Prolonged release matrix tablet of pyridostigmine bromide: Formulation and optimization using statistical methods

Noushin Bolourchian[1,2], Maryam Rangchian[1] and Seyed Mohsen Foroutan[1,2]
[1]School of Pharmacy, [2]Pharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract: The aim of this study was to design and optimize a prolonged release matrix formulation of pyridostigmine bromide, an effective drug in myasthenia gravis and poisoning with nerve gas, using hydrophilic - hydrophobic polymers via D-optimal experimental design. HPMC and carnauba wax as retarding agents as well as tricalcium phosphate were used in matrix formulation and considered as independent variables. Tablets were prepared by wet granulation technique and the percentage of drug released at 1 (Y_1), 4 (Y_2) and 8 (Y_3) hours were considered as dependent variables (responses) in this investigation. These experimental responses were best fitted for the cubic, cubic and linear models, respectively. The optimal formulation obtained in this study, consisted of 12.8 % HPMC, 24.4 % carnauba wax and 26.7 % tricalcium phosphate, had a suitable prolonged release behavior followed by Higuchi model in which observed and predicted values were very close. The study revealed that D-optimal design could facilitate the optimization of prolonged release matrix tablet containing pyridostigmine bromide. Accelerated stability studies confirmed that the optimized formulation remains unchanged after exposing in stability conditions for six months.

Keywords: Pyridostigmine bromide, matrix tablet, D-optimal design, optimization.

INTRODUCTION

Pyridostigmine bromide, a quaternary ammonium compound, inhibits cholinesterase activity reversibly and is mainly used in the treatment of myasthenia gravis and paralytic ileuses (Sweetman, 2007). Also, it could be useful as a prophylaxis agent against the nerve gas poisoning (Kluwe *et al.*, 1990; Sweetman, 2007). It is a water soluble deliquescent compound (Moffat *et al.*, 2004) with a short elimination half-life (about 3.7 hours) after oral use (Hardman and Limbrid, 1995) which makes the frequent dosing necessary. Therefore, prolonged release dosage form of this drug could reduce incidences of adverse drug reactions and be useful in the improvement of the patient compliance.

Drug-embedded matrix tablet containing a mixture of drug, retarding polymer and other additives is considered as one of the methods for delivering the drug into the systemic circulation in a prolonged manner (Reza *et al.*, 2003). Both hydrophilic and hydrophobic polymers have been used as retarding agents in the preparation of matrix tablet (Maswadeh *et al.*, 2006).

HPMC as a hydrophilic pH-independent polymer has been extensively used in the preparation of oral controlled release delivery systems (Barakat *et al*., 2009; Petrovic *et al*., 2009; Basak *et al*., 2010). The gel layer formed by this polymer in contact with biological fluids can retard the release of drug. However rapid diffusion of dissolved highly water soluble drug through this hydrophilic gel network restricts using of hydrophilic matrix alone for extending the drug released. Therefore including hydrophobic polymer in matrix system for water soluble drugs is suggested (Kuksal *et al*., 2006). A number of studies have been carried out regarding the use of binary mixtures of hydrophilic and hydrophobic polymers (Boyapally *et al*., 2009; Chakraborty *et al*., 2009). Studies showed that a combination of HPMC and hydrophobic waxy agents resulted in desirable sustained drug release profiles (Hayashi *et al.*, 2005). Therefore in this study a combination of HPMC and carnauba wax was used to prepare the highly water soluble drug, pyridostigmine bromide, prolonged release matrix tablet. Huang *et al.* (2007) used extrusion-spheronization and fluid-bed technique to prepare coated HPMC-pellets of this drug as sustained release formulation. In the present study, the wet granulation technique as a simpler method was used to prepare the prolonged release system of pyridostigmine bromide.

Designing an optimal formulation with suitable drug release behavior in a short period of time is an essential issue in the formulation of prolonged release systems. For this purpose, D-optimal mixture design was used to optimize the formulation variables, which is considered as a useful approach in the development as well as optimization of various drug delivery systems. In a mixture experiment, the components of a mixture are considered as independent factors and the response is assumed to depend on the proportions of the ingredients. Due to simultaneous study of the effect of different variables on the responses and possible inter-relationship among them, experimental design allows obtaining highest information with the smaller number of experiments (Furlanetto *et al*., 2006).

