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OPTIMIZATION AND EVALUATION OF CLARITHROMYCIN FLOATING TABLETS USING EXPERIMENTAL MIXTURE DESIGN

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Abstract: The purpose of the study was to prepare and evaluate clarithromycin (CLA) floating tablets using experimental mixture design for treatment of *Helicobacter pylori* provided by prolonged gastric residence time and controlled plasma level. Ten different formulations were generated based on different molecular weight of hypromellose (HPMC K100, K4M, K15M) by using simplex lattice design (a sub-class of mixture design) with Minitab 16® software. Sodium bicarbonate and anhydrous citric acid were used as gas generating agents. Tablets were prepared by wet granulation technique. All of the process variables were fixed. Results of cumulative drug release at 8th h (CDR 8th) were statistically analyzed to get optimized formulation (OF). Optimized formulation, which gave floating lag time lower than 15 s and total floating time more than 10 h, was analyzed and compared with target for CDR 8th (80%). A good agreement was shown between predicted and actual values of CDR 8 th with a variation lower than 1%. The activity of clarithromycin contained optimized formula against *H. pylori* were quantified using well diffusion agar assay. Diameters of inhibition zones *vs.* log10 clarithromycin concentrations were plotted in order to obtain a standard curve and clarithromycin activity.

Keywords: clarithromycin, floating tablets, modified release, experimental mixture design, *H. pylori*

The oral route is the most preferred form of drug administration for systemic action, having a high degree of patient compliance (1) and ease of use. However, oral route has several physiological difficulties such as variable nature of gastric emptying system, inability to localize the drug delivery system (DDS) in desired regions of the gastro-intestinal system (GIS), shorter residence time and incomplete drug release of DDS (2). As a result of above factors, a shorter residence time and incomplete drug release leads to unpredictable bioavailability of DDS. Thus, control of the placement for DDS in a specific part of GIS according to absorption window may increase gastric residence time and bioavailability of DDS by preventing drug release from reaching desired absorption site of GIS (3). There have been several systems developed by researchers to increase the retention time of a DDS in the stomach (2). The examples of these are floating systems (4), swelling and expanding systems (5, 6), modified-shape systems (5–7), high density systems (8), and other delayed gastric emptying devices (2, 9). The floating drug delivery system is used commonly among these delivery systems.

Helicobacter pylori was discovered in 1984 (10) and then it has been considered to be the most frequent bacterial infection worldwide to cause ulcer disease, gastric cancer and MALT-lymphoma (11). Complete eradication can't be achieved due to insufficient amount of antibiotics in gastric mucosa and short residence time of DDS's (12). Thus, eradication of *H. pylori* requires high concentration of antibiotics in gastric mucosa and availability of DDS in stomach for longer durations.

CLA is a semi-synthetic antimicrobial macrolide antibiotic, which was discovered and patented by Taisho Pharmaceutical Co. Ltd. Japan in 1980 (12). CLA is the most preferred molecule, due to the lowest MIC value, proper pharmacokinetic properties and high efficiency on monotherapy of *H. pylori* (12). Moreover, CLA is stable in gastric environment and pH, and further (13), it has short half-life (14). Above properties of CLA make it a proper candidate for modified release drug delivery systems for the treatment of *H. pylori*.

Traditional drug development strategies require more runs of experiments, time and money to achieve desired product quality while providing

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Table 1. Composition of formulations tested.

Formulation Code	CLA (mg)	HPMC K100 (mg)	HPMC 4M (mg)	HPMC K15M (mg)	PVP K30 (mg)	$NaHCO_3$ (mg)	Citric acid (mg)	Cellulose, microcrystalline PH 101 (mg)	Talc (mg)	Magnesium stearate (mg)	Total (mg)
F 1	250	143.00	-	-	20	98	13	109	10	7	650
F 2	250	23.83	95.33	23.83	20	98	13	109	10	7	650
F 3	250	95.33	23.83	23.83	20	98	13	109	10	7	650
F 4	250	71.50	71.50	-	20	98	13	109	10	7	650
F 5	250	23.83	23.83	95.33	20	98	13	109	10	7	650
F 6	250	-	143.00	-	20	98	13	109	10	7	650
F 7	250	71.50	-	71.50	20	98	13	109	10	7	650
F 8	250	47.67	47.67	47.67	20	98	13	109	10	7	650
F 9	250	-	-	143.00	20	98	13	109	10	7	650
F 10	250	-	71.50	71.50	20	98	13	109	10	7	650
OF	250	91.13	31.36	20.51	20	98	13	109	10	7	650

less process and product understanding. In pharmaceutical field, it's possible to evaluate the influence of process inputs on finished product quality by 'design of experiment' (DOE), which is also expressed as 'experimental design'. By performing adequate literature search and risk assessments, well-designed experiments provide helpful insight, reliable results and understanding of cause-effect relationship, with fewer runs than haphazard and unplanned experiments. In general, fractional factorial and central composite designs are used preferred to evaluate the influence of the process variables as temperature, pressure, time etc. on production properties. Another main type of DOE application concerns the preparation and modification of mixtures (15). The mixture design is considerable for investigation of relative proportions of components in pharmaceutical formulation development (16). In this study, experimental mixture design was used to determine the optimum relative proportions of polymers in formulation.

