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Developing, Optimization and In vitro Evaluating of Three-layers Floating Dipyridamole Film in Hard Gelatine Capsule

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ABSTRACT

Dipyridamole's bioavailability decreases with increasing gastric pH. To overcome this problem it was planned to prepare three-layered floating Dipyridamole films by floating enhancers and release controlling polymers for remain buoyant in the stomach. It focuses on the development and in vitro evaluating of the floating film in hard gelatine capsule through the design of experiment using different hydrophilic polymers, hydroxypropylmethylcellulose (HPMC) K4M and hydroxyethylcellulose (HEC). The amounts of HPMC K4M and HEC were independent variables, affecting film formulations found as 0.242 g and 0.337 g, respectively. The thickness of films, swelling index and percent of drug release in 4th hour were dependent variables found as 2.44±0.09 mm, 137.9%±1.11% and 67.07%±3.28%, respectively. In conclusion, the best fitted kinetic model was the Higuchi model where the drug release was controlled by diffusion, and optimized floating film formulation could be offered as a promising strategy to increase the bioavailability of Dipyridamole.

Keywords: Dipyridamole, Design Expert, Floating film, In vitro release, Swelling index

INTRODUCTION

Extended drug delivery systems are increasingly attracted due to reduction in dose frequency, decreasing of fluctuations in plasma concentration level and

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raising patient compliance. During the development of these dosage forms may get into the some difficulties such as the inability to complete absorption of drug in therapeutic diversity, and it is difficult to remain in the desired and adequate period in the stomach[1]. For this reason, there is an increased interest in the floating dosage forms[2] and gastroretentive dosage forms[3].

Floating systems are the part of the extended-release systems and extremely hopeful approaches if there is an unexpectable gastric emptying time problem and the active ingredient has low gastric residence time. Especially if the drug has low solubility and low bioavailability, floating systems may be a different way of new applications. Therefore, as the absorption of the drug increases the effectiveness of the dosage form increases, and it is possible to provide benefits targeting the specific region[4].

As gastric transition time is increased, fluctuations in plasma drug concentration levels can be controlled more effectively with these systems. As a result, good gastroretentive behavior can be achieved. And also, this makes it easier to obtain prolonged drug release period[5].

Dipyridamole is a BSC Class II weakly basic drug that is absorbed in the upper intestine and it has pH-dependent solubility. Its bioavailability decreases with increasing gastric pH. It is used for the treatment of angina pectoris because of preventing effect from the myocardial infarctions and thrombosis. This effect is provided by phosphodiesterase 5A inhibition so prevents aggregation and blocking the reuptake of adenosine via red blood cells. It also scavenges the free radicals that inactivate cyclooxygenase, leading to the inhibition of platelet activation and thrombin generation[6].

HPMC K4M is a bioadhesive material and used for coating agent, controlled-release agent, extended-release agent, film-forming agent, granulation aid, modified-release agent, mucoadhesive, release-modifying agent, solubilizing agent, stabilizing agent, suspending agent, sustained-release agent, tablet binder, thickening agent, viscosity-increasing agent. It is widely used in oral, ophthalmic, nasal, and topical pharmaceutical formulations[7].

HEC is a nonionic, water-soluble polymer used for coating agent, suspending agent, tablet binder, thickening agent, viscosity-increasing agent in pharmaceutical formulations[7].

Experimental researches need a long process, attention and self-sacrifice. They are carried out by experimental design methods recently. So researchers can minimize raw materials, labor and time consumption. That is why they can achieve correct results quickly with less raw materials and there is no need for

lots of energy or labor. One of the most preferred experimental design program Design Expert is a software for high accuracy data in scientific studies. It explains multifactor data and offers interpretation results. For the optimization, it brings out polynomial equations and investigates the response over the experimental data[8].

In this research paper, a new formulation of floating dipyridamole three-layers film was developed and evaluated. Floating systems or dynamically controlled systems are low-density systems and can float over the extended period of time due to remain on the surface of the stomach fluid without being affected by gastric emptying time. Thus, the fluctuations in plasma active substance concentration can better be controlled as it increases the residence time in the stomach.[5] For this reason, preparing the floating formulation of Dipyridamole will bring many advantages for its absorption. The more amount of drug remains in absorption region, the more amount of drug absorbed. An equal amount of active ingredient was added for each layer to fit the desired amount of active ingredient into the dosage form, that is why three-layered film was prepared. Two different polymers were used during the preparation. HPMC K4M (Hypromellose) was responsible for delaying release and, HEC (Cellosize HEC, Natrosol)[9] was responsible for floating in stomach because of its low density and structural integrity.

METHODOLOGY

Materials

Dipyridamole was kindly donated by Sanovel Pharmaceuticals, HPMC K4M and HEC were obtained from Ashland, glycerine and ethyl alcohol were purchased from Merck. All other chemicals and reagents used in this study were of analytical grade.