**Corresponding author:* e-mail: bolourchian@sbmu.ac.ir

The aim of the present study was to develop and optimize the formulation variables of pyridostigmine bromide prolonged release matrix tablet prepared by hydrophilic and hydrophobic polymers using D-optimal mixture design and to evaluate the influence of different mixture compositions on dependent variables (responses).

MATERIALS AND METHODS

Materials

The active ingredient, pyridostigmine bromide, was purchased from Alborz Darou Co. (Iran). The other materials were as follows: polyvinylpyrrolidone (PVP10) and carnauba wax (Sigma Aldrich, Germany), HPMC K4M (Colorcon, UK), tricalcium phosphate, magnesium stearate, colloidal silicon dioxide, phosphoric acid and triethyl amine (Merck, Germany), sodium 1-octane sulphonic acid (HPLC grade) (Acros organics, Belgium).

Tablet preparation

After choosing formulation ingredients and method of tablet preparation during preliminary studies, tablet matrices containing pyridostigmine bromide were made by the method of wet granulation. In the first step the drug was homogeneously blended with adequate amount of carnauba wax (hydrophobic retarding agent), HPMC (hydrophilic polymer) and tricalcium phosphate (filler) after passing through the no. 35 (500 µm) screen sieve. Pyridostigmine bromide is a deliquescent compound and incorporation of amorphous hygroscopic excipients (such as tricalcium phosphate) in tablet formulation seems to be useful in inhibition of active ingredient hydration and therefore better stability of the formulation (Airaksinen, 2005). The obtained mixture was then granulated using alcoholic solution of PVP (10 % w/v), passed through no. 14 mesh screen and dried at 40°C. Colloidal silicon dioxide and magnesium stearate were added to the prepared granules and mixed for 3 min prior to compression by a single punch tablet press machine (Erweka AR 4100, Germany) having 12 mm flat-faced punch and die set.

Experimental design

In the present study, statistical experimental design was used in order to get more information about the effect of formulation components on drug release and to obtain the optimum formulation through minimum time and expenses. Considering properties of study and related design space, mixture D-optimal model was selected with three independent variables including the percentage of HPMC (X_1), carnauba wax (X_2) and tricalcium phosphate (X_3). It must be considered that in a mixture design modification of the amount of each component can not be done independently (Jin, 2008) and the proportions of the components (independent factors) must sum to 100% (Mura *et al*., 2005). In the present study, fixed drug content (30 %), magnesium stearate (1 %), silicon dioxide (1%) and PVP (4%) were used in tablets preparation. Therefore, the experimental range lay between 0 and 64 % (w/w). Table 1 shows the experimental ranges and constraints for independent variables (mixture components) based on the preliminary experiments as well as the applicable percents of the components in oral pharmaceutical formulations.

Table 1: Experimental ranges for independent variables and constraints

Independent variable	Constraint (%)
X_1 = HPMC	0-35
X_2 = Carnauba wax	0-50
X_3 = Tricalcium Phosphate	14-59
$X_1 + X_2$	5-50
$X_1 + X_2 + X_3$	64

To identify the release pattern and ensure complete drug release, the percentage of drug released at 1 (Y_1), 4 (Y_2) and 8 (Y_3) hours were considered as responses (dependent variables). Table 2 shows dependent variables and their acceptable ranges which were determined according to the data achieved from commercial Mestinon® retard tablet. Based on the performed design, 22 formulations (runs) including 4 replicate formulations (table 3) were randomly arranged by Design-Expert® software (version 7, Stat-Ease Inc., USA).

Table 2: Dependent variables and the constraints applied on responses

Response	Constraint (%)
Y_1 (Drug released at 1 hr)	43-47
Y_2 (Drug released at 4 hr)	73-81
Y_3 (Drug released at 8 hr)	85-94

In Vitro drug release studies

The release rate of pyridostigmine bromide from all tablets was determined using USP dissolution testing apparatus II, paddle method, (Erweka DT 6R, Germany) at 50 rpm. The dissolution medium consisted of 900 ml phosphate buffer solution (pH 7.2), at 37 ± 0.5°C. Aliquots (5 ml) were withdrawn from the vessels at specific time intervals and replaced with same amount of fresh medium. Withdrawal samples were analyzed using UV spectrophotometer (CECIL CE2021, UK) at 270 nm for pyridostigmine bromide content. The average of three determinations was calculated and mean cumulative percentage of drug released was plotted against time of release. In order to simulate the *in vivo* conditions, the optimum formulation was also tested under continuous dissolution method considering 1 hour in 0.1 N HCl and the rest of experiment carried out in phosphate buffer solution (pH 7.2). The method of study was the same as explained above.

