

Mathematical Optimization and Investigation on Polymeric Blend of Chitosan and Hydroxy Propyl Methyl Cellulose K4M for Sustained Release of Metronidazole

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Abstract

Background: Sustained release gastro retentive drug delivery systems enable prolonged and continuous input of the drug to the gastrointestinal tract and improve the bioavailability of medications that are characterized by the narrow therapeutic window. **Aim:** The objective of this study encompasses the application of the response surface approach in the development of pharmaceutically active hydrodynamically balanced system (HBS)-containing metronidazole (MN). **Materials and Methods:** Experiments were performed according to a 3² factorial design to evaluate the effects of gel forming polymers, low molecular weight chitosan, and medium molecular weight chitosan on the buoyancy and time taken for 60% drug release ($t_{60\%}$). The effect of the two independent variables on the response variables was studied by surface response curves and contour plots generated by the Minitab-17 software. **Results:** The drug-excipients interaction studies performed by differential scanning calorimetry revealed drug polymer compatibility, and hence, formulations were prepared by physical blending of MN and polymers by encapsulation into hard gelatin capsules. *In vitro* buoyancy study and drug release study in the gastric environment showed the efficacy of the HBS to remain gastro retentive (buoyant) for a longer period and simultaneously sustained the release of the drug. **Conclusion:** Thus, a conclusion might be brought forward that the present HBS could be an ideal system for stomach specific sustained delivery of MN and would be useful to patients where the prolonged therapeutic action on the infection site caused by microorganisms is required.

Key words: Buoyancy, chitosan, hydrodynamically balanced system, mathematical optimization, metronidazole

INTRODUCTION

Gastric emptying of dosage forms is an extremely variable process and ability to prolong and control the emptying time is a valuable asset for dosage forms, which reside in the stomach for a longer period than conventional dosage forms.^[1-3] Scintigraphic studies determining gastric emptying rates revealed that orally administered controlled release dosage forms are subjected to 2 complications, primary of short gastric residence time, and unpredictable gastric emptying rate. Hence, gastro retentive systems can remain in the gastric region for several hours, and consequently, prolongs the gastric residence time of drugs.^[3-6]

Several difficulties are faced in designing controlled release systems for better

absorption and enhanced bioavailability. One of such difficulties is the inability to confine the dosage form in the desired area of the gastrointestinal tract (GIT).^[1,2] Gastro retentive drug delivery systems are defined as systems that increase the retention of a per-oral dosage form in the stomach offering numerous advantages for drugs exhibiting an absorption window in the GIT; drugs that are poorly soluble in the alkaline medium, and drugs that are intended for local action on the gastroduodenal wall.^[7,8]

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Over the last three decades, various approaches have been made to design gastro retentive delivery systems including floating systems,^[9] modified shape systems,^[10] swelling and expanding systems,^[11] bioadhesive systems,^[12,13] and high-density systems.^[14]

Hydrodynamically balanced systems (HBS) are single-unit dosage forms, containing one or more gel forming hydrophilic polymers. The polymers are mixed with drug and usually administered via gelatin capsule and are so designed to prolong the stay of the dosage form in the gastrointestinal tract so as to enhance the absorption of the drug. Figure 1 illustrates the working principle of HBS, which shows that the capsule rapidly dissolves in the gastric fluid at body temperatures, via hydration and swelling of the surface polymers to produce a floating system for a prolonged period of absorption. Further, the drug release is controlled by the formation of a hydrated boundary at the surface. Continuous erosion of the surface allows water to penetrate into the inner layers, maintaining surface hydration, and buoyancy.^[15,16] Chitosan fulfills all the polymeric attributes that are essential to achieving a high level of retention at applied and targeted sites via mucoadhesive bonding. The mucoadhesive property of chitosan is due to the electrostatic interaction of the protonated amino group in chitosan with negatively charged silicic acid residues in mucin (the glycoprotein that composes the mucus). This interaction takes place very close to the mucosal surface and thus possesses potential to confer significant gastro retention of formed hydrogel into GIT. In addition, the hydroxyl and amino groups may interact with mucus via hydrogen bonding. To remain in the stomach for a prolonged period, the dosage form must have a bulk density of <1.^[17-19]

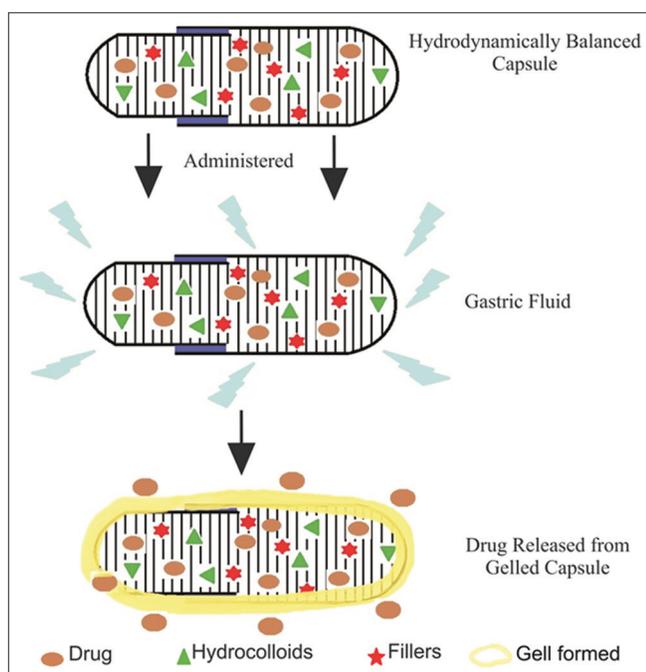


Figure 1: Working principle of hydrodynamically balanced system

Factorial designs, dealing with factors in all possible combinations, are considered to be the most efficient in estimating the influence of individual variables and their interactions using nominal experiments. The applicability of factorial design in the development of pharmaceutical formulation has helped in understanding the link between the independent variables and the responses to them. The independent variables are manageable, whereas responses are dependent. This supports the process of optimization by rendering an empirical model equation for the response as a function of the different variables. The technique needs minimum experimentation and time, thus establishing far more cost-effective formulation than the conventional methods of formulating dosage form.^[20]

