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Research Article

pH TRIGGERED DRUG DELIVERY OF ORNIDAZOLE FROM EUDRAGIT COATED CHITOSAN MICROSPHERE: FORMULATION, OPTIMIZATION AND INVITRO EVALUATION

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ABSTRACT

The purpose of the present studies was to prepare and characterize and evaluate the colon targeted microsphere of ornidazole for the treatment and management of Amoebasis. The microsphere was prepared by the Emulsion dehydration method. The microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach. The prepared microspheres were evaluated for Surface morphology, entrapment efficiency, drug loading, micrometric properties and *in-vitro* drug release. Micrometric properties showed good flow properties and packability of prepared micorspheres.

Keywords: Emulsion dehydration method, Microspheres, Amoebiasis.

INTRODUCTION

Microspheres are sometimes referred to as micro particles. Microspheres can bemanufactured from various natural and synthetic materials. Various natural and synthetic polymers used are Agarose, carrageenan, chitosan, starch, albumin, collagen, poly alkyl cyano acrylates, poly anhydrides, poly methyl methacrylate etc. The idea behind Microspheres forColon specific drug delivery system is intended because it may reduce the Systemic side effect because of low dose of the drug. The absorption of the poorly absorbed drug is increase because of increase retention time in the colon[1, 2].

Ornidazole is used for the management of amoebiasis. Amoebiasis is an infection caused by *Entamoebahistolytica*with or without symptoms (WHO 1969). Synonyms include entamoebiasis, amoebiosis, amoebic dysentery or bloody flux.The aim of the study was to develop colon targeted Microsphere of Ornidazole using Chitosan, and Eudragit S-100 as carriers in the treatment of amoebiasis.

Significance of this Research Investigation

Increase the absorption and bioavailability of the drug via delayed release formulation. Utilize the non-toxic and biodegradable nature of Chitosan that makes it safer for patients as compared to other synthetic polymers it is also economical.Reduce the dose and administration frequency.Reduce the incidences of adverse drug reaction.

MATERIALS AND METHODS

Ornidazole, Chitosan and eudragit S-100 was purchased from the balaji pharmaceutical Pvt. Ltd. The Span-80, Liquid paraffin and Acetone was obtained from the central drug store.

Preparation of microspheres[3]

Microspheres were prepared by Emulsion dehydration method. Accurately weighed ornidazole and chitosan were dissolved in 1% glacial acetic acid and stirred to solublize. This drug polymer solution was dispersed in liquid paraffin containing 1.25% wt/vol span 80 stirred at 1000rpm for 30 min to form uniform emulsion. Then acetone was added in order to dehydrate the chitosan dropletscontinuously

stirred for 1hrs. Microspheres were formed which were dried overnight and kept in air tight container for further studies.

Coating of Microspheres

Chitosan microsphere was coated with eudragit S-100 by Solvent evaporation method. Chitosan microsphere was dispersed in Eudragit coating solution prepared by dissolution of 500mg of Eudragit S-100 in 10 ml of Ethanol: Acetone (2:1). Finally, the coated microspheres were filtered. Washed with n-Hexane, and dried in desiccators[4].

Evaluation of Microspheres

- **Micromeritic properties**[5]
- **Bulk density**

Bulk density is determined by following formula.

Bulk density =*M/Vb*

M = Mass of microspheres (g),
Vb= volume of microspheres (after three tapping)

- **Tapped density**

Tapped density is determined by following formula.

Tapped density = *m/vt*

m = mass of microspheres (g),
Vt= volume of microspheres (final tapped volume)

- **Carr's Index**

It is determined by following formula.

$$\textbf{Carr's Index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100$$

- **Hausner ratio**

It is determined by following formula.

$$\textbf{Hausner ratio} = \frac{\text{tapped density}}{\text{buk density}}$$

Angle of repose

Determination of angle of repose of chitosan microspheres were carried out by employing fixed funnel method.

Angle of repose $\theta = \text{Tan}^{-1}(h/r)$

H=height of pile,
R= radius of pile.

- **Percentage yield**

The measured amount was divided by total amount of all non volatilecomponent which were used for the preparation of microspheres.