The aim of this study was to develop a floating drug delivery system containing CLA. To optimize the amount of different grades of polymers and to achieve desired dissolution ratio at 8th h, experimental design was performed by mixture design methodology, which has not been widely used for pharmaceutical formulation development. The physical and analytical characteristics of formulations were evaluated.

MATERIALS AND METHODS

CLA, cellulose microcrystalline PH 101, povidone K30, sodium bicarbonate, anhydrous citric acid, talc and magnesium stearate were donated by Deva Holding, Turkey. HPMC (hypromellose) K100, HPMC K15M and HPMC K4M were donated by Colorcon, Turkey. Folin Ciocalteu's phenol reagent was purchased from Merck, Turkey. *Helicobacter pylori* ATCC 43504 was purchased from DSMZ, Germany. Mueller Hinton agar (KKMHA) was donated by Ant Teknik and Organik Laboratuvarlarý, Turkey. All chemicals were of analytical grade.

Preparation of CLA tablets

CLA, cellulose microcrystalline PH 101, povidone K30, sodium bicarbonate and different grades of HPMC were mixed in V-shaped mixer for 20 min and sieved through 0.5 mm sieve. All ingredients were mixed and granulated with adequate amount of ethyl alcohol in laboratory scale

high shear mixer (MicroGral™, Belgium). Wet mass was dried in oven at 55°C until obtaining final loss of drying (LOD) value of 2–4%, using moisture analyzer, Mettler Toledo (Switzerland), at 105°C. Dried granules were sieved through 0.841 mm sieve, mixed with anhydrous citric acid, and then mixed with talc and magnesium stearate for further 3 min in V-shaped mixer. Final mixture was compressed with Piccola rotary press machine (England) using 11.9 mm round-biconvex punches. The final crushing strength values of tablets were obtained in the range of 80–95 N.

Experimental design

The total amount of three different grades of HPMC (K100, K4M and K15M) was fixed at 143 mg (22%) in the formulations. Simplex lattice design (augmented, degree 2) was used to generate and arrange the amount of dependent variables (K100, K4M and K15M) in formulations by Minitab 16® software (LEADTOOLS © 1991–2004, LEAD Technologies, Inc., USA.). The target was set as 80% CDR at 8th h due to the dissolution study result of marketed product URCLAR, which was reported by Patel et al. (14). Ten different design points and the formulations are described in Table 1 and Figure 1. After getting results, the data were fitted into Minitab 16® software and statistically analyzed using analysis of variance (ANOVA). The statistical model was validated by preparation and dissolution analysis of optimized formulation (OF), which was generated by Response Optimizer tool of the software.

Measurement of tablet properties

The weight variations of tablets were determined according to the PhEur. The diametrical tablet crushing strength was evaluated using a tablet hardness tester (Erweka, Germany). Tablet diameter and thickness were measured using a digital micrometer with a sensitivity of 0.01 mm (Bestool-Kanon, Japan)

***In vitro* buoyancy studies**

In vitro buoyancy studies were performed according to method described by Gambhire et al. (17). Briefly, tablets of each formulation were kept in 100 mL beaker containing 0.1 mol/L HCl. The time taken for tablet to rise to surface was determined and reported as floating lag time (FLT). The total floating time (TFT) was reported by determining the constantly remaining time of the tablet on the medium surface.

***In vitro* dissolution studies**

USP Dissolution Test Apparatus Type II was used for *in vitro* dissolution tests. The dissolution test was performed using 500 mL of 0.1 M HCl, at 37°C and 100 rpm. At various time intervals, a sample of 5 mL was withdrawn and replaced with equal volume of fresh medium (n = 3). The samples were filtered through 0.45 µm filters. Two mL of samples were diluted up to 10 mL with 2 mL of Folin-Ciocalteu's phenol reagent (diluted 1 : 2 with distilled water), 2 mL of 20% sodium carbonate solution and 0.1 M HCl and mixed properly. The absorbances of the samples were measured at 760 nm using Lambda 25 UV/Vis double-beam spectrophotometer (Perkin-Elmer, USA). The drug concentrations in the samples were calculated by using standard calibration curve (14).

