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FORMULATION AND EVALUATION OF MICROSPHERE MUCOADHESIVE DRUG DELIVERY SYSTEM OF ANTIAMOEBIC DRUG

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ABSTRACT

The purpose of the work was to formulate and evaluate *in vitro* parameters of mucoadhesive metronidazole microspheres for the potential delivery of the drug to the colon. Mucoadhesive microspheres of metronidazole for colon targeting were prepared by the emulation-solvent evaporation method using span-80 as a emulsifying agent. The results of the preliminary trials indicate the drug: polymer ratio affected the characteristics of the mucoadhesive microspheres.The mucoadhesive microspheres were prepared in different drug: polymer (Metronidazole: Eudragit RL) ratio of 1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5. Mucoadhesion of microspheres was achieved by coating the microspheres with the 5%Chitosan solution, the resulting mucoadhesive microspheres were filled in the hard gelatin capsule shell followed by coating with Eudragit RL coating solution. The mucoadhesive microspheres were further evaluated for micromeritics study, particle size and surface characteristic, percentage drug content, encapsulation efficiency, *in vitro* Wash-off test for mucoadhesion and *in vitro* drug release study in the three different pH(0.1 N HCL, Phosphate buffer pH 6.8 and Phosphate buffer pH 7.4) for 12 hours. The mucoadhesive microspheres were further studied for *in vitro* release kinetics and drug release mechanism. The best formulation batch exhibited highest drug entrapment efficiency of 86.81%, particle size of 58.9µm with almost spherical shape and free flowing properties, 70.33% of mucoadhesion after 5 hours, and followed Zero order rate release with non Fickian-Diffusion mechanism with 82.77% of drug release at the end of 12 hours.

INTRODUCTION

MUCOADHESIVE MICROSPHERES [1, 2]

Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1-1000 µm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively[3]. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery[4]; but coupling of mucoadhesive properties to microspheres has additional advantages,[5] *e.g.* efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio[6], a much more intimate contact with the mucus layer, specific targeting of drug to the absorption site achieved by anchoring plant lectins[7], bacterial adhesions and antibodies, *etc*[8]. on the surface of the microspheres. Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye[9], nasal cavity and urinary and gastrointestinal tract[10], thus offering the possibilities of localized as well as systemic controlled release of drugs[11]. Application of mucoadhesive microspheres to the mucosal tissues of ocular cavity[12], gastric and colonic epithelium is used for administration of drugs for localized action[13]. Prolonged release of drugs and a reduction in frequency of drug administration to the ocular cavity can highly improve the patient compliance[14]. The latter advantage can also be obtained for drugs administered intra-nasally due to the reduction in mucociliary clearance of drugs adhering to nasal mucosa.[15] Microspheres prepared with mucoadhesive and bioerodible polymers undergo selective uptake by the M cells of Payer patches in gastrointestinal (GI) mucosa.[16] This uptake mechanism has been used for the delivery of protein and peptide drugs[17], antigens for vaccination and plasmid DNA for gene therapy[18]. Moreover, by keeping the drugs in close proximity to their absorption window in the GI mucosa[19]. The mucoadhesive microspheres improve the absorption and oral bioavailability of drugs like furosemide and riboflavin.[20] The concept of a non-invasive single shot vaccine, by means of mucosal immunization[21], offers controlled release of antigens and thus forms another exquisite application of mucoadhesive microspheres.[22]

METHODOLOGY: PREPARATION OF MUCOADHESIVE MICROSPHERES BY EMULSION-SOLVENT EVAPORATION TECHNIQUE[33,34,35]

Accurately weighed quantities of the polymers were (Eudragit RL) dissolved in 20 ml of acetone. Weighed quantity of Metronidazole (drug) (previously passed through the sieve # 150) was then dispersed in the above polymer phase and it was stirred for 2 hours. Then it was emulsified with the 100 ml of liquid paraffin containing 1% w/v of Span 80 with continuous stirring at 800 rpm under a mechanical stirrer. The stirring was continued for 2 hours to ensure complete evaporation of acetone. The microspheres were then separated from liquid paraffin by filtration using Whatmann filter paper No. 44, washed three times with 50 ml of petroleum ether, and air dried for 12 hours. These resultant microspheres were further coated with 5% of Chitosan solution and dried for 12 hours. These mucoadhesive microspheres were filled in the hard gelatin capsule shell and the shell was coated

with Eudragit RL solution by dipping and drying method. to exactly target the colon All the formulations of microspheres were prepared in the same way.

The composition of Eudragit RL contains: Eudragit RL (8%), Dibutyl phthalate (2%), in the 80% of methanol solution.

CALCULATION OF CONTROLLED RELEASE DOSE [61]

Required dose = conventional dose $(1+0.693\times\tau / t_{1/2})$: τ = Duration of Dose

Required dose = $200(1+0.693\times12/6)$ $t_{1/2}$ = Half life of drug

Required dose = 477 mg of Metronidazole

Table No. 1

COMPOSITION OF FORMULATIONS OF MUCOADHESIVE MICROSPHERES OF NATEGLINIDE

Formulation Code	Metronidazole (mg)	Eudragit RL(mg)	Chitosan solution
MF_1	500	500	5% w/v
MF_2	500	750	5% w/v
MF_3	500	1000	5% w/v
MF_4	500	1250	5% w/v
MF_5	500	1500	5% w/v
MF_6	500	2000	5% w/v

RESULTS

PRE FORMULATION STUDIES FOR DRUG AND CARRIER INTERACTION

a) Fourier Transform Infrared Spectrophotometry (FTIR)

Infrared spectra for pure Metronidazole and for the physical mixture of Metronidazole and all the polymers were determined to check the intactness of the drug in the polymer mixture using SHIMADZU (FTIR 410) by disc method. The following table shows the wave number for the characteristic bands in the IR spectra of pure Metronidazole.

Table No. 2

Wave number in cm^{-1}	Characteristic bands
3219.54	-OH- (Stretching Vibration)
1535.88 and 2139.38	C=N (Stretching Vibration)
1369.02	Aromatic 3^0 amine (C-N Vibration)
1535.88 and 1428.81	Aromatic C-C (Multiple bond stretching)
1369.02	Aromatic Nitro Compounds

FTIR spectra for pure drug, for the carriers and for the physical mixture of both are shown in **Figure No 1 to 4.**

