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Research Article

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Preparation and *invitro* evaluation of diltiazem hydrochloride microspheres by using eudragit RL100, eudragit RS100 as polymers

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ABSTRACT

The objective of this research was to prepare a Gastro Retentive drug delivery system (GRDDS) of Diltiazem hydrochloride. In the present study, preparation of Diltiazem HCl Microspheres was formulated using Eudragit RL100 and Eudragit RS100 as the rate controlling polymer, alone by solvent evaporation methods with an aim to prolong its release. Six formulations prepared by using different drug to polymer ratios, were evaluated for relevant parameters. Particle size analysis, drug encapsulation efficiency, Invitro release studies were performed. The mean particle size of prepared hollow microspheres increased but the drug release rate from the microspheres decreased as the polymer concentration increased. Depending upon the drug to polymer ratio, the entrapment efficiency were found to range between 97.73± 0.27, 91.28± 0.38, 95 .8± 0.25, 85.21± 0.36, 87.84± 0.27, 88.12± 0.28 respectively. In-vitro evaluation of Floating Drug Delivery System (FDDS), prediction of the drug release, and polymers concentration to match target release profile was investigated. In vitro studies were carried out at different pH for a period of 12 hours. The scanning electron microscopic study indicated that the microspheres were spherical in shape and the drug remained dispersed in the polymer matrix at amorphous state. Drug polymer interaction was absent as evidenced by FT-IR.

Keywords: Diltiazem hydrochloride, mucoadhesive microspheres, solvent evaporation technique, Eudragit RL100 and RS100, in vitro evaluation.

INTRODUCTION

Diltiazem HCl, a benzodiazepine, voltage sensitive ca $^{2+}$channel blocker with a high therapeutic potential but with a very short biological half life was encapsulated within microsphere. Diltiazem is a calcium ion influx inhibitor (calcium entry blocker or calcium ion antagonist). The antihypertensive, antianginal and antiarrhythmic effects of Diltiazem is believed to be related to its specific cellular action of selectively inhibiting transmembrane influx of calcium in cardiac muscle, coronary arteries, and systemic arteries and in cells of the intra cardiac conduction system.[1] Given orally, 90–100% of Diltiazem is absorbed, but due to high first pass metabolism, bioavailability is much lower (40–60%), half life is 4-5 hours (with chronic dosages) and not cleared by hemodialysis.[2] Diltiazem hydrochloride are easily absorbed from gastrointestinal tract (GIT) and have a short half-life are eliminated quickly from blood circulation.[3] This drug undergoes substantly hepatic first pass effect it shows to oral bioavailability 40%. So they require frequently dosing to avoid these drawback, the oral sustained control release formulation have been developed in an attempt to release the drug surely in to the GIT and maintained an

effective drug concentration in the serum for longer period of time. Administration of conventional tablet of Diltiazem Hydrochloride has been reported to exhibit fluctuations in plasma drug level resulting either in side effect of reduction in drug concentration at receptor side, also the maintenance of constant plasma concentration of cardiac vascular drug is important in ensuring the designed therapeutic response. again since the half life of Diltiazem HCl is 3-4 hrs multiple dose of drug need to maintained constant plasma concentration for good therapeutic response and improve patients compliance .Hence the objective of study was made to develop control release microsphere system of Diltiazem hydrochloride using polymer like Eudragit RL100, RS100 which will controlled the released of drug increase the bioavailability of drug and dos e decreasing the dosing frequency of drug. The Eudragit are biocompatible copolymers which were synthesized from acrylic and methacrylic acid esters. These polymers are well tolerated by the skin and have been used in the formulation of dosage forms especially matrix tablets for oral sustained release [4,5] and in tablet coating. They have also been used in the microencapsulation of drugs [6, 7, 8].

EXPERIMENTAL SECTION

Materials

Diltiazem Hcl was received as a gift from M/s Microlabs, Bangalore, India. Eudragit RL100, RS100 were obtained Gift sample from Dr.Reddy's Lab, Hyderabad, India. All other reagents and solvents used were of pharmaceutical or analytical grade.

Methods

Preformulation Studies of Pure Drug:

Identification of pure drug:

Identification of Diltiazem Hydrochloride was carried out by Infrared Absorption Spectroscopy.

