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Original Research Article

Optimization of a Novel Oral Colon Delivery System of Indomethacin Using Full Factorial Design

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Abstract

Purpose: *To develop and optimize indomethacin (IDM) matrix tablets for specific colon drug delivery.*
Methods: *Indomethacin matrix tablets containing hydrogenated castor oil (HCO), and pectin (PEC) were prepared by hot fusion method. A 3^2 full factorial design was used to investigate the combined effect of two independent formulation variables, X1 and X2, namely, the amount of HCO and PEC, respectively. Their effect on IDM release from the matrix tablets in acidic medium (0.1 N HCl) and phosphate buffer (pH 6.8), were analyzed and optimized. A contour plot was also applied to graphically represent the effect of the independent variables on drug release in pH 6.8 medium at 2 h (Y1) and 24 h (Y2), and the time required for 25 % drug release (Y3) as dependent variables.*
Results: *The optimized IDM matrix tablets showed almost total retardation of drug release in acidic medium and prolonged sustained release in pH 6.8 medium over 24 h. The correlation coefficient (R^2) value for Y1, Y2 and Y3 were 0.99850, 0.9980 and 0.9970, respectively, indicating good correlation between dependent and independent variables. Differences between the coefficients for Y1, Y2 and Y3 were significant ($p < 0.05$), and hence contributed significantly to the prediction of the independent variables.*
Conclusion: *The findings indicate that successful design, development, and optimization of IDM matrix tablets for colon delivery has been achieved.*

Keywords: *Indomethacin, Hydrogenated castor oil, Pectin, Factorial design, Matrix tablets, Colon delivery system*

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INTRODUCTION

Oral colon-specific drug delivery system (CDDS) has been developed as one of the site-specific drug delivery systems. This delivery system comprises a combination of one or more controlled release mechanisms leading to hardly any release of the drug in the upper part of the gastrointestinal (GI) tract, but rapid release in the colon following oral administration [1-3].

Polysaccharides are widely used in oral drug delivery systems because of the simplicity to obtain the desired drug delivery system and drug release profile, by the control of cross-linking, insolubility of crosslinked beads in gastric environment and broad regulatory acceptance [4-6]. The inability of the GIT enzymes to digest certain plant polysaccharides is taken advantage in developing a colon-specific drug delivery system. Various polysaccharides are being evaluated for colon targeting such as pectin

(PEC), guar gum, gum ghatti, dextran, chitosan, and xylan [7,8].

Indomethacin (IDM) is a nonsteroidal anti-inflammatory drug (NSAID) that can exhibit chemoprotective effects against tumors and reduce the risk of colon cancer [9-11]. Despite recent advances in NSAIDs formulations, the design of targeted delivery systems to improve the efficacy and reduce side effects of NSAIDs continues to be a focus of much research [12-14].

This study was aimed at the design and development of controlled release matrix tablets of IDM using hydrogenated castor oil (HCO) and PEC, with the aid of 3^2 full factorial design, to achieve a colon targeted 24 h release profile *in vitro*.

EXPERIMENTAL

Materials

Indomethacin (γ- polymorphic form) (IDM) was kindly supplied by Pharco Pharmaceuticals, Alexandria, Egypt. Also used were pectin (PEC, BDH Co, England). hydrogenated castor oil (HCO, Girnar Industries, Gujarat, India, as well as talc and magnesium stearate (BDH Chemicals Ltd, Poole, UK). All other chemicals used were of reagent grade.

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectrum of IDM and physical mixtures (1:1:1) of IDM with other excipients (PEC and HCO) as well as different formulations prepared by hot fusion method, namely F4 and F6, were performed to find out any possible drug- excipients interaction using KBr pellet method using Perkin-Elmer FTIR series (model- 1615) spectrophotometer between 4000-450 cm^{-1}.

