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Application of Factorial Design for the Development of Site Specific Systems in the Management of Ulcerative Colitis.

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ABSTRACT

The objectives of the present investigation are to prepare and evaluate drug loaded sustained release matrix tablets for "ULCERATIVE COLITIS", using hydrophilic and hydrophobic polymers, by applying 2^3 factorial designs. The sustained release tablets of Mebeverine HCl were prepared employing different concentrations of HPMC K4M, HPMC K100M and Eudragit L100 in different combinations as a rate retarding polymer by wet granulation technique using 2^3 factorial designs. The quantity of polymers, HPMC K4M, HPMC K100M and Eudragit L100 required to achieve the desired drug release was selected as independent variables, X1, X2 and X3 respectively whereas, time required for 80% of drug dissolution (t_{80}%) was selected as dependent variables. Totally eight formulations were designed and are evaluated for hardness, friability, diameter, thickness, % drug content, *In-vitro* drug release and *In-vivo* studies. From the Results it was concluded that all the formulation were found to be within the Pharmacopoeia limits and the *In-vitro* dissolution profiles of all formulations were fitted in to different Kinetic models, the statistical parameters like intercept (a), slope (b) & regression coefficient (r) were calculated. Polynomial equations were developed for $t_{80\%}$. The formulation (F9) containing three polymers in optimized level using 2^3 factorial designs showed high $t_{80\%}$ value of 12 hours. The selected formulation (F9) follows Higuchi's kinetics, and the mechanism of drug release was found to be Anomalous type (Non-Fickian, n=0.896).

Keywords: Mebeverine HCL, 2^3 factorial designs, HPMC K4M, HPMC K100M and Eudragit L100.

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INTRODUCTION

Drug delivery in conventional dosage forms often suffers from the drawbacks of repeated drug administration and large fluctuations in drug blood levels. The frequency with which a rapidly absorbed and distributed drug must be given in a conventional dosage form is dependent upon intrinsic properties of the drug, viz. elimination half-life ($t_{1/2}$) [1].Sustained release dosage forms are designed to complement the pharmaceutical activity of the medicament in order to achieve better selectivity and longer duration of action including improved therapeutic effect, increased patient compliance by reducing dosing frequency and decrease in incidence and /or intensity of adverse effect by a constant blood concentration. Sustained release matrix tablet is relatively easy to fabricate by incorporating drug molecules into a matrix in slowly disintegrating or inert porous material containing a hydrophilic rate controlling polymer [1].

Mebeverine hydrochloride is one of the musculotropic anti-spasmodic agent used for the symptomatic treatment of abdominal pain, bowel disturbances and intestinal discomfort associated with Irritable Bowel Syndrome. Mebeverine hydrochloride exerts an anti-spasmodic effect by reducing the sodium ion permeability of smooth muscles cells and also indirectly reducing potassium ion efflux, thus avoiding hypertonia1. Mebeverine hydrochloride is highly soluble in water and is readily absorbed into the systemic circulation from upper GIT. It has mean plasma half time of 2.5 hrs. A dose of 135 mg Mebeverine appears to provide effective relief from the symptoms of irritable bowel syndrome but higher frequency of administration of drug may lead to high plasma concentration, resulting in to systemic side effects like decreased heart rate and blood pressure. Sustained release oral drug delivery systems are designed to achieve therapeutically effective concentrations of drug in the systemic circulation over an extended period of time, thus achieving better patient compliance and allowing a reduction of both the total dose of drug administered and the incidence of adverse side effects. Considering this aspect, it is desirable to develop a 12 hrs sustained release formulation of Mebeverine hydrochloride [2].

The aim of this present work is to formulate a sustained release [3] tablet of Mebeverine by wet granulation method using various polymers such as HPMC (K4M and K100M). Eudragit L100 and applied the different kinetic models to study the drug release mechanisms.

A 2^3 full factorial design was employed to investigate the effect of three independent variables (factors), i.e. the amounts of HPMC K4M, HPMC K100M and Eudragit L100. On the dependent variables, i.e. $t_{80\%}$ (Time taken to release 80% of drug, dosage form, first order rate constant respectively)

MATERIALS AND METHODS

Materials

Mebeverine hydrochloride was received as gift sample from Dr. Reddy's Laboratories, Hyderabad, India. HPMC K4M, HPMC K100M and Eudragit L100 were procured from colorcon Asia Pvt Ltd, Goa. Other excipients such as Micro crystalline cellulose, Magnesium stearate, were procured from S.D. Fine Chem. Ltd., Mumbai.

Formulation Development of Mebeverine hydrochloride Sustained Release Tablets

The factorial design is a technique that allows identification of factors involved in a process and assesses their relative importance. In addition, any interaction between factors chosen can be identified. Construction of a factorial design involves the selection of parameters and the choice of responses [4, 5].