Drug - Excipients Compatibility Studies

Compatibility of Diltiazem Hydrochloride with the respective polymers that is Eudragit RL100 and RS100,[9] and physical mixture of main formulation was established by Infrared Absorption Spectral Analysis (FTIR) (Fig 1,2,3) . Any changes in the chemical composition after combining with the excipients were investigated with IR spectra.

Fig 1 IR Spectrum of Diltiazem Hydrochloride in KBr

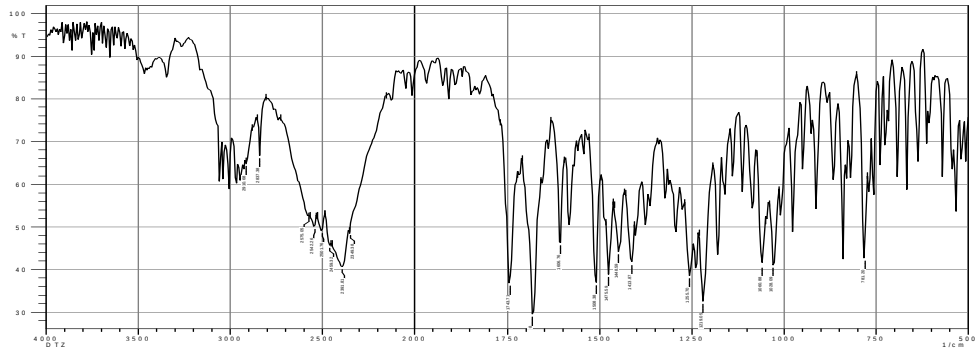

Fig 2 IR spectrum of DTZ.HCl, PF1 & FM1 (PF1 corresponding to Physical mixture &FM1 corresponding to Formulation (ERL100 = 1:1)

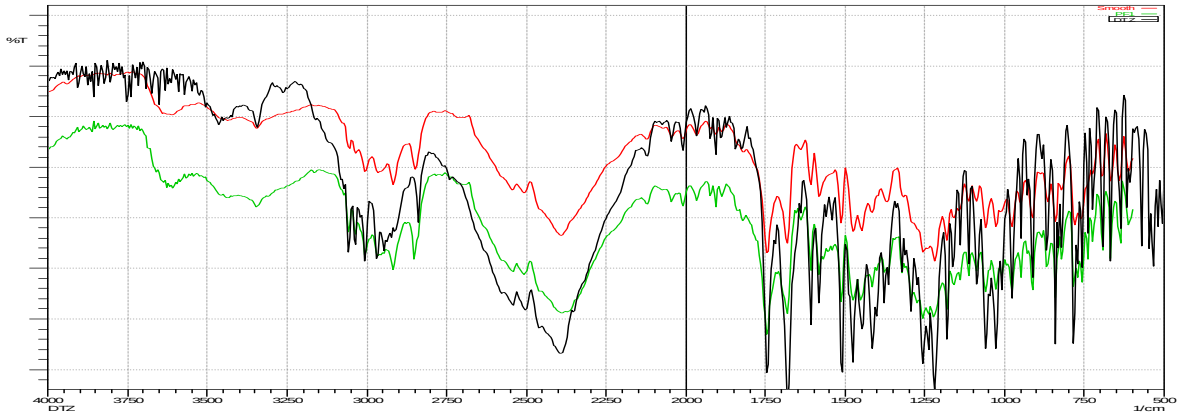

Fig 3 IR spectrum of DTZ.HCl, PF2, FM2 (PF2 corresponding to Physical mixture & FM2 Corresponding to Formulation (ERS100 = 1:1))