The current study aimed at developing and optimizing an HBS-containing metronidazole (MN) as a model drug, utilizing a computer aided optimization technique. Factorial 2-factor interaction model was employed to investigate the effect of low molecular weight chitosan (LMWC) and medium molecular weight chitosan (MMWC) as a gel forming polymers for formulating the HBS. This is due to the fact that the two important variables, that is, buoyancy (floating time) imparted by the incorporation of gel forming polymers and time taken to release 60% of drug from the dosage form, shall contribute effect on the nature and performance of the HBS.

MATERIALS AND METHODS

Materials

MN was obtained as a gift sample from J.B. Chemicals, Ankleshwar, India. LMWC, MMWC, and hydroxy propyl methyl cellulose (HPMC K4M) were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. All reagents used were of analytical grade.

Physicochemical investigation of the interaction

Designing any drug delivery system, it is necessary to give consideration to the compatibility of drug and polymer used within the system. Therefore, it is imperative to confirm that the drug is not interacting with polymer under experimental conditions and shelf life. Differential scanning calorimetry (DSC) analysis was performed to assess the interaction between the drug and the polymers used in the development of HBS.

DSC study was carried out on pure substances (MN, LMWC, MMWC, and HPMC K4M) and their physical mixtures. 10 mg of the physical mixture was dispensed by triturating all the ingredients gently in the mortar and pestle. Weighed quantities of the mixture were placed on the aluminum pans of the apparatus (PerkinElmer Pyris Diamond DSC) equipped with Pyris – Instrument Managing Software for computing the heat flow from the sample. Samples were

heated at a scanning rate of 10°C over the range of 40-300°C with 20 mL/min of nitrogen gas flow.

Experimental design

A 3² factorial design was employed where the amount of two polymers (factors) were varied at two levels as hypothesized by the design. The amount of LMWC (A) and MMWC (B) was selected as factors and studied at two levels. Table 1 summarizes the nine experimental runs studied, their factor combinations, and the translation of the coded level to the experimental units employed during the study. Buoyancy or floating time (Y_1) and time taken for the release of 60% of drug ($t_{60\%}$ or Y_2) were taken as the response variables.

Preparation of HBS capsule containing MN

Single-unit capsules were prepared by physically blending MN and HPMC K4M alone or in combination with other polymers in a Double Cone Blender for 15 min followed by encapsulation in hard gelatin capsules.^[21] The composition of 9 experimental batches is in accordance with the experimental design elaborated in Table 2 except on one point that HPMC K4M being a hydrophilic polymer it is responsible for early onset of gelation, hence it was incorporated in every formulation at a fixed concentration of 16.66 %w/w of polymers. HPMC K4M is responsible for interaction by the formation of a hydrogen bond with LMWC and MMWC so as to attain the objective of the study.

In vitro evaluation of HBS capsule

Prepared HBS capsules were evaluated for buoyancy, drug content, *in vitro* drug release studies.

In vitro buoyancy studies

Prepared capsules were immersed in 0.1 M HCl (pH 1.2) in USP paddle type apparatus at 50 rpm. The floating lag time and time for which the capsules remained buoyant was observed.^[21]

Effect of release modifiers

To achieve the objective of the study, LMWC and MMWC were used as MN release modifiers at concentrations mentioned in Table 2. These polymers were physically blended separately with HPMC K4M and MN and filled into the hard gelatin capsules.

Determination of drug content of capsules

Drug content was determined by emptying 10 same formulations filled into hard gelatin capsules as completely as possible. A powder equivalent to average weight was added to 100 mL of 0.1 M HCl (pH 1.2) at 37°C ± 0.5°C followed by stirring for 1 h at 500 rpm. The solution was filtered through 0.45 µ membrane filter, diluted suitable,

Table 1: Factor combination as per 3² factorial designs

Trial number	Coded factor levels	
	Factor 1	Factor 2
1	-1	-1
2	0	1
3	1	1
4	1	-1
5	1	0
6	-1	1
7	0	0
8	0	-1
9	-1	0

Translation of coded levels in actual units

Coded levels	-1	0	1
A: LMWC (%w/w of polymers)*	16.66	0	33.33
B: MMWC (%w/w of polymers)*	16.66	0	33.33

*Low and high coded levels were chosen on the basis of preliminary trials conducted for efficient buoyancy. LMWC: Low molecular weight chitosan, MMWC: Medium molecular weight chitosan

Table 2: Composition of HBS capsules containing MN along with and without release modifiers

Formulation code	HPMC K4M (%w/w)	LMWC (%w/w)	MMWC (%w/w)	MN (in mg)
F1	16.66	16.66	16.66	190
F2	16.66	0	33.33	190
F3	16.66	33.33	33.33	190
F4	16.66	33.33	16.66	190
F5	16.66	33.33	0	190
F6	16.66	16.66	33.33	190
F7	16.66	0	0	190
F8	16.66	0	16.66	190
F9	16.66	16.66	0	190

LMWC: Low molecular weight chitosan, MMWC: Medium molecular weight chitosan, MN: Metronidazole, HPMC: Hydroxy propyl methyl cellulose, HBS: Hydrodynamically balanced system

and the absorbance of the resultant solution was measured spectrophotometrically at 277.40 nm.