A selected two level, three factor experimental designs (2^3 factorial designs) describe the proportion in which the independent variables HPMC K4M, HPMC K100M and Eudragit L100 were used in formulation of Mebeverine hydrochloride sustained release (SR) Tablets. The time required for 80% ($t_{80\%}$) drug dissolution was selected as dependent variables. Polynomial equations were developed for ($t_{80\%}$).

The two levels of factor X_1 (HPMC K4M) at a concentration of 100 mg and 150 mg. Two levels of factor X_2 (HPMC K100M) at a concentration of 75 mg and 150 mg. Two levels of factor X_3 (Eudragit L100) at a concentration of 50 mg and 100 mg was taken as the rationale for the design of the Mebeverine hydrochloride SR tablet formulation. Totally eight Mebeverine hydrochloride sustained release tablet formulations were

prepared employing selected combinations of the three factors i.e X_1, X_2 and X_3 as per 2^3 Factorial and evaluated to find out the significance of combined effects of X_1, X_2 and X_3 to select the best combination and the concentration required to achieve the desired prolonged/ sustained release of drug from the dosage form [7].

Preparation of Mebeverine hydrochloride Sustained Release Tablets:

Eight different tablet formulations were prepared by wet granulation technique as reported. The composition of 230 mg Mebeverine of the drug, polymer(HPMC K4M,K100M) and Eudragit L100 and filler talc was dry mixed thoroughly and sufficient volume of granulating agent (5%w/v ethonolic solution of PVP-K90). Ethanolic solution of PVP was added slowly. After enough cohesiveness was obtained, feeded in wet granulator and the mass was sieved. The granules were dried at 55ºc for 1 hour. These granule mixtures was blended with magnesium stearate (1.6%w/w) as lubricant, the appropriate and then compressed using a 16 station tablet compression machine round, flat-faced punches of 12.5 mm diameter and die set. All compressed tablets were stored in air tight container at room temperature for the study. The detailed compositions of various formulations prepared employing 2^3 factorial designs.

Experimental Design:

Experimental design utilized in present investigation for the optimization of polymer concentration such as, concentration of HPMC K4M was taken as X_1 concentration of HPMC K100M was taken as X_2 and concentration of Eudragit L100 was taken as X_3. Experimental design was given in the Table No: 1 and 2.

Table 1: Levels of Factors

Factor	Low level	High level
A	-	+
B	-	+
C	-	+

Table 2: Two- level-3-factor- full-factorial Experiment design pattern

Run	Combination	Factors		
		A	B	C
1	(1)	-	-	-
2	A	+	-	-
3	B	-	+	-
4	AB	+	+	-
5	C	-	-	+
6	AC	+	-	+
7	BC	-	+	+
8	ABC	+	+	+

EVALUATION PARAMETERS

Pre-formulation Studies

Fourier Transform Infrared Spectroscopy

The Fourier transform infra-red analysis was conducted for the structure characterization. FTIR spectra of the pure drug, polymers and formulations. Approximately 5mg of samples were mixed with 50mg of spectroscopic grade KBr; samples were scanned in the IR range from 1125 to 2955.20cm^{-1}, with a resolution of 4 cm^{-1}.

Differential scanning colorimeter (DSC)

DSC thermo gram of Mebeverine HCl and physical mixture of drug and polymers are shown in Figure No.4**,** 5. DSC thermo gram of pure drug has shown a melting endotherm at 135.7 °C with normalized energy.

The thermo gram of physical mixture showed that the Mebeverine HCl melting onset temperature decreased to 124.40° C because of the presence of polymers in the physical mixture.

Pre-compression studies of Mebeverine sustained release matrix tablets

Angle of repose

Angle of repose ranged from31.07^{O} to 34.89^{O}. The results were found to be below 25^{0} and hence the blend was found to have excellent flow property. (Table No: 4)

Table 4: Results of Pre-compression studies of Mebeverine HCL sustained release matrix tablet

Parameter	F1	F2	F3	F4	F5	F6	F7	F8
Bulk density	3.87	3.27	3.00	4.012	4.329	3.996	4.32	4.329
Bulkiness	0.28	0.305	0.332	0.249	0.23	0.250	0.23	0.230
Angle of repose	31.79	32.83	31.81	34.89	31.07	33.80	31.24	34.51
Carr's index	11.06	12.34	13.87	13.87	14.17	11.17	14.98	13.27
Hausner's ratio	1.18	1.16	1.14	1.14	1.120	1.17	1.27	1.15
%Drug content (uniformity)	98.59	99.12	98.41	98.76	98.59	98.41	99.5	99.23

Bulk density and tapped density

Bulk and tapped densities are used for the measurement of compressibility index. The LBD and TBD ranged from 0.29 to 0.63 and 0.41 to 0.570 respectively. (Table No: 4)

Compressibility index :(Carr's index)

Compressibility index ranged from 11.06% to 14.98% the blend was found to have free flowing property as the result were found to be below16 %.(Table No: 4)

Hausner's ratio

The hausner ratio ranged from 1.12to 1.18 the result indicate the free flowing properties of the granules. (Table No: 4)

Drug Content Uniformity

The drug content in a weighed amount of granules blend of all SR formulations ranged from 98.41% to 99.5%

Post-compression studies of Mebeverine sustained release matrix tablets

Hardness test

The hardness of all batches ranged from 4.68 to 5.13 kg/cm^2

Friability test

The percentage friability of all batches ranged from 0 to 0.57%.

