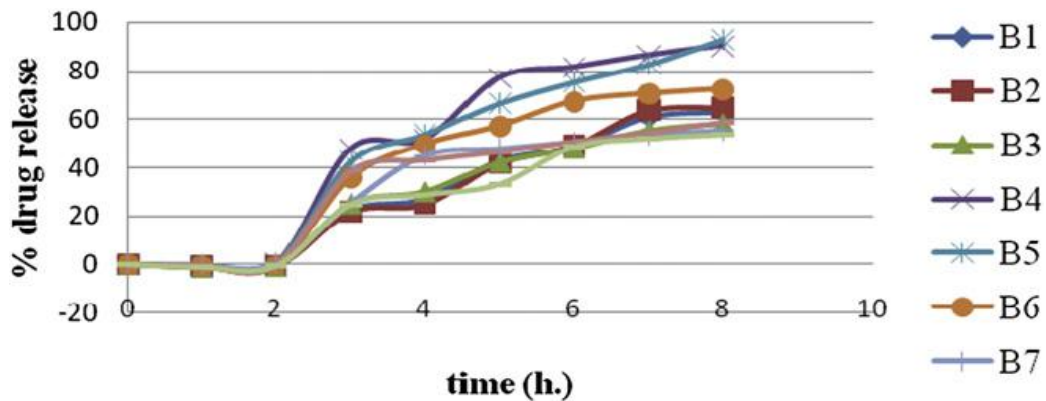**Figure 5: DSC RESULTS.**

Figure 6: Drug Release Profile of different batches of Microspheres.
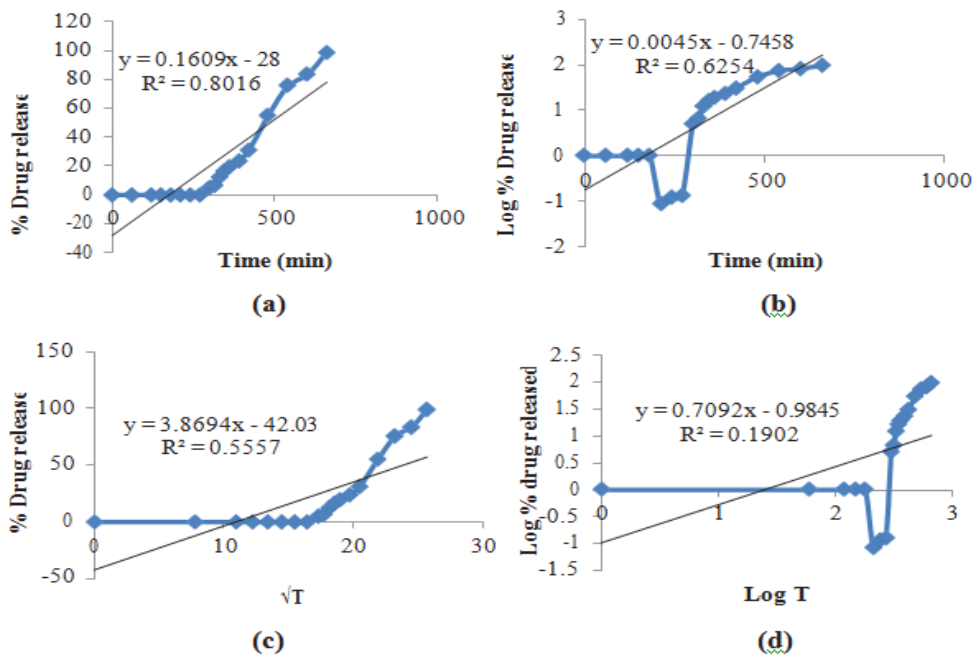

Figure 7: Release kinetics of Tinidazole microspheres.

CONCLUSION

Eudragit microspheres of tinidazole were successfully prepared by emulsion solvent evaporation technique. The results shown in Table indicates that optimum concentration of surfactant (1. 8 ml) and stirring speed (2000 rpm) showed higher percent of entrapment efficiency while change in stirring speed up to optimum range and change the surfactant concentration up to optimum range change the percent entrapment efficiency (Table 4).

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