In vitro drug release studies

In vitro release of MN from the HBS capsule was performed in USP dissolution apparatus type II at 50 rpm. Evaluation of drug release was performed using 900 mL of 0.1M HCl (pH 1.2) at 37°C ± 0.5°C. At predetermined intervals, 1 mL aliquot was withdrawn and replenished with an equal volume of fresh dissolution media to maintain the sink conditions perfectly. Withdrawn samples after suitable dilutions were analyzed spectrophotometrically (UV/Vis spectrophotometer, 1800 Shimadzu, Japan) at 277.40 nm.

Scanning electron microscopy (SEM) studies

The optimized formulation F3 was studied for the surface morphology by SEM (Supra 40 VP, Zeiss, Germany). The formulation was subjected to dissolution containing dissolution medium 0.1 M HCl. After 1 h of dissolution and at the end of 10 h, the gel formed by the HBS was taken out and dried to remove water. The samples (gold coated) were placed on a specimen holder made up of copper with the help of double-sided adhesive tape, and then, it was analyzed for surface topography at an accelerating voltage of 8-15kV.

Drug release kinetics and mechanism

Different kinetic models (zero order, first order, and Higuchi's model) were applied to the release data to interpret the drug release kinetics and to know the mechanism of drug release from these HBS capsules with the help of Equations (1-3).

$$\text{Zero order equation: } Q=Q_0-k_0t \quad (1)$$

$$\text{First Order equation: } L_n Q=L_n Q_0-k_1 t \quad (2)$$

$$\text{Higuchi's equation: } Q=k_H t^{1/2} \quad (3)$$

In these equations, Q_0 is the initial drug concentration, Q is the amount of drug released at time t , and k_0 , k_1 and k_H are the rate constant for zero order, first order, and Higuchi's model, respectively.^[22]

To confirm the exact mechanism of drug release from HBS capsules, the data were fitted according to the Korsmeyer–Peppas model. Korsmeyer *et al.* used a simple empirical equation to describe the general solute release behavior from controlled release polymer matrices:^[23]

$$M_t/M_\infty=K t^n$$

Where, M_t/M_∞ is the fraction of drug released, K is the kinetic constant, t is release time, and n is the diffusional exponent for drug release. The value of n gives an indication of the release mechanism: When $n = 1$, the release rate is independent of time (zero order, case II transport). $n = 0.5$ stands for Fickian diffusion and when $0.5 < n < 1.0$, diffusion and non-Fickian transport are implicated. Finally, when $n > 1.0$, super case II transport is apparent. n is the slope value for $\log (M_t/M_\infty)$ versus \log time curve.^[23]

Optimization data analysis

Various response surface methodology (RSM) computations for the current optimization study were performed employing Minitab-17 software. Statistical second order model, including interaction and polynomial terms, was generated for all the response variables. The general form of the model is represented as in the following:

$$Y=\beta_0+\beta_1A+\beta_2B+\beta_3AB+\beta_4A^2+\beta_5B^2+\beta_6A^2B+\beta_7AB^2+\beta_8A^2B^2 \quad (4)$$

Where β_0 , the intercept, is the arithmetic average of all quantitative outcomes of nine runs, β_1 - β_8 are the coefficient computed from the observed experimental values of Y , and A and B are the coded levels of the independent variable(s). The terms AB and A^2 and B^2 are the interaction and polynomial terms, respectively. The main effects (A and B) postulate the average result of changing one factor at a time from its low to high value. The interaction term (AB) shows how the response changes when two factors are changed accordingly. The polynomial terms (A^2 and B^2) symbolize nonlinearity.^[20]

The polynomial equation was used to draw conclusion after considering the intensity of coefficient and the mathematical sign it carries, that is, positive or negative. A positive sign signifies synergesis. The statistical validity of the polynomials was established on the basis of ANOVA provided in the Minitab-17 software. The level of significance was considered at $P < 0.05$. Furthermore, three-dimensional response surface graphs and contour plots were generated by the Minitab-17 software.

RESULTS AND DISCUSSION

HBS is the simplest gastro retentive dosage forms, composed of hard gelatin capsules filled with a mixture of gel forming polymeric substances and an active pharmaceutical ingredient. After immersion into solution (*in vitro*) or swallowing (*in vivo*), the shell of the swollen hydrogel is formed. It controls the release rate of the drug, and it maintains the appropriate integrity of the HBS and low apparent density of the systems, ensuring flotation. Such systems are best suited for drugs having a better solubility in an acidic environment and for the drugs having a specific site of absorption in the upper part of the small intestine.^[19,24]

Drug-excipient interaction

Selection of polymers in HBS requires the knowledge of their interaction with other polymers as well as with the therapeutic molecule used in the system. DSC analysis of pure MN showed a sharp endothermic peak at 164.68°C (which was around its actual melting point 160°C), which represents the melting of the MN [Figure 2a]. A broad endothermic bend in thermogram showed in Figure 2b from 40°C to 110°C for HPMC K4M can plausibly be attributable to the vaporization of the moisture present in the sample. The DSC thermogram of LMWC showed a broad endothermic peak at 157.92°C, which indicates the glass transition temperature of the polymer [Figure 2c]. Thermogram of MMWC showed a broad peak at 40-80°C over a large temperature range is attributed to water loss due to evaporation of absorbed water, and this represents the energy required to vaporize water present in the samples [Figure 2d]. Peak disappearance in the DSC thermogram of