Figure No 1

FTIR SPECTRA FOR METRONIDAZOLE

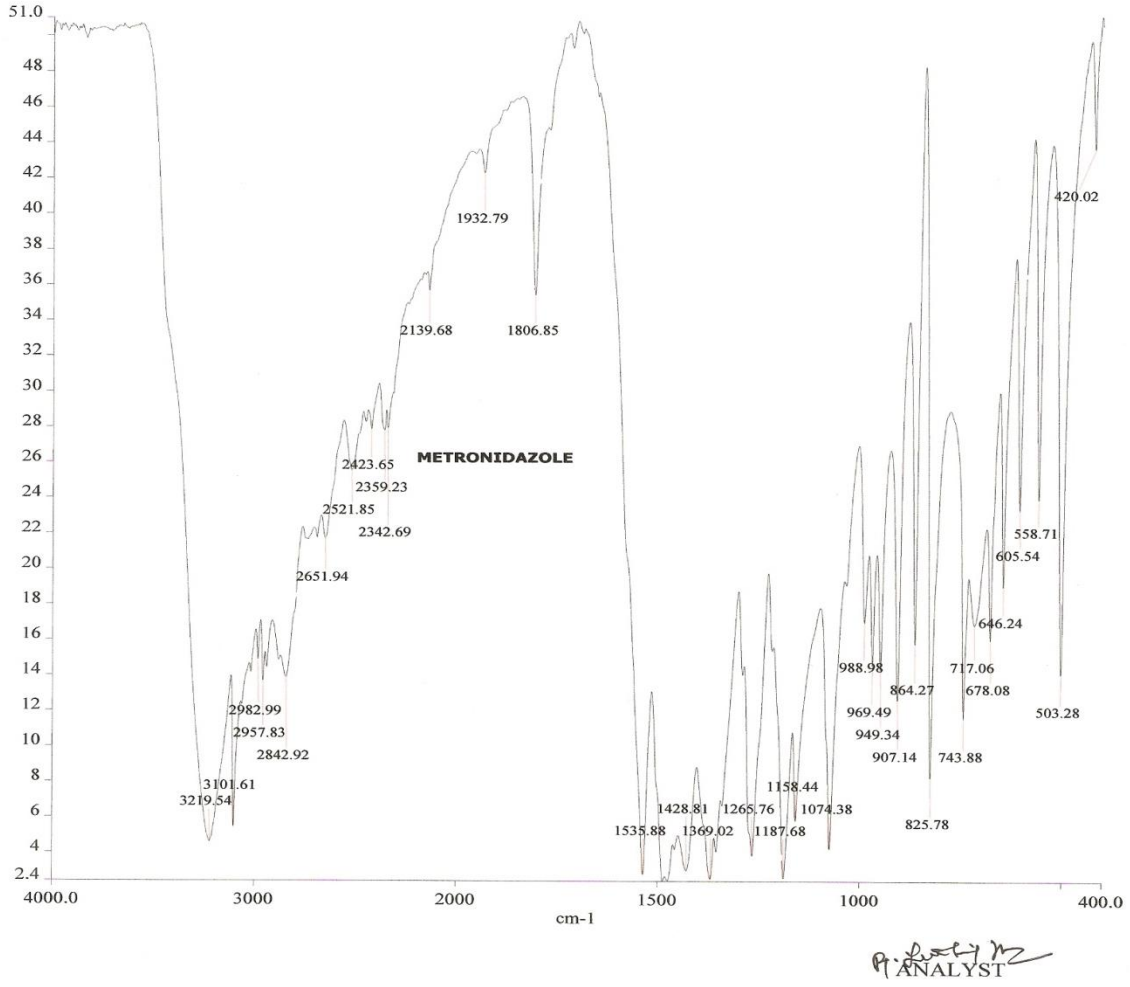


Figure No 2

FTIR SPECTRA FOR METRONIDAZOLE + EUDRAGIT RL

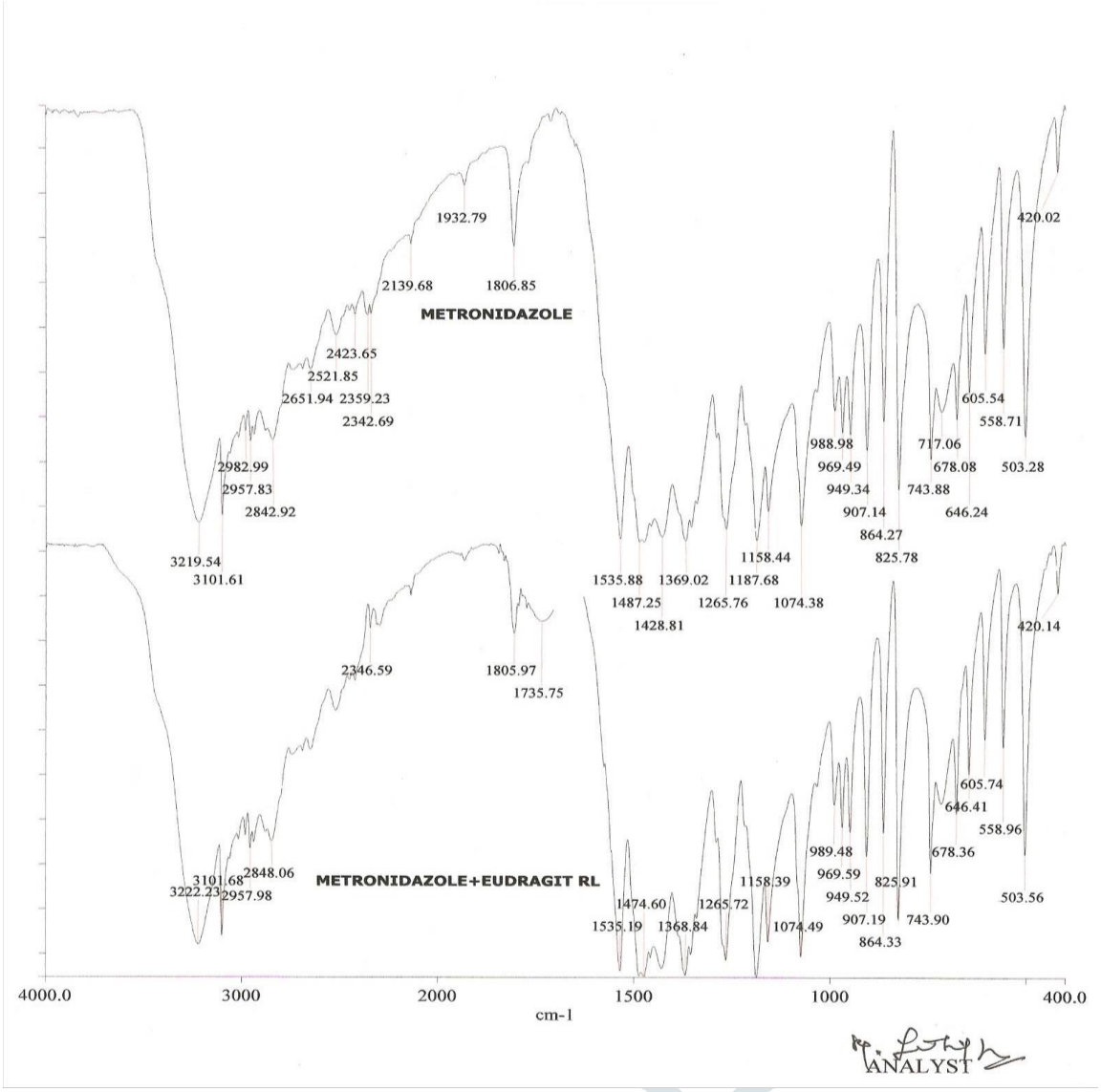


Figure No 3

FTIR SPECTRA FOR METRONIDAZOLE + CHITOSAN

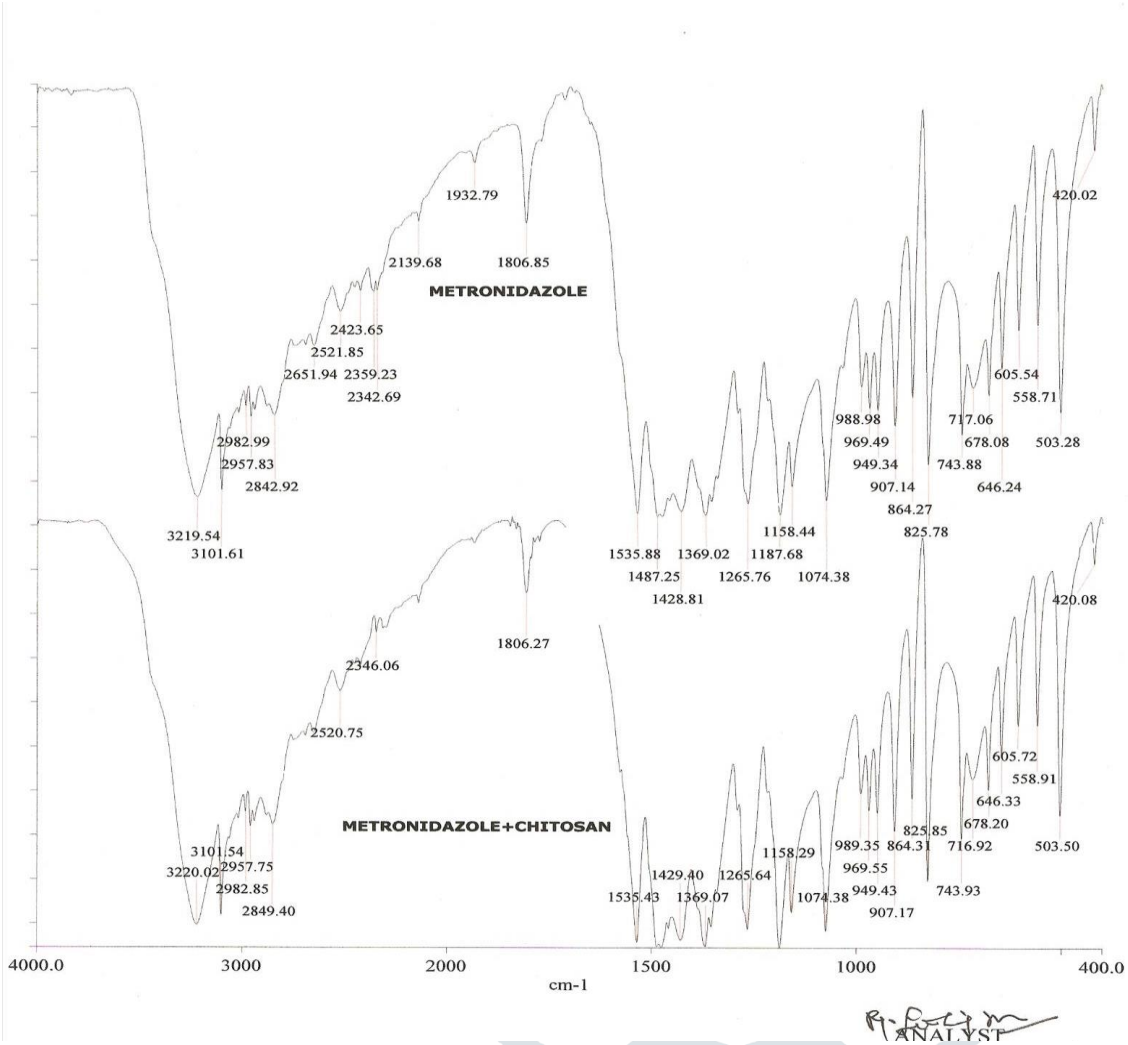
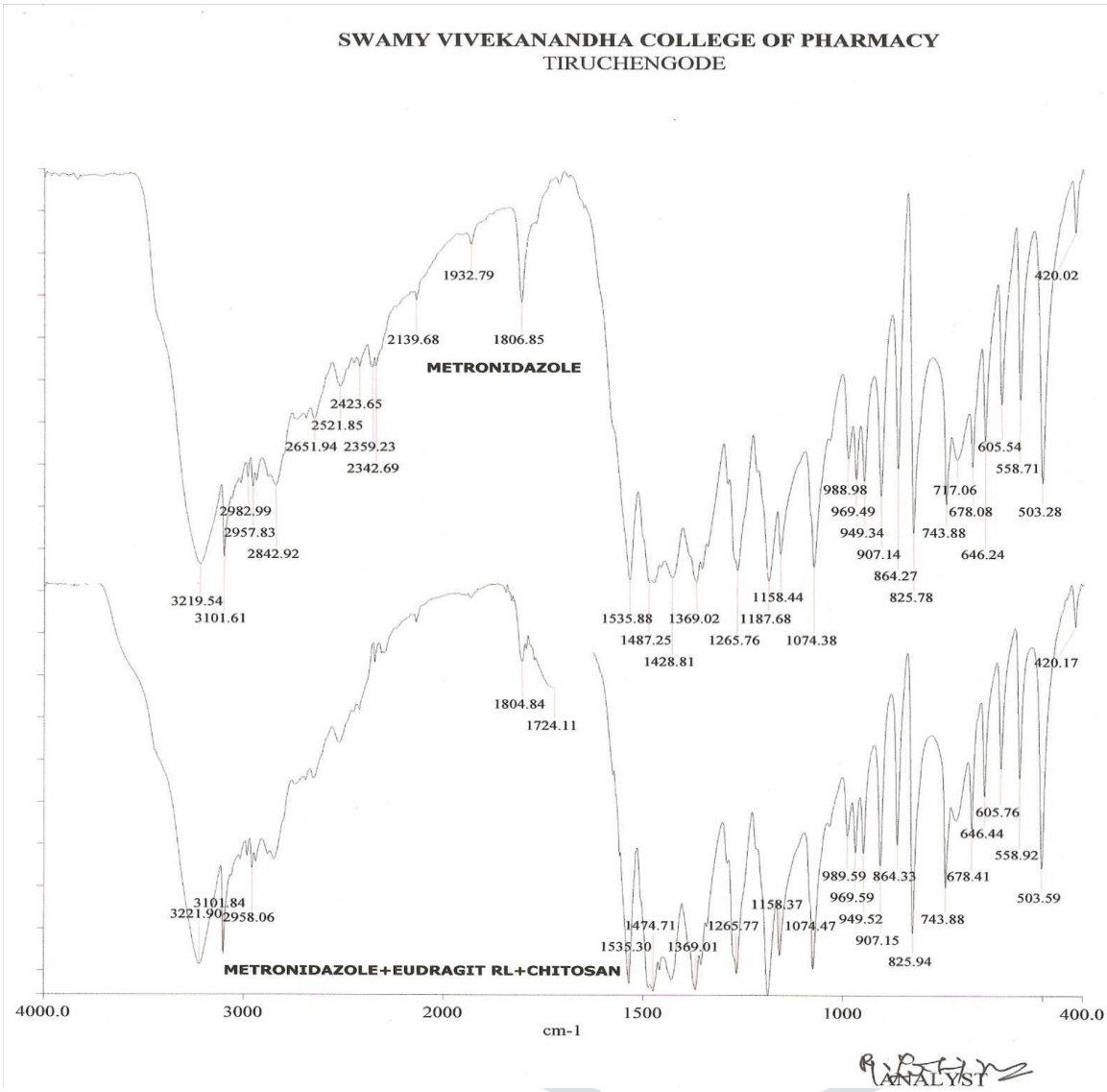


Figure No 4

FTIR SPECTRA FOR METRONIDAZOLE + EUDRAGIT RL + CHITOSAN



b) Differential scanning colorimeter (DSC)

DSC thermogram of Metronidazole and physical mixture of drug and polymers are shown in **Figure No. 9 to 12**. DSC thermogram of pure drug has shown a melting endotherm at 167.50°C.The thermogram of physical mixture showed that the Metronidazole melting onset temperature decreased to 164.37° C because of the presence of polymers in the physical mixture.