Weight variation

The percentage weight variations for all formulations are present in (Table No: 5).All the formulations passed weight variation test as per the pharmacopoeias limits of 5%

Table 5: Results of Post-compression studies of Mebeverine HCL sustained release matrix tablet

Parameter	F1	F2	F3	F4	F5	F6	F7	F8
Weight variation(mg)	595	590	593	591	597	595	596	595
% Friability	0.28	0.57	0.28	0.283	0	0.563	0	0.28
Hard ness test	5.05	4.81	4.80	4.85	4.68	5.13	5.01	5.07
Swelling study	77.89	78.47	78.11	76	81.3	80	79.7	84.04
% Drug content (uniformity)	96.04	96.39	97.88	97.44	98.32	97.36	97	96.65

Drug content uniformity:

Drug content was found to be uniform among the all formulations and ranged from 96.04 to 98.32%

Swelling Index

Swelling study was performed for all batches (F_1 to F_8) for 5 hours. The result is shown in the (Table No: 5) and swelling index against time (5 hours)

***In vitro* dissolution** [5]

The release of Mebeverine from the sustained release tablet was studied up to 12 hours in phosphate buffer as dissolution medium using a USP dissolution paddle assembly at 50 rpm and 37˚± 0.5˚C. An aliquot (1ml) was withdrawn at specific time intervals, filtered and diluted to 10ml with the dissolution medium, and drug content was determined by UV- visible spectrophotometer at 263nm. An equal volume of fresh dissolution medium was replaced to maintain the dissolution volume.

Dissolution studies were performed for a period of 12hrs and the value was taken. Cumulative percentage of drug release was calculated using an equation obtained from a standard curve.

Kinetic modeling of drug release

The dissolution profile of all the formulations was fitted in to zero-order, first-order, and Higuchi and Korsmeyer-peppas models to ascertain the kinetic modeling of drug release [9, 10].

Experimental colitis in rats

IBS syndrome, ulcerative colitis and crohon's disease represent chronic alteration of the GIT of unknown etiology perhaps involving immunological agents such as dinitrochlorobenzene, 2, 4, 6-trinitrobenzene, sulfonic acid and carrageenan. The *in vivo* study was approved by Institutional Animal Ethics Committee of Chalapathi Institute of Pharmaceutical Sciences (Approval No: 28/IAEC/CIPS/2014-15; dated 21/02/2015) Female Sprague Dawley rats weighing 150-200gm were used for carrageen induced experimental colitis. [11].

RESULTS AND DISCUSSION

Sustained release tablets of Mebeverine were prepared and optimized by 2^3 factorial designs in order to select the best combination of different rate retarding polymers, HPMC K4M, HPMC K100M and Eudragit L100 also to achieve the desired prolong/sustained release of drug from the dosage form. The three factorial parameters involved in the development of formulations are, quantity of HPMC K4M , HPMC K100M and Eudragit L100 polymers as independent variables (X_1,X_2 and X_3), and *In vitro* dissolution parameters such as $t_{80\%,}$ as dependent variables. Totally eight formulations were prepared using 2 levels of 3 factors and all the formulations containing 230 mg of Mebeverine Hydrochloride were prepared as a sustained release tablet dosage form by wet granulation technique as per the formulae given in Table No.3.The optimized formulation F9 formulated after applying 2^3 factorial designs were subjected for *in-vivo* studies.

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Table 3: Composition of Formulations of Mebeverine HCL sustained release matrix tablet

S.No	Ingredients(mg)	F1	F2	F3	F4	F5	F6	F7	F8
1.	Mebeverine HCL	230	230	230	230	230	230	230	230
2	HPMC K4M	100	100	150	150	100	100	150	150
3	HPMC K100M	75	100	75	100	75	100	75	100
4	Eudragit L100	50	50	50	50	100	100	100	100
5	Micro crystalline cellulose	140	115	90	65	90	65	40	15
6	Magnesium stearate	5	5	5	5	5	5	5	5
7	Total tablet weight	600	600	600	600	600	600	600	600

All the prepared tablets were evaluated for different post compression parameters, drug content and in-vitro dissolution. Good uniformity in drug content was found among different batches of the tablet. All the tablets formulation showed acceptable pharmacopoeia limit specifications for weight variation drug content, hardness, and friability.