% yield= Actual weight of product /total weight of drug and polymer * 100

- **Particle size analysis of microspheres**

The particle size analysis was done with the help of optical microscope using calibrated ocular micrometer. The mean particle size was calculated by measuring the diameter of 50 particles. The average particle size was determined using Edmondson's equation.

$$D = nd / n$$

Where, n =number of microspheres,
d= mean of the size range,
D = average particle size (in μm)
From the particle size analysis it was inferred that the microspheres were uniform in size and the size of the microspheres increased with the increase in the polymer concentration.

- **Drug entrapment efficiency**

In 100ml of volumetric flask 100mg of microspheres were crushed taken and dissolved with 6.8 pH phosphate buffer and stirred for 24 hrs. after stirring the solution was filtered through whatman filter paper and from the filtrate appropriate dilution were made and absorbance was measured at 317nm by using shimadzu 1700 UV spectrophotometer.

$$\%\text{Drug entrapment} = \frac{\text{amount of drug actually present}}{\text{theoratical weight of the drug}} X100$$

- **FTIR Spectroscopy**

FTIR spectra of Ornidazole, Chitosan, Eudragit S-100 and mixture of Ornidazole, Chitosan, Eudragit S-100 was taken by using Bruker Infrared spectrophotometer.

- ***In-vitro* drug release**

The in vitro release of drug from the micro particles filled in enteric coated gelatin capsules was carried out in basket type dissolution apparatus for all the batches. In the dissolution test the micro particles were firstly subjected to a pH 1.2 buffer for 2 hours and then to a pH 6.8 for next 10 hrs. The volume of the dissolution media was maintained at 900 ml with constant stirring (100 rpm) and the temperature was maintained at 37±0.50C. After a time interval of 1 hr. sample were withdrawn and replaced with fresh media immediately after sampling. The samples withdrawn were analyzed for the drug content by scanning the sample at 262 nm using UV spectrophotometer (Shimadzu UV1800)

- **Kinetics and Mechanism of Release Analysis**

To study the release kinetics, the data obtained from in vitro drug release studies were plotted in various kinetic models.

- **Zero order**

as percent drug release versus time describes concentration independent drug release rate from the formulation. It is calculated by following equation

$$C = k_o t \qquad (1)$$

Where k_o is the zero order rate constant expressed in units of concentration/time and t is the time in hours.

- **First order**

as log percent drug remaining versus time describes concentration dependent drug release from the system. its equation is given below

$$\text{Log } C = \text{Log } Co - kt/2.303 \qquad (2)$$

Where Co is the initial concentration of the drug and k is the first order rate constant.

- **Higuchi matrix model**

As percent of drug release versus square root versus time describes the release of drugs based on ficikan diffusion as a square root of time dependent process from swell able in soluble matrix. Its equation is given below

$$Q = kt^{1/2} \quad (3)$$

Where k is the constant reflecting the design variables of the system.

- **Korsmeyerpeppas model**

as log percent drug release versus log time describes drug release from a polymeric system. Its equation is given below

$$M_t / M_\infty = k_{KP} t^n \quad (4)$$

RESULT AND DISCUSSION

➢ **Micromeritic properties**

The values of micromeritic properties Carr's index (9.51 -16.83), Hausner ratio (1.10 - 1.20), angle of repose (29.36 – 39.65) of chitosan micropsheres indicates the excellent to fair flow properties of microspheres.

➢ **FTIR Spectroscopy**

The FTIR spectra of Ornidazole, Chitosan, Eudragit S-100 were taken. The FTIR of mixture of Ornidazole, Chitosan ,Eudragit S-100 (1:1:1) Showed all the peak of pure drug spectra which showed absence of drug and excipients interaction.

➢ **Percentage yield**

The percentage yield of chitosan microspheres was found to be 85 to 90%. The percentage yield of chitosan microspheres decrease with increase in concentration of the polymer.