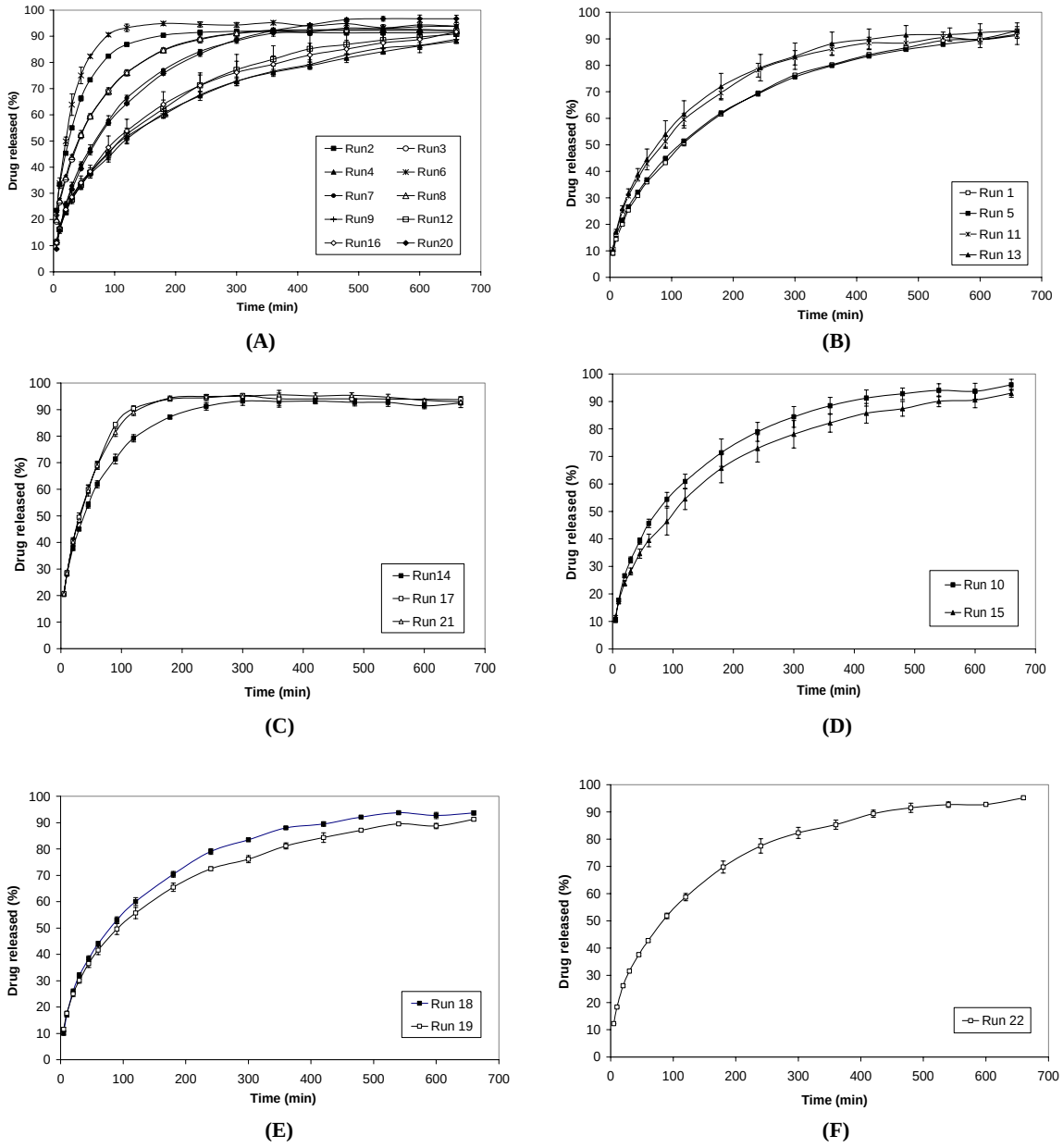


Fig. 1: Release profiles of the design formulations: (A): Vertex points, (B): Cent edge points, (C): Third edge points, (D): Trip blend points, (E): Axial CB points, (F): Center points (n=3)

Drug assay and uniformity of dosage unit

Twenty drug containing random tablets of optimal formulation were powdered, dissolved in phosphate buffer solution (pH=7), and assayed by HPLC (Merck Hitachi, Germany) for drug content after centrifugation. The column was L1 (RP18) and the mobile phase was prepared by sodium 1-octan sulphonic acid, triethylamine and acetonitrile using phosphoric acid for pH adjustment at 3 (USP, 2008), with the flow rate of 2 mL min^{-1}. UV detector at 270 nm was used to detect the drug. All assays were performed in triplicate and at room temperature.