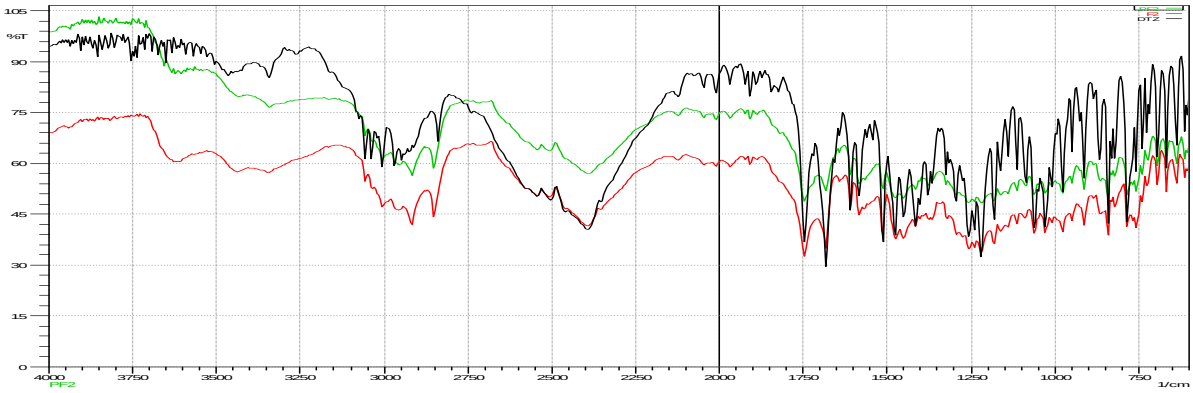

Table 1 Wave- number of different functional groups present in Diltiazem. HCl

Code	Composition	Peak for Diltiazem hydrochloride				
		O-CH_3 C-H sretch (cm^{-1})	Aromatic C-H Stretch (cm^{-1})	Amine HCl N-H stretch (cm^{-1})	Acetate C=O stretch (cm^{-1})	Lactam C=O stretch (cm^{-1})
DTZ. HCl	Diltiazem Hcl	3057.27	2837.38	2391.81	1743.71	1681.98
FM1 PF1	Formulation FM1	3057.27 3057.27	2850.88 2839.31	2360.95 2362.88	1745.64 1745.64	1681.98 1685.84
FM2 PF2	Formulation FM2	3057.27 3055.35	2850.88 2850.88	2387.95 2366.74	1745.64 1745.64	1681.98 1683.91

Preparation of Diltiazem Hcl microspheres

Diltiazem hydrochloride loaded microspheres were prepared by solvent evaporation method. [10] The formulation of microspheres (table 2,3). Diltiazem hydrochloride and each polymer mixture were dissolved completely in acetone-methanol mixture by stirring at 500rpm with magnetic stirrer. Magnesium stearate was added and the mixture was stirred with magnetic stirrer at 500 rpm in ice-bath at 10^{o} C for 10 minute. Above mixture was poured into the liquid paraffin previously cooled at 10^{o}C, while it was being stirred by mechanical stirrer at 1000 rpm.Resulting emulsion was stirred at 35^{o}C for 4 hours using mechanical stirrer and the organic solvent, acetone-

methanol were removed completely by evaporation. Solidified microspheres were filtered through Whatmann filter paper (No.1), washed six times with 50 ml n-hexane. Dried under vacuum at room temperature for 12 h and stored in desiccators containing calcium chloride.

Table 2 Formulations of Diltiazem hydrochloride Microspheres prepared with different Polymers and Polymer mixtures (Drug: Polymer =1:1)

Contents of Formulations	FM1	FM2	FM3
Diltiazem hydrochloride (gm)	2.0	2.0	2.0
Eudragit RL100(gm)	2.0	-	1.0
Eudragit RS100(gm)	-	2.0	1.0
Magnesium Stearate (gm) (Dispersing Agent)	0.300	0.300	0.300
Methanol (ml)	3.0	3.0	3.0
Acetone (ml)	7.0	7.0	7.0
Liquid paraffin (ml)	100	100	100

Table 3 Formulations of Diltiazem hydrochloride Microspheres prepared with different Polymers and Polymer mixtures (Drug : Polymer =1:2)

Contents of Formulations	FM4	FM5	FM6
Diltiazem hydrochloride (gm)	2.0	2.0	2.0
Eudragit RL100 (gm)	4.0	-	2.0
Eudragit RS100(gm)	-	4.0	2.0
MMagnesium Stearate (gm) (DispersingAgent)	0.600	0.600	0.600
Methanol (ml)	6.0	6.0	6.0
Acetone (ml)	14.0	14.0	14.0
Liquid paraffin (ml)	200	200	200

Evaluation of microspheres

Particle Size analysis

The particle size of microspheres was determined by optical microscopy method; approximately 100 microspheres were counted for particle size using a calibrated optical microscope. The microspheres were uniformly spread on a slide. The particle size of the microsphere was measured, along the longest axis and the shortest axis (cross shaped measurement). Average of these two readings was given as mean diameter of particles. The diameter of a minimum number of 100 microspheres in each batch was calculated.