➢ **Particle size analysis**

The particle sizes observed for chitosan microspheres different batches prepared (F1, F2, F3, F4 and F5) were found to 105.49 ± 2.24 µm, 110.9 ± 1.29 µm, 124.1 ± 1.31µm, 130.5 ± 1.29µm and 138.6 ± 1.45 µm. Whereas Eudragit S-100 coated chitosan microspheres The particle sizes observed for different batches prepared (F1, F2, F3, F4 and F5) were found to 121.2 ± 1.24 µm, 132.45 ± 0.29 µm, 140.1 ± 1.21µm, 148.5 ± 1.29µm and 155.6 ± 1.56µm. The table 3 give the particle size analysis.

➢ **Drug entrapment efficiency**

The drug entrapment efficiency of chitosan microspheres was found to be 65.7 to 86.5%. That showed good entrapment efficiency of drug.

➢ ***In-vitro* drug release of Eudragit S-100 Chitosan microspheres**

The results of in-vitro dissolution study of Eudragit S-100 coated microspheres showed that release of the drug from the microspheres in the stomach was 6.01% to 8.02% and in the 5hrs there is increase in drug release from the microspheres because at that time formulation were exposed to pH 7 which is the pH for the solubility of eudragit S-100. In the 24hrs 88.96% to 97.88% to of drug was released from the formulations. The F3 batch showed higher drug release of 97.88%.

Data obtained from *in vitro* release study was utilized for release kinetics. The values of *in-vitro* release were attempted to fit into various mathematical model i.e. zero order, first order, korsmeyerpeppas and higuchi matrix. Kinetic data obtained from *in vitro* release profiles of different formulations of colon targeting ornidazole loaded chitosan microspheres are given in table 4.

The values were compared with each other for model fitting equation. Based on highest regression value (r), formulation gave good fit to the first order kinetics. The *in vitro* kinetic plots are given in figure 4A, 4B, 4C, 4D. The *in vitro* kinetic data subjected to log time drug release transformation plot (Peppas model) revealed the fact that the drug release follows a super case II transport with diffusion exponent (n) value >1.

➢ **SEM analysis**

The morphology of microspheres was examined by scanning electron microscopy which showed the Smooth surface of the microspheres.

CONCLUSION

In this Research work the results shows that of entrapment efficiency of drug was found to be 65.4 to 86.45%. The percentage yield of the formulation was found to be 90 to 85%. The flow properties of the microspheres showed excellent to good flow property. Whereas the F3 batch showed maximum in-vitro drug release 98.71%. The SEM analysis of microspheres showed smooth surface. So the Multiparticulate delivery System can be potential approach to be used as colon drug delivery.

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Table 1: Formulation of Microspheres

S. No	Formulation Code	Amount of Chitosan (gm)	Amount of drug (gm)	1% glacial acetic acid (ml)	Liquid paraffin (ml)	Span 80 (% w/v)	Acetone (ml)
1.	F1	1	1	40	50	1.25	50
2.	F2	2	1	40	50	1.25	50
3.	F3	3	1	40	50	1.25	50
4.	F4	4	1	40	50	1.25	50
5.	F5	5	1	40	50	1.25	50

Table 2: Evaluation of Chitosan microspheres

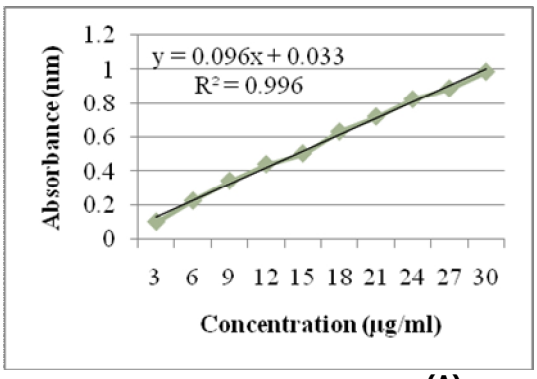
S. No	PROPERTY	F1	F2	F3	F4	F5
1.	BULK DENSITY (gm\ml)	0.67	0.64	0.60	0.61	0.56
2.	TAPPED DENSITY (gm\ml)	0.75	0.73	0.69	0.69	0.68
3.	CARR'S INDEX	9.51	11.89	13.86	12.21	16.83
4.	HAUSNER RATIO	1.10	1.14	1.16	1.13	1.20
5.	ANGLE OF REPOSE	29.36	30.96	33.28	34.56	36.56
6.	PERCENTAGE YIELD (%)	90	86.66	87.5	88	85
7.	DRUG ENTRAPMENT (%)	65.7 ± 0.95	75.4 ± 1.00	86.5 ± 0.55	79.6 ± 0.75	72.4 ± 0.56
8.	PARTICLES SIZE OF UNCOATED MICROSPHERES (μm)	105.49 ± 2.24	110.9 ± 1.29	124.1 ± 1.31	130.5 ± 1.29	138.6 ± 1.45
9.	PARTICLES SIZE OF COATED MICROSPHERES (μm)	121.2 ± 1.24	132.4 ± 0.29	140.1 ± 1.21	148.5 ± 1.29	155.6 ± 1.56