***In Vitro* Release Studies**

The in-vitro drug release characteristics were studied in 900 ml of 0.1N HCL for first 2 hours and 900 ml of P^H 6.8 for rest of hours, using USP XXIII dissolution apparatus type II (paddle) method. The difference in burst effect of the initial time is a result of the difference in the viscosity of the polymeric mixtures. Dortunc and Gunal have reported that increased viscosity resulted in a corresponding decrease in the drug release, which might be due to the result of thicker gel layer formulation[18.] The *In -vitro* dissolution data of Mebeverine SR formulations was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equations, Higuchi's and Korsmeyer-Peppas models to assess the mechanism of drug release.

Table 6: *In vitro* drug release for oral sustained release Tablets of Mebeverine HCL

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	10.79	9.33	10.43	9.45	10.30	10.35	9.87	8.33
2	16.51	17.39	17.91	13.39	15.84	15.62	13.59	15.87
3	38.93	26.46	32.74	29.70	29.26	27.27	31.71	35.38
4	46.02	39.96	40.59	35.13	37.34	32.59	39.63	47.65
5	54.44	45.61	49.26	47.62	43.89	45.87	45.68	51.19
6	61.32	53.58	54.84	53.86	57.27	54.96	53.25	56.58
7	67.53	60.50	58.29	59.97	62.81	57.31	57.34	63.49
8	72.36	64.67	66.43	63.34	65.47	59.73	62.29	66.28
9	77.82	70.84	72.76	72.90	69.93	61.67	67.49	69.57
10	80.59	74.67	77.69	76.71	75.42	63.07	74.07	72.07
11	84.41	82.37	80.93	79.89	79.03	74.13	79.52	78.47
12	86.73	84.74	82.57	81.71	81.43	80.63	81.83	80.34

The results of linear regression analysis including regression coefficients are summarized in Table No: 7. It was observed from the above that dissolution of all the tablets followed first order kinetics with co-efficient of determination (R^2) values above 0.953. The values of r of factorial formulations for Higuchi's equation was found to be 0.987which shows that the data fitted well to Higuchi's square root of time equation confirming the release followed diffusion mechanism. Kinetic data also treated for Peppas equation, the slope (n) values ranges from 0.837- 0.929 that shows Non-Fickian diffusion mechanism. Polynomial equations were derived for, $t_{80\%}$ values by backward stepwise linear regression analysis. The dissolution data of factorial formulations F1 to F8 are shown in Polynomial equation for 2^3 full factorial designs is given in Equation

$$Y = B_0 + B_1(x_1) + B_2(x_2) + B_3(x_3) + B_{12}(x_1x_2) + B_{13}(x_1x_3) + B_{23}(x_2x_3) + B_{123}(x_1x_2x_3)$$

Where, Y is dependent variable, B0 arithmetic mean response of nine batches, and B1 estimated co-efficient for factor X_1. The main effects (X_1, X_2 and X_3) represent the average result of changing one factor at a time from its low to high value. The interaction term (X_1, X2 and X_3) shows how the response changes when

three factors are simultaneously changed. The polynomial terms (X_1^2, $X2^2$ and X_23) are included to investigate non-linearity.

Table 7: Kinetic Analysis of *In – Vitro* Release Rates of Sustained Release Tablets of Mebeverine HCL

F.code	Zero-order plots		First-order plots		Higuchi's plots	Korsemeyer plots	
	K_0	(R^2)	K_1	(R^2)	(R^2)	(n)	(R^2)
F1	8.479	0.903	-0.106	0.996	0.942	0.859	0.949
F2	7.815	0.953	-0.101	0.984	0.923	0.904	0.988
F3	7.899	0.941	-0.119	0.992	0.941	0.848	0.981
F4	7.747	0.957	-0.061	0.991	0.916	0.929	0.972
F5	7.747	0.946	-0.055	0.953	0.926	0.875	0.980
F6	7.170	0.936	-0.073	0.954	0.923	0.837	0.974
F7	7.604	0.952	-0.112	0.987	0.929	0.886	0.965
F8	7.816	0.983	-0.078	0.970	0.939	0.893	0.941

The data demonstrate that both X_1 (amount of HPMC K4M) and X_2 (amount of HPMC K100M) and X_3 (amount of Eudragit L100) affect the time required for drug release ($t_{80\%}$). From the results it can be concluded that, and increase in the amount of the polymer leads to decrease in release rate of the drug and drug release pattern may be changed by appropriate selection of the X_1, X_2 and X_3 levels. The final best (Optimized) formulation (F9) is compared with F1-F8.