Figure No. 5

DSC Spectra for pure drug of Metronidazole

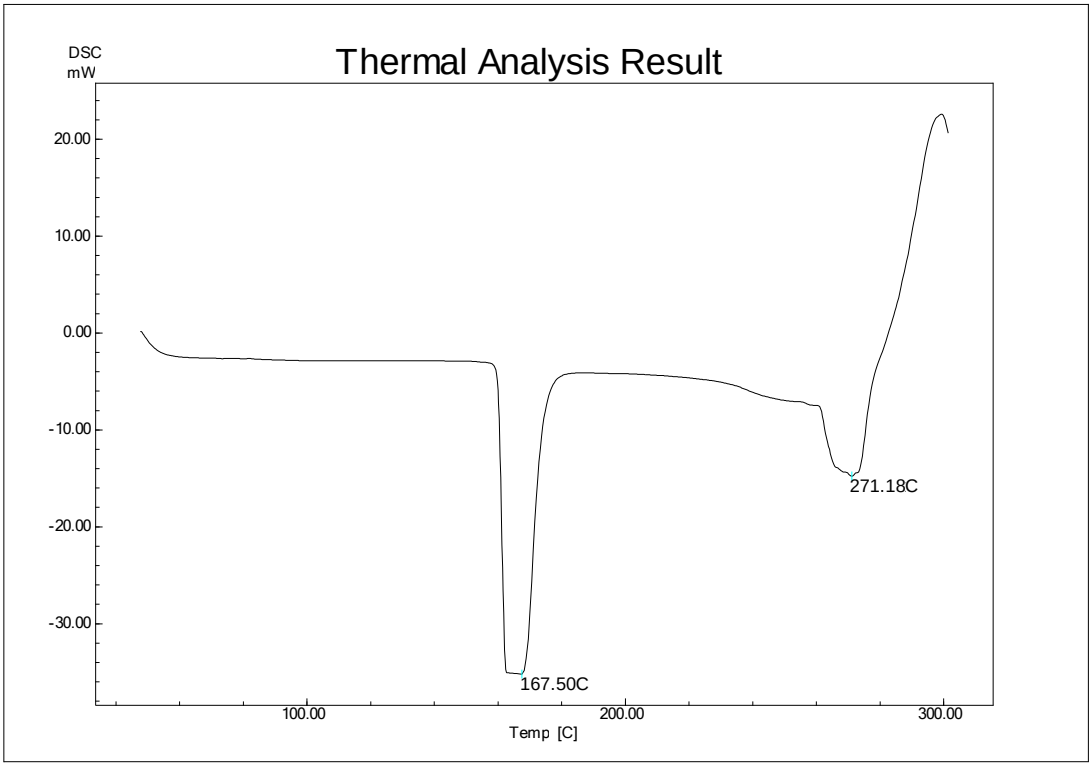


Figure No 6

DSC Spectra for physical mixture of pure drug (Metronidazole) + Eudragit RL

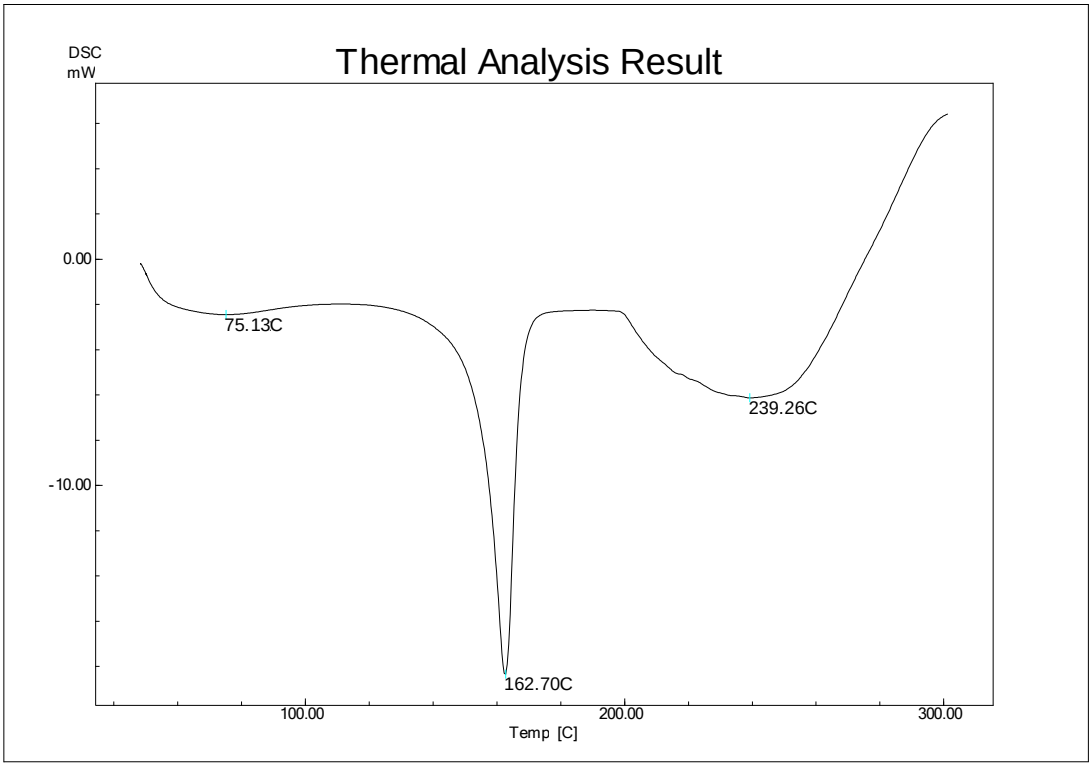


Figure No. 7

DSC Spectra for physical mixture of pure drug (Metronidazole) + Chitosan

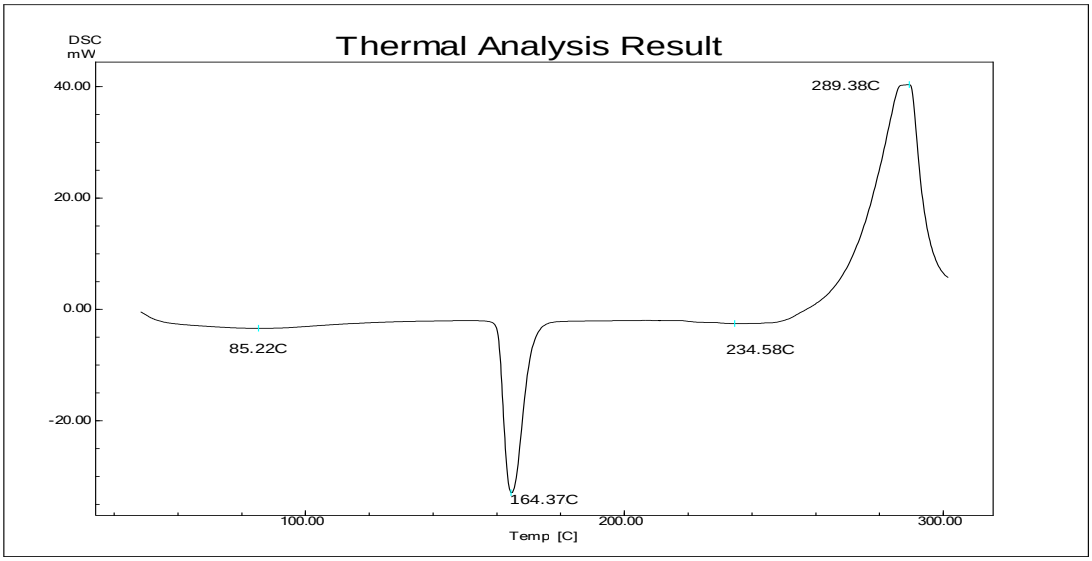
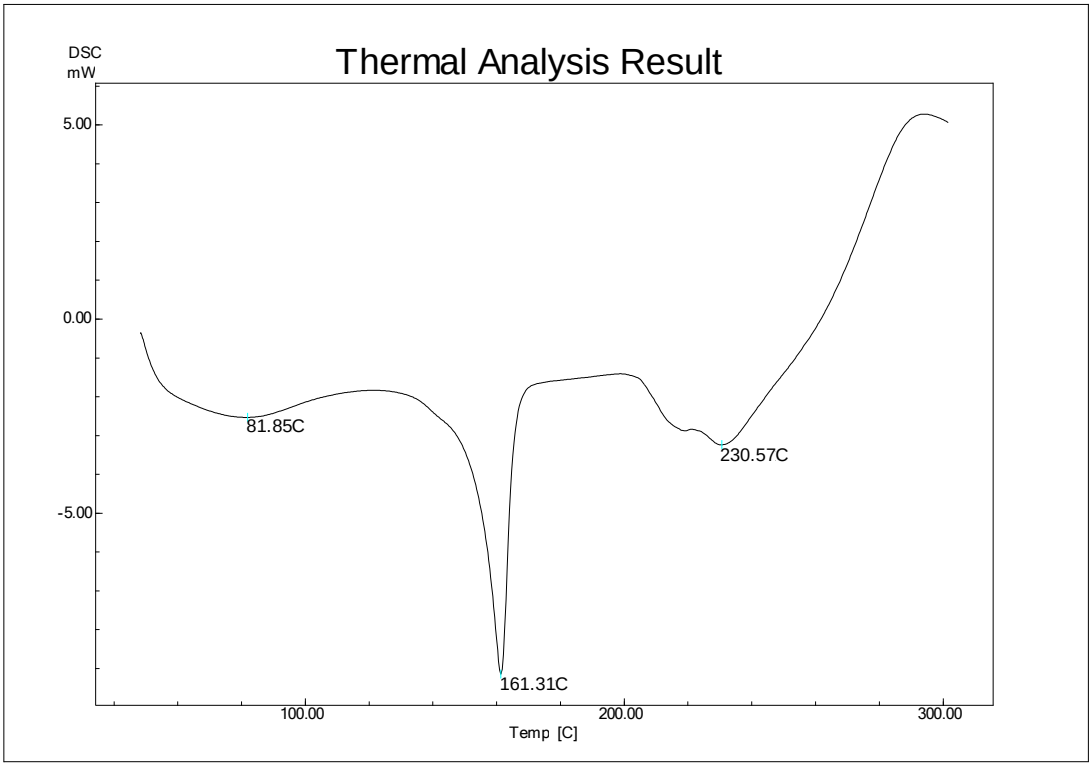


Figure No 8

DSC Spectra for physical mixture of pure drug (Metronidazole) + Eudragit RL + Chitosan



CONSTRUCTION OF STANDARD CURVE FOR METRONIDAZOLE

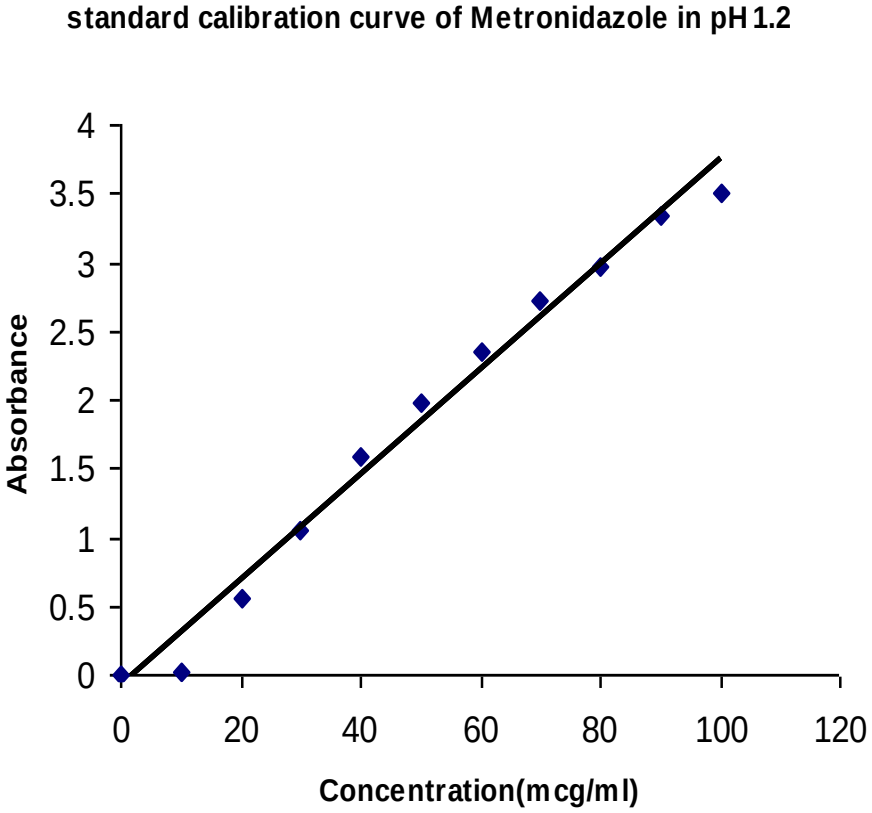
Table No.3

CALIBRATION CURVE FOR THE ESTIMATION OF METRONIDAZOLE IN 0.1 N HCl

Sl. No	Concentration (µg/ml)	Absorbance in 0.1N HCl
1.	0	0
2.	10	0.025
3.	20	0.563
4.	30	1.057
5.	40	1.579
6.	50	1.980
7.	60	2.346

8.	70	2.713
9.	80	2.978
10.	90	3.349
11	100	3.503
Slope		0.0383
Correlation Coefficient		0.9862

Figure No. 9

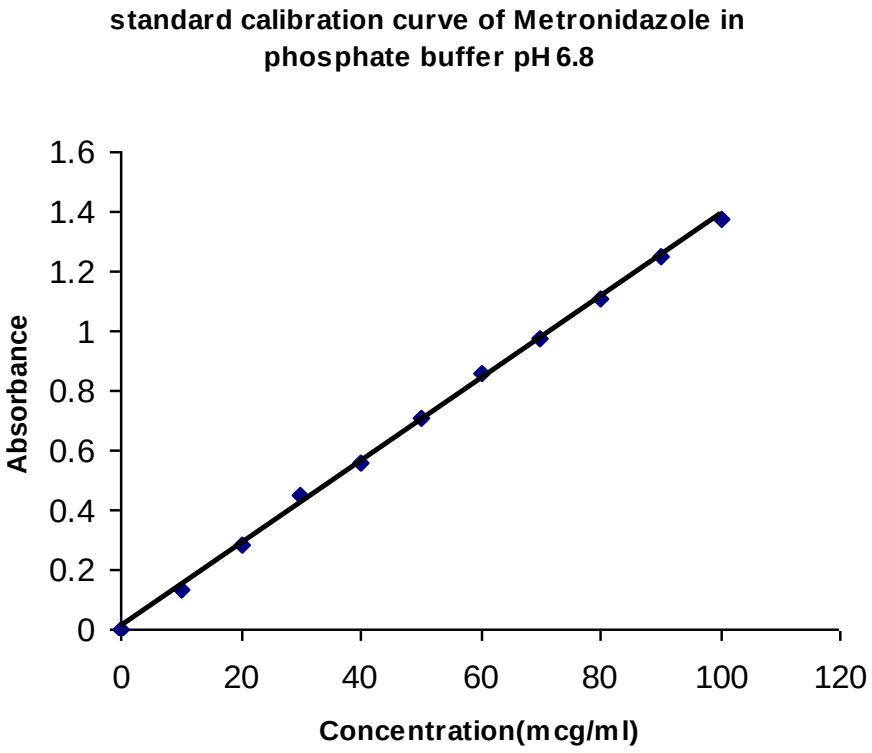


CALIBRATION CURVE FOR THE ESTIMATION OF METRONIDAZOLE IN PHOSPHATE BUFFER PH 6.8

Table No:4

Sl. No	Concentration (µg/ml)	Absorbance in Phosphate buffer pH6.8
1.	0	0
2.	10	0.136
3.	20	0.279
4.	30	0.446
5.	40	0.561
6.	50	0.710
7.	60	0.859
8.	70	0.978
9.	80	1.111
10.	90	1.248
11	100	1.371
Slope		0.0138
Correlation Coefficient		0.9992

Figure No. 10



CALIBRATION CURVE FOR THE ESTIMATION OF METRONIDAZOLE IN PHOSPHATE BUFFER PH 7.4

Table No: 5

Sl. No	Concentration (µg/ml)	Absorbance in Phosphate buffer pH6.8
1.	0	0
2.	10	0.136
3.	20	0.279
4.	30	0.446
5.	40	0.561
6.	50	0.710
7.	60	0.859
8.	70	0.978
9.	80	1.111
10.	90	1.248
11	100	1.371
Slope		0.0138
Correlation Coefficient		0.9998

Figure No 11

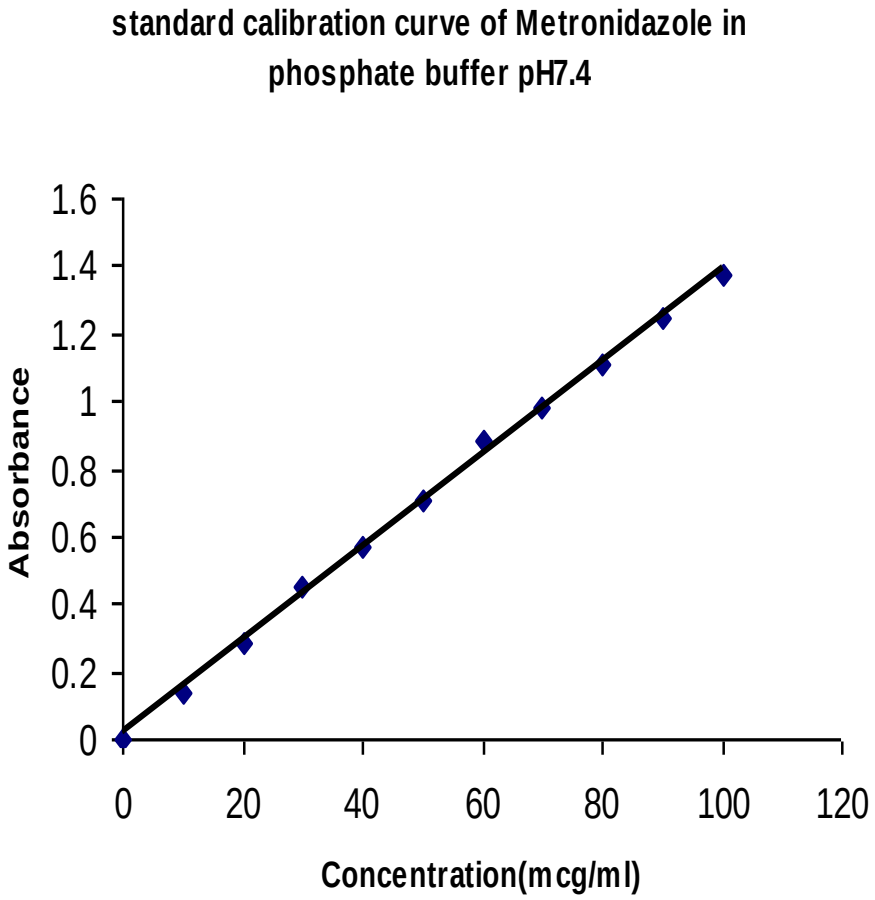

standard calibration curve of Metronidazole in phosphate buffer pH7.4
Absorbance
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
Concentration(mcg/ml)

Table No. 6

DATA FOR PERCENTAGE YIELD OF FORMULATIONS OF MUCOADHESIVE MICROSPHERES OF METRONIDAZOLE

Formulation code	Percentage yield (%)
MF_1	72.66
MF_2	70.27
MF_3	70.81
MF_4	71.64
MF_5	72.54
MF_6	79.30

Table No 7

DATA FOR PARTICLE SIZE OF FORMULATION OF MUCOADHESIVE MICROSPHERES OF METRONIDAZOLE

Formulation code	Average particle size(µm)
MF 1	58.9
MF 2	80.6
MF 3	96.1
MF 4	116.2
MF 5	153.7
MF 6	205.1

Table No. 8

DATA FOR ANGLE OF REPOSE OF FORMULATIONS OF MUCOADHESIVE MICROSPHERES OF METRONIDAZOLE

Formula code	Angle of repose $\theta=\tan^{-1}(h/r)$ Mean ± S.D *(n=3)*
MF_1	24° 70' ± 0.53
MF_2	25° 23' ± 1.10
MF_3	25° 24' ± 1.36
MF_4	25° 30' ± 1.32
MF_5	25° 08' ± 0.88
MF_6	23° 72' ± 0.51

Table No. 9

DATA FOR BULK DENSITY OF FORMULATIONS OF MUCOADHESIVE MICROSPHERES OF METRONIDAZOLE

Formula code	Bulk Density gm/cm^3 ± SD
MF_1	0.559 ± 0.033
MF_2	0.574 ± 0.013
MF_3	0.484 ± 0.018
MF_4	0.543 ± 0.007
MF_5	0.568 ± 0.016
MF_6	0.599 ± 0.010