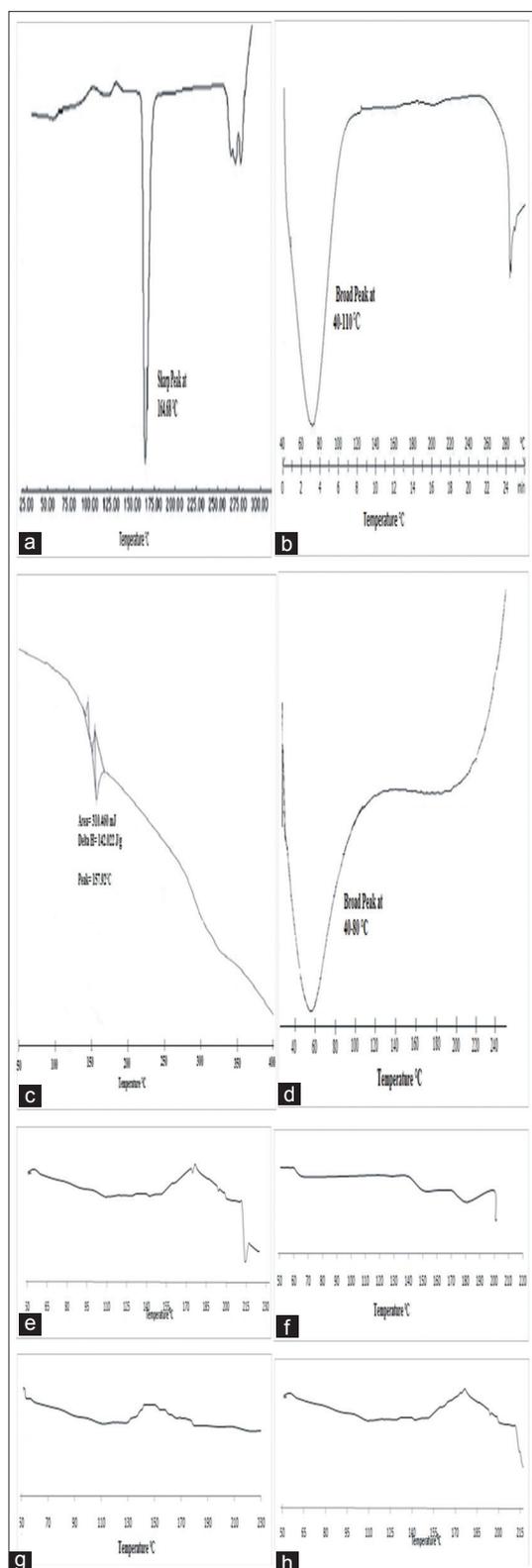


Figure 2: Differential scanning calorimetry thermogram of pure ingredients and their mixtures. (a) Pure metronidazole (MN). (b) Pure hydroxy propyl methyl cellulose (HPMC) K4M. (c) Pure low molecular weight chitosan (LMWC). (d) Pure medium molecular weight chitosan (MMWC). (e) Physical mixture of MN + HPMC K4M. (f) MN+HPMC K4M + LMWC. (g) MN + HPMC K4M + MMWC. (h) MN + HPMC K4M + LMWC + MMWC

physical mixture containing MN and polymers [Figure 2e-h] indicates that the drug was molecularly dispersed in the matrix of polymeric blend. Overall, Figure 2a-h did not show any major or unwanted interaction for the formulation of MN-based HBS system.

Effect of formulation variable on buoyancy (Y_1)

Table 3 listed the values of various response parameters of the nine optimization formulations. The constant and regression coefficients for Y_1 (buoyancy or floating time) were as follows:

$$Y_1 = 2.167 + 0.667A + 1.167B + 1.500A^2 + 2.000B^2 + 0.375A*B \quad (5)$$

The polynomial quadric model was found significant with an F value of 23.73 ($P = 0.013$). Equation 5 indicated that AB , A^2 , B^2 were significant model terms. The combination effect of factors A and B could further be elucidated with the help of surface response curve and contour plots [Figure 3a and b]. However, the steeper ascent in the response surface with MMWC (B) than the LMWC (A) was clearly perceptible from both the plots, indicating that the effect of MMWC was comparatively more pronounced than that of LMWC. From this, the conclusion can be drawn that the buoyancy might be changed by appropriate selection of the levels of A and B .

From the *in vitro* buoyancy studies, it was observed that all formulations exhibited immediate buoyancy with no lag time. When HBS formulations filled into hard gelatin capsules and placed in 0.1 M HCl, the disruption of capsule shell begins, and it was observed that as the dissolution medium penetrated through the disrupted capsule shell, the outer layer of the polymer matrix hydrated to form gel. As confirmed from Figure 3a and b, all formulations except F2, F3, and F6 failed to remain buoyant for up to 5 h, hence these formulations were found to be optimized. HPMC K4M alone (F7) remain buoyant for up to 2.5 h after that the formed gel was got burst

Table 3: Response parameters for MN-loaded HBS prepared as 3^2 factorial design

Formulation code	Floating time or buoyancy (Y_1) (h)	$t_{60\%}$ (Y_2) (h)
F1(-1,-1)	4	3.5
F2(0,1)	5	4.5
F3(1,1)	8	6
F4(1,-1)	4.5	4
F5(1,0)	4.5	3
F6(-1,1)	6	5.5
F7(0,0)	2	2.5
F8(0,-1)	3.5	3.5
F9(-1,0)	3	2.5