Preparation of indomethacin solid dispersions by hot fusion method

Hydrogenated castor oil was melted in porcelain evaporating dish using a water bath at 60 °C. Indomethacin and pectin were mechanically mixed to form homogenous mixture and then this mixture was added with continuous stirring (HCO, PEC and the drug were used in the required ratios for each preparation) to get a homogeneous dispersion. The obtained molten mass was then allowed to cool down and solidify. Subsequently, the mass was ground, pulverized and passed through a 60-mesh sieve (< 300 µm). The obtained powders were stored in desiccators at room temperature until use.

Preparation of indomethacin matrix tablets

The solid dispersion was directly compressed using a single punch tablet machine (Erweka, Germany) fitted with 9 mm diameter normal flat punches and die sets. Relatively constant tablet hardness was held around 10 kg. An amount of solid dispersion equivalent to 50 mg of IDM was compressed after the addition of 3 % w/w (of tablet weight) of lubricant (Talc: Magnesium stearate 9:1). Tablet weight was 300 ± 5 mg. Tablets were then subjected to *in vitro* release study.

In vitro release studies

The *in vitro* release study of IDM tablets was performed using USP apparatus II (Erweka, Germany) fitted with paddle (50 rpm) at 37 ± 0.5 °C using 0.1 N HCl (pH 1.2, 900 mL) as a dissolution medium for the first 2 h, followed by pH 6.8 phosphate buffer solutions for further 10 h. At the predetermined time intervals, 5 mL samples were withdrawn and replaced with fresh preheated dissolution medium, then the withdrawn samples were filtered through a 0.45 µm membrane filter and assayed spectrophometrically at 270 nm using a UV spectrophotometer (Shimadzu, Kyoto, Japan). Cumulative drug release was computed from a standard calibration curve. The dissolution profile of all batches were fitted to various models including zero order, first order [15], Higuchi [16, 17], Korsmeyer and Peppas [18] to ascertain the kinetics of drug release (Equations 1-4, respectively).

$C = K_0 t$ …….. (1)

where, K_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$Log\ C = LogC_0 - K_1 t/2.303$ …… (2)

where C_0 is the initial concentration of drug and K_1 is first order constant.

$Q = K_H t^{1/2}$ …… (3)

where, KH is the constant reflecting the design variables of the system.

$M_t/M_\infty = Kt^n$ …… (4)

where M_t/M_∞ is fraction of drug released at time t, K is the release rate constant.

Optimization of variables using 3^2 full factorial design

A 3^2 full factorial design was employed to systematically study the joint influence of the effect of independent variables X_1 and X_2 on the dependent variable. In this design, 2 factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations. The amounts of HCO (X_1) and PEC (X_2) were selected as independent variables in 32 full factorial design, while Y1, Y2 and Y3 (% drug release after 2, 24 h and time required to release 25 % drug, respectively) were taken as dependent variables. The formulation layout for the factorial design batches (F1-F9) is shown in Table 1.

Response surface analysis

Two dimensional (2-D) contour plot and three dimensional (3-D) surface response plots where constructed based on the model polynomial function using Minitab program version 17. These plots are very useful to see interaction effect on the factor of the response.

Statistical analysis

The results were analyzed by using Graph Pad software version 6. One way analysis of variance (ANOVA) with Tukey's multiple comparisons post hoc was used to test for significant differences, and differences were considered significant at $p < 0.05$.

RESULTS

Compatibility of IDM with the polymers

Figure 1 demonstrates the characteristic IR peaks of pure IDM which were compared with peaks obtained from the respective physical mixture (1:1:1) as well as with F4 and F6. It was observed that characteristic peaks of IDM appears with identical or with minor differences, at frequencies 3370.33 and 1717.14 cm^{-1} corresponding to carboxylic O−H and C=O stretch, respectively. The spectrum shows also characteristic peaks at 2961.65 cm^{-1} (C-H stretching vibrations), 1691.83 cm^{-1} (C = O stretching vibrations), 1234.15 cm^{-1} (asymmetric aromatic O-C stretching), 1086.55 cm^{-1} (symmetric aromatic O-H stretching) and 1479.50 cm^{-1} (C-C stretching) [19]. It can be seen that the peaks of the prepared formulations F4 and F6 as well as the physical mixture are the sum of the characteristic peaks of the drug and the corresponding excipients used. The spectra of Fig. 1 indicated the compatibility between the drug and the two polymers used for preparation of IDM matrix tablets by hot fusion technique.