SEM Photographs of Microspheres

Instrument used Lieca stereomicroscope EZ4D and Magnified 10x20x .

Micromeritic properties of microspheres
The floating microspheres are characterized by their micromeritic properties such as bulk density, compressibility index, Hausner's ratio and angle of repose.

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Determination of percentage yield
The prepared microspheres were collected and weighed.[11] The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

$$\text{Yield (\%)} = \frac{\text{Weight of microspheres}}{\text{Total expected weight of drug and polymer}} \times 100$$

Determination of entrapment efficiency (%)
50 mg of the microspheres were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N Hcl repeatedly. [12]The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N Hcl. The solution was filtered and the absorbance measured after suitable dilution spectrophotometrically (UV 1700, Shimadzu, Japan) at 236 nm against appropriate blank.

The entrapment efficiency (%) was calculated according to the following relationship

$$\text{Entrapment efficiency (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \text{x100}$$

In- Vitro Release Study of the Microspheres
Dissolution studies were carried out by using USP XXIII dissolution test apparatus (Basket) method. Capsules were placed in a basket so that the capsule should be immersed completely in dissolution media but not float. In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were sequentially used referred to as sequential pH change method.[13] When performing experiments, the pH 1.2 medium was first used for 2 hrs (since the average gastric emptying time is 2 hrs) then removed and the fresh pH 7.4 phosphate buffer saline (PBS) was added. After 3 hrs (average small intestinal transit time is 3 hrs) the medium was removed and fresh pH 6.8 dissolution medium was added for subsequent hrs. 900ml of the dissolution medium was used at each time. Rotation speed was 100 rpm and temperature was maintained at 37±0.5 ◦C. 5ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 237 nm, by UV absorption spectroscopy.

RESULTS AND DISCUSSION

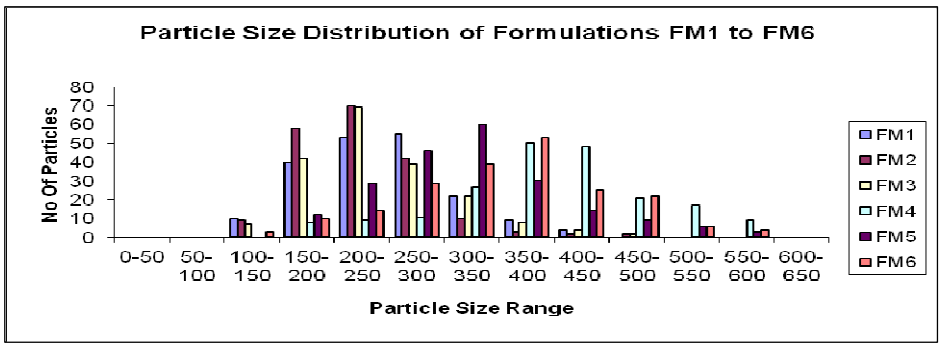
Particle Size analysis
It has been observed that the particle size increases with increasing polymer amount.

The increase in the mean size with increasing polymer concentration was attributed to the fact that higher concentration of polymer in the sample leads to increase in viscosity of the dispersed phase, which results in formation of bigger droplets and also, fusion of semi-formed particles and producing an overall increase in the size of the microspheres. Eudragit RL100 microspheres and Eudragit RS100 microspheres prepared with the same polymer concentration did not show any significant variation in their mean size(Table 4.Fig 4)

Table 4 Particle Size Distribution of Formulations FM1 to FM6

Range(μM)	FM1	FM2	FM3	FM4	FM5	FM6
0-50	0	0	0	0	0	0
50-100	0	0	0	0	0	0
100-150	10	9	7	0	0	3
150-200	40	58	42	8	12	10
200-250	53	70	69	9	29	14
250-300	55	42	39	11	46	29
300-350	22	10	22	27	60	39
350-400	9	3	8	50	30	53
400-450	4	2	4	48	14	25
450-500	0	2	2	21	9	22
500-550	0	0	0	17	6	6
550-600	0	0	0	9	3	4
600-650	0	0	0	0	0	0