MN: Metronidazole, HBS: Hydrodynamically balanced system

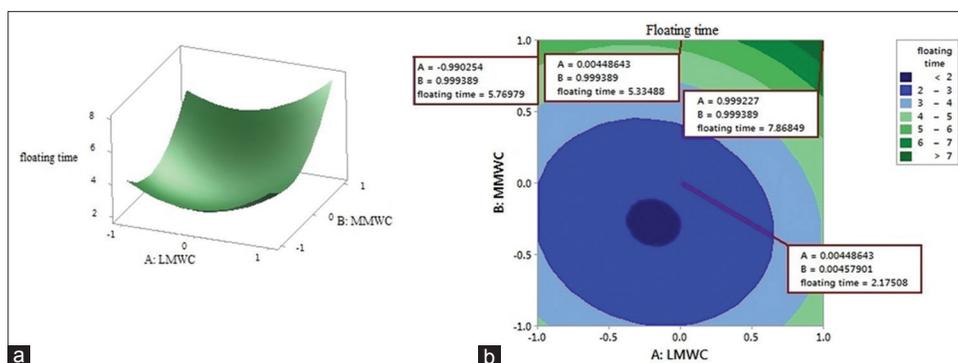


Figure 3: (a) Response surface plots showing the influence of medium molecular weight chitosan and low molecular weight chitosan on the floating time or buoyancy (Y1). (b) Corresponding contour plot showing the relationship between various levels of the two factors (data mentioned in the rectangular boxes shows the desired effect)

and mixed with dissolution media; this could be attributed to the weak gel network formed due to the hydrophilicity of HPMC K4M. Figures 4-11 showed the floating behavior of HBS capsules in the time range of 1-10 h, respectively. For efficient buoyancy, swelling of the polymer was very vital. Further, there must be a balance between swelling and water acceptance. In our case, during swelling of the hydrophilic cellulose derivative (HPMC K4M), the macromolecular chains absorb water leading to an expansion of the network formed and to the formation of a quasi-equilibrium structure. This three-dimensional network structure usually is held together by physical chain entanglements, hydrogen bonds, tie junctions, or tie points produced by various types of forces. Upon further absorption of water, these gels may start disentangling, indicating a competitive phenomenon of swelling and dissolution. Beyond that time, HPMC K4M gel thicknesses were no longer sustained.^[25-27] Hence, the addition of release modifiers such as LMWC and MMWC are necessary to incorporate into formulation along with HPMC K4M. HPMC K4M in combination with LMWC or MMWC forms hydrogen bond, which exhibited good floating behavior throughout the experiment. Chitosan forms gel in the acidic medium, swelling of chitosan polymers resulted in increase in bulk volume. The air entrapped in the swollen chitosan, and hydrogen bonding with HPMC K4M maintains the density less than unity which ultimately confers buoyancy to the dosage forms.^[28-32]

Drug content of capsules

Drug contents of all formulations were determined UV spectrophotometrically and were found to be in the range of 98-99%. Table 4 showed the drug content and drug remaining in the gel matrix. The experiment was conducted in triplicate.

Effect of formulation variables on *in vitro* drug release

$t_{60\%}$ is an important variable for assessing drug release from the dosage form, indicating the amount of drug available at

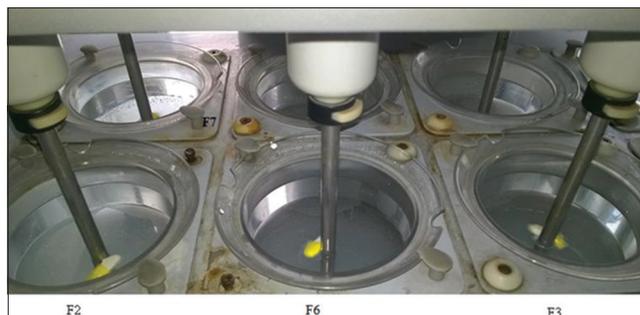


Figure 4: F2, F3, F6, and F7 remained buoyant in the 1st h

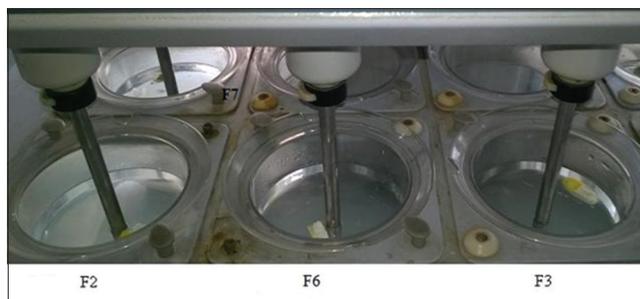


Figure 5: F2, F3, F6, and F7 starts to gel in the 2nd h

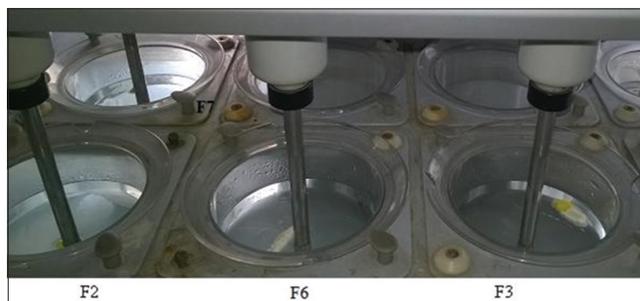


Figure 6: F7 is dissolved while F2, F3, and F6 are buoyant after 3 h

the site of absorption. The parameter was dependent on the formulation variables. Table 3 listed the values of various response parameters of the nine optimization formulations. The quadratic model for $t_{60\%}$ (Y_2) was found to be significant