Preliminary trial

In a preliminary study, batches containing various concentrations of HCO alone and in combination with the other polymer (PEC) were prepared to check their influence on in vitro characterization of compressed matrix tablets. It was found that as the concentration of HCO increased, the drug release for upper GIT was retarded till 6.79 %. It was found that tablets containing combinations of HCO with PEC retarded the drug release in pH 1.2 and gave faster drug release in pH 6.8.

In vitro drug release

Figure 2 illustrates the release profile of IDM from the different matrix tablets prepared by direct compression of the solid dispersion of the drug into the polymers.

Table 1: Composition and the experimental design of factorial design batches

Batch code	Variable levels in coded form		Dependent variables		
	X_1	X_2	Y1	Y2	Y3 ($T_{25\%}$)
F1	-1*	-1**	0.78	76.00	50.00
F2	-1	0**	0.98	66.20	47.32
F3	-1	+1**	0.72	54.70	68.03
F4	0*	-1	1.20	70.00	39.60
F5	0	0	2.00	59.27	71.00
F6	0	+1	2.50	42.81	81.00
F7	+1*	-1	0.30	67.54	79.00
F8	+1	0	0.30	55.86	42.00
F9	+1	+1	0.00	41.43	40.00

-1 125 mg/tablet for X_1, -1** 62.5 mg/tablet for X_2; 0* 166.66 mg/tablet for X_1, 0** 83.33 mg/tablet for X_2; +1* 187.5 mg/tablet for X_1, +1** 125 mg/tablet for X_2*

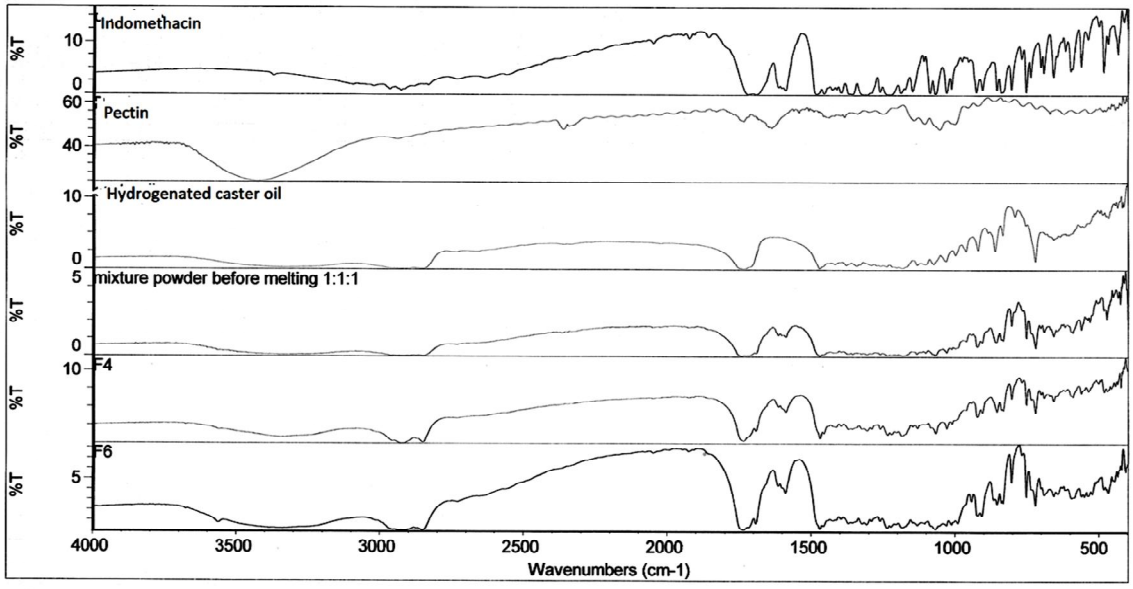

Figure 1: IR spectra of indomethacin, pectin, hydrogenated castor oil, their physical mixture in the ratio of 1:1:1 and after fusion (formulations F4 and F6)