Kinetic mechanism of drug release

The release mechanisms of CLA from floating tablets were determined by calculating the correlation coefficients after application of release data to Korsmeyer-Peppas equation (18).

Well diffusion agar assay of optimized formulation

H. pylori ATCC 43504 was incubated in Mueller Hinton agar (KKMHA) including 5% of sheep blood at 37°C during 3 days under micro-aerophilic conditions. Bacteria suspension was prepared in physiological saline according to Mc Farland 2 opacity after incubation. Hundred µL of suspension was spread over the KKMHA medium, which was guided for inoculation. Hundred µL of dissolution samples, which were withdrawn at 1st, 2nd, 4th, 6th and 8th h and filtered through 0.45 µm filters, were inoculated into guides in KKMHA medium. Inoculated mediums were incubated at 37°C during 3 days under micro-aerophilic conditions. Diameters of inhibition zones *vs.* log10 CLA concentrations were plotted in order to obtain a standard curve. The inhibition zones were measured by caliper and the concentration of the samples were calculated by using standard calibration curve. Results were presented as the average value of 6 samples, which were studied in different two separate days (3 samples per day) (19–21).

RESULTS AND DISCUSSION

Weight variations, hardness, thickness, floating lag time (FLT), and total floating time (TFT) were evaluated and are reported in Table 2. Target

Table 2. Physical evaluation results of formulations tested.

Formulation	Weigh variation (average weigh; mg; % RSD)	Hardness (N; average)	Thickness (mm)	FLT (s)	TFT (h)	Mass integrity at 8th h
F 1	648; 0.36	84	6.870 ± 0.020	max. 13	> 4	-
F 2	649; 0.43	85	6.930 ± 0.040	max. 5	> 8	+
F 3	651; 0.38	86	6.865 ± 0.075	max. 6	> 8	+
F 4	651; 0.40	86	6.915 ± 0.025	max. 7	> 8	+
F 5	651; 0.48	86	6.965 ± 0.015	max. 9	> 8	+
F 6	652; 0.43	87	6.935 ± 0.015	max. 8	> 8	+
F 7	652; 0.47	88	6.990 ± 0.020	max. 9	> 8	+
F 8	649; 0.32	86	6.895 ± 0.025	max. 13	> 8	+
F 9	652; 0.41	87	6.895 ± 0.015	max. 6	> 8	+
F 10	651; 0.35	91	6.895 ± 0.035	max. 15	> 8	+
OF	651; 0.49	81	6.935 ± 0.015	max. 12	> 8	+

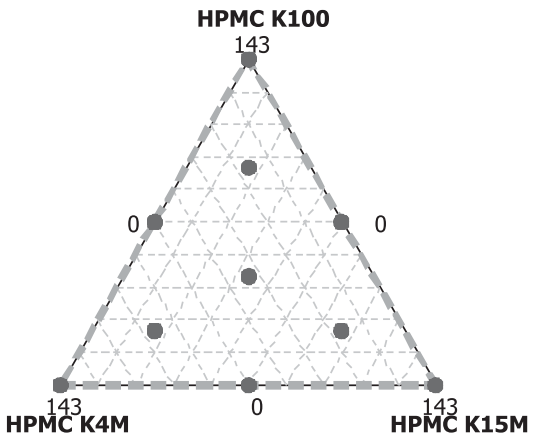

Figure 1, Simplex design plots in amounts

for average weight was set at 650 mg for all formulations. As reported in Table 2, average tablet weights were varied between 648 and 652 mg. All of the formulations proved good uniformity with % RSD lower than 0.5 when pharmacopeia limits were considered as ± 5%.

A study to determine the influence of factors on dissolution of hydrophilic polymer matrix system was performed by Saeio et al. (22). In that study, it was shown that increasing hardness values caused a decrease in porosity of polymer matrices and increasing compaction values had a high ability to retard water penetration into the core of tablet. The dissolution was slowed as a result of retarded water penetration. As the same with the mentioned study, the average hardness of the formulations was kept in the range of 81 and 91 N in our study as shown in Table 2. The hardness and tablet weights were almost similar for all of the formulations tested. Thus, no effect was considered for these physical properties between formulations to cause any variation of their dissolution behavior.

It is mandatory to use gas generating agents and hydrophilic polymers in formulation to achieve short FLT and long TFT. The buoyancy and floating are two expressions explained as reduction of the density lower than gastric fluid as a result of gas generation in dissolution media and the entrapment of generated gas by gel forming layer (by hydrated polymers). Shorter FLT is maintained by gas generating agent, and longer buoyancy (TFT) is maintained by hydrophilic polymers. In this study, sodium bicarbonate and anhydrous citric acid were used as gas generating agents. Gambhire et al. (17) reported that an increase of sodium bicarbonate decreases (alone as gas generating agent) floating lag time and excessive amounts of sodium bicarbonate causes pore formation in the tablet surface, which led to rapid hydration of polymer and rapid drug release from the system. It was also reported that 10% sodium bicarbonate concentration was essential to achieve FLT of 4 to 5 min (17). On the other hand, reduction in FLT was reported by Danki et al. (23) with the addition of citric acid to the formulation with FLT values of 24, 20 and 15 s.