Based on USP guideline, 10 tablets of the optimized formulation were weighted accurately and tested individually for uniformity of dosage unit as above. The acceptance value (AV) was calculated based on the US Pharmacopoeia 31 (USP, 2008).

Tablets physical properties evaluation

Tablets hardness (n=6) of optimized formulation was measured using Erweka TBH 28 instrument (Germany). The friability test (Erweka TA 63974, Germany) was conducted on 11 tablets according to the USP 31 (2008).

Table 3: Experimental plan for D-optimal design and results

Run	Variable factor (%)			Response (%)		
	HPMC (X_1)	Carnauba (X_2)	Tricalcium Ph. (X_3)	Y_1	Y_2	Y_3
1	20.00	0.00	44.00	35.96	69.55	86.59
2	0.00	5.00	59.00	73.36	91.49	91.27
3	0.00	50.00	14.00	59.23	88.77	92.19
4	35.00	15.00	14.00	38.17	67.61	81.68
5	20.00	0.00	44.00	36.82	69.22	85.95
6	0.00	5.00	59.00	82.35	94.55	94.81
7	5.00	0.00	59.00	46.91	84.17	93.1
8	0.00	50.00	14.00	59.48	89.04	92.54
9	35.00	0.00	29.00	37.28	67.24	82.93
10	11.67	23.33	29.00	45.65	78.96	92.79
11	17.50	32.50	14.00	42.83	78.17	88.4
12	35.00	0.00	29.00	37.81	71.23	86.86
13	17.50	32.50	14.00	44.5	79.13	91.43
14	0.00	35.00	29.00	61.88	91.24	92.7
15	25.00	5.00	34.00	39.45	72.92	87.34
16	35.00	15.00	14.00	38.32	71.16	85.11
17	0.00	20.00	44.00	69.13	94.48	94.07
18	7.50	9.50	47.00	44	79.05	92.13
19	25.00	14.50	24.50	41.6	72.5	87.1
20	5.00	0.00	59.00	45.92	83.28	96.38
21	0.00	20.00	44.00	68.87	94.93	95.37
22	15.00	14.00	35.00	42.71	77.49	91.51

X_1: HPMC, X_2: carnauba wax, X_3: tricalcium phosphate

Optimized formulation was also tested for the moisture content (n=9) using Karl Fischer method (Mettler DL 77 titrator KVDV 705, Switzerland).

Kinetics evaluation

The release data obtained for the optimum formulation was fitted to zero order ($Q_t=k_0.t$) (Najib and Suleiman, 1985), first order ($\ln (100-Q_t) = \ln 100-k_1.t$) (Desai *et al.*, 1966) and Higuchi model ($Q_t=k_H.\sqrt{t}$) (Higuchi, 1963) to ascertain the release kinetics.

Furthermore, in order to characterize the release mechanism, the Korsmeyer-Peppas semi-empirical model ($Q_t=k_{KP}.t^n$) was applied (Korsmeyer *et al.*, 1983).

Stability studies

In order to carry out the accelerated stability studies, optimal formulation tablets were kept at 45°C and 75% relative humidity (RH) for 6 months after packing in suitable primary packaging. Various tablet properties including hardness, friability, drug assay, uniformity of dosage unit, moisture content as well as drug release were analyzed at the end of each month.

Similarity factor (f_2) was also calculated to compare the release data of the optimized formulation after conducting the stability study using the following equation (Yuksel *et al*., 2000):

$$f_2 = 50 \log \{[1+ (1/n) \sum_{n-1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\}$$

In the above equation n is the number of time points, R_t and T_t are the mean percentage of drug dissolved in the reference and test formulations, respectively.

RESULTS

The concentration of mixture components used to prepare the 22 formulations (runs) and the relevant responses were depicted in table 3. Fig. 1 shows the release profiles of pyridostigmine bromide from designed formulations.

Different models were studied to describe the relationship between independent variables and responses. Statistical parameters related to the selection of appropriate models are depicted in table 4.