Table No. 10

DATA FOR PERCENTAGE DRUG CONTENT OF FORMULATIONS OF MUCOADHESIVE MICROSPHERES OF METRONIDAZOLE

Formula code	Percentage Drug Content (% ± SD)
MF_1	77.99 ± 0.995
MF_2	72.08 ± 0.721
MF_3	77.11 ± 0.821
MF_4	75.39 ± 0.823
MF_5	71.97 ± 0.121
MF_6	83.37 ± 0.845

S.D= Standard deviation

Table No. 11

DATA FOR PERCENT ENTRAPMENT EFFICIENCY OF FORMULATIONS OF MUCOADHESIVE MICROSPHERES OF METRONIDAZOLE

Formula code	Theoretical drug content in %	Practical drug content in %	Entrapment Efficiency in %
MF_1	10.51	9.13	86.81
MF_2	9.60	8.08	84.12
MF_3	10.50	8.57	81.60
MF_4	9.89	7.85	79.35
MF_5	11.121	8.70	78.21
MF_6	10.38	8.04	77.41

Table No. 12

DATA FOR *IN VITRO* WASH-OFF TEST FOR MUCOADHESION IN 0.1N HCl

Formula code	Mean Percentage of microspheres adhering to tissue *(n=3)*					
	0.1N HCl					
	0.5hr	1hr	2hrs	3hrs	4hrs	5hrs
MF_1	75.67 (3.27)	72.67 (3.46)	71.61 (3.69)	69.33 (3.77)	68.46 (3.94)	66.33 (4.03)
MF_2	79.33 (5.41)	78.622 (7.39)	76.67 (7.77)	75.33 (2.99)	74.00 (5.26)	72.67 (6.19)
MF_3	74.33 (3.15)	73.66 (6.54)	72.67 (6.93)	72.00 (6.25)	70.67 (3.69)	68.33 (7.53)
MF_4	84.67 (2.55)	82.67 (2.66)	81.33 (5.68)	79.00 (5.00)	78.27 (7.77)	76.33 (7.90)
MF_5	71.67 (3.27)	70.67 (3.46)	69.10 (6.25)	68.66 (3.69)	66.33 (3.77)	65.00 (6.67)
MF_6	70.33 (2.84)	70.00 (5.00)	68.67 (7.77)	6733 (2.99)	65.67 (3.09)	65.10 (3.15)

Table No. 13

DATA FOR *IN VITRO* WASH-OFF TEST FOR MUCOADHESION IN PHOSPHATE BUFFER PH 6.8

Formula code	Mean Percentage of microspheres adhering to tissue *(n=3)*					
	Phosphate buffer pH 6.8					
	0.5hr	1hr	2hrs	3hrs	4hrs	5hrs
MF_1	78.67 (3.69)	77.33 (3.77)	76.27 (3.94)	75.57 (3.04)	73.46 (3.94)	71.33 (4.03)
MF_2	81.67 (7.77)	78.33 (2.99)	77.00 (5.26)	74.00 (5.20)	72.00 (5.26)	70.67 (6.19)
MF_3	70.67 (6.93)	68.00 (6.25)	67.67 (3.69)	64.47 (3.60)	62.67 (3.69)	68.33 (7.53)
MF_4	80.33 (5.68)	79.00 (5.00)	76.54 (7.77)	72.67 (7.77)	70.27 (7.77)	68.33 (7.90)
MF_5	69.00 (6.25)	67.62 (3.69)	65.33 (3.77)	63.33 (4.77)	60.33 (3.77)	58.00 (6.67)
MF_6	68.67 (7.77)	65.33 (2.99)	64.67 (3.09)	61.67 (3.09)	58.67 (3.09)	56.10 (3.15)

Table No. 14

DATA FOR *IN VITRO* WASH-OFF TEST FOR MUCOADHESION IN PHOSPHATE BUFFER PH 7.4

Formula code	Mean Percentage of microspheres adhering to tissue *(n=3)*					
	Phosphate buffer pH 7.4					
	0.5hr	1hr	2hrs	3hrs	4hrs	5hrs
MF_1	80.67 (3.69)	77.33 (3.77)	75.27 (3.94)	74.57 (3.04)	73.46 (3.94)	70.33 (4.03)
MF_2	79.67 (7.77)	77.33 (2.99)	76.00 (5.26)	74.25 (5.20)	72.00 (5.26)	70.01 (6.19)
MF_3	70.67 (6.93)	68.00 (6.25)	67.67 (3.69)	64.47 (3.60)	61.67 (3.69)	59.33 (7.53)
MF_4	82.33 (5.68)	79.00 (5.00)	76.54 (7.77)	73.67 (7.77)	71.27 (7.77)	68.33 (7.90)
MF_5	69.00 (6.25)	67.62 (3.69)	66.33 (3.77)	64.33 (4.77)	63.33 (3.77)	61.00 (6.67)
MF_6	78.67 (7.77)	75.33 (2.99)	74.67 (3.09)	71.67 (3.09)	69.67 (3.09)	66.10 (3.15)

Numbers in parenthesis indicates the coefficient of variance (CV) (or) percentage relative standard deviation (%RSD).

CV = (Standard Deviation / Mean)*100

Time (hrs)	Cumulative % of Drug release			Mean ±SD
	1	2	3	
0	0	0	0	0 ± 0
1	0	0	0	0 ± 0
2	0	0	0	0 ± 0
3	6.426	15.996	13.262	11.895 ± 3.34
4	11.074	20.645	17.910	16.543 ± 3.30
5	23.106	32.677	29.942	28.575 ± 3.21
6	33.415	42.986	40.251	38.884 ± 3.31
7	35.726	45.296	42.562	41.194 ± 3.35
8	45.447	55.017	52.283	50.916 ± 3.39
9	53.582	63.152	60.418	59.051 ± 3.29
10	62.729	72.299	69.565	68.197 ± 3.32
11	74.364	83.934	81.200	79.833 ± 3.33
12	77.303	86.874	84.140	82.772 ± 3.36

Table No. 15

***IN VITRO* DRUG RELEASE PROFILE FOR FORMULATION MF1**

Figure No. 12

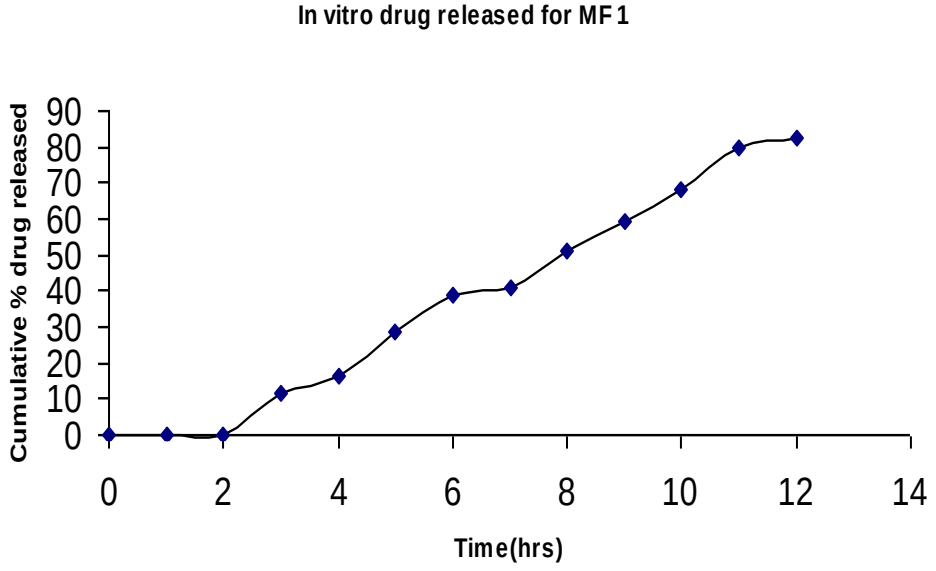


Figure No. 13

Zero order plot for MF1

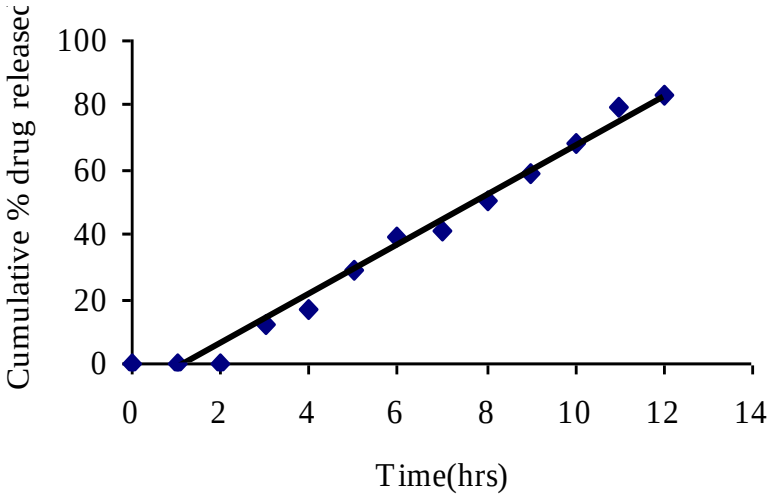


Figure No 14

First order plot for MF1

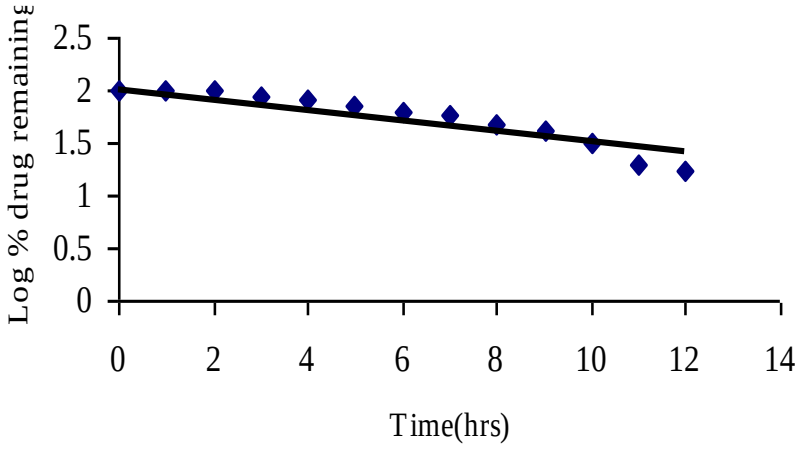


Figure No 15

Korsmeyer-peppa's plot for MF1

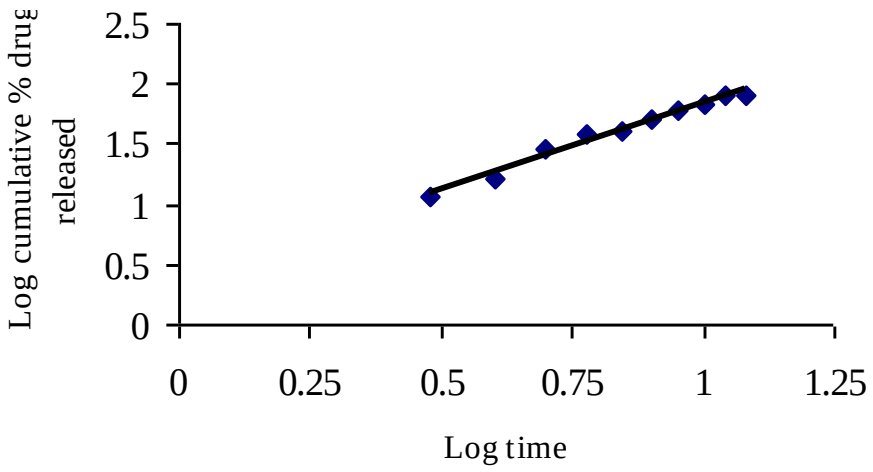


Figure No 16

Higuchi plot for MF1

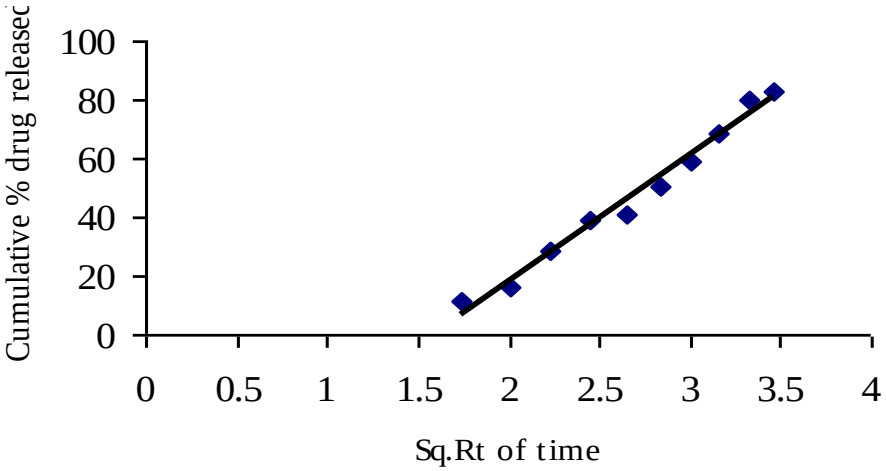


Table No. 16

***IN VITRO* DRUG RELEASE PROFILE FOR FORMULATION MF2**

Time (hrs)		Cumulative % of Drug release			Mean ±SD
		1	2	3	
0		0	0	0	0 ± 0
1		0	0	0	0 ± 0
2		0	0	0	0 ± 0
3		6.227	15.798	13.063	11.696 ± 4.91
4		9.584	19.155	16.420	15.053 ± 4.90
5		22.039	31.610	28.876	27.508 ± 3.98
6		33.688	43.251	40.525	39.157 ± 4.92
7		43.943	53.513	50.779	49.412 ± 4.95
8		52.543	62.113	59.379	58.012 ± 4.99
9		65.422	74.993	72.258	70.891 ± 4.94
10		74.131	83.702	80.968	79.600 ± 4.89
11		75.895	85.466	82.731	81.364 ± 4.97
12		76.168	85.739	83.005	81.637 ± 4.93