Fig 4 Particle Size Distribution of Formulations FM1 to FM6



SEM Photographs Of Microspheres

It shows all microspheres were almost spherical in shape and No aggregation of microspheres had taken place. (Fig 5, 6, 7)

Fig 5 Formulation FM1

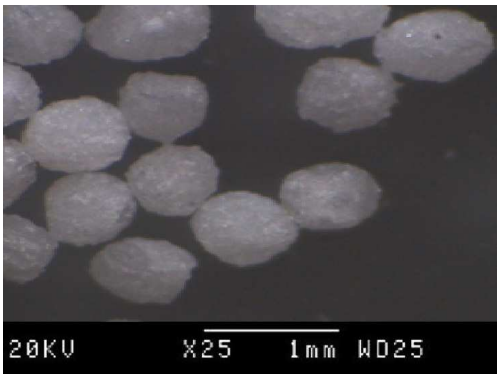


Fig 6 Formulation FM2

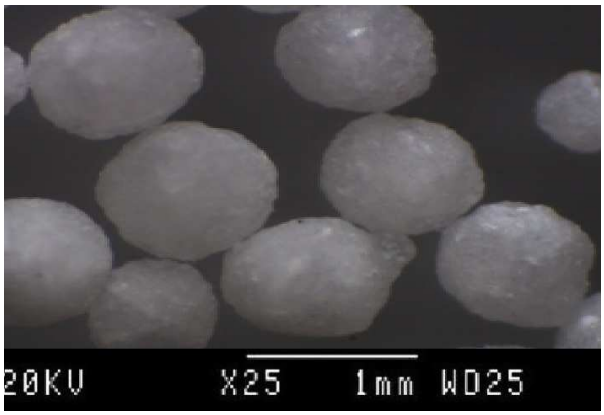


Fig 7 Formulation FM3

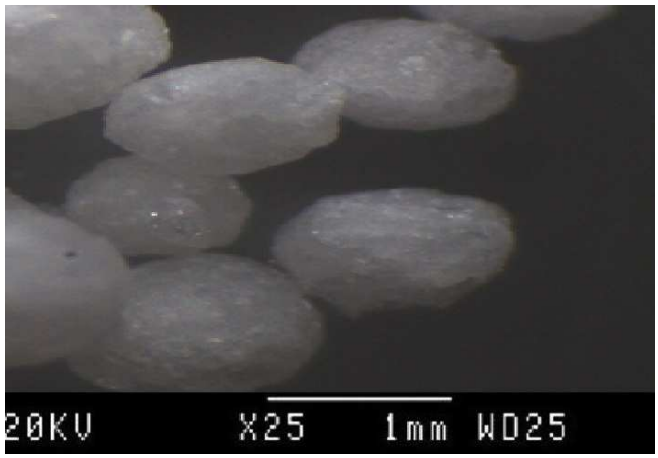


Table 5 Micromeritic properties of microspheres

Batch Code	Bulk Density (gm/ml)	Carr's Index	Hausner's ratio	Angle of repose (θ)
Diltiazem Hcl	0.167 ±0.01	24.38±0.16	1.43 ±0.07	***
FM 1	0.286±0.01	6.12±0.12	1.06 ±0.05	21.82±0.20
FM 2	0.301±0.01	8.82±0.16	1.11 ±0.02	22.86 ±0.62
FM3	0.304±0.02	9.0 ±0.09	1.10 ±0.06	23.30 ±0.55
FM4	0.312 ±0.01	11.5±0.20	1.13 ±0.07	25.55 ±0.40
FM5	0.321±0.02	12.17±0.19	1.14±0.04	27.29 ±0.38
FM6	0.360±0.03	13.21±0.20	1.16±0.08	28.32 ±0.45

Percentage Yield Values and Entrapment Efficiencies of Formulations

Diltiazem hydrochloride loaded microspheres having a fairly high yield (76.48 – 88.94%) were obtained. The entrapment efficiencies ranged from 86.11 – 98.73%. The incorporation efficiency of formulations, FM1 – FM3 was less than formulations FM4 – FM6. The highest incorporation efficiency of formulation having drug: polymer ratio 1:2 can be explained through the fact that the amount of polymer in per unit drug is greater than that in other formulations.(Table 6.Fig 8)