Figs. 2a-c show the response surface plots predicted from the cubic model for Y_1 and Y_2 and linear model for Y_3. In these graphs, the response is shown as the function of X_1: HPMC, X_2: carnauba wax and X_3: tricalcium phosphate. The results of analysis of variance (ANOVA) of dependent variables are presented in table 5.

DISCUSSION

According to the drug release profiles, presented in fig. 1, drug release from matrices containing no HPMC was very fast, but inclusion of HPMC in the formulation slowed down the release rate. It means that HPMC has a major role in retarding the drug release from matrices compare to the hydrophobic carnauba wax.

With regard to the results of data analysis, cubic, cubic and linear models were accepted for description the relationship between independent variables and Y_1, Y_2 and Y_3 as dependent variables, respectively (table 4). Smaller PRESS (predicted residual sum of square) obtained for the chosen model comparing to the other considered models, indicates how well the selected model fits the data (Huang *et al.*, 2005).

Based on the statistical analysis (ANOVA), the chosen model was fitted to the data for the responses Y_2 (drug released at 4 hours) and Y_3 (drug released at 8 hours) ($p < 0.05$). On the other hand, according to the Box-Cox plot and for more precise analysis, a transformation was needed for the response data of the drug released at 1 hour (Y_1) and therefore $Y_1^{-2.09}$ was used in the cubic model.

The mathematical models generated for the responses Y_1, Y_2 and Y_3 are as follows:

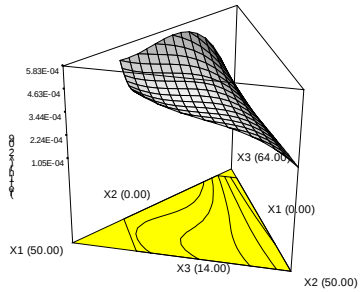
$$Y_1^{-2.09} = (8.459E\text{-}004)\,X_1 + (1.970E\text{-}004)\,X_2 + (9.796E\text{-}005)\,X_3 - (4.485E\text{-}004)\,X_1X_2 + (2.368E\text{-}004)\,X_1X_3 + (3.932E\text{-}005)\,X_2X_3 + (7.043E\text{-}004)\,X_1X_2X_3 - (7.559E\text{-}004)\,X_1X_2(X_1\text{-}X_2) - (1.905E\text{-}003)\,X_1X_3(X_1\text{-}X_3)$$

$$Y_2 = 60.25\,X_1 + 88.93\,X_2 + 94.88\,X_3 - 35.34\,X_1X_3 + 71.20\,X_1X_3(X_1\text{-}X_3)$$

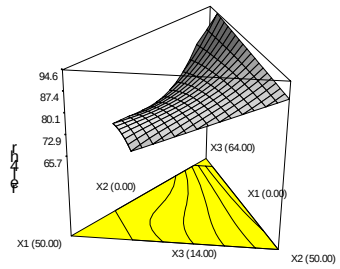
$$Y_3 = 80.11\,X_1 + 93.43\,X_2 + 94.46\,X_3$$

Referring to the ANOVA table (table 5) $X_1X_2(X_1\text{-}X_2)$ and $X_1X_3(X_1\text{-}X_3)$ were significant terms in case of Y_1 ($p < 0.05$) which means that in various HPMC concentrations, different optimum points for carnauba wax and tricalcium phosphate could be achieved. The mathematical equation obtained for Y_2 shows that the interaction between HPMC and tricalcium phosphate (X_1X_3) has a negative effect on the dependent variable. The results also confirmed that all three independent variables have linear and positive relationship with Y_3. According to the last equation, it seems that increasing the amount of HPMC, resulted in higher drug released at 8 hours. But it must be considered that in the mixture design, application of higher amount of one component is along with using lower percentages of the other components (carnauba wax and tricalcium phosphate) which have higher value of coefficients in the equation than HPMC and therefore have more influence on Y_3. It must be considered that drug release from tablets at the late hours of dissolution study, depends more on the percentage of drug remained intact in matrix structure compared to the first hours of the release study.