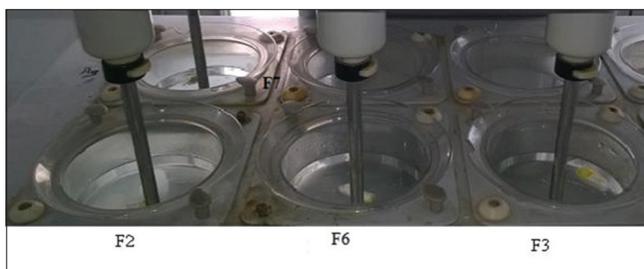


Figure 7: F2, F3, and F6 remained buoyant in the 4th h. Gel formed by F2 starts to loosen its strength, whereas F3 and F6 have firmed gel

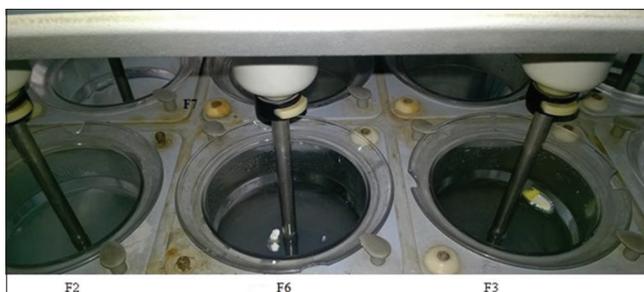


Figure 8: F2 is dissolved. Gel formed by F6 starts to loosen its strength while F3 is buoyant in 5th h

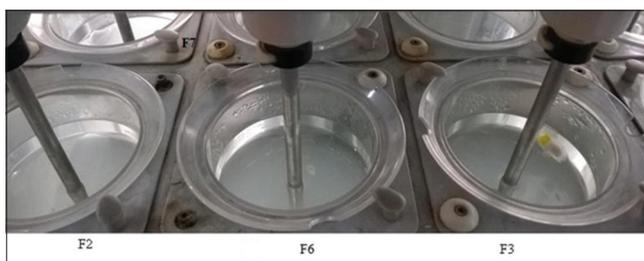


Figure 9: F6 is dissolved while F3 is buoyant in 6th h



Figure 10: Gel formed by F3 has firmed strength after 7 h of study

($P = 0.022$) with an F value of 16.13. Here also, the steeper ascent in the response surface with MMWC (B) than the LMWC (A) was clearly perceptible from both the plots, indicating that the effect of MMWC was comparatively more pronounced than that of LMWC. Consider the following:

$$Y_2 = 2.278 + 0.250A + 0.833B + 0.583A^2 + 1.833B^2 + 0.000A^2B \quad (6)$$

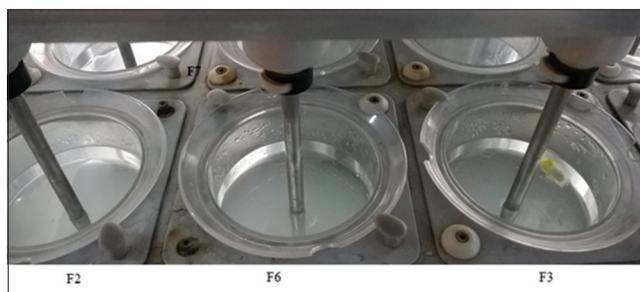


Figure 11: F3 was remained buoyant for up to 10 h

Table 4: Drug contents in various HBS formulations

Formulation code	Drug content (%)	% Drug release	Drug remaining in the gel matrix (%)
F2	98.23±1.31	72.44±1.14	27.56±2.42
F3	99.24±1.06	78.01±1.07	21.99±1.58
F6	98.12±1.63	57.12±1.99	35.92±2.89
F7	99.13±1.02	98.91±2.39	1.09±1.76

HBS: Hydrodynamically balanced system

The combined effect of factors A and B could further be elucidated with the help of surface response curve and contour plots [Figure 12a and b]. A clear effect was observed with increase concentration of MMWC at all levels of LMWC. An increase in the concentration of MMWC resulted into a decrease in drug release. The phenomenon behind this is explained in the succeeding paragraphs.

After considering the effect of variables (LMWC and MMWC) on the buoyancy and $t_{60\%}$, three formulations were optimized, i.e., F2, F3, and F6 from the surface response curve and contour plots [Figures 3a and b, 12a and b], which fits on the objective of the research. Hence, these formulations were characterized for *in vitro* release studies and to found the statistical significance these formulations were compared with the F7 (HPMC K4M only).

In vitro release studies were carried out in 0.1M HCl (pH 1.2), and it was observed that as the imbibition of the acidic dissolution medium into the capsule shell, formation of gel layer around the polymer matrix was initiated. Initially, drug particles located at the surface of the polymer matrix dissolved and released rapidly. Thereafter, it was expected that the drug release would retard as drug particles located at successively increasing distances from the surface of the polymer matrix will be dissolved and released through the gel layer. All formulations except F7 are buoyant for at least 5 h and are capable of sustaining the release of MN from HBS capsules even though the solubility of MN in water was very high.