Methods

Preparation of floating films

The solvent casting method was used to prepare three-layers of floating film formulations[10]. HPMC K4M and HEC were used as a film-forming agent and glycerine was added to the formulation as a plasticizing agent. The amounts of HPMC K4M and HEC included in the formulation were obtained from the design expert software (Table 1).

For the preparation of the first layer of film formulation, half of the amount of HPMC K4M was12 weighed, and 3.5 mL distilled water was added on the polymer, after this the solution left to swell overnight by solvent casting. After that,

200 mg dipyridamole weighed and dissolved in 4.5 mL ethyl alcohol at 40°C in an ultrasonic bath (Bandelin Sonorex-Germany) and poured on to the solution followed by the addition of 1.2 mL glycerine, then the solution was allowed to dry at 37°C for 12 hours. For the second layer, the amount of HEC was weighed and 7 mL distilled water added to the polymer, and it left overnight. Then 200 mg dipyridamole was weighed and dissolved in 4.5 mL ethyl alcohol at 40°C in an ultrasonic bath and poured to the HEC followed by the addition of 1.2 mL glycerine. Then the solution poured to the first layer. This two-layered film was allowed to dry at 37°C for 12 hours. Then, the third layer has prepared with the same method as the first layer. The flow chart of this method is given in Fig. 1. After the drying of the third layer, one film has formed. This film then divided into four equal parts. Each part of them contains 150 mg dipyridamole.

Table 1. Experimental design matrix

Formula	Factor1 (HPMC K4M) mg	Factor2 (HEC) mg
F1	0.27	0.36
F2	0.14	0.36
F3	0.27	0.23
F4	0.36	0.27
F5	0.40	0.36
F6	0.36	0.45
F7	0.18	0.45
F8	0.27	0.49
F9	0.18	0.27
F10	0.27	0.36
F11	0.27	0.36
F12	0.27	0.36
F13	0.27	0.36

*Glycerine (1.2 mL), ethyl alcohol (4.5 mL) and distilled water (7 mL) were added in all formulations.

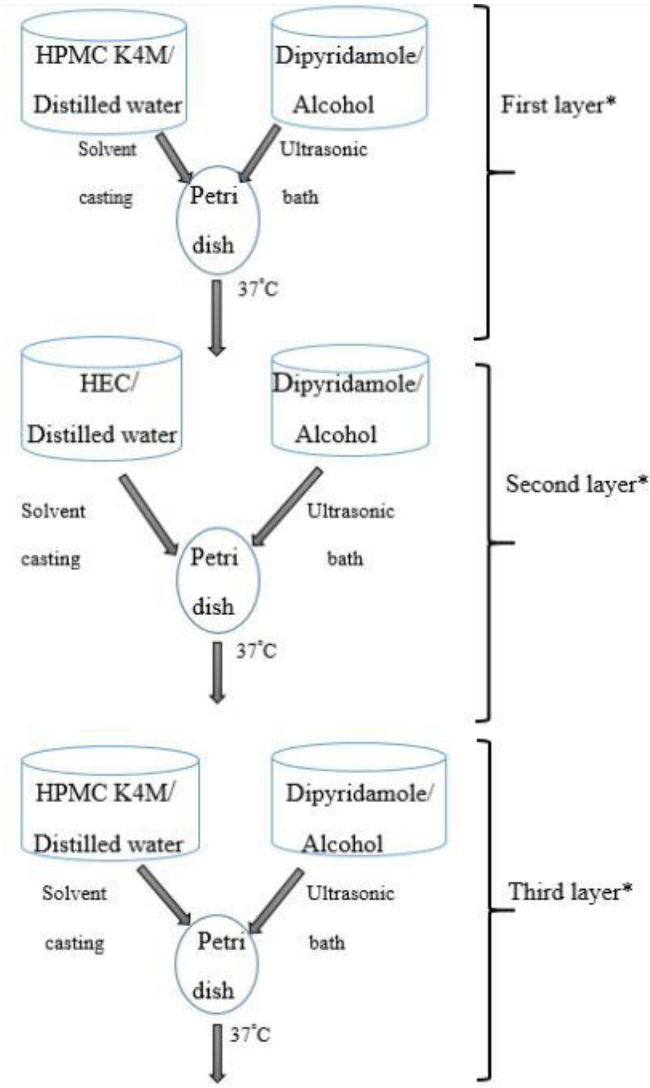

Figure 1. Flow chart of films to be prepared (*Each layer includes 1.2 mL glycerine)

Experimental Design

Film formulations were optimized by the response surface methodology (RSM). According to preliminary studies and literature review, X_1 (HPMC K4M) and X_2 (HEC) were selected as independent variables. X_1 was entered into the design expert software between 0.18 g and 0.36 g, while X_2 was entered between 0.27 g and 0.45 g. Plasticizer concentration was kept constant in all formulations. Independent variables were studied at 5 different levels (-α, -1, 0, +1, +α) using central composite design (CCD). To improve accuracy of the method, the α

value of 1.41 was determined (α value was chosen 1.41 to provide the the rotatability and orthogonality of the design) and total 13 experiments were carried out with 4 factorial points, 4 axial points and 5 replications of the central point (Table 2).