***In Vivo* Studies**

Histopathology report

In the control group animals colon section the simple columnar epithelium, lamina propria, and muscularis mucosa with absorptive and goblet cells were visible. The multiple serial sections studied in the disease control group (the histopathology of the diseased colon with histamine induced ulcerative colitis) shows colon epithelium with irregular ulcerations with collection of inflammatory cells extending into the sub epithelium and muscularis mucosa. From the multiple serial sections of treated group animals it is evident that there is improvement of repair response which is observed with corrected histology of all four layers of the colon.

Table 8: *In-vitro* Drug Release Profile of Extra Design Check Point

Time in hrs	F9
0	0
1	9.32
2	17.54
3	35.59
4	50.55
5	53.24
6	59.17
7	65.49
8	69.83
9	71.57
10	76.33
11	78.67
12	80.9

Table 9: *In- Vitro* Release Kinetic Data for Extra Design Check Point

Formula code	$t_{80\%}$	*Zero order*		*First order*		*Higuchi's*	*Korsemeyer plots*	
		K_0	*r*	K_1	*r*	*r*	*n*	*r*
F_9	*11.9*	*7.316*	*0.973*	*-0.109*	*0.988*	*0.939*	*0.893*	*0.941*

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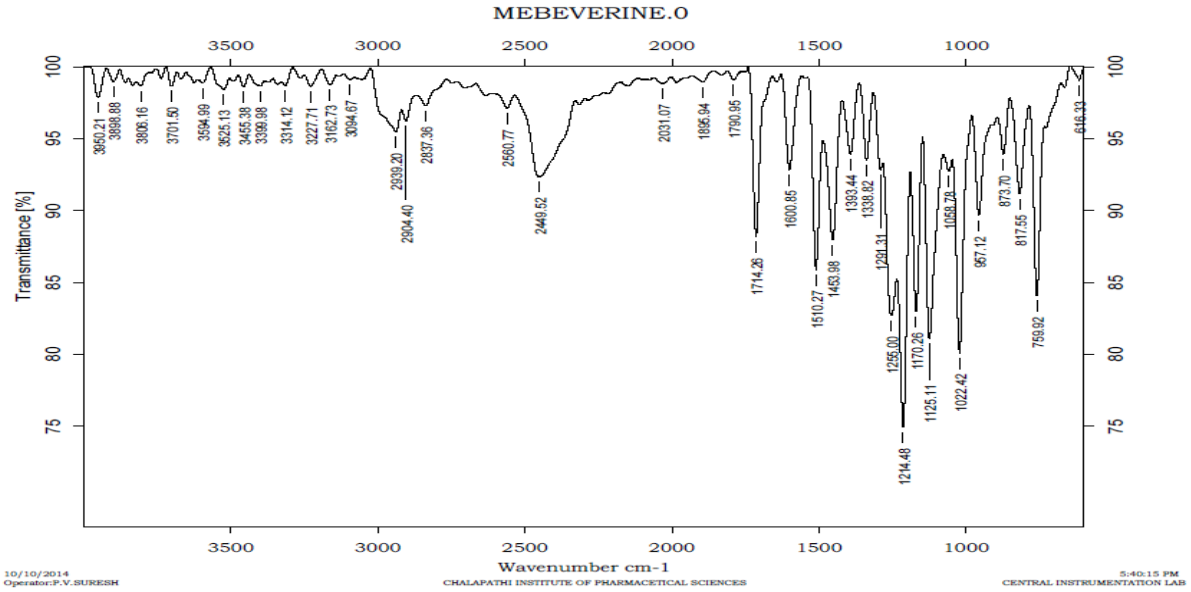