Table 6 Percentage Yield Values and Entrapment Efficiencies of Formulations

Formulation code	Percentage Yield (%)	Theoretical Drug Content (%)	Actual Drug Content (%)* ± S.D.	Entrapment Efficiencies (%)* ± S.D.
FM 1	78.51	45.35	39.05± 0.18	97.73± 0.27
FM 2	79.32	45.35	40.22± 0.14	91.28± 0.38
FM3	81.13	45.35	41.74± 0.16	95.8± 0.25
FM4	89.47	29.3	26± 0.07	85.21± 0.36
FM5	91.53	29.3	26.86± 0.11	87.84± 0.27
FM6	94.14	29.3	28.63± 0.07	88.12± 0.28

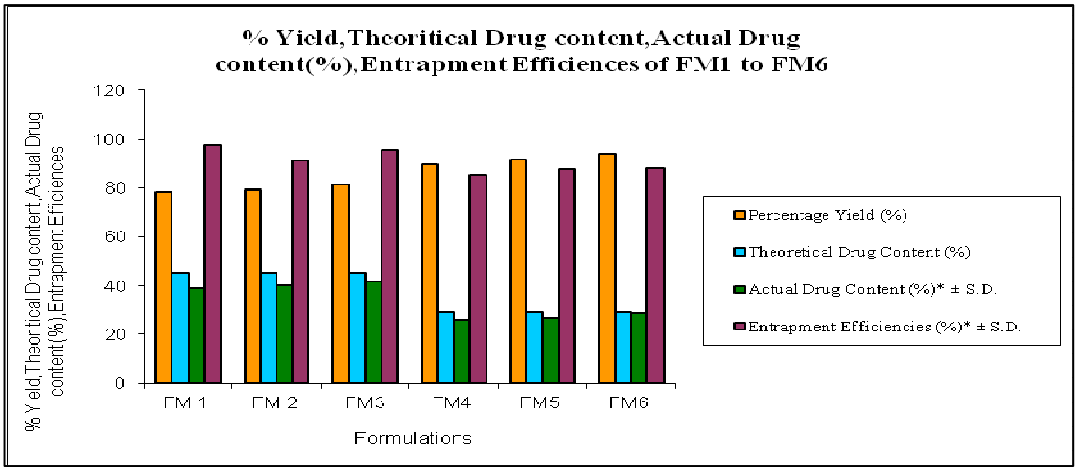


Fig 8 Percentage Yield Values and Entrapment Efficiencies of Formulations

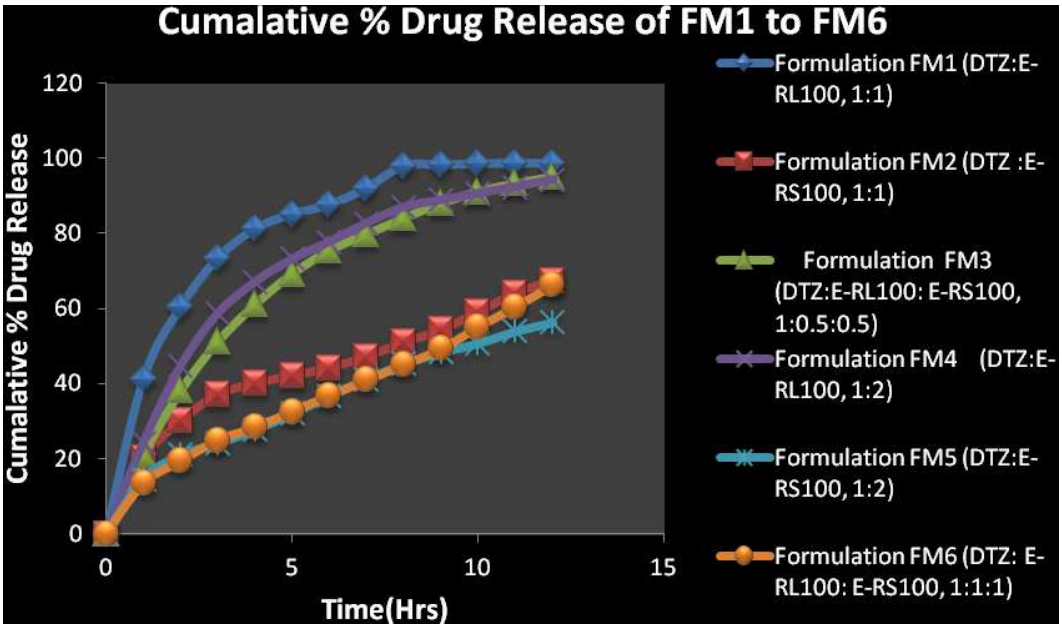
In- Vitro Release Study of the Microspheres