Table 2. Selected variables in central composite design

	Variables	Level of variables				
		-1.41	-1	0	1	1.41
A	HPMC K4M(mg)	0.14	0.18	0.27	0.36	0.40
B	HEC (mg)	0.23	0.27	0.36	0.45	0.49

Y1 (thickness), *Y2* (swelling index) and *Y3* (drug release) were chosen as dependent variables.

Design-Expert software was used for experiment design and statistical analysis. Response was predicted by the quadratic polynomial equation:

$$Y: \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$$

Where Y is predicted response, X_1 *and* X_2 independent variables, β_0 is the arithmetic mean response of the all runs, β_1 *and* β_2 are the predicted coefficients for the dependent factors X_1 *and* X_2, respectively. X_1X_2 displays how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are used to evaluate nonlinearity.

Analysis of variance (ANOVA) was applied to interpret the effect of independent variables on the dependent variables, and $p<0.05$ was considered to be statistically significant. Optimal responses were determined considering minimum thickness and maximum swelling index and in vitro release values. The optimized formulation was prepared in triplicate, and the obtained experimental results were compared to the predicted values.

Characterizations of floating films

Thickness of films

The thickness of films was measured at different points by using dial caliper (Japan). The average thickness, standard deviation and RSD% were calculated.

Swelling index of films

The swelling behavior of polymers was determined by the water uptake study. 3 cm x 5 cm x 3 cm sponge was soaked with 0.1 N HCl, and placed in a petri dish. Because the sponge is constantly wet, about 1 cm high 0.1 N HCl was added into the petri dish. A filter paper was placed on the upper part of the sponge. It is

made sure that the filter paper was completely wet with buffer. The setup was kept 15 minutes to stabilize before the experiment. Wet paper and the dry film were weighed separately, then it was placed on the setup. A glass fan closed to prevent the system airing. The initial mass of the setup is recorded. For the first six hours, the mass of the setup was recorded, with one-hour interval. The difference between the first weight and "t" time weight was figured out then water absorption capacity was calculated by Equation 1[11]. The experiment was repeated three times for each formulation and standard deviation values were determined.

Swelling index = $(W_t - W_o) / W_o$ x100 Equation 1

W_t = weight of film at t time

W_o = Initial weight

Drug content of films

Three-layer films put in 100 ml alcohol and stirred continuously using a mechanical stirrer (Kika Werke RT15-Germany) at the 40±2°C and the samples were withdrawn at the end of three hours and the drug content was determined spectrophotometrically at 283 nm. The experiment was carried out three times.

Mechanical properties of films

Mechanical properties of optimum film formulation (n=3) were performed using a texture analyzer (TA.XTPlus, Stable Micro Systems-UK) with a load cell of 5 kg. The film (1x4 cm) was placed between clamps at distance of 2 cm. Clamps were removed from each other with a constant crosshead speed of 0.5 mm/s until breakage of the films. The tensile strength and elongation at break (%) were calculated using the following Equations[12].

Tensile strength=Breaking force (N) / Cross-sectional area of film (cm^2) Equation 2

Elongation at break=Increase in length at breaking point (mm)/initial film length (mm) x 100 Equation 3

Dissolution of floating films

Dissolution test was performed in 900 mL 0.1 N HCl at 37°C±0.5. USP apparatus II (Sotax-Switzerland) was used, and the rotation speed was 50 rpm. At the predetermined time intervals samples were withdrawn, and replaced with the same amount of fresh buffer mediums[13]. Schematic illustration of the apparatus used for dissolution studies of films is depicted in Fig. 2. The absorbance of samples was analyzed with required dilutions spectrophotometrically at 283

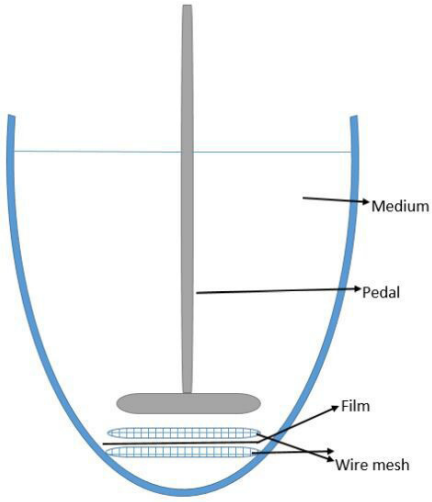

Figure 2. Schematic diagram of film and wire meshes in dissolution wessel

Kinetic models on drug release data

Drug release kinetics have a pivotal role in the field of drug delivery since they get profound vision into the mechanism from the dosage forms. For this reason, zero order, first order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell release kinetic models were examined to understand drug release kinetics. Kinetic models were evaluated by the coefficient of correlation (r^2). The highest degree of r^2 values determines the best appropriate kinetic model. All calculations were carried out according to the following kinetic equations.