Table 3: In-vitro dissolution studies of Eudragit S-100 Coated Chitosan Microspheres

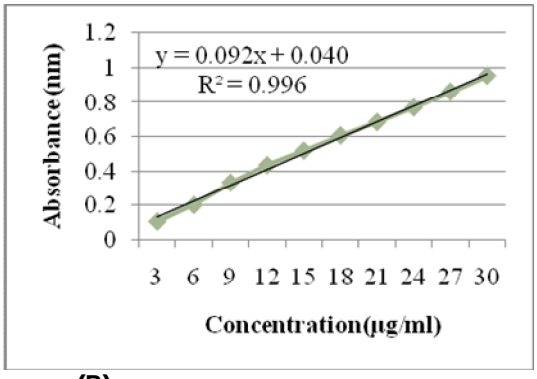
S. No	Time (hours)	F1 (%)	F2 (%)	F3(%)	F4 (%)	F5 (%)
1.	2	8.02	7.56	6.01	6.23	6.94
2.	5	24.66	22.48	18.45	20.62	21.45
3.	7	42.46	38.6	36.54	37.54	34.55
4.	9	71.26	68.24	55.46	57.61	55.41
5.	12	84.56	77.55	65.66	68.45	66.45
6.	15	88.11	83.32	78.25	79.54	78.56
7.	18	88.66	86.45	88.25	85.12	83.25
8.	21	89.24	88.21	94.55	87.12	86.45
9.	24	90.12	92.11	97.88	89.54	88.96

Table 4: Kinetic data obtained from I*n Vitro* Release Profile for colon Targeting ornidazole loaded chitosan microspheres

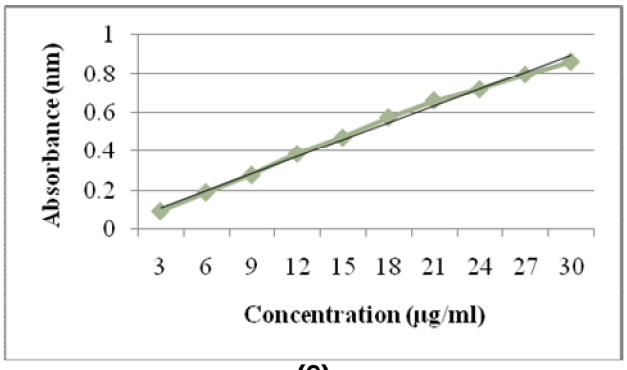
Formulation	R^2				n values
	Zero order	First order	Higuchi matrix	Peppas Model	
F1	0.750	0.859	0.750	0.800	0.996
F2	0.824	0.969	0.824	0.820	1.029
F3	0.954	0.945	0.954	0.867	1.152
F4	0.899	0.987	0.899	0.840	1.093
F5	0.913	0.989	0.913	0.858	1.045



(A)