According to above studies, the concentration of sodium bicarbonate and citric acid were kept constant as 15.08% and 2.00%, respectively. The FLTs and TFTs of all formulations were observed

Table 3. Dissolution results of formulations tested.

Formulation	Composition (mg)			Cumulative drug release (%; n = 3)				
	K100	K4M	K15M	1st hour ± %RSD	2nd hour ± %RSD	4th hour ± %RSD	6th hour ± %RSD	8th hour ± %RSD
F 1	143.00	-	-	14.8 ± 4.6	49.4 ± 6.0	82.8 ± 1.6	108.5 ± 2.5	108.1 ± 1.6
F 2	23.83	95.33	23.83	5.8 ± 12.3	13.9 ± 8.8	36.7 ± 3.2	59.4 ± 6.6	70.3 ± 7.2
F 3	95.33	23.83	23.83	7.9 ± 18.0	22.4 ± 6.2	43.7 ± 3.1	68.0 ± 3.8	82.5 ± 3.5
F 4	71.50	71.50	-	9.0 ± 19.0	16.9 ± 7.1	42.7 ± 4.9	62.9 ± 3.3	76.2 ± 2.3
F 5	23.83	23.83	95.33	4.0 ± 18.1	10.2 ± 12.6	32.4 ± 10.2	46.7 ± 7.8	62.0 ± 5.5
F 6	-	143.00	-	5.4 ± 10.6	11.7 ± 9.0	35.6 ± 6.8	56.9 ± 5.9	65.3 ± 4.3
F 7	71.50	-	71.50	3.5 ± 16.0	12.0 ± 3.5	38.4 ± 4.5	66.4 ± 2.7	76.6 ± 2.5
F 8	47.67	47.67	47.67	4.9 ± 11.5	12.4 ± 10.4	35.6 ± 6.2	62.3 ± 4.0	71.5 ± 2.9
F 9	-	-	143.00	3.6 ± 19.7	11.7 ± 2.7	27.2 ± 3.0	48.1 ± 6.7	53.0 ± 5.8
F 10	-	71.50	71.50	3.8 ± 15.6	10.2 ± 7.2	30.1 ± 6.6	53.0 ± 3.9	57.2 ± 2.8
OF	91.13	31.36	20.51	8.1 ± 14.2	23.4 ± 5.7	42.8 ± 5.9	61.6 ± 5.9	79.4 ± 7.9

as maximum 15 s and more than 8 h, respectively, which are reported in Table 2. The FLT of optimized formula was observed as 12 s (Table 2, Figure 2). Only F1 was disintegrated and lost mass integrity after 4th h (Table 2). It was our assumption that F1 only contained 22% of HPMC K100 concentration and its molecular weight and viscosity were not able to provide system integrity due to higher swelling property.

Similar FLT and TFT results were observed for all formulations except for F1. The results were complied with Danki et al. (23). Short FLT and long TFT obtained in this study can be explained as follows. First, immediate generation of CO_2 occurs from gas generating agent with the interaction of dissolution media and generated CO_2 is entrapped by swollen polymer matrices. Then, as a result of expansion of polymer matrix, tablet density decreases lower than that of the dissolution media and this phenomenon leads to longer TFTs.

The objective of the study was to design a formulation whose dissolution rate was 80% at 8th h. HPMC K100, HPMC K4M and HPMC K15M were used as release modifying agents to determine the ideal concentration of different grades of polymers in desired formulation. The maximum polymer level was set at 22% for all formulations. *In vitro* dissolution studies were performed on ten different formulations. Comparative dissolution data and dissolution profile graphics are shown in Table 3 and Figure 3.

Formulation F1 disintegrated within 4 h and showed maximum CDR at 6th h (see Table 2 and 3). It can be explained that low viscosity of HPMC K100 couldn't provide a robust gel layer and sustain the generated CO_2 for longer times due to earlier polymer chain relaxation than that of HPMC K4M and HPMC K15M. The rest of formulations kept their integrity until 8 h.