Zero-order model: $Q_t=k_0t$ Equation 2

where Q is the amount of drug released at time t and k_0 is the zero-order release rate constant.

First-order model: $LogQ_t = LogQ_0 - k_1t$ Equation 3

where Q is the amount of drug release at time t and K is the first-order rate constant.

Higuchi-diffusion model

$Q_t = KHt^{1/2}$ Equation 4

where Q is the amount of drug released to the membrane (in mg) at time (t) in minutes. KH is the Higuchi square root of time release constant.

Korsmeyer-Peppas model $Ct/C\infty = Ktn$ Equation 5

where $Ct/C\infty$ is a fraction of drug released at time t, K is the release constant,

and n is the release exponent.

Hixson-Crowell diffusion release model $Q_0^{1/3} - Qt^{1/3} = K_{HC}t$ Equation 6

where Q t/Q is the amount of released drug at time t, K is the constant comprising the structural and geometric characteristics of the formulations, and n is the release of exponent[14].

In vitro buoyancy studies of films

The in vitro buoyancy includes floating lag time and total floating time[15]. In this study, disintegration time of capsules and time of rising to the surface of dissolution medium were floating lag time. The total floating time study was carried out at 37±0.5°C in filled 900 mL 0.1 N HCl dissolution wessel. The turbulence was created in it due to the pedals[10]. The floating behavior was observed. The floating film at different time intervals is depicted in Fig. 3.

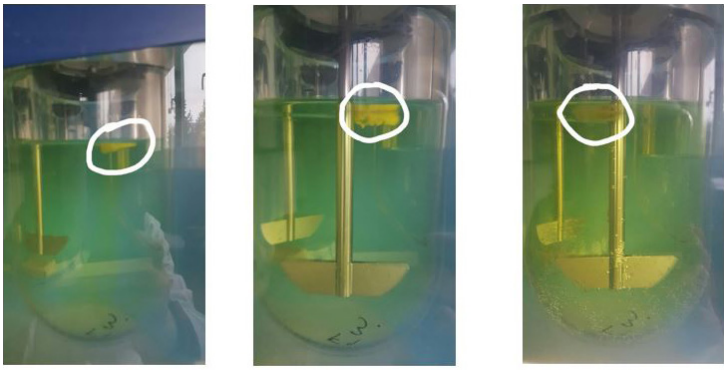

Figure 3. The floating film in 0.1 HCl (a) Initial time (b) 4th hour (c) 8th hour

Floating films into hard gelatine capsule

The prepared films were encapsulated for ease of oral use. For this purpose, the dried 6 cm diameter of film in which area is 28.26 cm² was removed from the petri dish and divided into 4 equal parts. One of them inserted into hard gelatine capsule (size 000-CAPSUGEL) as follows (Fig. 4). Each of them contains 150 mg Dipyridamole.

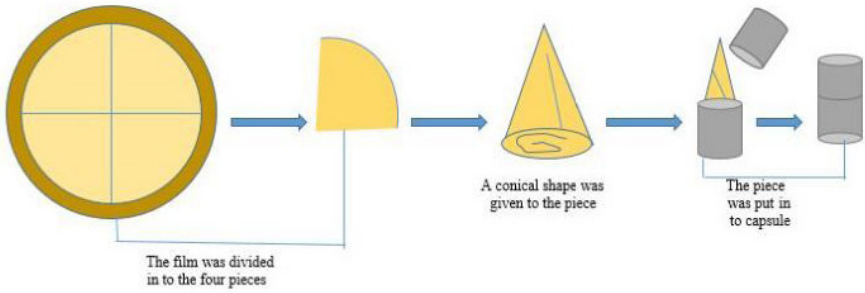

Figure 4. Schematic pattern of three-layers film into hard gelatine capsule

Test on hard gelatine capsules

Disintegration time of hard gelatine capsules

Disintegration time of floating film in hard gelatin capsules (n=6) was determined using disintegration apparatus (Sotax CH-4123, Switzerland). The beaker was filled with 0.1 N HCl. The temperature was maintained at 37° ±0.5°C. The disintegration times noted are the times at which the capsules ruptured, which assists minimize the uncertainty associated with determining the disintegration times based on "complete disintegration". Accordingly, these times are described as the times at which first visible cracks in the capsule shell appear.

Statistical Analysis

All the experiments were carried out in at least triplicate and the resulting data were presented as the mean±standard deviation (SD). Statistical analyses were performed using one-way ANOVA with Tukey's *post hoc* test. A difference with $p<0,05$ was considered to be statistically significant.