The release profile of IDM from the 9 batches showed drug release in pH 6.8 ranged from 0-2.5 % after 2 h while, drug release after 24 h ranged from 41 % to 76 %. The release of the drug after 24 h for all the tablets prepared was based upon the mechanical mixtures of IDM and pectin. However, tablets prepared using high concentration of HCO (187.5 mg) showed IDM release of < 67 % after 24 h whereas, the tablets prepared with medium (166.66 mg) and low (125 mg) concentrations of HCO released about 70 and 76 % of the drug, respectively. As the amount of HCO increased from 125 mg (F1, F2 and F3) to 187.5 mg (F7, F8 and F9), the drug release was significantly decreased ($p < 0.05$). The in vitro release profiles of drug from all these formulations can be best expressed by Higuchi equation as the correlation coefficient (R^2) values were the highest (0.820 for F4 to 0.923 for F5) as compared with the other release kinetic models studied.

Full factorial design

According to the 3^2 factorial designs, various trial formulations of IDM matrix tablets were prepared by direct compression method using ingredients stated in Table 1. The results of the regression analysis indicated that these models were significant for all response parameters (Table 2). The minitab-17 program provided suitable polynomial model equations involving individual main factors and interaction factors after fitting these data.

A statistical model incorporating interactive and polynomial terms was used to evaluate the response.

$$Y= b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4 X_1^2 + b_5X_2^2 + b_6X_1^2X_2 + b_7X_1X_2^2 + b_8X_1^2X_2^2 \quad \text{..... (5)}$$

where, Y is the dependent variable, b_0 is the intercept representing the arithmetic mean response of the nine runs, and b_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high values. The interaction terms (X_1X_2) show how the response changes when 2 factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate nonlinearity.

The data from Fig 2 and data of Table 1 indicate that the drug release profile is strongly dependent on the selected independent variables. The fitted equations relating the responses, Y1, Y2 and Y3 to the transformed factor are shown in Table 2.

The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries whether it is negative or positive. Table 2 shows the results of regression analysis which was performed to identify insignificant factors. Data were analyzed using Minitab 17 program.