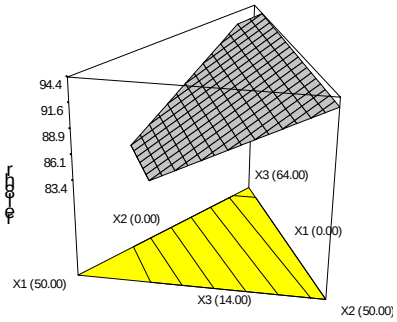
Based on fig. 2a-c, application of higher percentage of HPMC in tablet formulation may result in lower drug release at 1, 4 and 8 hours which was predictable due to the retarding effect of this polymer. In addition, using higher concentration of carnauba wax with lower amount of HPMC seems to have a little effect in sustaining the release of pyridostigmine bromide from matrices.



(A)



(B)



(C)

Fig. 2: 3D response surface plots: A) drug released at 1 hour, B) drug released at 4 hours, C) drug released at 8 hours, responses are shown as the function of X_1: HPMC, X_2: carnauba wax and X_3: tricalcium phosphate with respect to the total of 64%.

Table 4: Fit summary data for selected models

Dependent variable	Selected model	R^2	Adjusted R^2	Predicted R^2	PRESS [a]	Prob>F
Y_1	Cubic	0.996	0.993	0.986	6.479 E-009	<0.0001
Y_2	Cubic	0.980	0.965	0.928	136.92	0.0163
Y_3	Linear	0.808	0.788	0.739	92.37	<0.0001

[a]: Predicted residual sum of squares

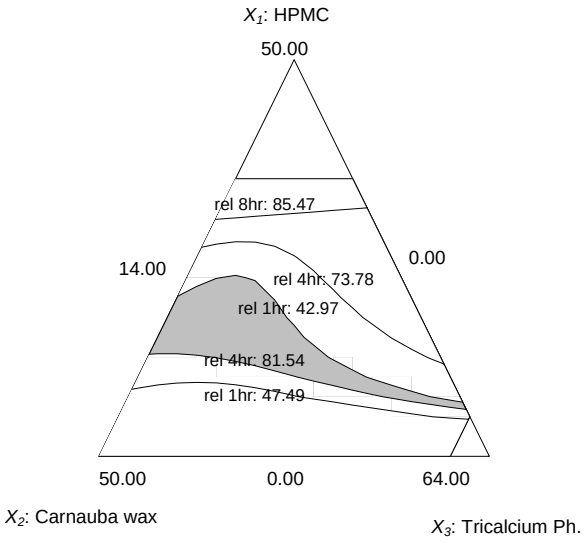


Fig. 3: The overlay plot showing the desired area of all three responses containing optimum formulations in gray; X_1: HPMC, X_2: carnauba wax and X_3: tricalcium phosphate

Table 5: Analysis of variance (ANOVA) of dependent variables

Source of variation	Sum of squares	Degree of freedom	Mean square	F-ratio	p-value
Y_1					
X_1X_2 [a]	9.31E-010	1	9.31E-010	6048	0.0244
X_1X_3	2.86E-010	1	2.86E-010	1.99	0.1814
X_2X_3	1.06E-010	1	1.06E-010	0.74	0.4049
$X_1X_2X_3$	3.75E-010	1	3.75E-010	2.61	0.1299
$X_1X_2(X_1-X_2)$	2.14E-009	1	2.14E-009	14.93	0.0020
$X_1X_3(X_1-X_3)$	1.48E-008	1	1.48E-008	103.25	<0.0001
Residual	1.87E-009	13	1.44E-010		
Lack of Fit	4.58E-010	5	9.15E-011	0.52	0.7559
Y_2					
X_1X_3	107.79	1	107.79	35.59	<0.0001
$X_1X_3(X_1-X_3)$	116.70	1	116.70	38.54	<0.0001
Residual	51.48	17	3.03		
Lack of Fit	31.49	9	3.50	1.40	0.3230
Y_3					
Residual	68.06	19	3.58		
Lack of Fit	37.11	11	3.37	0.87	0.5943

[a] X_1: HPMC, X_2: carnauba wax, X_3: tricalcium phosphate

Table 6: Validation step: optimized levels for independent variables and comparative values of predicted and observed responses for numerically optimized formulations