Figure No. 17

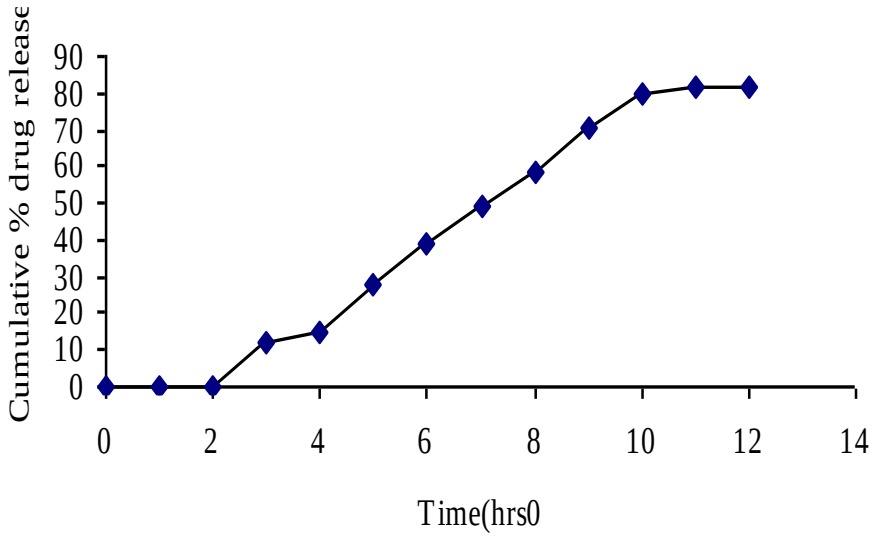


Figure No.18

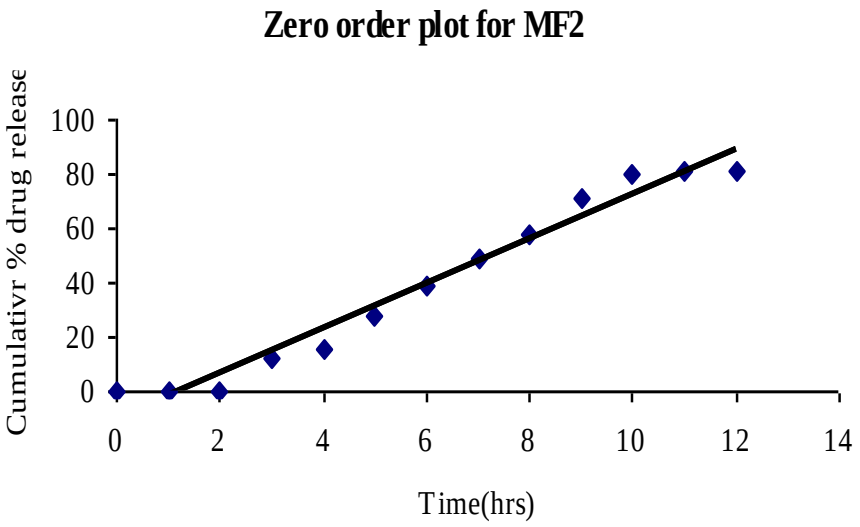

Zero order plot for MF2
Cumulativr % drug release
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Time(hrs)

Figure No.19

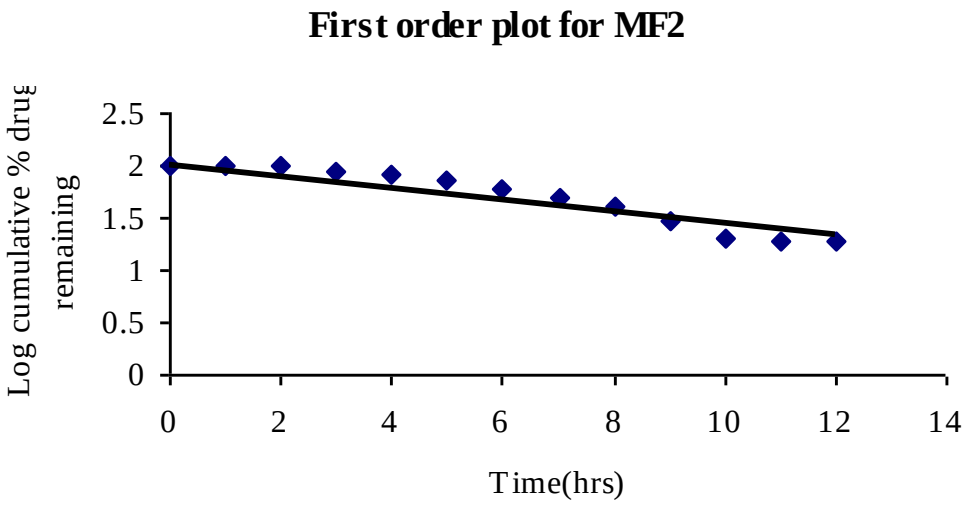
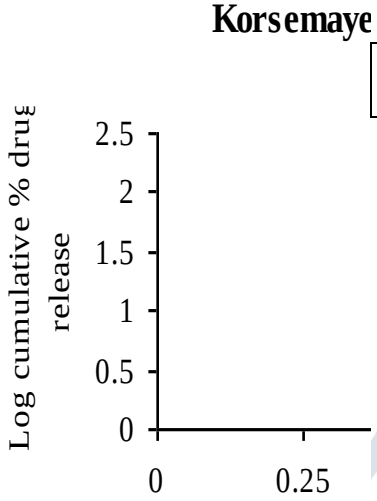

First order plot for MF2
Log cumulative % drug remaining
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
14
Time(hrs)

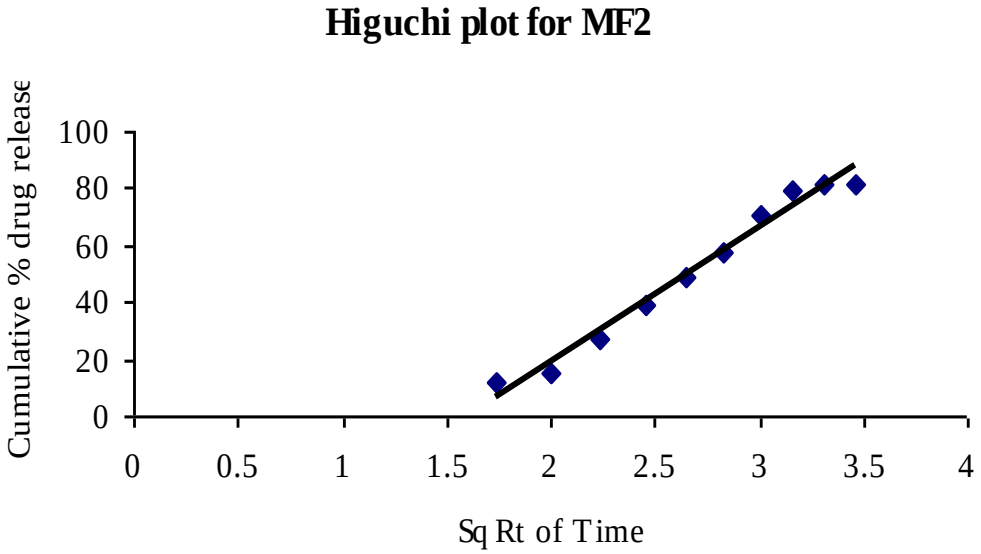
Figure No. 20



Time (hrs)	Cumulative % of Drug release	Mean ±SD

Table No. 17

***IN VITRO* DR RELEASE PROFILE FOR FORMULATION MF 3**



	1	2	3	
0	0	0	0	0 ± 0
1	0	0	0	0 ± 0
2	0	0	0	0 ± 0
3	6.227	15.798	13.063	11.696 ± 2.71
4	10.951	20.522	17.787	16.420 ± 2.71
5	21.766	31.337	28.602	27.235 ± 2.74
6	32.595	42.165	39.431	38.063 ± 2.78
7	41.208	50.779	48.044	46.677 ± 2.76
8	49.056	58.627	55.892	54.525 ± 2.69
9	58.586	68.156	65.422	64.055 ± 2.78
10	64.424	73.995	71.260	69.893 ± 2.74
11	70.426	79.997	77.262	75.895 ± 2.73
12	73.707	83.278	80.544	79.176 ± 2.77

Figure No. 21

In vitro drug release for MF3

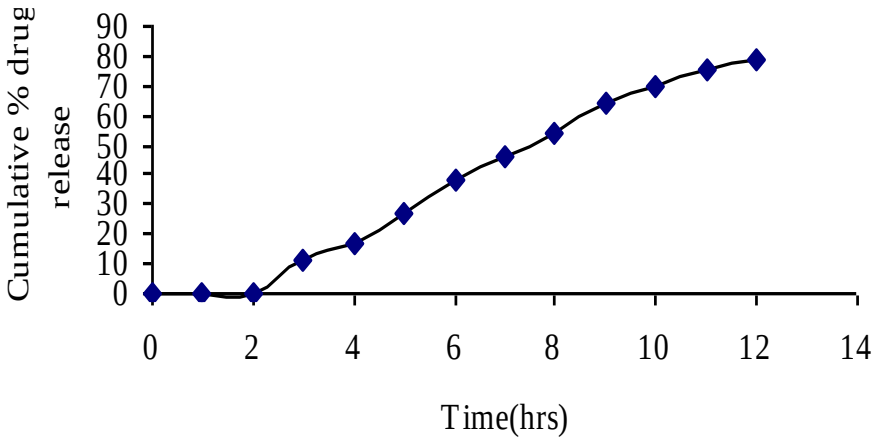


Figure No. 22

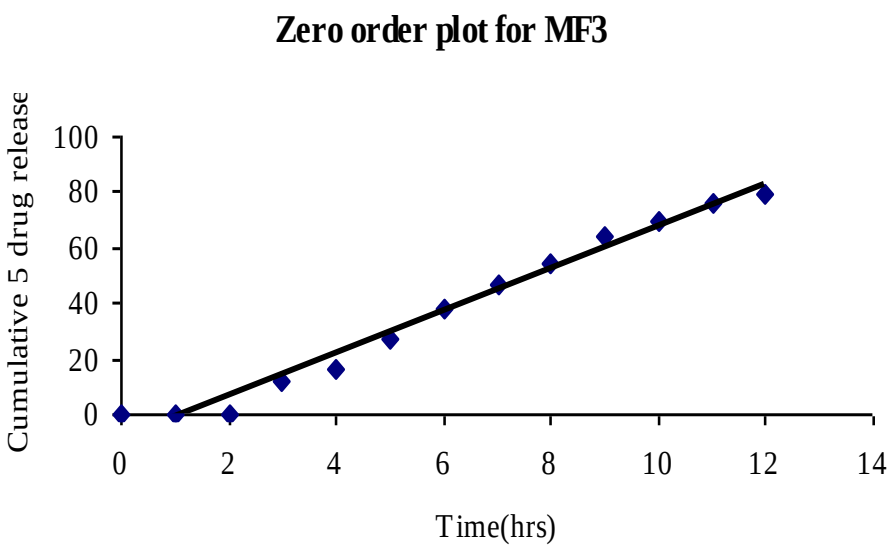


Figure No. 23

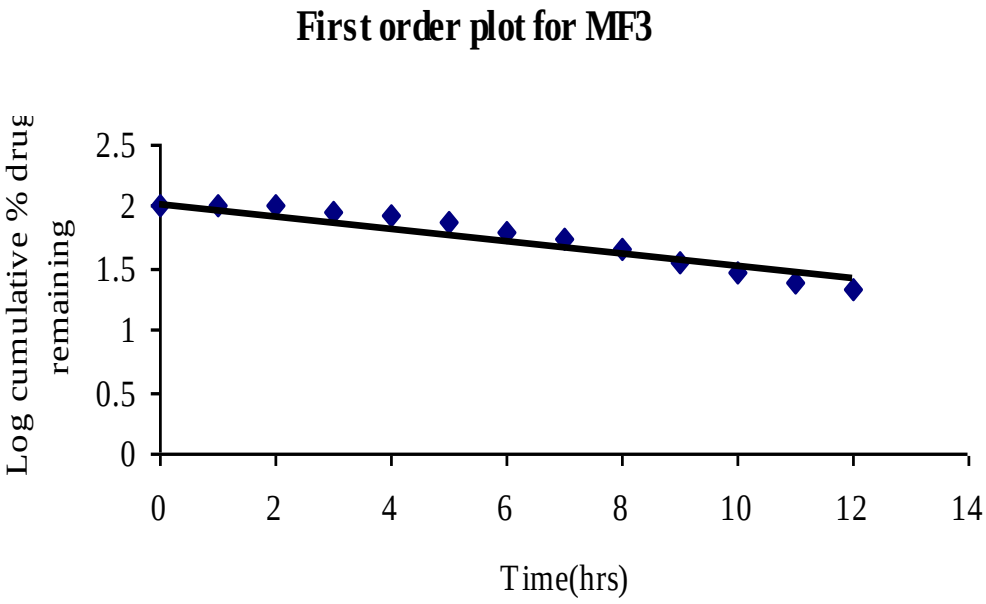


Figure No. 24

Korsmeyar-Peppa's plot for MF3

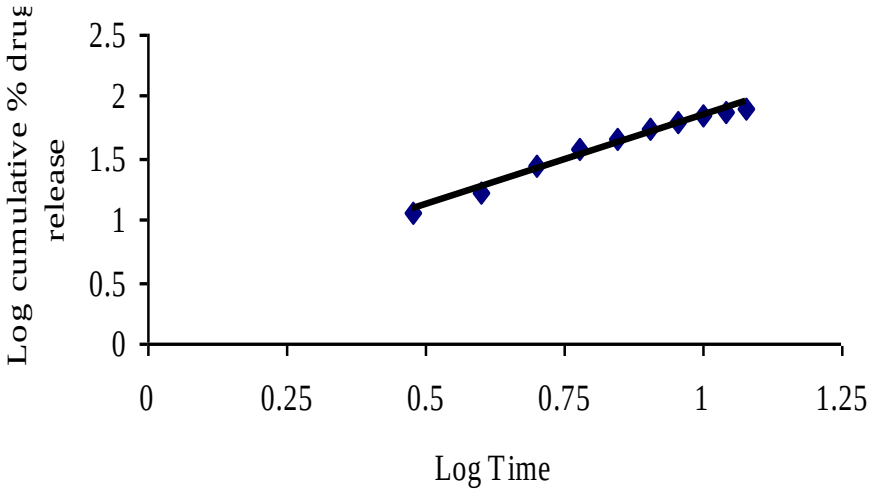
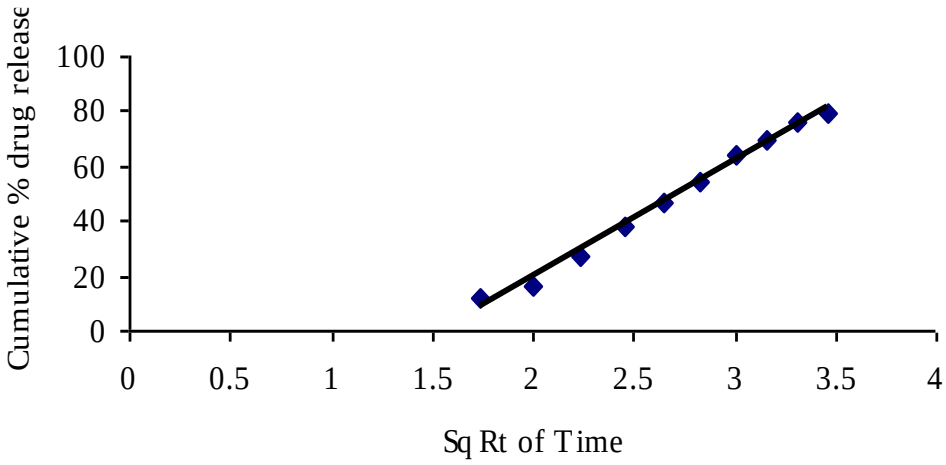