Formulation F7, which contains only HPMC K4M releases 98.91% ± 2.39% of MN in 4 h. It may be attributed to the

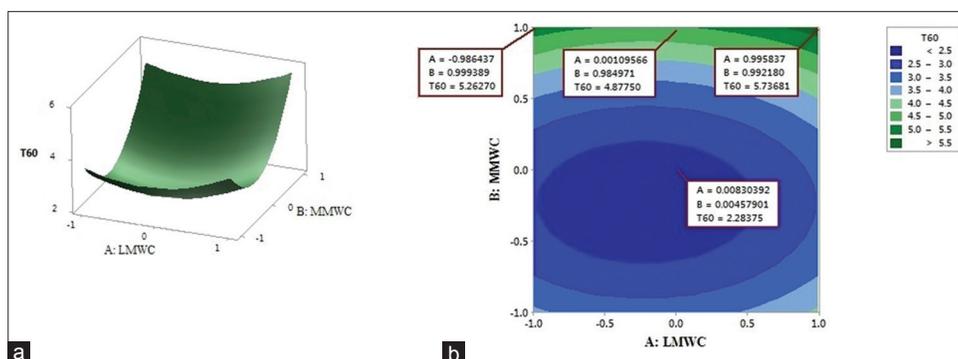


Figure 12: (a) Response surface plots showing the influence of medium molecular weight chitosan and low molecular weight chitosan on the $t_{60\%}$ (Y2). (b) Corresponding contour plot showing the relationship between various levels of the two factors (data mentioned in the rectangular boxes shows the desired effect)

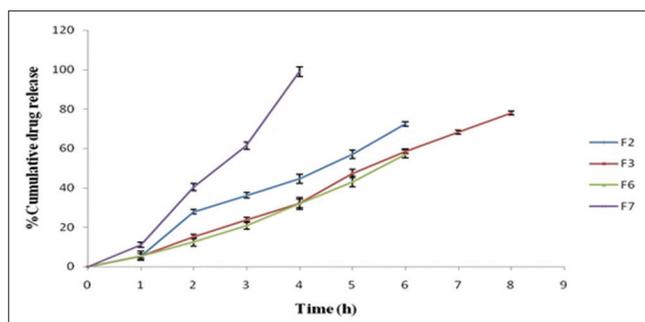


Figure 13: Percentage cumulative drug release from various formulations

Table 5: Cumulative percentage drug release

Time (h)	F2 (%)	F3 (%)	F6 (%)	F7 (%)
0	0	0	0	0
1	5.57±2.20	5.5±2.15	5.57±1.20	11.14±1.30
2	27.86±1.12	15.32±1.14	12.53±2.12	40.40±1.92
3	36.22±1.32	23.68±1.28	20.89±1.98	61.30±1.98
4	44.58±2.23	32.04±2.10	32.04±2.89	98.91±2.39
5	57.12±2.14	47.36±1.92	43.18±2.74	-
6	72.44±1.14	58.51±1.15	57.12±1.99	-
7	-	68.26±1.05	-	-
8	-	78.01±1.07	-	-

absorption of dissolution medium by the macromolecular chains of HPMC K4M strong enough which leads to the expansion of three-dimensional network and disentanglement of polymeric chains causes weakened density and strength of gel layer, resulting in rapid erosion and burst release of the drug. Table 5 and Figure 13 showed the % cumulative drug release.

However, in case of formulation F2, the polymer matrix was made up of HPMC K4M: MMWC (16.66:33.33) which got hydrated within 2 h and led to the formation of gel, releases 72.44% ± 1.14% of MN with less standard deviation, in 6 h (Table 5 and Figure 13 showed the % cumulative drug release).

While formulations F3 (HPMC K4M: LMWC:MMWC; 16.66:33.33:33.33) and F6 (HPMC K4M: LMWC:MMWC; 16.66:16.66:33.33) got hydrated within 1 h and tends to form firm gel enough to sustain the release (78.01% ± 1.07% and 57.12% ± 1.99%, respectively) of MN over the time period of 8 and 6 h, respectively. The early onset of gelation was attributed to the formation of hydrogen bond between HPMC K4M and chitosan; as the acidic dissolution medium penetrates deeper to the gel surface layer, the amino group in LMWC and MMWC got protonated and swells which resulted in increase of bulk volume and provides sufficient buoyancy, whereas due to high viscosity of MMWC the matrices become nonporous and as the dissolution proceeds the diffusion path length for the drug increases which sustained the overall release of MN. As evident from the drug release profile and *in vitro* buoyancy studies, F3 (HPMC K4M: LMWC:MMWC; 16.66:33.33:33.33) was considered as the optimized formulation since the gel formed was firm enough to sustain the release of drug MN.

SEM studies

The optimized formulation, i.e., F3 was subjected to SEM studies. Since F3 shows buoyancy for 10 h, therefore, SEM was done after 1 h and 10 h of dissolution. The resulting micrographs of the SEM studies were shown in Figure 14. These images were compared in respect of the morphological characters to speculate the mechanism of drug release and floating. From SEM studies, it was clear that after 1 h of dissolution, the surface showed some pores, cracks, and tortuosities which varied with the time of exposure of matrix to the dissolution medium. This change in surface is due to the erosion of the polymer. On the other hand, after 10 h, the solvent front reached to the center of the polymer matrix as the polymer coat was eroded. The micrographs at this time point showed a network in the swollen polymer matrix through which drug diffused to the surrounding dissolution medium. Thus, it was concluded that the drug release from the HPMC K4M and chitosan matrix might be due to the erosion of polymer followed by a diffusion mechanism.