Formulation	Variable factor [a]			Response (%)								
				Y_1			Y_2			Y_3		
	X_1	X_2	X_3	Pred [b]	Obs[c, d]	PE[e]	Pred	Obs	PE	Pred	Obs	PE
O_1	13.90	23.70	26.40	44.83	45.49 ±3.23	1.47	78.58	80.90 ±4.83	2.95	89.98	93.96 ±2.22	4.42
O_2	13.32	23.32	27.36	44.93	46.16 ±2.43	2.74	78.79	83.33 ±3.15	5.76	90.16	95.57 ±1.91	6.00
O_3	12.83	24.43	26.74	45.24	45.12 ±1.92	-0.27	79.23	80.44 ±2.45	1.53	90.27	93.44 ±1.66	3.51
O_4	12.64	23.21	28.16	45.11	46.91 ±0.84	3.99	79.13	83.46 ±3.44	5.47	90.35	95.41 ±1.72	5.60

[a] X_1: HPMC, X_2: carnauba wax, X_3: tricalcium phosphate
[b] Predicted values; [c] Observed values; [d] Mean ± SD; [e] Predicted error

Table 7: Physicochemical characteristics of the optimized formulation (O_3) after conducting accelerated stability studies (45°C, 75 % RH)

Dependent variable	Time (month)				
	0	1	2	3	6
Y_1 (%) (n=6)	47.57±3.01 [b]	44.83±2.09	42.29±2.17	42.78±1.60	44.93±1.52
Y_2 (%) (n=6)	80.86±3.31	76.88±3.05	74.95±2.48	77.67±4.40	80.50±3.52
Y_3 (%) (n=6)	91.94±2.83	91.76±1.59	89.36±.074	91.32±2.77	91.31±1.80
f_2 (%) (n=6) [a]	-	68.13	83.94	64.43	76.06
Drug assay (%) (n=3)	103.1±1.8	100.6±0.8	100.2±0.1	103.6±1.7	96.14±0.46
Hardness (KP) (n=6)	11.20±0.98	12.48±0.58	12.77±0.71	13.67±0.79	12.67±0.75
Friability (%) (n=11)	0.544	0.561	0.631	0.641	0.660
Thickness (cm) (n=10)	0.531±0.010	0.541±0.003	0.539±0.001	0.538±0.003	0.519±0.005
Uniformity of dosage unit (AV) (n=10)	3.46	4.04	3.73	7.57	0.91
Maximum deviation from average weight (%) (n=10)	1.12	0.98	0.87	1.70	0.85
Moisture content (%) (n=9)	2.18±0.37	1.98±0.23	1.77±0.93	2.07±0.38	2.76±0.49

[a]: Calculated compare to freshly prepared tablets (Time= 0), [b]: Mean ± SD

For graphical optimization, an all over optimum region was achieved by the combination of the surface plots for all three responses which could be helpful to obtain the defined targets for all responses simultaneously. Fig. 3 shows the overlay plot containing the desired area of all three responses, based on the applied constraints, including optimum formulations.

In numerical optimization, four optimum checkpoint formulations (O_1-O_4) with higher desirability, found by multiple criteria optimization were selected (table 6). In the next step, the selected formulations were prepared and evaluated regarding the responses. Values of predicted and observed responses, reported in table 6, were compared using predicted error calculation by the following equation (Huang *et al*., 2005):

Predicted error (%) = (observed value – predicted value) × 100 / predicted value

Based on the results, predicted error for all three dependent variables was not more than 6%, which confirms the efficacy of D-optimal technique used in this study for the optimization of prolonged release pyridostigmine bromide tablet (Anderson *et al*., 1998).

Among the formulations (O_1-O_4) the highest correlation (lowest predicted error) for all three responses was detected for O_3 which was selected as the optimum formulation for further studies. Fig. 4 shows the release profile of pyridostigmine bromide from the optimum formulation (O_3) in different medium. It is observed that using dissolution medium with different pHs did not affect the drug release pattern significantly due to the nonionic nature of the retarding agents in the formulation. According to kinetics evaluation, the release data for the optimized formulation was best fitted with Higuchi model (R^2 = 0.975, P value of deviation from linearity test =

0.931) compared with zero and first order (R^2 = 0.943 and 0.949, respectively). This is in agreement with published reports that drug release from HPMC matrices is based on diffusion and Higuchi model (Sung *et al*., 1996; Bettini *et al*., 2001; Garg and Gupta, 2009).

In addition, the release exponent (n) calculated for the optimized formulation by Korsmeyer-Peppas equation was about 0.4 (R^2 = 0.976), indicating Fickian (case I) release mechanism.