Figure No. 25

Higuchi plot for MF3



Time (hrs)	Cumulative % of Drug release			Mean ±SD
	1	2	3	
0	0	0	0	0 ± 0
1	0	0	0	0 ± 0
2	0	0	0	0 ± 0
3	16.030	6.460	9.194	10.561 ± 4.09
4	19.155	9.584	12.318	13.686 ± 4.11
5	31.747	22.176	24.911	26.278 ± 4.16
6	43.259	33.688	36.423	37.790 ± 4.17
7	51.599	42.028	44.763	46.130 ± 4.14
8	59.461	49.890	52.625	53.992 ± 4.13
9	69.387	59.816	62.551	63.918 ± 4.18
10	73.584	64.014	66.748	68.115 ± 4.15
11	80.817	71.246	73.981	75.348 ± 4.17
12	83.388	73.817	76.551	77.919 ± 4.11

Table No. 18

***IN VITRO* DRUG RELEASE PROFILE FOR FORMULATION MF4**

Figure No. 26

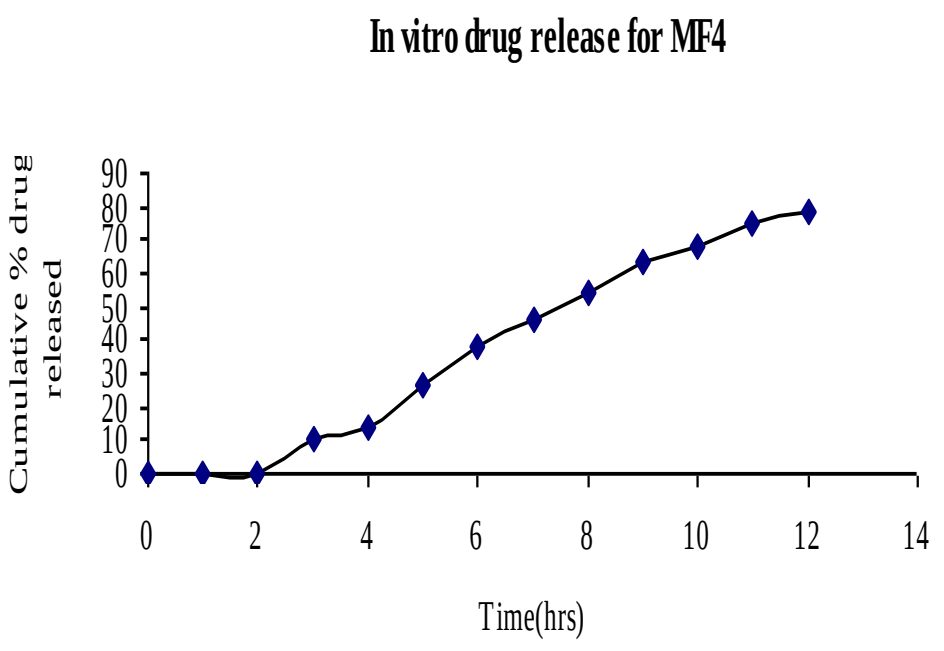


Figure No. 27

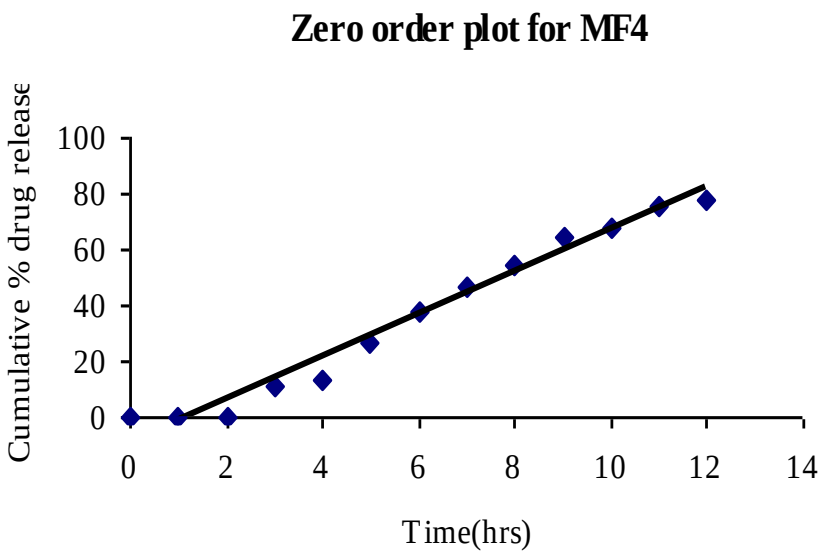


Figure No. 28

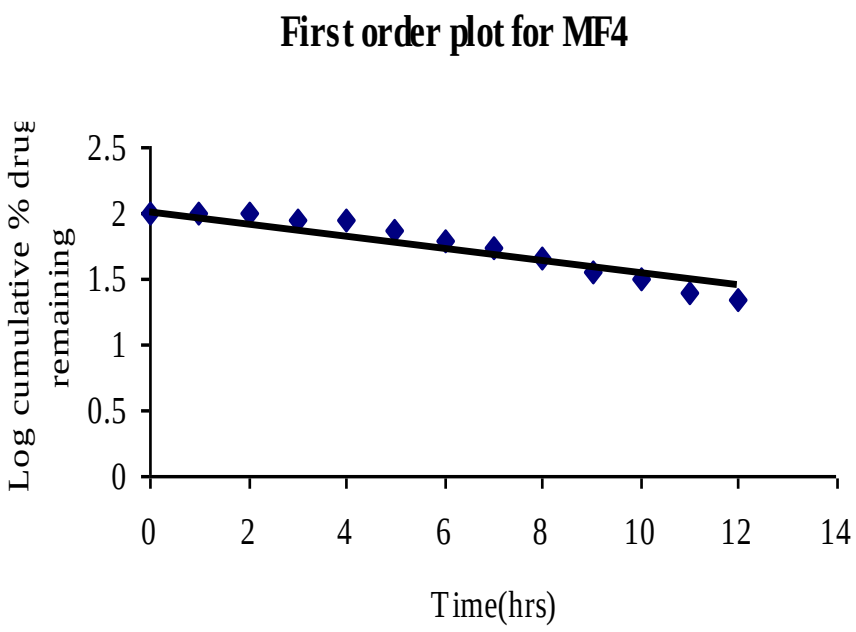


Figure No. 29

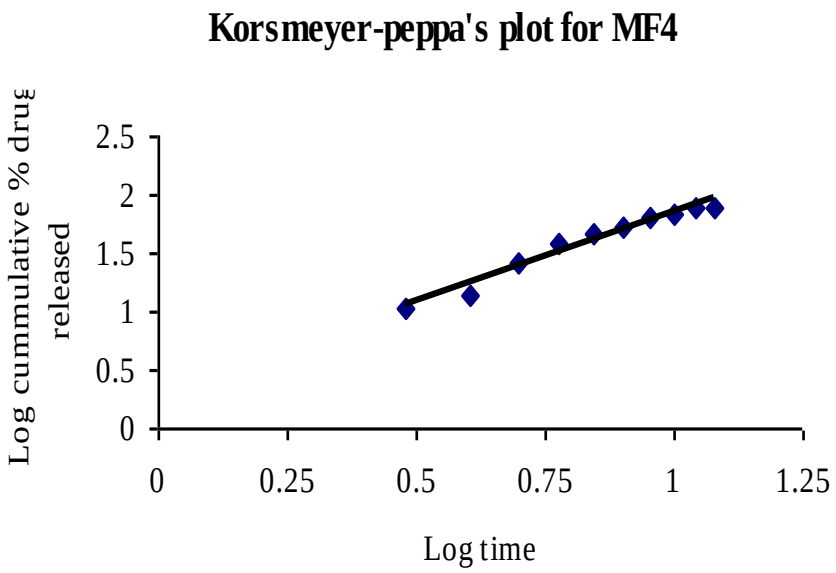


Figure No. 30

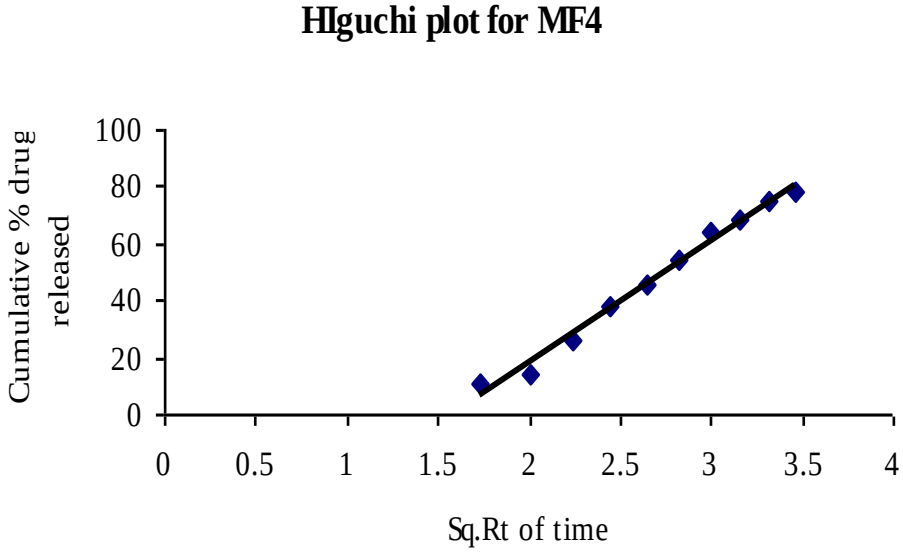


Table No. 19

Time (hrs)	Cumulative % of Drug release			Mean ±SD
	1	2	3	
0	0	0	0	0 ± 0
1	0	0	0	0 ± 0
2	0	0	0	0 ± 0
3	6.391	9.126	15.962	10.493 ± 4.26
4	9.174	11.908	18.744	13.275 ± 4.24
5	19.442	22.176	29.012	23.543 ± 4.27
6	31.596	34.331	41.167	35.698 ± 4.25
7	39.021	41.755	48.591	43.122 ± 4.26
8	46.199	48.933	55.769	50.300 ± 4.27
9	54.347	57.082	63.918	58.449 ± 4.25
10	60.596	63.33	70.166	64.697 ± 4.33
11	69.879	72.614	79.450	73.981 ± 4.29
12	72.381	75.116	81.952	76.483 ± 4.19