Figure 1: FT-IR spectrum of pure Mebeverine

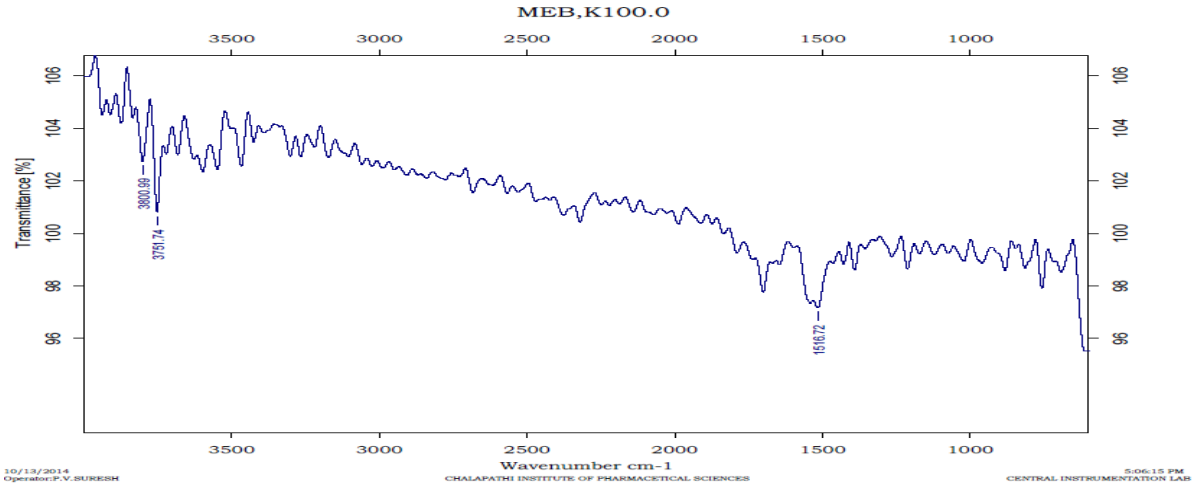

Figure 2: FT-IR spectrum of Mebeverine+HPMCK100M

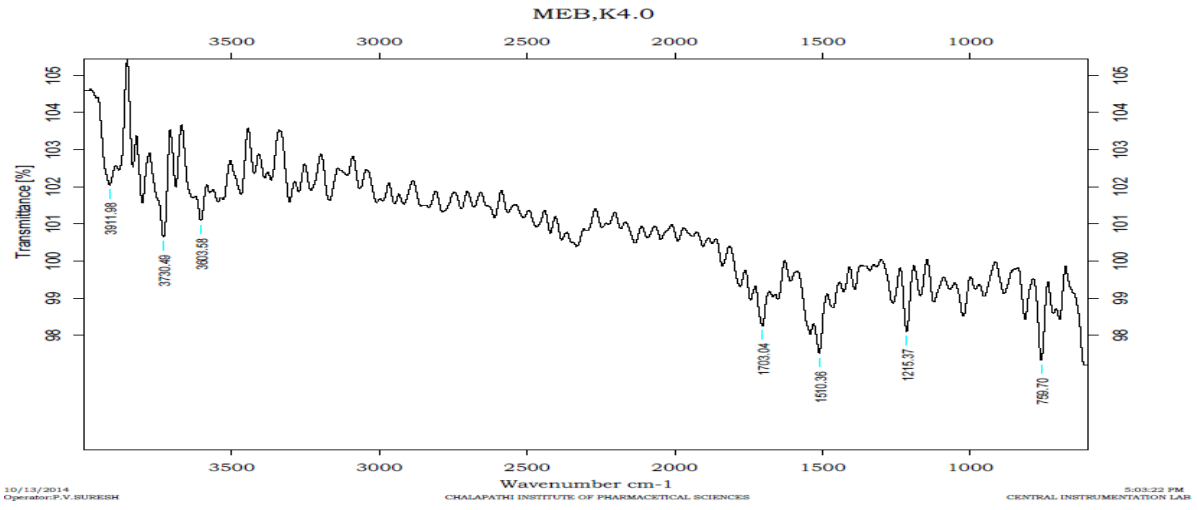

Figure 3: FT-IR spectrum of Mebeverine+HPMCK4M

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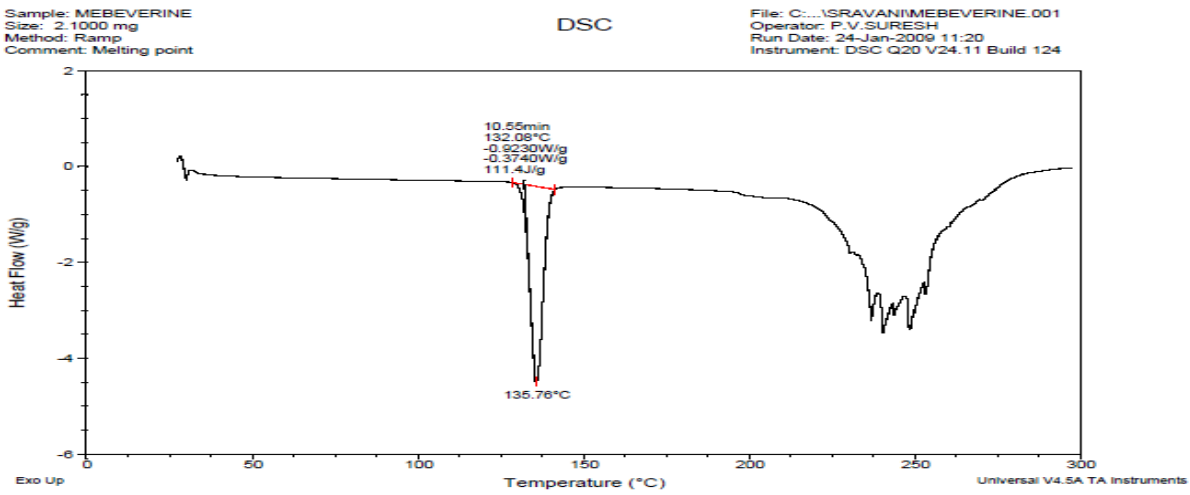