RESULTS and DISCUSSION

Results of test on floating film formulations

Influence of independent variables on thickness

As indicated in Table 3, the thickness of the film was found to be in the range from 1.90 to 3.26 mm. ANOVA results in Table 4 indicated that the amount of HPMC K4M and HEC amounts in the formulation were mainly affected (p value<.001) film thickness. When the 3D response surface graph in Fig. 7A is examined that thickness of film increases with increasing amounts of film forming polymers[16].

The following equation was used to predict the thickness of film:

$$Y1: 2.04 + 0.27 X_1 + 0.23 X_2 + 0.08 X_1 X_2 + 0.17 X_1^2 + 0.3 X_2^2$$

where *Y1* is thickness value, while X_1 and X_2 are the coded values of HPMC K4M and HEC, respectively. The model F value (17.47) and low P-value (0.001) implied the significance of the model equation as displayed in Table 4. The lack of fit of the model (P=0.103) was not significant.

Table 3. The results of in-vitro characterization study of film formulations

Formulation Code	Average thickness of films (mm)	Average drug content (%)	Swelling index (%)	In-vitro release for 4th hour (%)
F1	2.05±0.14	93.42±0.87	149.49±8.70	71.94±5.54
F2	2.16±0.24	97.06±2.35	97.05±1.81	74.40±6.65
F3	2.47±0.44	93.62±0.73	109.35±2.04	74.37±3.60
F4	2.45±0.47	92.58±1.69	89.62±7.63	72.02±5.27
F5	2.66±0.11	92.43±1.21	73.87±1.88	62.60±4.06
F6	3.26±0.54	97.37±0.68	115.21±3.50	65.53±0 .94
F7	2.39±0.12	91.26±1.11	79.15±2.90	73.12±3.08
F8	2.86±0.36	89.32±2.04	104.2±5.81	68.26±3.79
F9	1.90±0.44	89.96±1.15	109.31±1.57	67.93±5.54
F10	2.07±2.07	97.37±1.25	124.15±7.30	66.92±6.37
F11	2.08±2.08	92.37±0.50	134.45±6.70	69.44±0.73
F12	2.04±2.04	92.87±0.11	137.56±8.45	74.37±10.20
F13	1.98±1.98	96.45±2.18	141.71±5.65	74.09±2.90

Table 4. ANOVA Results for response *Y1*

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.78	5	0.36	17.47	0.0008	significant
A-X1	0.57	1	0.57	27.79	0.0012	
B-X2	0.43	1	0.43	21.05	0.0025	
AB	0.026	1	0.026	1.26	0.2991	
A2	0.21	1	0.21	10.3	0.0149	
B2	0.63	1	0.63	30.99	0.0008	
Residual	0.14	7	0.02			
Lack of Fit	0.14	3	0.045	29.71	0.1034	not significant
Pure Error	6.12E-03	4	1.53E-03			
Cor Total	1.92	12				

Influence of independent variables on swelling index

The swelling index was calculated by Equation 1 and the results were shown in Fig. 5. When the time increased it was also increased depended on the hydration. Generally, all formulations have good swelling character because of the polymers' natures. HPMC K4M and HEC are good gel-forming, highly swellable and matrix-forming agents. It was observed to have increased swelling index depending on time. It was found to be in the range from 73.87% to 149.49% (Table 3). ANOVA results in Table 5 indicated that the change in the amount of HPMC K4M ($p<0.001$) had more influence than the change in the amount of HEC (P:0.068) on the swelling index.

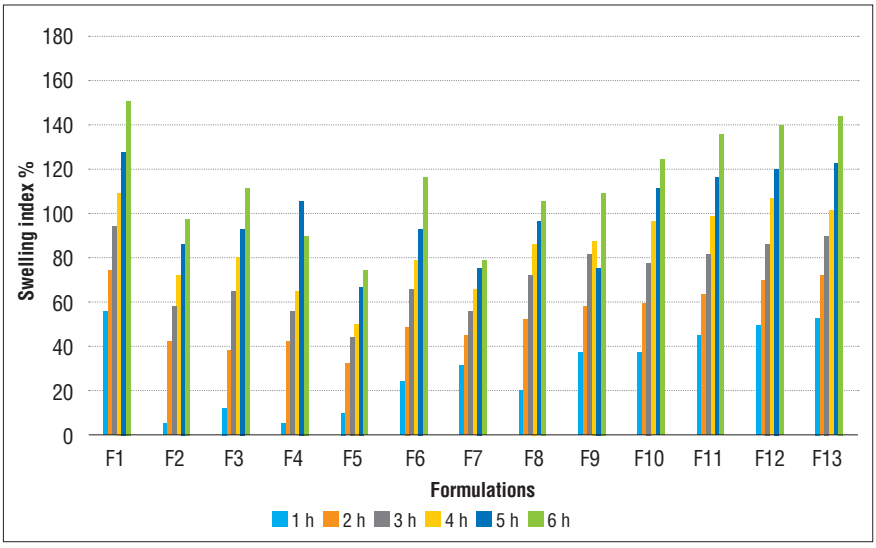

Figure 5. Swelling index cumulative column graph

The following equation was used to predict the swelling index:

$$Y2: 137.47 - 2.05\,X_1 - 1.48\,X_2 + 13.94\,X_1 X_2 - 25.45\,X_1^2 - 14.8\,X_2^2$$

Where $Y2$ is swelling index value, while X_1 and X_2 are the coded values of HPMC K4M and HEC, respectively. The model F value (13.26) and low P-value (0.001) implied the significance of the model equation as displayed in Table 5. Lack of fit of the model (P=0.418) was not significant.