The release of Diltiazem hydrochloride from different formulations depended on the type of polymer and the ratio of the polymer in the formulations. The release of Diltiazem hydrochloride from microspheres of Eudragit L-type was more as compared to Eudragit S-type. This was due to the presence of more functional quaternary ammonium groups (10%) in L-type than S-type (5%).It is also observed that as the amount of polymer in the formulation increased, the drug release decreased. It can be explained on the basis that as the polymer amount increases, the matrix wall of microspheres become thicker. A burst effect of drug release can be observed on the various formulations. The burst effect can be attributed to the presence of non-encapsulated drug particles on the surface of the microspheres. The burst effect of drug release also depended upon the drug : polymer ratio. From the figure of release profile, it can be observed that burst effect of drug release is more in formulations having drug : polymer ratio 1:1, while in the formulation having drug : polymer ratio 1:2, burst effect is less. Withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 236 nm, by UV absorption spectroscopy.(Table 7,Fig 13)

Table 7 Cumulative Percent Released Diltiazem hydrochloride from Microspheres FM1 toFM6

S. NO.	TIME(hrs)	Formulation FM1 (DTZ:E-RL100, 1:1)	Formulation FM2 (DTZ :E-RS100, 1:1)	Formulation FM3 (DTZ:E-RL100: E-RS100, 1:0.5:0.5)	Formulation FM4 (DTZ:E-RL100, 1:2)	Formulation FM5 (DTZ:E-RS100, 1:2)	Formulation FM6 (DTZ: E-RL100: E-RS100, 1:1:1)
		1:1			1:2		
1	0	0	0	0	0	0	0
2	1	41.1	20.02	20.91	24.27	14.6	13.36
3	2	60.29	30.05	37.93	44.68	20.97	19.39
4	3	73.42	37.06	51	58.67	24	24.89
5	4	81.15	40.09	60.96	67.05	27.71	28.34
6	5	85.11	42.15	69.14	73.21	31.94	32.58
7	6	87.6	44.21	75.35	77.72	36.77	36.81
8	7	92.11	47.22	79.74	82.76	41.12	40.92
9	8	97.99	51	83.99	87	45.25	45.06
10	9	98.2	54.3	87.93	88.91	48.24	49.23
11	10	98.56	59.13	91.08	90.65	50.72	55.09
12	11	98.6	64.14	93.18	92.41	53.9	60.48
13	12	98.64	67.51	94.98	94.34	56.27	66.23

Fig 13 Cumulative Percent Release of Diltiazem hydrochloride Microspheres (FM1 to FM6)



CONCLUSION

In this study, Diltiazem HCl was successfully encapsulated into two structurally different Eudragit polymers. By using an optimal proportion of magnesium stearate as droplet stabilizer, uniform and reproducible microspheres could be prepared. . SEM studies of the formulation were carried out for the confirmation of shape and surface morphology of microsphere. SEM revealed that microsphere was discrete and spherical in shape with porous outer surface. The encapsulation efficiencies were successfully increased with Eudragit polymers which range 88-97 % and the release rate of Eudragit microspheres exhibit a lag time at the initial release and the best release was observed with formulation Eudragit RL100. On the basis of, particle size, drug content, Scanning Electron Microscopy, IR-study, *in-vitro* release studies FM1 and FM4 was selected as an optimized formulation. Hence, finally it was concluded that the prepared microspheres be considered as one of the promising formulation technique for deliver the drug and at a control rate for prolonged period of time and hence in management of angina pectoris.

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