Figure 4: DSC Spectra for pure drug of Mebeverine HCL

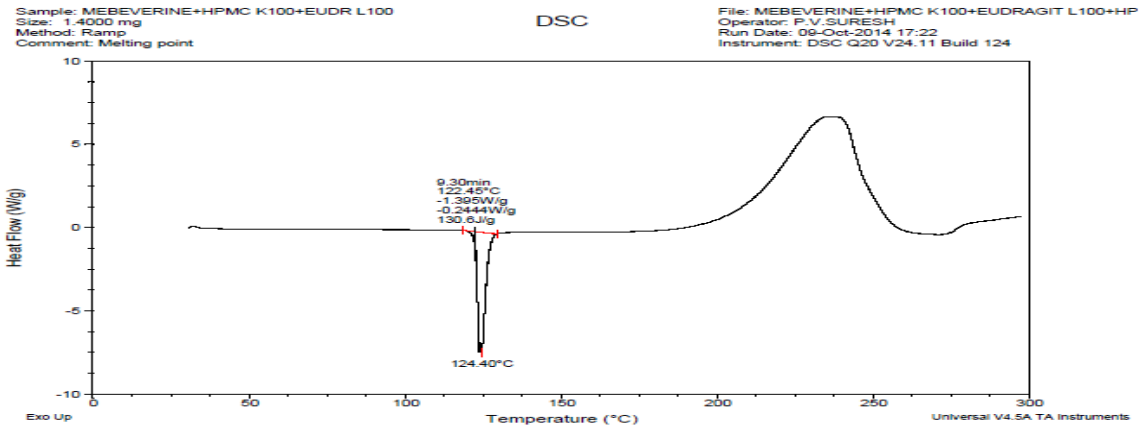

Figure 5: DSC Spectra for pure drug of Mebeverine HCL +HPMC (K4M, K100M) +EUDR L 100

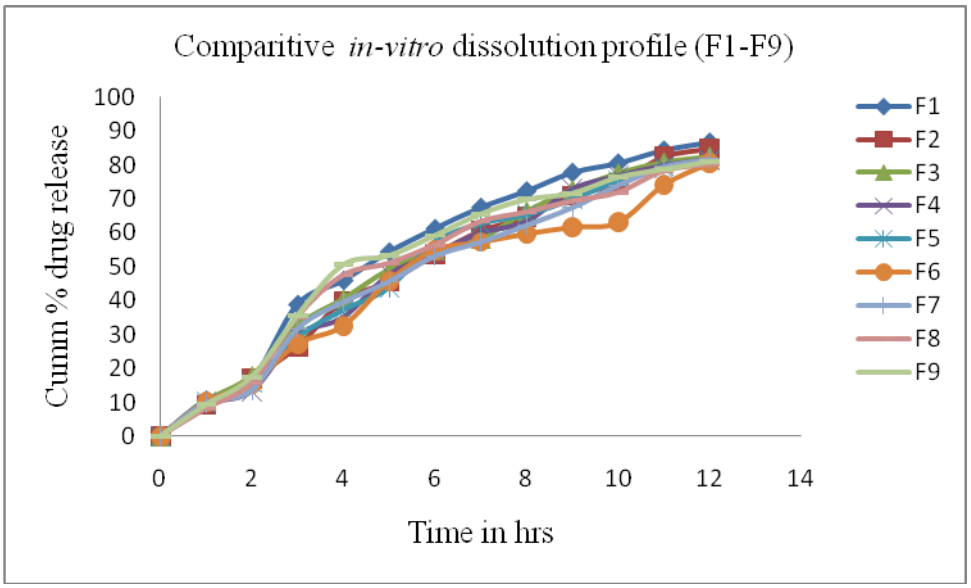

Figure 6: *In-Vitro* Drug Release Profile of F1-F9

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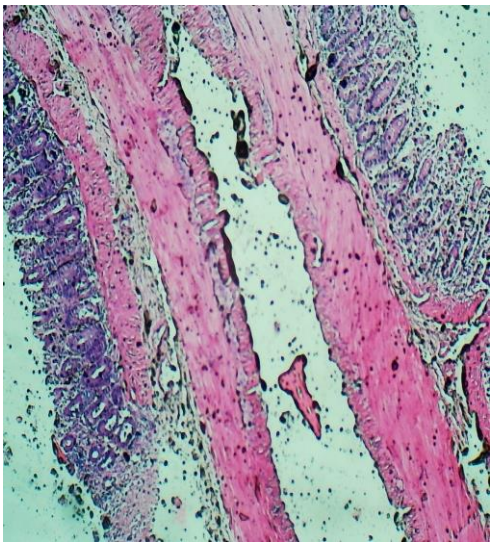