Table 5. ANOVA Results for response *Y2*

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	6267.88	5	1253.58	13.26	0.0019	significant
A-X1	33.67	1	33.67	0.36	0.0003	
B-X2	17.56	1	17.56	0.19	0.0682	
AB	777.02	1	777.02	8.22	0.0241	
A2	4507.44	1	4507.44	47.69	0.0002	
B2	1523.19	1	1523.19	16.12	0.0051	
Residual	661.64	7	94.52			
Lack of Fit	312.63	3	104.21	1.19	0.4182	not significant
Pure Error	349.01	4	87.25			
Cor Total	6929.51	12				

It has been reported that HPMC matrices were effected more than Carbopol matrices on percentage swelling[17]. HPMC K4M has a positive effect on swelling index due to its hydrophilicity and swellability[18,19]. The hydroxypropyl groups in the HPMC K4M indicate more affinity to water molecules. This behavior can be observed clearly in Fig. 7B as an increase in HPMC K4M amount resulted in increased swelling index until a value near 0.27 g. The increase in the amount of HPMC K4M after this value led to a decrease in the swelling index possibly due to the presence of more physical entanglements between film-forming polymers[20-22].

Influence of independent variables on in vitro release profile

Since the drug release profiles of all formulations reached the plateau value in the first 4 hours (Fig. 6), the cumulative drug release values at 4th hour were chosen to evaluate the effect of the independent variables on the drug release profile.

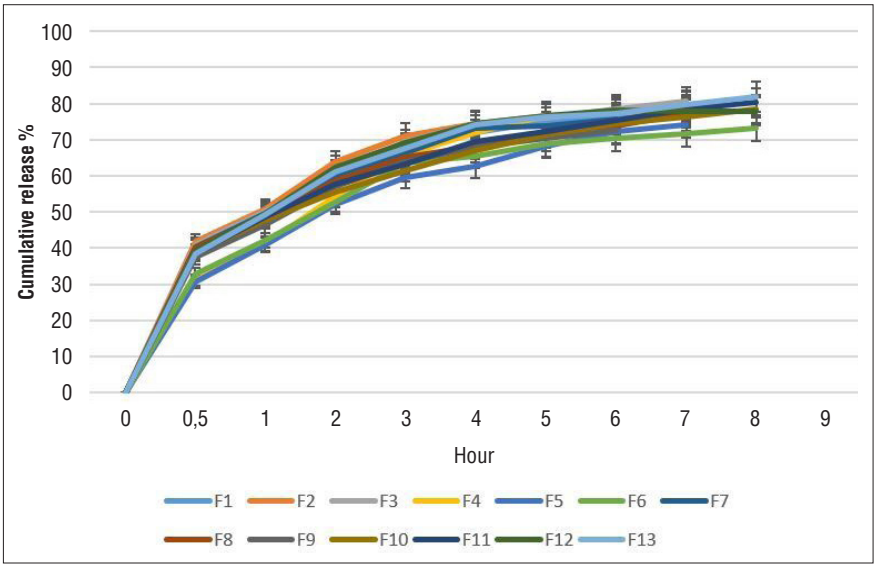

Figure 6. *In vitro* release profiles of formulations (n=6)

As indicated in Table 3, the in vitro drug release was found to be in the range from 62.6% to 74.4%. ANOVA results in Table 6 indicated that the change in the amount of HPMC K4M ($p<0.005$) had more influence than the change in the amount of HEC (P:0.299) on the drug release profile. When the 3D response surface graph in Fig. 7C is examined that the amount of drug released of the formulations decreases with increasing amounts of HPMC K4M[23,24].

The following equation was used to estimate the in vitro drug release:

$$Y3: 71.35 - 2.52\,X_1 - 1.24\,X_2 - 2.92\,X_1 X_2 - 1.49\,X_1^2 - 0.083\,X_2^2$$

where $Y3$ is in vitro release value, while X_1 and X_2 are the coded values of HPMC K4M and HEC, respectively. The model F value (12.3) and low P-value (0.001) implied the significance of the model equation as displayed in Table 6. Lack of fit of the model (P=0.497) was not significant.