Optimal formulation (O_3) was kept in accelerated stability conditions and then the physical properties of tablets were studied (table 7). Fig. 5 represents the release profile of pyridostigmine bromide from those tablets during stability studies. After exposing to the stability conditions for six months, the drug content of tablets did not show any significant changes (96.1-103.6%) which confirms that the formulation remains stable during the study. Other tablet properties such as hardness, friability and weight variation were all in the acceptable range. Moisture content of tablets also did not change during stability studies.

Calculation of the acceptance value (AV) is a method to evaluate the uniformity of dosage units. AV was equal to 3.46 for the freshly prepared tablet and 0.91-7.57 for tablets after conducting stability test. Since the maximum

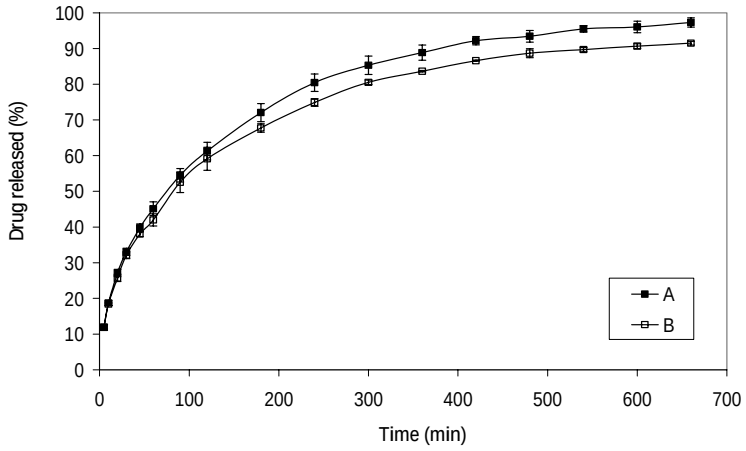


Fig. 4: Drug release profile of the optimum formulation in A) phosphate buffer solution (pH 7.2) and B) first hour in 0.1 N HCl and then in phosphate buffer solution (pH 7.2) for the rest of experiment (n=6)

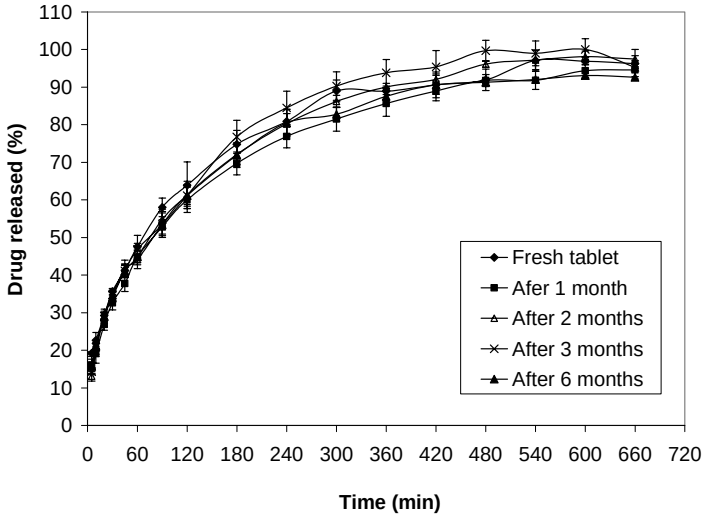


Fig. 5: Drug release profiles of optimum formulation tablet after conducting the accelerated stability test (n=6).

allowed acceptance value is 15 (USP 2008), therefore, tablets were considered uniform due to lower AV values.

Based on the statistical analysis (ANOVA), there was not any significant difference for the drug released at the 1st, 4th and 8th hours of dissolution study from the optimum formulation after exposing to the stability conditions ($p > 0.05$). The similarity factor calculated for the tablets compared to freshly prepared ones was in the range of 64.4-83.9, which confirms that the release pattern of tablets remains constant after six months keeping in accelerated stability conditions.

CONCLUSION

In conclusion, the prolonged release matrix tablet of pyridostigmine bromide with desirable physical properties could be designed successfully by D-optimal mixture method. The quantitative effect of mixture variables on drug released at 1, 4 and 8 hours of dissolution study could be predicted by cubic, cubic and linear models, respectively. The optimized formulation containing hydrophilic-hydrophobic polymers showed suitable tablet properties as well as desirable release profile without any significant variation during stability studies. As a conclusion, a mixture of HPMC and carnauba wax used as retarding agents in tablet preparation could prolonged the dissolution of pyridostigmine bromide, a deliquescent compound, which is helpful in reducing dosing frequency and improving patient compliance.

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