Figure 7: Control-1

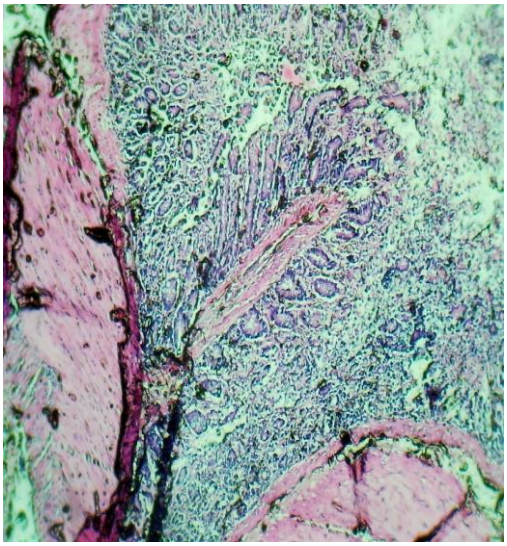

Figure 8: Control-2

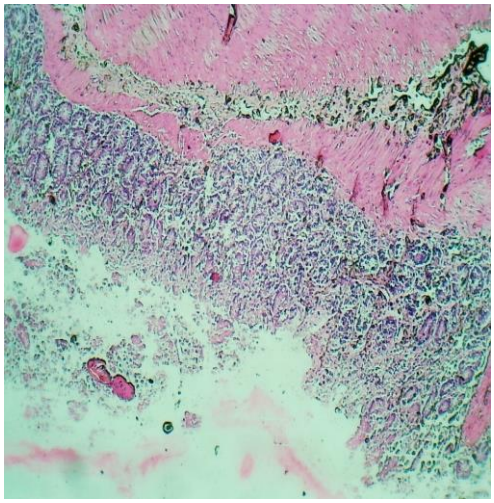

Figure 9: Disease Control-1

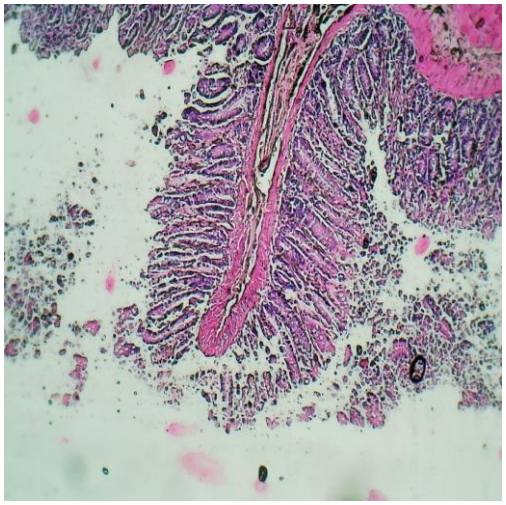

Figure 10: Disease Control-2

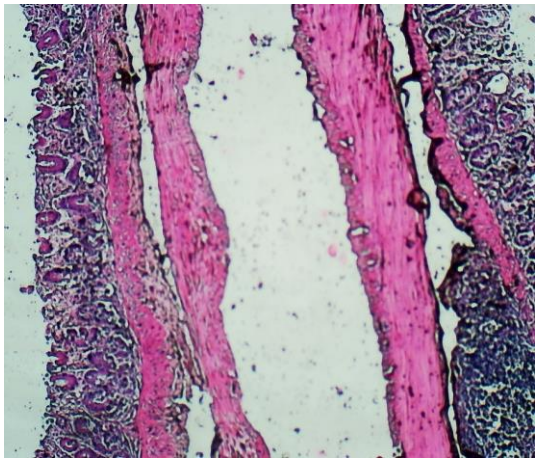

Figure 11: Test-1

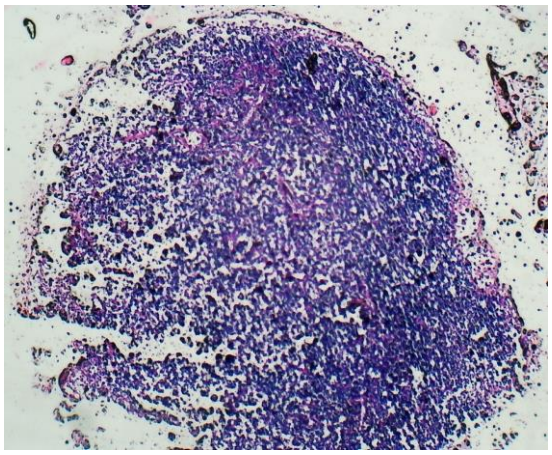

Figure 12: Test-2

CONCLUSION

The present research work envisages the applicability of hydrophilic polymers such as HPMC K4M, HPMC K100M and PH dependent polymers such as Eudragit L100 in the design and development of sustained release tablet formulations of Mebeverine HCL utilizing the 2^3 factorial designs. From the results it was clearly understand that as the polymer concentration increases the release rate of drug was retarded and both of these polymers can be used in combination since do not interact with the drug which may be more helpful in achieving the desired sustained release of the drug for longer periods.

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