When we compare the release profiles of F1, F6 and F9 (Table 3, Figure 3) at the maximum concentration of polymers alone, the increase of molecular weight of the polymer decreases the CDR at all sampling times. Contrary to this phenomenon, similar results were obtained from CDR 1st h and CDR 2nd h of F7, F9, and F10 (3.5% ± 16 RSD and 12.0% ± 3.5 for F7; 3.6% ± 19.7 RSD and 11.7% ± 2.7 RSD for F9; 3.8% ± 15.6 RSD and 10.2% ± 7.2 RSD for F10, respectively) (Table 3). It was

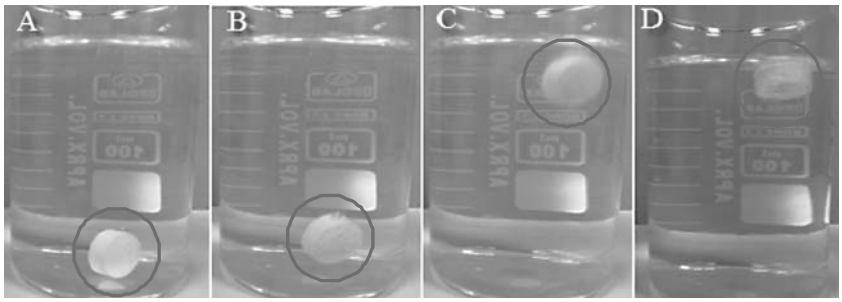

Figure 2. Photographs of optimized formulation in 0.1 M HCl (A: 0; B:4th s; C: 9th s; D:12 th s)

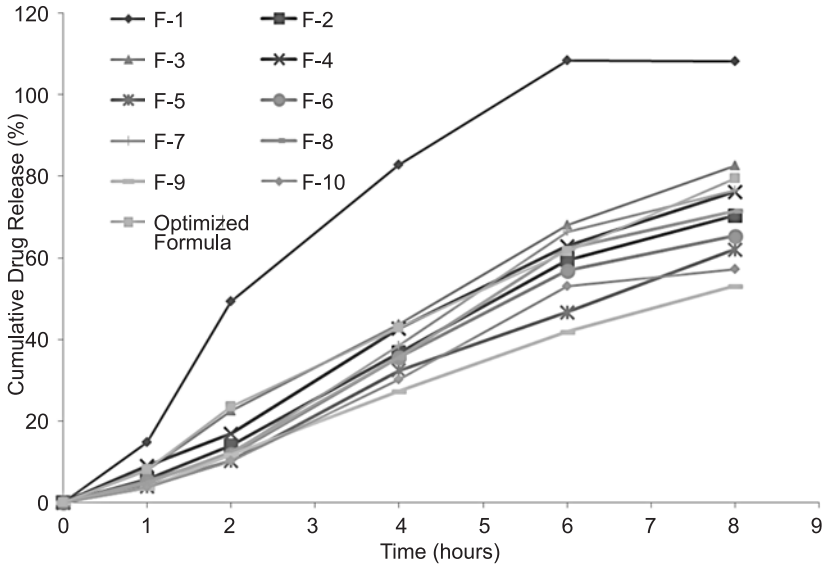

Figure 3. Comparison of *in vitro* dissolution profiles of formulations tested

observed that an increase of HPMC K15M concentration in polymer blend didn't decrease the CDR 1st h and CDR 2nd h significantly. This can be explained by less polymer-solvent interaction and polymer chain relaxation of HPMC K15M due to high molecular weight at first and second hours. The obtained results were complied with Patel et al. (14). In their study, unexpectedly, the dissolution of the system was increased by the enhancement of HPMC K15M instead of HPMC K4M. The increase of dissolution ratio was explained as a result of delaying on unwinding of the polymer chains and reduction of the gelling rate of the system due to higher molecular weight and lower flexibility of HPMC K15M than that of HPMC K4M.

Formulation F5, F9, and F10, which had higher molecular weight polymers than that of the other formulations, showed 62% for F5; 53% for F9; and 57% for F10 release of CLA, respectively, at 8th h (Table 3, Figure 3). HPMC K15M was found to be more effective for retarding the drug release of CLA from DDSs than other polymers at later dissolution stages. An increase of high molecular weight polymer in the blend decreased the drug release from DDS. Higher polymer concentrations with higher molecular weight and viscosity increased the gel formation and diffusion pathway of drug at later stages and this resulted in a reduction of drug release.

Formulations F2, F3, F4 and F8 showed 70.3, 82.5, 76.5 and 71.5% CDR at 8th h, respectively, as presented in Table 3. The highest drug release was detected for F3, which had the highest HPMC K100 amount as 95.33 mg (Table 3). By the way, the formulation F2 showed the lowest drug release, which had lowest HPMC K100 amount as 23.83 mg (Table

3). It was detected that an increase of relative proportion of HPMC K100 amount in the total polymer mixture caused enhancement in CDR 8th.