Table 6. ANOVA results for response *Y3*

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	112.94	5	22.59	12.3	0.0013	significant
A-X1	50.94	1	50.94	5.18	0.0048	
B-X2	12.35	1	12.35	1.26	0.2994	
AB	34.11	1	34.11	3.47	0.1049	
A2	15.45	1	15.45	1.57	0.2503	
B2	0.048	1	0.048	4.86E-03	0.9464	
Residual	68.86	7	9.84			
Lack of Fit	28.61	3	9.54	0.95	0.4974	not significant
Pure Error	40.25	4	10.06			
Cor Total	181.8	12				

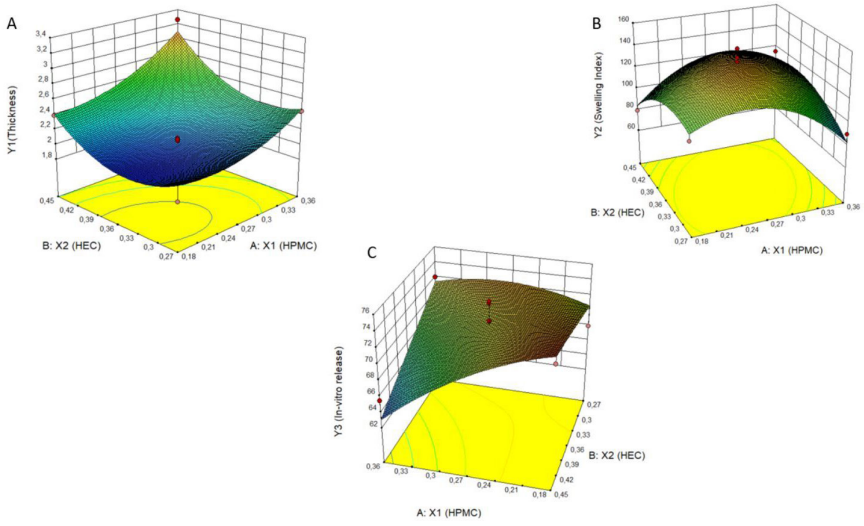

Figure 7. 3D response surface plots for (A) thickness, (B) swelling index, and (C) in vitro release as a function of HPMC and HEC amounts.

HPMC K4M has higher crosslinking degree, this hydrophilic matrix agent was supported the extended release period and floatation[25]. HPMC was used to make Alfuzosine floating beads, delaying release in a region-specific manner[26]. Gastroretentive dosage form was developed for cefuroxim axetil with HPMC and drug release was prolonged to 12 hours[27]. Domperidone floating tablet study showed that HPMC was effected on drug release rate and diffusion coefficient significantly[28]. Floating gastroretentive delivery system was prepared for fexofenadine

hydrochloride and HPMC which showed good floating properties in this study[29]. Also, HEC has good swelling properties and floating behavior[30].

The optimum floating film formulation prolonged drug release to 9 hours and its total floating time is 9 hours also. If the drug remains longer in the absorption region, the more drug is absorbed. Its percent drug release was found 85.78% in 9th hour. In this hour, buffer reaches the depths of matrices of rapidly hydrating polymers. When HPMC K4M contacted with buffer, it absorbs water which causes the polymer to swell. Due to this swelling, a viscous gel was formed around matrices. This matrice is resistant to water penetration. The drug which is inside matrice is released by diffusion due to this gel layer. The drug release is prolonged as the diffusion pathway increases, when the gel layer thickness increases[31].

Results of in vitro release kinetic studies

The results of release kinetic analyses of floating films are shown in Table 7. The release mechanism was calculated by finding the r^2 value for each model. The highest coefficient of correlation (r^2) value shows the most suitable kinetic model of drug release profile[32]. As seen from Table 7, drug release was found to be best fitted by Higuchi model for all formulations. It is pointed that the drug release happens from matrix as a square root of time-dependent process and diffusion controlled.

Polymeric systems commonly releases drug through Fickian diffusion. Atenolol floating sustained release matrix tablet was prepared that drug release kinetic was Higuchi and mechanism was diffusion. Furthermore, Metronidazole floating matrix tablet was developed which uses the same mechanism and has the same kinetic[33,34].

Table 7. Results of different kinetic models' r^2 values

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Model name	r^2												
Zero order	0.740	0.708	0.727	0.848	0.789	0.773	0.717	0.701	0.724	0.718	0.722	0.737	0.746
First order	0.893	0.873	0.891	0.964	0.908	0.893	0.875	0.858	0.867	0.872	0.877	0.898	0.904
Higuchi	0.940	0.922	0.931	0.987	0.964	0.956	0.926	0.916	0.931	0.923	0.928	0.939	0.943
Hixson–Crowell	0.847	0.823	0.842	0.934	0.872	0.857	0.827	0.809	0.823	0.824	0.830	0.850	0.857
Korsmeyer–Peppas	0.549	0.515	0.523	0.669	0.605	0.589	0.515	0.493	0.526	0.498	0.515	0.547	0.553
	rss*												
Zero order	18734.1	18646.3	18721.2	10432.6	14654.9	16438.8	18138.1	18723.3	17495.5	18123.9	17481.5	16187.4	15983.1
First order	7128.5	7274.1	7122.4	3945.5	4123.6	5809.5	6128.4	6165.4	6105.4	5993.6	5984.3	5913.5	5843.7
Higuchi	440.1	465.3	459.1	286.4	305.1	314.6	476.3	487.1	391.4	406.3	394.9	319.4	295.1
Hixson–Crowell	10165.1	11654.4	10879.4	8756.5	11870.9	12136.4	14781.9	19767.8	14436.9	14315.7	13917.4	13773.7	13175.1
Korsmeyer–Peppas	40583.5	48982.3	46447.3	38415.8	42715.8	46881.5	48912.5	51925.6	47176.4	53751.8	48691.8	41829.8	40198.4