IN VITRO DRUG RELEASE PROFILE FOR FORMULATION MF5

Figure No. 31

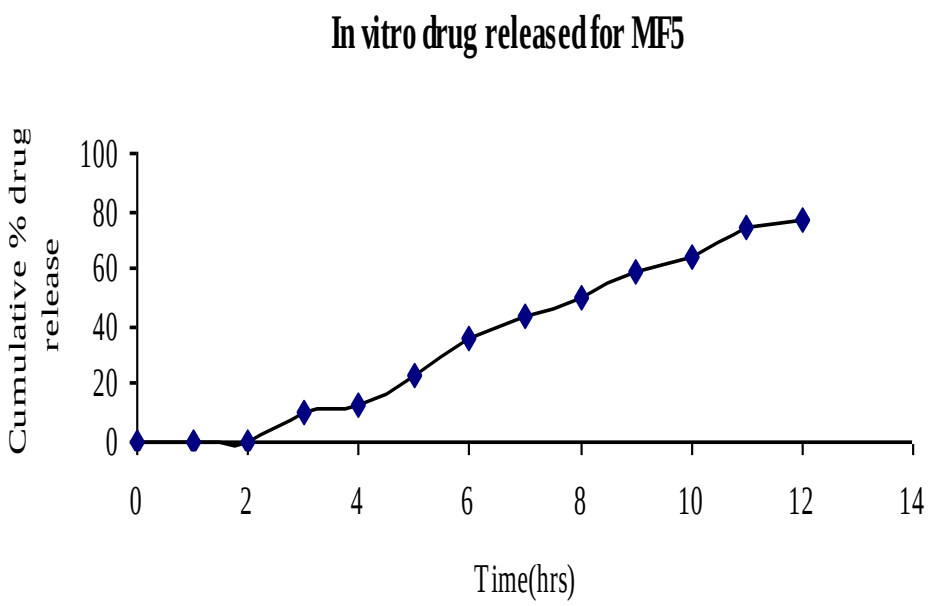


Figure No. 32

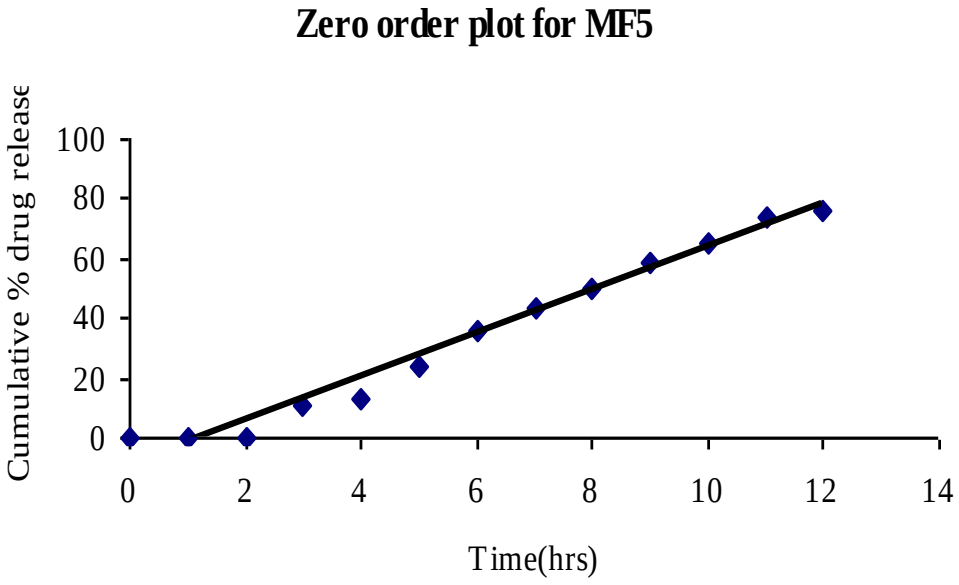


Figure No. 33

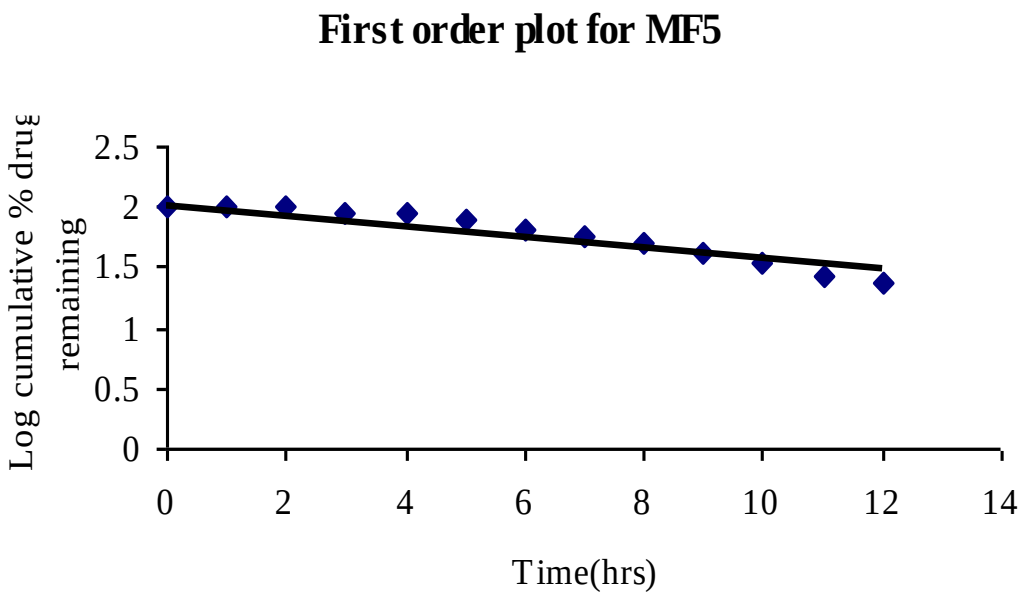


Figure No. 34

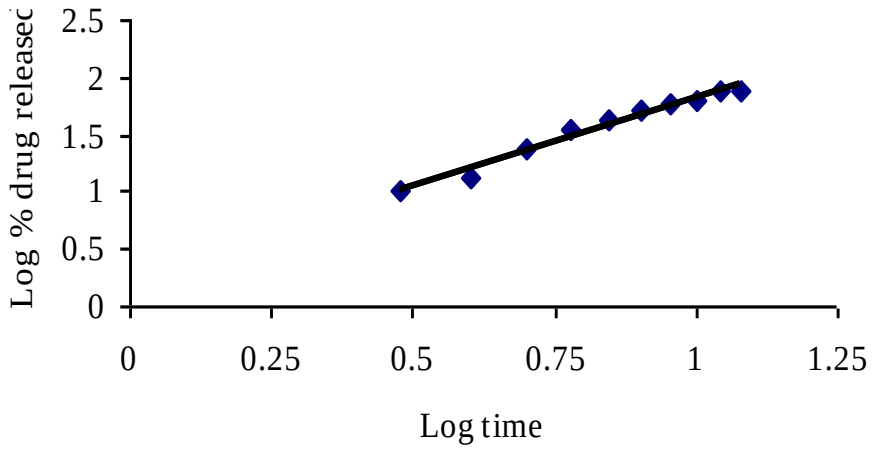


Table No 23

***IN VITRO* DRUG RELEASE PROFILE FOR FORMULATION MF6**

Cumulative % drug released

100
80
60
40
20
0

Time (hrs)	Cumulative % of Drug release			Mean ±SD
	1	2	3	
0	0	0	0	0 ± 0
1	0	0	0	0 ± 0
2	0	0	0	0 ± 0
3	14.048	4.477	7.212	8.579 ± 3.68
4	18.295	8.7243	11.458	12.826 ± 3.65
5	28.099	18.528	21.263	22.630 ± 3.62
6	40.484	30.913	33.647	35.015 ± 3.64
7	50.232	40.661	43.396	44.763 ± 3.58
8	55.181	45.611	48.345	49.712 ± 3.67
9	62.961	53.390	56.125	57.492 ± 3.62
10	68.690	59.119	61.853	63.221 ± 3.61
11	77.182	67.611	70.345	71.713 ± 3.60
12	80.448	70.877	73.612	74.979 ± 3.57