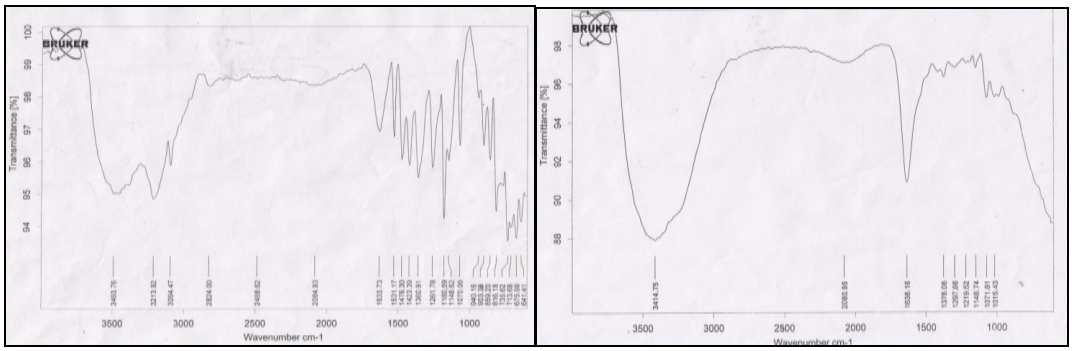


(B)

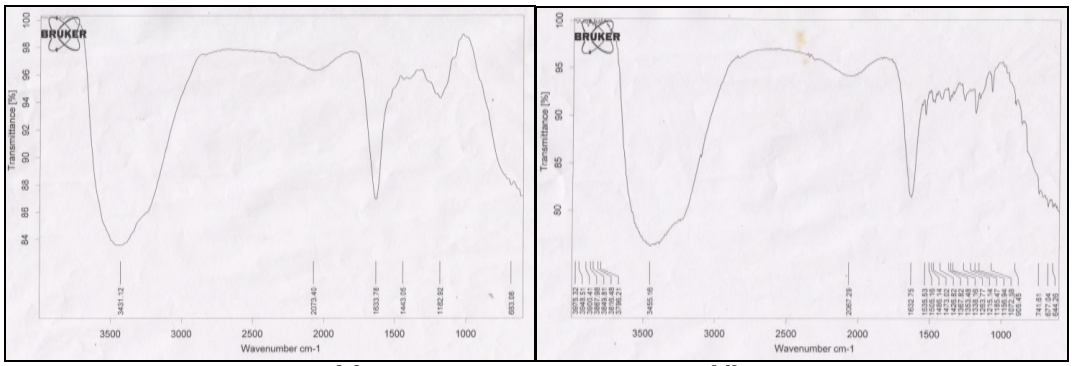


(C)

Fig. 1: Calibration curve of Ornidazole in (A) 0.1N Hcl (B) 6.8 pH Phosphate buffer and (C) 7.4 pH Phosphate buffer

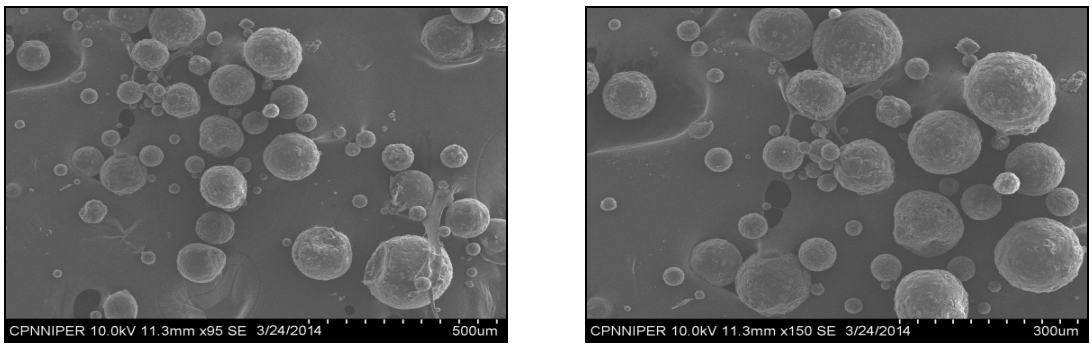


(a) (b)



(c) (d)

Fig. 2: FTIR spectrum of (a) Ornidazole (b) Chitosan (c) Eudragit S-100 and (d) Mixture of Ornidazole, Chitosan and Eudragit S-100 (1:1:1)



(1) (2)