*The values of the sum of squared residuals

Optimization study results

The optimum film formulation was determined by Design-Expert software based on the obtained results from CCD study. Desired limits were set considering the minimum thickness, maximum swelling index and in vitro dissolution values. After the statistical calculations performed by the software, the X1 and X2 quantities which are the critical parameters to be entered into the formulation were determined as 0.242 g and 0.337 g, respectively. The optimized formulation was prepared in triplicate to evaluate the model accuracy for the optimum conditions. As shown in Table 8 the experimental responses were found to be in close agreement with the predicted responses. The concordance between the results showed the importance and validity of the model.

Table 8. Predicted and experimental values of the optimized formulation (n=3)

Response	Predicted Value	Experimental Value	Prediction Error (%)
Thickness (mm)	2.39	2.44±0.09	2.09
Swelling Index (%)	135.91	137.9±1.11	1.46
In-vitro Release (%)	68.02	67.07±3.28	1.40

Drug Content of Floating Film Formulation

In order to determine the homogeneity of the Dipyridamole amount in different parts of the film, drug content determination studies were performed. The results achieved from the pieces taken from different parts of the film indicate that the Dipyridamole is highly recovered from the formulation (92,4%±0,92%) and has a homogeneous distribution in the film (Table 9).

Mechanical Properties of Floating Film Formulation

Tensile strength is one of the important properties for defining the mechanical performance of the material. As indicated in Table 9, the tensile strength value of optimum floating film formulation was found to be 2.65±0.162 N/cm^2. Elongation at break is the ratio between altered length and initial length after breakage of the film formulation. The elongation at break value of optimum floating film formulation was found to be 37.21%±0.875%. According to results obtained from the study of mechanical proporties, optimum three-layer of floating film formulation represents the capability of film formulation to maintain changes of shape without fracture formation.

Table 9. The results of in-vitro characterization study of optimum floating film formulations

Release kinetics and parametres		Optimum value
Korsmeyer–Peppas	r^2	**0.8203**
Higuchi		**0.9679**
First order		**0.9481**
Zero order		**0.8298**
Hixson–Crowell		**0.9174**
Average Thickness (mm)		**2.42±0.31**
Drug content (%)		**92.4±0.92**
Swelling index (%)		**137.9±1.11**
Tensile strength (N/cm^2)		**2.65±0.162**
Elongation at break (%)		**37.21±0.875**

In-vitro buoyancy studies

The in vitro buoyancy analysis was conducted at 37° ±0.5°C. The floating film at different time intervals is depicted in Fig.3. Optimum film formulation in hard gelatin capsules showed sufficient buoyancy time of 9 hours of, as desired.

Results of films in hard gelatine capsules

Disintegration time

Disintegration time is a significant method to determined the quality of the oral dosage forms. It is a process of the oral dosage whereby disintegrates into smaller particles before dissolution happens. During the in vitro disintegration test, the rupture of the capsules was visually evaluated. Their disintegration time was observed less than 4.5 minutes of all formulations. It is easier to disintegrated because gelatin is a globular protein and low molecular weight.

In this research, three-layers of Dipyridamole floating film formulations were optimized with an experimental study using RSM. The floating films were produced using solvent casting method. The effect of the interaction of two independent variables (the amounts of HPMC K4M and HEC) on respective dependent variables (the thickness of films, the swelling character of polymers and the percent of *in-vitro* release in the 4th hour) were investigated. It was observed that experimental results and predicted values showed similar results. Polymers showed good swelling characteristic in the 6th hour. HPMC K4M is more effective on swelling than HEC because of its polymeric features (independence from the medium' pH and its chains disentangled from the matrix through hydration). If the amount of polymer in floating film increases, the thickness of the film increases. The drug release rate slows with increasing amount of polymer in formulations. The release was fitted to Higuchi kinetic model for each formulations. Optimum formulation was obtained in 9 hours of buoyancy time and prolonged release in 0.1 N HCl. The film was cut four quarter-circle then one of them with a conical shaped inserted into a hard gelatine capsule. This provided us with ease of oral use. In conclusion, floating film in a hard gelatine capsule is an innovative approach for remain buoyant in the stomach. With this approach it is possible to overcome the bioavailability problem in high pH values of the Dipyridamole.

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