Figure No. 35

In vitro drug released for MF6

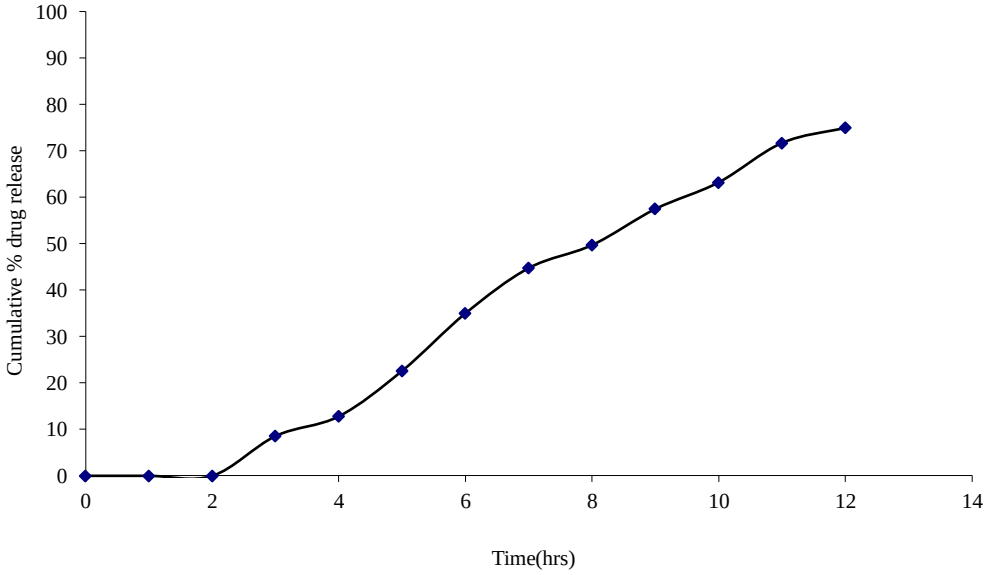


Figure No. 36

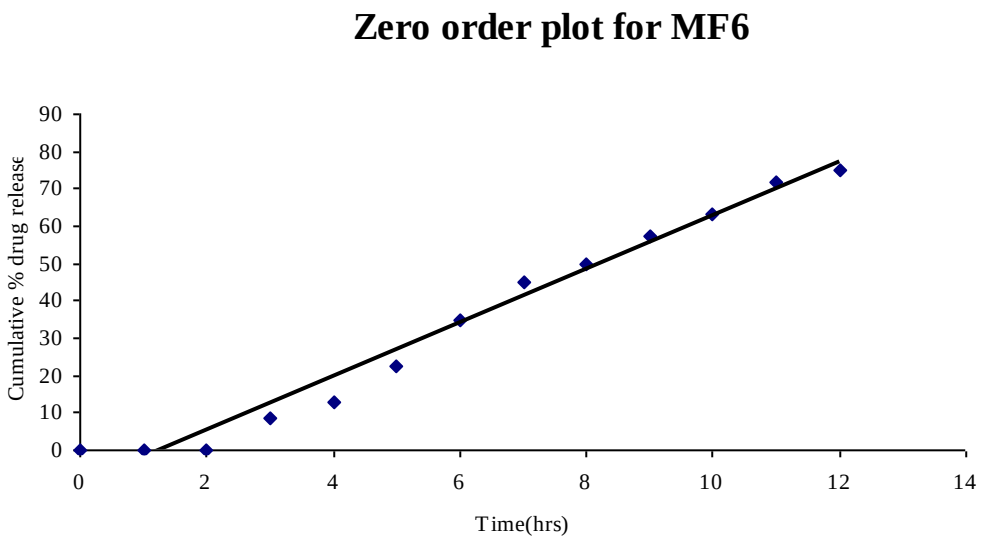


Figure No. 37

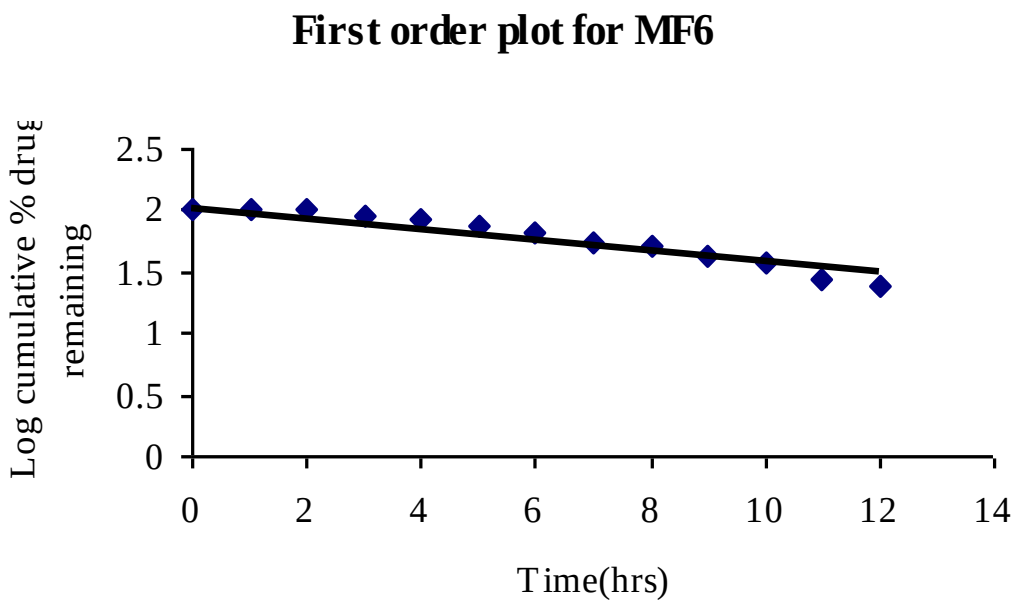


Figure No. 38

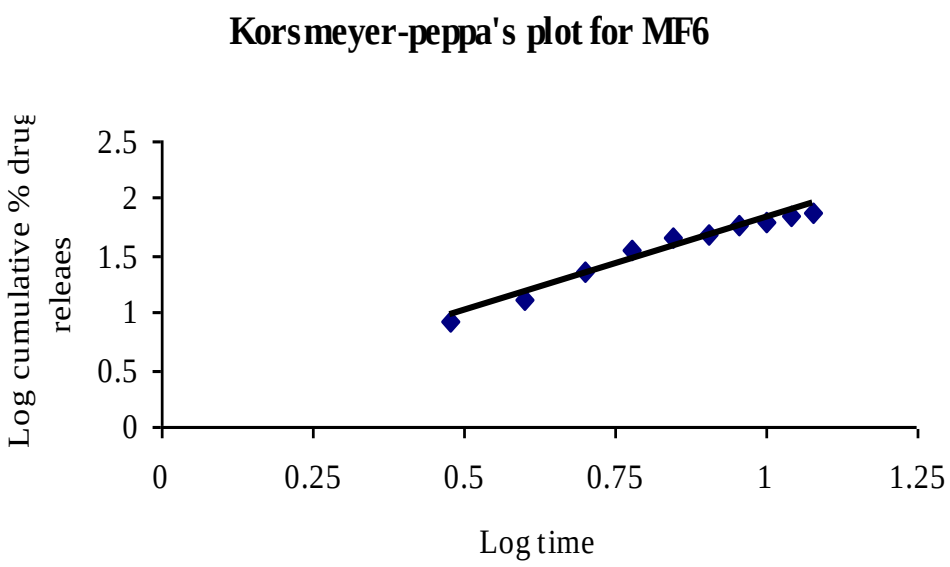


Figure No. 39

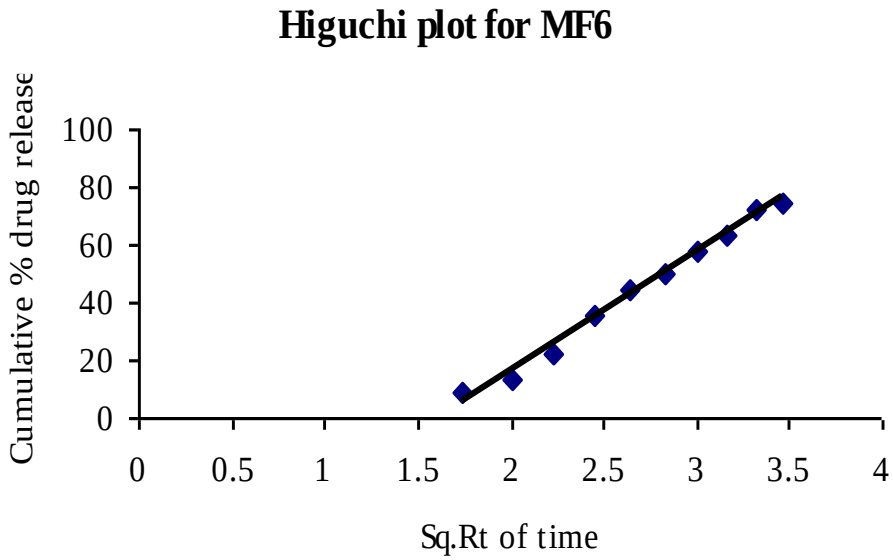


Figure No. 40

Comparative in vitro drug released plots for MF1 to MF6

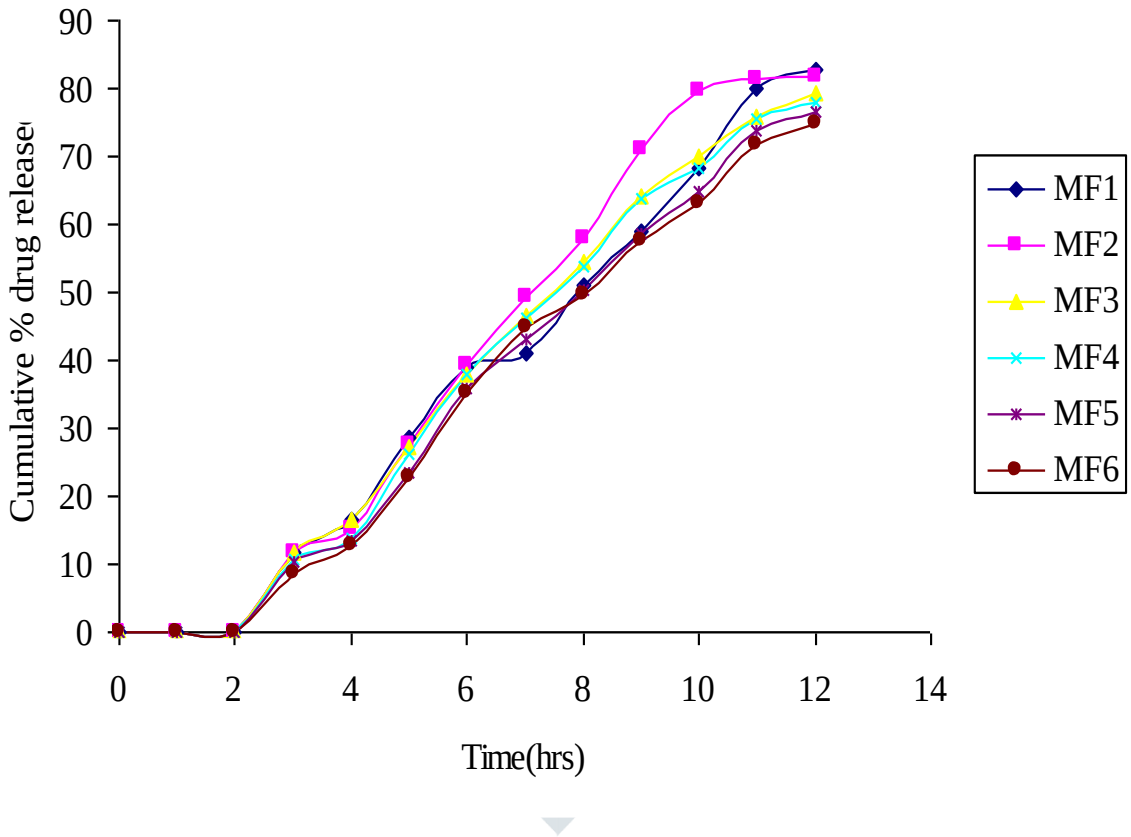


Table No 24

***IN VITRO* KINETIC DATA FOR MF1 TO MF6**

r-Correlation coefficient

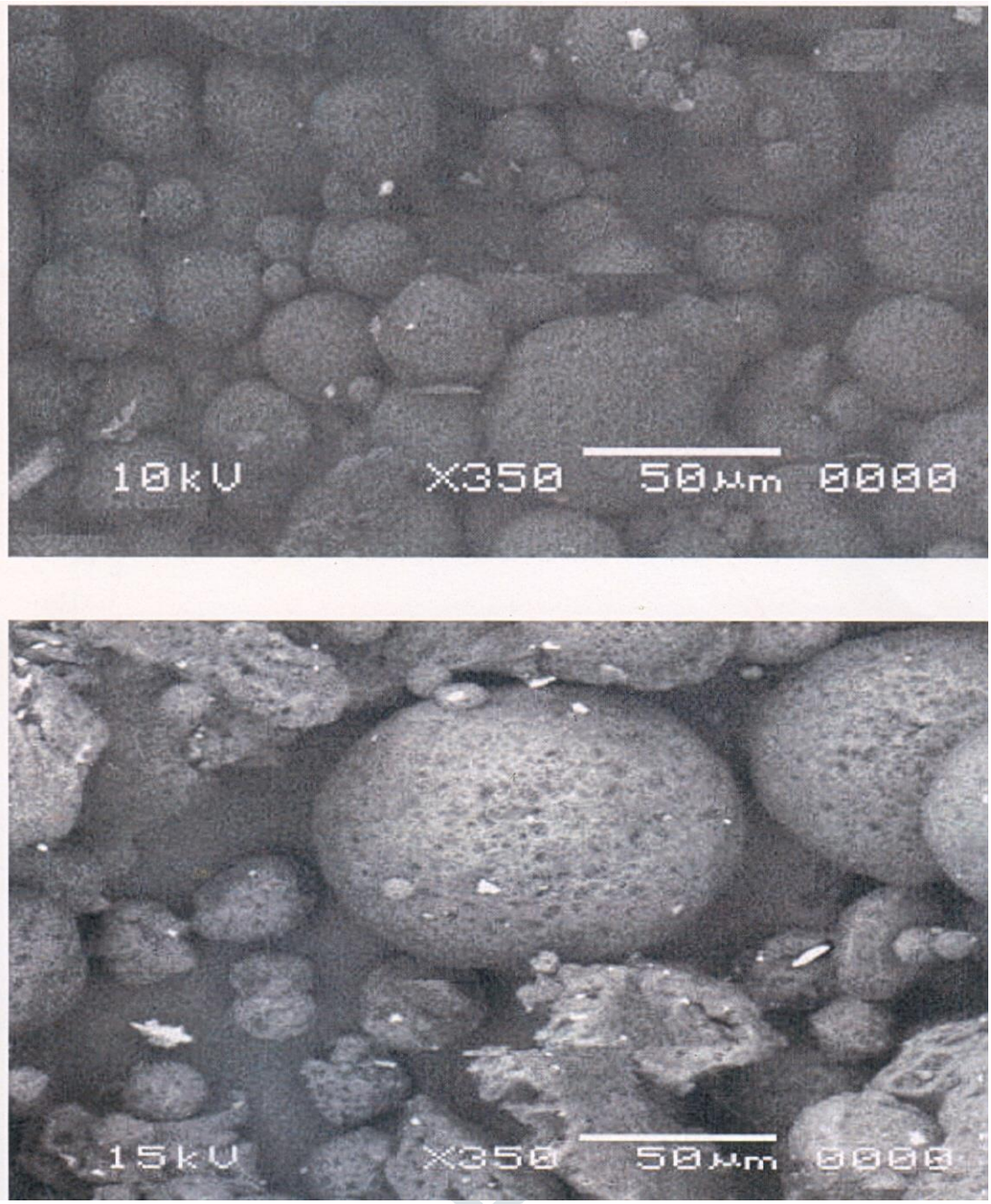
Formula code	Zero order	First order	Higuchi's	Korsmeyer-Peppa's
	r	r	r	r
MF_1	0.9822	0.8421	0.9829	0.9849
MF_2	0.9696	0.8651	0.9778	0.9689
MF_3	0.9709	0.8929	0.9827	0.9793
MF_4	0.9767	0.8903	0.9896	0.9666
MF_5	0.9795	0.8792	0.9898	0.9757
MF_6	0.9770	0.8865	0.9917	0.9702

SCANNING ELECTRON MICROGRAPH (SEM) OF THE PREPARED MUCOADHESIVE MICROSPHERES OF METRONIDAZOLE FORMULATION

SURFACE VIEW OF THE PREPARED MUCOADHESIVE MICROSPHERES

Figure No. 41

Figure No. 42



DISCUSSION

In this present work efforts have been made to develop mucoadhesive microspheres of Metronidazole using emulsion solvent evaporation technique using Eudragit RL along with mucoadhesive polymer Chitosan.

FT-IR Studies

Figure No 8 gives the FT-IR spectra of Metronidazole + Eudragit RL + Chitosan. In FT-IR Spectra of Metronidazole powder, -OH- group showed stretching vibration at the frequency of 3219.54 cm^{-1}, the C=N stretching band was observed at 1535.88 and 2139.38 cm^{-1}, the Aromatic 3^0 Amine was found at 1349.02 cm^{-1},

Aromatic C-C Multiple bond stretching was found at 1535.88 and 1428.81 cm^{-1} and the peak at 1369.02 showed the presence of the Aromatic Nitro Compounds. The FTIR spectral analysis showed that there was no appearance or disappearance of any characteristic peaks of pure drug Metronidazole in the physical mixture of drug and polymer, which confirms the absence of chemical interaction between drug and polymers.

DSC Studies

DSC provides information about all physical properties of sample as Crystalline or Amorphous nature and demonstrates the possible interaction between Drug and other Polymers. The thermal behavior of Metronidazole, Eudragit RL and Chitosan are shown in Figure No 12, According to Thermogram, Metronidazole produced sharp Endothermic peak at 167.50^0C which conformed crystalline form of the drug.

DSC curves of the Eudragit RL and Chitosan Exhibited an Endothermic peaks at 162.70 and 164.37^0 C, that has been attributed to the evaporation of water.

The thermogram of the physical mixture of Drug and Polymers showed that there was no interaction between drug and polymers.

Percentage Yield

The percentage yield of microspheres of all formulations was found in the range of 70.27% to 79.30% which is shown in Table No 9.

Morphology and Particle size

The microspheres prepared by solvent evaporation method were found to be discreet, spherical, free flowing and it was observed by Scanning Electron Microscopy (SEM) Figure No 47 and 48. The size of the mucoadhesive microspheres was determined by the optical microscopy method. The microspheres were found to be uniform in size with a size range of 58.9µm to 205.1µm which is shown in Table No10. The prepared microspheres were considered more suitable for colon targeting, mucosal retention and penetration which suggesting that the coating was well completed under the present conditions.

Micromeritics studies

The angle of repose was determined by funnel method. The angle of repose was found in the range of $23^0$72' to $25^0$30' which revealed that the microspheres of all the batches (MF1 to MF6) had good flow characteristics and flow rates (Table No.11.)

. The bulk density was in the range of 0.484gm/cm^3 to 0.599gm/cm^3 were shown in Table No 12.

Drug content analysis and Entrapment efficiency

The drug content values of mucoadhesive microspheres were found in the range of 71.97% to 83.37%, the determination of drug content showed that even if the polymer composition was changed the process was highly efficient to give microspheres having maximum drug loading. Table No.13. The result indicates that the 20 to 30% of drug leached out of microspheres, however the high drug dose is required for the treatment of amoebiasis, this drug content was considered acceptable.

The drug entrapment efficiency was found in the range of 77.41% to 86.81% which is shown in Table No.14.

***In vitro* wash off test for mucoadhesion**

Mucoadhesive Microspheres of Metronidazole exhibited good mucoadhesive properties in the *in vitro* wash off test. The results of wash off test were shown in Table No 15, 16 and 17.The MF1 formulation has more adhesive strength than others because of small particle size and surface area which is favoured by preparation and evaluation of mucoadhesive microspheres of Indomethacin[28].

***In vitro* drug release studies**

Metronidazole release from the microspheres was studied in 0.1N Hydrochloric acid as a simulated gastric fluid for first 2 hours, for next three hours in the phosphate buffer pH 6.8 as a simulated intestinal fluid and up to 12 hours in phosphate buffer pH 7.4 as a simulated colonic fluid by using USP XXIII basket type dissolution tester.

The drug release was retarded by increasing the polymer concentration due to increased viscosity and strength of matrix formed due to EUDRAGIT RL and CHITOSAN. The solubility of Eudragit RL is fully depended on the pH of the medium, it will dissolve at the range of pH 6 to pH 8 so the successful targeting to the colon will be achieved. Chitosan provides good mucoadhesion property for better efficacy of the drug on the colonic mucosa by increasing the colonic transit time due to sticking to the mucous of the colon.

In vitro drug released at the end of 12 hours showed that MF1 released the 82.77% of Metronidazole Table No 18, MF2 released 81.63% of Metronidazole Table No 19, MF3 released 79.17% of Metronidazole Table No 20, MF4 released 77.91% of Metronidazole Table No 21, MF5 released 76.48% of Metronidazole Table No 22, MF6 released 74.97% of Metronidazole Table No 23, so the drug release from microspheres was decreased by increasing the polymer concentration because the drug release mainly depends on the composition and amount of the polymer used. Since this finding is in the favour of releasing the Albendazole from the polymeric matrix of Eudragit RL[36].

***In vitro* drug release kinetics**

For all the formulation MF1 to MF6 the kinetic drug released data were shown in the Table No 24, For the first order kinetic the **r** values were found in the range of 0.8421 to 0.8929, For the zero order kinetic the **r** values found in the range of 0.9696 to 0.9822, so all formulations showed the zero order drug release kinetic, among them formulation MF1 showed best **r** value (0.9822) for the zero order kinetic.

Mechanism of drug release

In order to understand the complex mechanism of drug release from the mucoadhesive microspheres, the *in vitro* Metronidazole release data were fitted to korsmeyer-peppa's release model and interpretation of **r** values enlightens in understanding the release mechanism from the dosage form. The **r** values thus obtained were ranged from 0.9666 to 0.9849. All the formulations exhibited anomalous (non-fickian transport) diffusion mechanism. The drug release was diffusion controlled as the plot of Higuchi's model was found to be linear (**r** > 0.9778). These formulations are also showed as good '**r**'values of zero order kinetics indicating the Metronidazole release from these mucoadhesive microspheres were by both diffusion and erosion. It shows that the result was found in present study is strongly supported by the previous work carried out on preparation and optimization of metoclopromide mucoadhesive microspheres.[27].

The formulation MF1, Drug: polymer ratio (1:1) was selected as BEST formulation with 82.77% of drug released at 12th hours.

CONCLUSION

In the present work efforts have been made to design and evaluate mucoadhesive microspheres of Metronidazole and the results obtained in the study have been summarized below.

All the formulations exhibited anomalous (non-fickian transport) diffusion mechanism and follow zero order kinetic. The formulation MF1 (Metronidazole-500 mg, Eudragit RL-500 mg, Chitosan 5% w/v solution) was selected as best formulation; with 82.77% of controlled drug release at the end of 12 hours with best mucoadhesion properties,hance such a design can be used for colon targeted drug delivery of metronidazole to eradicate the parasites from the colonic region

Finally it is concluded that by increasing polymer concentration the drug release from microspheres will be slow.

Success of the *in vitro* drug release studies recommends the product for further *in vivo* studies in detail for its viability in clinical practice.

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