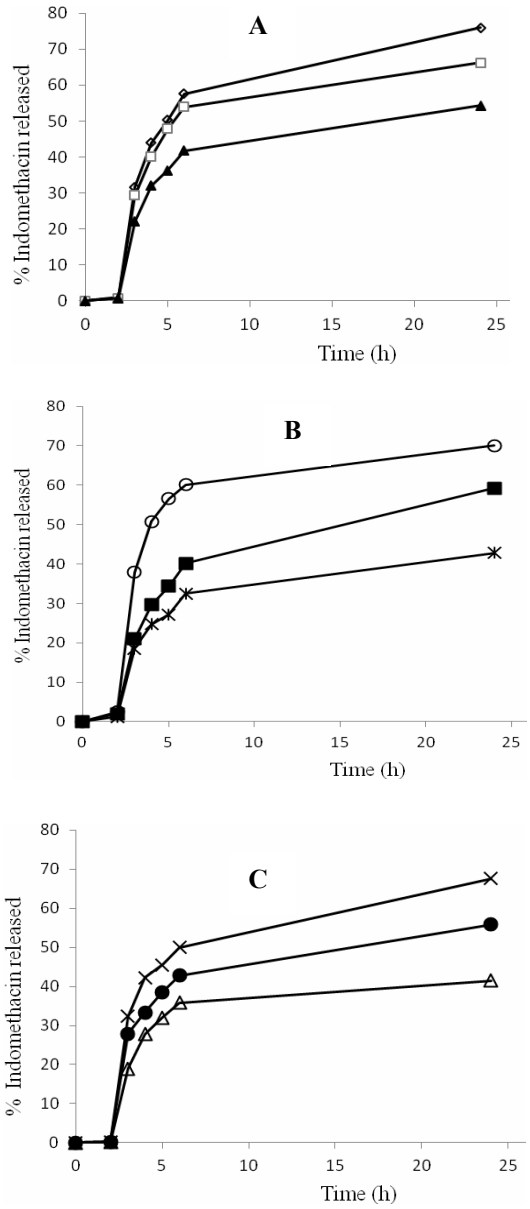

Fig 2: Release profiles of (A) IDM matrix tablets ◊F1, □F2 and ▲F3; (B) IDM matrix tablets ○F4, ■F5 and ∗F6; (C) IDM matrix tablets ×F7, ●F8 and △F9. Test performed in 0.1 N HCl for 2h and thereafter in phosphate buffer (pH 6.8)

R^2 value for Y1, Y2 and Y3 are 0.99850, 0.9980 and 0.9970, respectively, indicating good correlation between dependent and independent variables. The significance levels of the coefficients in the Y1, Y2 and Y3 were found to be significant at $p < 0.05$ hence contribute significance information to the prediction of the independent variables.

Factorial equation for Y1

$$Y1 = 29.5992 - 0.84475\ X_1 - 13.0447\ X_2 + 2.64943\ X_1X_2 + 13.4493\ X_1^2 + 8.1597\ X_2^2 + 11.6856\ X_1^2X_2 - 2.67114\ X_1X_2^2 - 18.6842\ X_1^2X_2^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Factorial equation for Y2

$$Y2 = 59.2694 + 0.67001\ X_1 - 13.5987\ X_2 + 2.35862\ X_1X_2 + 7.5979\ X_1^2 - 2.8694\ X_2^2 + 5.31173\ X_1^2X_2 - 8.38236\ X_1X_2^2 - 6.3578\ X_1^2X_2^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Factorial equation for Y3

$$Y3 = 70.9987 - 2.65975\ X_1 + 20.6960\ X_2 - 14.2558\ X_1X_2 - 26.3390\ X_1^2 - 10.7008\ X_2^2 - 25.9392\ X_1^2X_2 + 2.89951\ X_1X_2^2 + 25.3029\ X_1^2X_2^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Response surface analysis

Three-dimensional response surface plots and their corresponding contour plots to estimate the effects of the independent variables (factors) on each response investigated were presented in Fig. 3A, B and C. The three-dimensional response surface plots and corresponding contour plots relating drug release indicated the deceased values of Y1, Y2 and increased values of Y3 with the increment of the two independent variables X_1 and X_2 (amounts of HCO, and PEC in IDM matrix tablets).

Table 2: Results of regression analysis

	Y1		Y2		Y3	
	Coefficient	***P-value***	***Coefficient***	***P-value***	***Coefficient***	***P-value***
Intercept	29.5992	0.0001	59.2694	0.000	70.9987	0.000
X_1	-0.84475	0.0002	0.67001	0.0001	-2.65975	0.0003
X_2	-13.0447	0.0001	-13.5987	0.0003	20.6960	0.0001
X_1X_2	2.64943	0.0001	2.35862	0.000	-14.2558	0.0001
X_1^2	13.4493	0.0002	7.5979	0.0001	-26.3390	0.000
X_2^2	8.1597	0.0001	-2.8694	0.0003	-10.7008	0.0002
$X_1^2X_2$	11.6856	0.0001	5.31173	0.0002	-25.9392	0.0001
$X_1X_2^2$	-2.67114	0.0001	-8.38236	0.0001	2.89951	0.0001
$X_1^2X_2^2$	-18.6842	0.0001	-6.3578	0.0001	25.3029	0.0001
R^2	0.99850		0.9980		0.997	