Drug release kinetics depends on several factors such as swelling rate, rate of penetration of water through the matrix, rate of dissolution of the drug, rate of diffusion of the drug through the swelled material and erosion of the matrix. Obviously, some of the above processes take place simultaneously (24). The release data of different formulations were analyzed using the linear regression to determine the release mechanism according to Korsmeyer-Peppas equation presented below (25):

$$Mt / M\infty = K \cdot t^n$$

where Mt / M8 is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The "n" value is used to characterize different release for cylindrical shaped matrices.

In this model, the value of n characterizes the release mechanism of drug shown as follows; 0.45 = n corresponds to a Fickian diffusion mechanism, 0.45 < n < 0.89 to non-Fickian transport, n = 0.89 to Case II (relaxational) transport, and n > 0.89 to super case II transport (26, 27).

To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release *versus* log time; "*n*" values for all formulations were found to be higher than 0.89, which are presented in Table 4. The release exponent data indicated that all of the formulations showed super case II transport.

Mixture experiments are a special class of response surface experiments in which the product under investigation is made up of several components or ingredients. Designs for these experiments are useful because many product design and development activities in industrial situations involve formulations or mixtures (16). Minitab provides simplex centroid, simplex lattice, and extreme vertices designs for mixture experiments. Specifically, extreme vertices design was used when components had upper and lower limits. There were no constraints for the total amount of polymer mixture and augmented, degree 2 simplex lattice design had adequate coverage of the experimental region of interest as shown schematically in Figure 1. The total amount of three different grades of HPMC (K100, K4M and K15M) was fixed at 143 mg (22%) in the formulations. HPMC K100, HPMC K4M and HPMC K15 M (given as X_1, X_2, and X_3, respectively) were chosen as inter-dependent variables. Each point in Figure 1 indicates a formulation with different amounts of polymers. For example, the tips of the triangle represent the maximum amounts per grade of polymers and the center point represents the mixture of equally blended amounts of polymers. Cumulative drug release at 8th h (CDR 8th) was chosen as dependent variable (Y = response). The release results were statistically analyzed by using stepwise-analyze method as a function of Minitab

Table 4. Results of model fitting of drug release.

Formulation	Korsmeyer – Peppas	
	R^2	n
F 1	0.917	0.947
F 2	0.993	1.243
F 3	0.987	1.118
F 4	0.993	1.074
F 5	0.991	1.358
F 6	0.988	1.271
F 7	0.986	1.529
F 8	0.992	1.349
F 9	0.986	1.289
F 10	0.987	1.374
OF	0.982	1.068

Table 5. Summary of regression analyses for response (CDR 8th).

Terms	Coefficients	SE Coefficients	t	p	VIF
HPMC K100	108.11	2.118	*	*	2.057
HPMC K4M	64.11	1.922	*	*	1.694
HPMC K15M	56.19	1.845	*	*	1.561
HPMC K100 × HPMC K4M	-35.31	9.536	-3.70	0.021	1.982
HPMC K100 × HPMC K15M	-19.95	9.579	-2.08	0.106	2.000
HPMC K100 × HPMC K4M × HPMC K15M	-73.22	30.049	-2.44	0.071	1.21

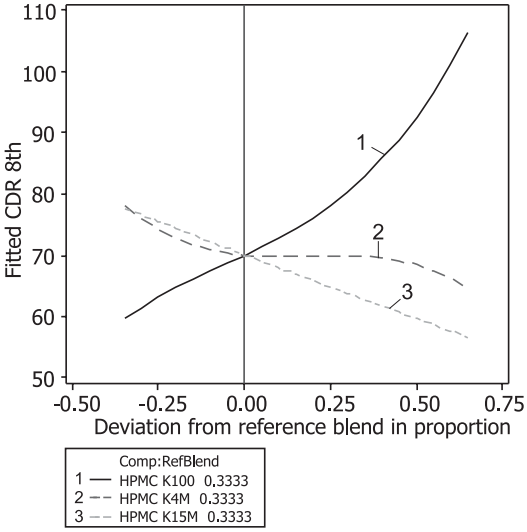

Figure 4. Cox response trace plot indicating the influence of the polymers on CDR 8th

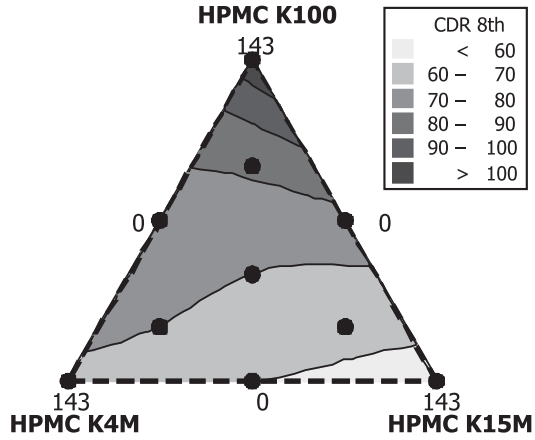

Figure 5. Mixture contour plot indicating the influence of the polymers on CDR 8th