Statistical analysis

The results of ANOVA for the dependent variables [Table 6] demonstrated that the model was significant for all response variables. The analysis of variance of the linear and quadratic regression models and a higher F value of the models reveal that the models are highly significant. For the better correlation between the observed and predicted values, the values of R^2 are need to be closer to 1. Here, the values of $R^2 = 0.975$ and 0.964 for responses Y_1 and Y_2 showed an excellent correlation between the experimental and predicted values. The P values are used as a tool to check the significance of each of the

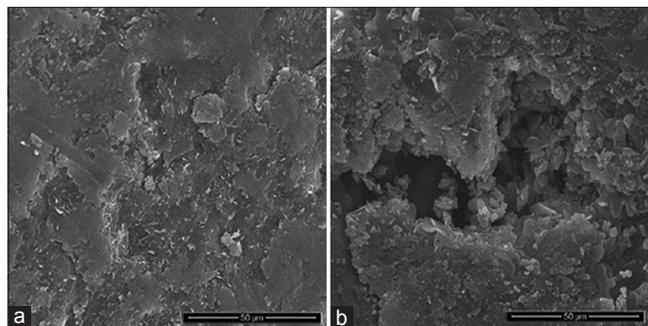


Figure 14: Scanning electron microscopy micrographs (at $\times 1000$) taken after (a) 1 h of dissolution, (b) 10 h of dissolution

Table 6: Results of ANOVA for measured responses

Parameter	SS	df	MS	F	Significance F
Buoyancy or floating time (Y_1)					
Model	23.8958	5	4.7792	23.73	0.013
Residual	0.6042	3	0.2014	-	-
Cor. total	24.5000	8	-	-	-
	$R^2=0.975$; adj: 0.934				
$t_{60\%}$ (Y_2)					
Model	11.9444	5	2.38889	16.13	0.022
Residual	0.4444	3	0.14815	-	-
Cor. total	12.3889	8	-	-	-
	$R^2=0.964$; adj: 0.904				

df: Degree of freedom, SS: Sum of square, MS: Mean square, F: Fischer's ratio, ANOVO: Analysis of variance

coefficients, which, in turn, are necessary to understand the pattern of the mutual interactions between the best variables. The smaller the magnitude of p , the more significant is the corresponding coefficient.^[33] The parameter estimates and the corresponding P values [Table 6] suggest that among the independent variables LMWC (A) and MMWC (B) have a significant effect on the dependent variables (responses). The quadratic term of these two variables also has a significant effect.

Mechanism of drug release

The ability of the polymeric blend to swell when placed in dissolution medium (here, it is 0.1 M HCl) is one of the most revealing characteristics of a gel. The swelled gel exhibits exchange of dissolved substances to and from the gel together with the dissolution medium. This property of forming gel is at the origin of the potential use of gel forming swellable polymeric blend as a drug carrier. When the HBS formulations come in contact with the acidic dissolution medium, there occurs the absorption of dissolution medium and subsequent swelling or hydration which tends to create three distinct regions inside the gel matrix that may generate three moving fronts: A swelling front, an erosion front, and a diffusion front. Therefore, a combination of swelling, erosion, and diffusion may form the basis through which gel controls the drug release.^[34] Table 7 represents drug release kinetics from the formulations. Zero order kinetics (coefficient of correlation, R^2 in the range of 0.971 to 0.991) seemed to be the most appropriate model describing that the release rate from the formulations is independent of the concentration of the drug. On the other hand, n values for formulations F2, F3, F6, and F7 (1.336, 1.266, 1.298, and 1.549, respectively) were found to be >1 , indicated the super case-II transport mechanism for MN release which is possibly owing to the swelling, chain disentanglement with erosions/spaces in the polymeric chains of the blended polymers.

Besides the R^2 values, the selection of a most appropriate model for drug release kinetics was based on Akaike Information Criterion (AIC). The AIC is a measure of the goodness of fit of a particular model based on the maximum likelihood.^[35] When comparing several models for a given set of data, the model associated with the smallest value AIC is regarded as the best fit out of that set of models. The AIC

Table 7: Drug release kinetics data derived from various mathematical models for HBS formulations consisting of MN

Formulation code	Drug release kinetics				
	Zero order	First order	Higuchi	Korsmeyer–Peppas	n
F2	0.983	0.947	0.890	0.933	1.336
F3	0.991	0.937	0.867	0.996	1.266
F6	0.978	0.932	0.822	0.977	1.298
F7	0.971	0.688	0.810	0.988	1.549

MN: Metronidazole, HBS: Hydrodynamically balanced system

Table 8: Comparison of AIC values for various dissolution kinetics models

Formulation code	AIC values			
	Zero order	First order	Higuchi	Korsmeyer–Peppas
F2	33.41	129.92	68.73	36.55
F3	39.35	157.50	89.58	49.01
F6	32.20	124.32	64.42	38.38
F7	29.96	101.42	51.82	34.46

AIC: Akaike information criterion

is only appropriate when comparing models using the same weighting scheme.

$$AIC = n \cdot \ln(WSSR) + 2 \cdot p \quad (7)$$

Where, n is the number of dissolution data points (M/t), p is the number of the parameters of the model, and $WSSR$ is the weighed sum of the square of residues. Table 8 depicts AIC values for various models calculated by KinetDS-3.0 software. AIC values reconfirmed that the all the formulations followed zero order kinetics.

CONCLUSION

In conclusion, we reported here the formulation of MN-containing HBS produced following the design of experiments (Minitab-17) and optimized with the help of RSM involving the factors as percentage of gel forming polymer (LMWC and MMWC) and response taken as buoyancy and release ($t_{60\%}$) of the drug from HBS. The formulation coded as F2, F3, and F6 were found to be optimized with desirable buoyancy and optimum drug release in the gastric environment. However, the formulation F3 fulfills on all the criteria of the study as it was remained buoyant for 10 h, and second, it is the only formulation which sustained the release of MN for more than 8 h. The constituents of the HBS preparation had already been used in internal dosage additives and thus safe. From these findings, we can suggest that the present formulated HBS capsule containing MN can be reproduced with high predictability and shall be useful to patients where the prolonged therapeutic action on infection sites caused by the microorganism is required.

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