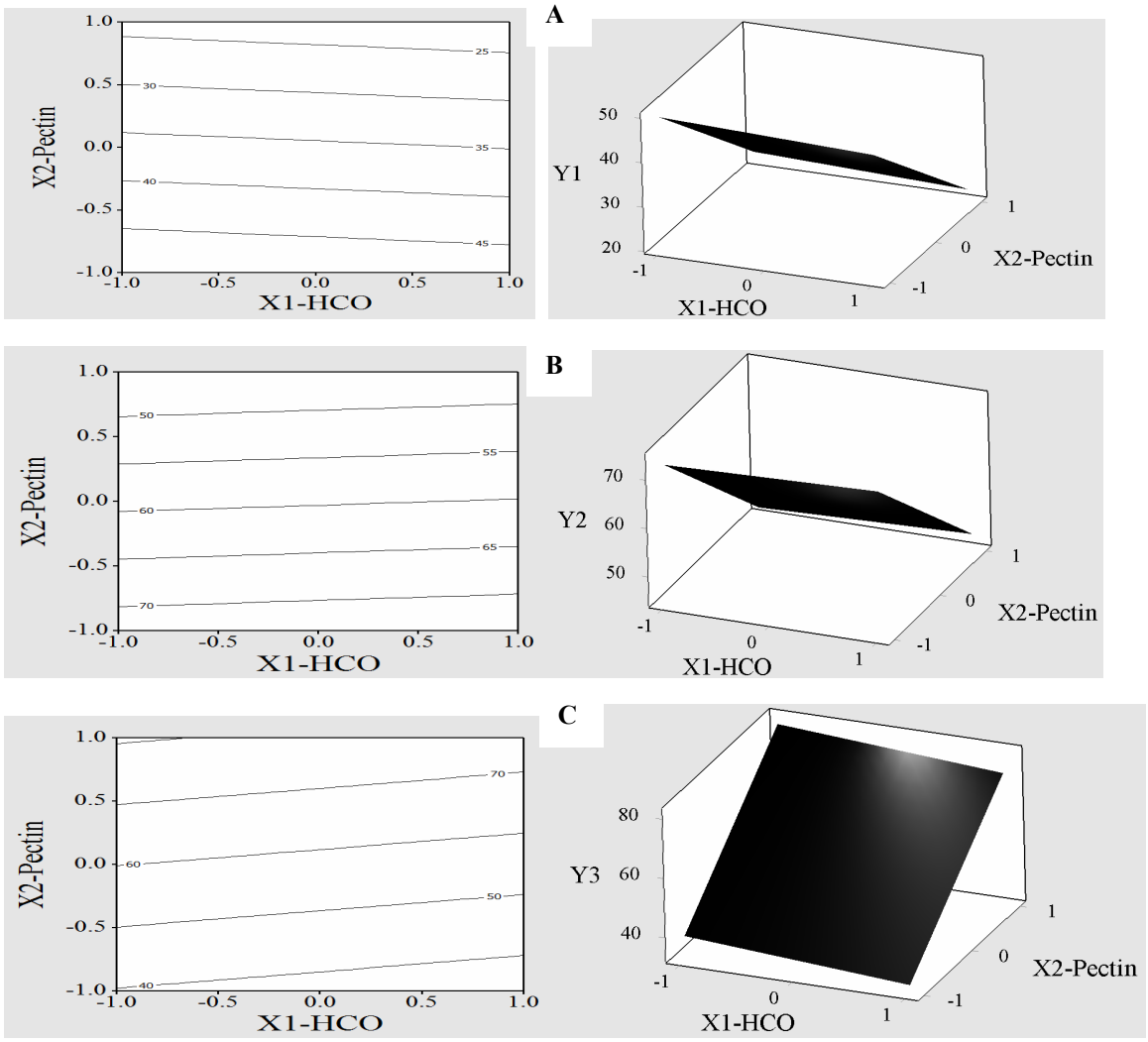

Fig 3: Response surface and contour plots for drug release after (A) 2 h (Y1); (B) 24 h (Y2); and (C) for time required for 25% drug release (Y3)

DISCUSSION

The slower release from the solid dispersion matrices using HCO alone can be due to almost complete coating of the IDM particles by HCO melted in the process of hot fusion. Further batches were prepared using combinations of HCO and PEC to check the synergistic effect of various combinations on the drug release.

On this preliminary study a factorial design was employed to find out the best combination of HCO and PEC that minimizes the further drug release in upper GIT and upon arrival to colon that releases the drug as quickly as possible.