16® software. The obtained model equation, unexplained variation (S), R-square (R-sq) and R-square adjusted (R-Sq adj.) given by Minitab 16® software are shown below:

Y (CDR 8th) = 108.11 × HPMC K100 + 64.11 × HPMC K4M + 56.19 × HPMC K4M – 35.31 × HPMC K100 × HPMC K4M – 19.95 × HPMC K100 × HPMC K15M – 73.22 × HPMC K100 × HPMC K4M × HPMC K15M

S = 2.1453; R-sq = 99.09%; R-sq (adj.) =: 97.94%

Both R-sq and Rs (adj.) values indicate that the model fits the data well. R-sq is generally considered as minimum 70% (28), however, in our study, we obtained R-sq as 99.09% which meant 99.09% of the total variation could be explained by the model and the model was reliable. Unexplained variation of the model was 2.1453 and this was low enough.

It was found that the interaction of HPMC K100 and HPMC K4M was statistically significant for CDR 8th because the *p*-value of the interaction of HPMC K100 and HPMC K4M was lower than 0.05, shown in Table 5 (16, 29).

VIF indicates the extent to which multicollinearity (correlation among predictors) is present in a regression analysis; n regression, multicollinearity refers to predictors that are correlated with other predictors. Moderate multicollinearity may not be problematic. However, severe multicollinearity is problematic because it can increase the variance of the regression coefficients, making them unstable and difficult to interpret (16).

Variance inflation factors (VIF) measure how much the variance of the estimated regression coefficients are inflated as compared to when the predictor variables are not linearly related. The following guideline is used to interpret the VIF (16):

For VIF = 1 predictors are not correlated, for 1< VIF <5 are moderately correlated and for VIF > 5 to10 they are highly correlated.

As presented in Table 5, VIF values of all factors were obtained below 5 (2.057 for HPMC K100M, 1.694 for HPMC K4M, 1.561 for HPMC K15M, 1.982 for interaction of HPMC K100M and HPMC K4M, 2.000 for interaction of HPMC K100M and HPMC K15M and 1.211 for interaction of three components). It signifies that all factors showed moderate multicollinearity, which was not considered to increase the variance of regression coefficients. The standard error of coefficient (SE coef.) is the standard deviation of the estimate of a regression coefficient. It measures how precisely the data can estimate the coefficient's unknown value. Its value is always positive, and smaller values indicate a more precise estimate (16); 't-value' measures the difference between an observed statistic and its hypothesized population parameter in units of standard error. A t-test compares this observed "t-value" to a critical value on the t-distribution with (n – 1) degrees of freedom, to determine whether the difference between the estimated and hypothesized values of the population parameter is statistically significant (16). Correspondences of t-values were presented as p-values, which indicate whether the factor is significant or not.

Table 6. Target limit, upper limit, lower limit, and desirability results to reach target response for CDR 8th.

Formulation	Composition		Composite desirability	Target (CDR 8 th)	Predicted value	Actual value	Variation
Optimized formula	K100	91.13%	0.9889%	80%	9.98%	79.4%	0.58%
	K4 M	31.36%					
	K15 M	20.51%					

Table 7. Comparative dissolution results of optimized formula using well diffusion agar assay and spectrophotometric method.

Analytical method	Cumulative drug release (%; n = 6)				
	1st hour ± %RSD	2nd hour ± %RSD	4th hour ± %RSD	6th hour ± %RSD	8th hour ± %RSD
Well diffusion agar method	6.3 ± 7.74	12.3 ± 3.95	22.1 ± 1.69	41.5 ± 0.54	76.3 ± 1.99
Spectrophotometric method	8.1 ± 14.2	23.4 ± 5.7	42.8 ± 5.9	61.6 ± 5.9	79.4 ± 7.9

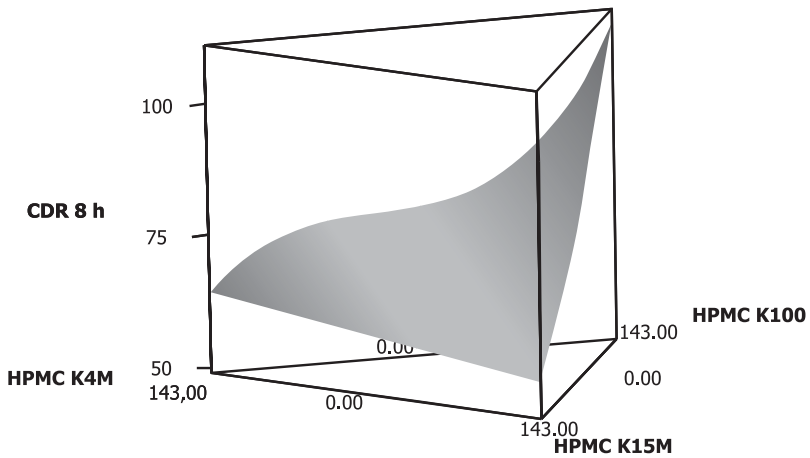

Figure 6. Mixture surface plot indicating the influence of the polymers on CDR 8th