Fig. 3: SEM images of ornidazole loaded chitosan microspheres of batch F3

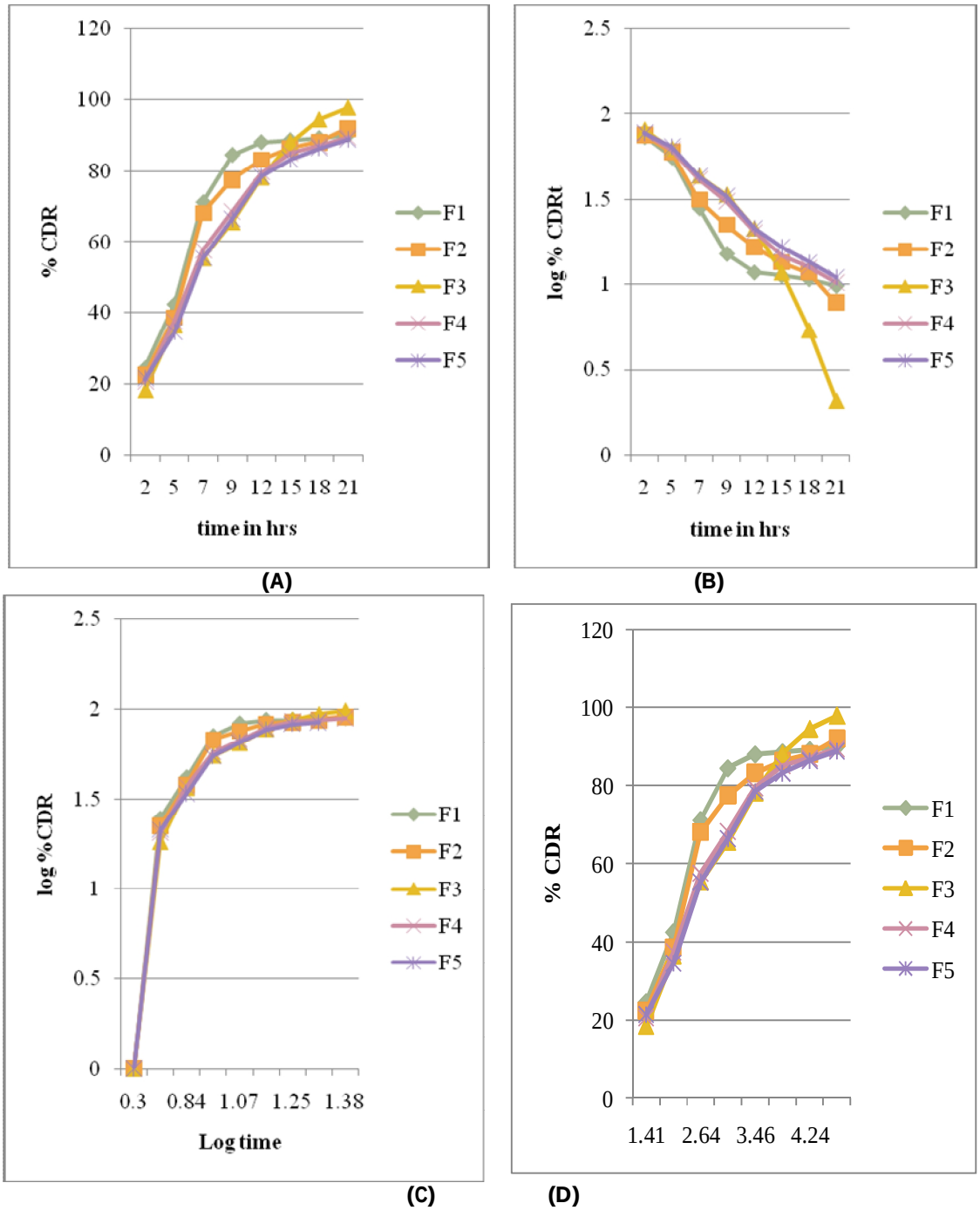


Fig. 4: A) Zero order kinetics B) First order kinetics C) Peppas Model D) Higuchi Model

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