In vitro drug release

It was expected that the penetration of the dissolution medium into the matrix will be low and hence, the release of the drug occurs at a slower rate. So, as the concentration of HCO increased the thickness of the drug particles coating increased leading to higher extent in drug release retardation.

From Fig. 3 it was concluded that at higher percentage of PEC, the tablets could remain intact in the physiological environment of stomach and small intestine but once tablets enter into the colon, it is acted upon by polysaccharidases, which degrade the PEC and hence promote release the drug in the colon.

The two criteria upon which the optimized formulations for colon delivery were selected are the percentage zero release in acidic medium (pH 1.2) and the slow release of the drug in pH 6.8. Formulations F7, F8 and F9 fulfilled the previous two requirements. The slow release *in vitro* indicated reasonable release *in vivo* in presence of the pectinase enzyme. It was reported that the drug release in presence of rate cecal content could be increased from 20 - 30 %

[20]. Consequently that 100 % drug release rate could be attained within 16 h, which is the normal residence time of a solid dosage form in the colon [21]. Therefore, it can be concluded that F7, F8 and F9 matrix tablets (Fig. 2C) would be considered as promising sustained release formulations of IDM for colon delivery. Higuchi's kinetics explains why the drug diffuses at a comparatively slower rate (0.043 for F4 and 0.083 for F6) as the distance for diffusion increases. All the formulations showed slope (n) values ranging from 0.494 for F4 to 0.618 for F6. The n values for all formulations indicated non-Fickian diffusion which refers to a combination of both diffusion and erosion controlled-drug release.

Traditionally, pharmaceutical formulators develop various formulations by changing one variable at a time but the method is time-consuming. However, many experiments not succeed in their purpose because they are not properly thought out and designed, and even the best data analysis cannot compensate lack of planning. Therefore, it is essential to understand the influence of formulation variables on the quality of formulations with a minimal number of experimental trials and subsequent selection of formulation variables to develop an optimized formulation using established statistical tools for optimization [22].

In case of Y1, the results of multiple regression analysis showed that both the coefficients b_1 and b_2 (coefficient of factors X_1 and X_2, respectively) bear a negative sign indicating a release retarding effect. It was anticipated that both matrix forming polymers would have synergistic retardant effect. The highly hydrophobic HCO and the gel forming polymer PEC ensured the expectation of very slow drug release rate. So increasing the amount of the two polymers in the formulation decreased the amount of drug released into the dissolution media. Depending on the value of the coefficient of factors, it can be stated that both polymers (X_1 and X_2) were responsible for the obtained value of Y1 but X_2 has more pronounced effect on drug release retardation.

The amount of drug released after 24 h is an important parameter for prominent drug release from sustained release matrix formulation. The data of the factorial equation for Y2 showed that the coefficient factor of X_2 is negative indicating a release retarding effect. The delay in drug release may be conditioned by the proportion of PEC in the formulations. As shown in Fig 2, as the amount of PEC increased in the tablet matrices, the drug release was decreased after 24 h.

The time required for 25 % drug release was selected for comparison between the different formulations. The coefficient factor of X_1 is negative indicating that Y3 was strongly dependent on HCO as a matrix forming material. The time needed for melting of the hydrophobic polymer was the primary factor affecting the initial drug release.

Response surface methodology is a widely proficient approach in the development and optimization of drug delivery devices [23,24]. The three-dimensional response surface plot is very useful in learning about the main and interaction effects of the independent variables (factors), whereas two-dimensional contour plot gives a visual representation of values of the response [23,24].

From the response surface analysis, it was found that the values decreased with the increase of two independent variables (amount of HCO and PEC). The higher viscosity due to increasing amount of hydrophilic polymer used, PEC may promote the formation of highly viscous gel upon contact with aqueous fluids of the dissolution medium, which would retard the drug release rate from these IDM matrix tablets.

CONCLUSION

Indomethacin matrix tablets for colon delivery containing a combination of the polymers, HCO and PEC, hold good promise for drug release retardation. Successful 3^2 full factorial design, development, and optimization of IDM tablets has been achieved in this study.

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