The coefficients of three interdependent variables were 108.11 for HPMC K100, 64.11 for HPMC K4M, and 56.19 for HPMC K15M indicating that HPMC K100 had the highest impact on dissolution. Negative coefficients indicated that two components were antagonistic towards one another. That meant that the mean acceptance score of mixture was lower than that of the one, which was obtained by calculating the simple mean of two acceptance scores (16).

Cox response trace plot (Fig. 4) shows the effect of each polymer on response reference to relative blend. If relative proportion of HPMC K100 (curve no 1, Fig. 4) increases in the mixture (and the other polymer proportions decrease), the CDR 8th increases. HPMC K100 had the steepest response trace and showed greatest effect on dissolution. If relative proportion of HPMC K4M (curve no. 2, Fig. 4) was increased in the mixture (and the other polymer proportions were decreased), the CDR 8th slightly decreased, remained horizontal and again decreased. The horizontal part of the trace for HPMC K4M indicated that there was no significant effect on response between these concentrations. HPMC K4M which had the shortest response trace was used with smaller ranges. If relative proportion of HPMC K15M (curve no. 3, Fig. 4) was increased in the mixture (and the other polymer proportions were decreased), the CDR 8th linearly decreased. According to Cox response trace plot (Fig. 4) it

could be concluded that while HPMC K100 had positive effect on CDR 8th, HPMC K4M and HPMC K15M had negative effect on CDR 8th.

The mixture contour plot, shown in Figure 5, provided a two-dimensional view where all points had the same response in the same shade regions connected with contour lines (Fig. 5). Mixture surface plot, shown in Figure 6, provided a three-dimensional view supplying a clearer picture of the surface. The area with highest release was located on the HPMC K100 edge of the plots both in mixture surface and contour plots (Figs. 5, 6). It was easy to visualize that CDR 8th was at peak value when mixture contained maximal amount of HPMC K100 and, in addition, CDR 8th had the lowest value when mixture contained maximal amounts of HPMC K15M. According to the obtained model (mentioned as an equation) from the statistical analysis, the optimized formulation, which was considered to have the best values of polymers, was generated by design optimizer tool of Minitab 16® software. In design optimizer, to obtain the target response for CDR 8th, desirability was considered to be close to '1'. The target limit, upper limit, lower limit, and desirability were set as 79.98%, 82%, 78% and 0.9889, respectively, in design optimizer tool (Table 6). Optimized formulation was evaluated to validate the ability of the model and so the CDR 8th value, which was presented as actual value in Table 6 (79.43%), was compared with predicted value 79.98%. The variation was found lower than 1.0% as shown in Table 6. A good agreement was shown between predicted and actual values of CDR 8th. By this way the release profile of the optimized formula was verified.

Since CLA is an antibiotic, inhibition activity of CLA has to be shown against *H. pylori*. The CDR results of withdrawn samples at various times intervals were calculated fitting the measured inhibition zone diameters in calibration curve. CDR results of optimized formulation, which were determined by well diffusion agar assay and comparison of average CDR values between spectrophotometric and well diffusion agar assay are presented in Table 7. The antimicrobial effectiveness of the CLA contained DDS was proved by the inhibition zones in mediums. It was observed that a slight difference occurred for CDR at the 1st and 8th h. In addition, a significant difference occurred for CDR of the 2nd, 4th and 6th h. This difference can be explained by logarithmical transformation of concentrations and as a result of the fact that even minor measurement differences of inhibition zones determined by calipers had a huge impact on calculations.

CONCLUSION

A promising formulation of gastro-resistant effervescent floating drug delivery system of clarithromycin could be developed by mixture design methodology using gel forming agents (hypromellose), gas generating agents, sodium bicarbonate and citric acid. Different grades of HPMC (K100, K4M and K15M) provide reduction on release ratio of the CLA. The values of 15.08% of sodium bicarbonate and 2% of citric acid were found to be sufficient to provide FLT lower than 30 s and TFT more than 8 h. Desired dissolution ratio was achieved generating a suitable formulation using statistical software Minitab 16®. The antibiotic efficiency of optimized formula was proved by well diffusion agar assay as well.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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