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Design and statistical optimization of circadian rhythm based press-coated tablets of Atomoxetine hydrochloride

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ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is a common neurological disease affecting 5-8 percent of school going children with symptoms persuading into adulthood in about 60 percent of cases. ADHD severity correlates positively with circadian delay i.e. retarded sleep timing and day time sleepiness, suggesting that treatment interventions focussed at advancing circadian phase may make better day time sleepiness. Hence, the present research work aimed to formulate circadian rhythm based press-coated tablets of atomoxetine hydrochloride in morning hours by increasing adrenal hormones in the brain. The core tablets formulated by direct compression and press coated with HPMC K100M and MCC and the release compared with natural polymers Guar gum (6000 cps) and Xanthan gum (1800 cps). The three level two factorial design and one-way Anova employed to evaluate the effect of natural and synthetic polymers on drug release and lag time. IR spectrophotometer study showed that all the excipients were compatible with the drug. The stability study carried out for the desired optimized formulation for a period of 3 months and showed insignificant difference.

Keywords: ADHD, Atomoxetine hydrochloride, Pulsatile, Circadian rhythm, Factorial design

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INTRODUCTION

The physiological process such as heart rate, blood pressure, plasma concentration of hormones, plasma proteins and enzymes display steadiness over time, drug delivery systems with constant release profiles have thus been favored. The wide research into circadian rhythms and their influence on biological systems has given rise to study of chronobiology and later chronotherapy, study of delivering drugs in synchrony with biological rhythms or precisely timed drug delivery systems required to correlate drug delivery with circadian rhythms to provide maximum therapeutic efficacy for chronotherapeutic diseases when most needed [1].

Attention-deficit/hyperactivity disorder (AD/HD) is a common neurobiological condition affecting 5-8 percent of school age children with symptoms persisting into adulthood in as many as 60 percent of cases (i.e. approximately 4% of adults)[2]. Circadian rhythm sleep disorder occurs in 70% of patients with ADHD and can negatively impact work, behavioral disorder and social performance as the chemical messengers do not work properly in these patients. ADHD severity correlates positively with circadian delay with delayed sleep timing and day time sleepiness, suggesting that treatment interventions for advancing circadian phase may improve day time sleepiness[3].

The present research work aimed to formulate pulsatile release tablets of atomoxetine hydrochloride to reset the circadian clock by ameliorating delayed circadian sleep disorders of ADHD by releasing the drug in the morning hours to improve the attention and decrease the hyperactivity by increasing the dopamine and nor-epinephrine in the brain.

In this work, 3^2 full factorial statistical experimental design central composite design (CCD)was employed to investigate the effect of two factors viz., swellable (synthetic / natural) and rupturable (synthetic / natural) polymers on lag time and 90% drug release of the delivery system .Further CCD design was evaluated by one-way ANOVA, multiple regression analysis(MRA), RSM and Contour plots[4]. In this work synthetic polymers compared with natural polymers as the synthetic polymers are toxic, expensive, have environmental related issue, requires long development time for synthesis in comparison to naturally occurring polymers. However the use of natural polymers for drug delivery is tremendous as they are economical, readily available, non-toxic,capable of chemical changes, biodegradable ,biocompatible and with few exceptions[5]. Hence, with the proposed delivery system, a new therapeutic dimension has been given to treat ADHD.

MATERIALS AND METHODS

Atomoxetine hydrochloride received from Swapnroop drugs & Pharmaceuticals, Maharashtra, India. Hydroxy propyl methyl cellulose HPMC K100M procured from Ozone International, Mumbai. Avicel PH-102, Mannitol, Sorbitol and Magnesium Stearate obtained from Loba chemicals, Mumbai. Polyvinylpyrollidone (PVP K 30), Lactose and Isopropyl alcohol obtained from SD Fine Chemicals, Mumbai. Xanthan gum 1800 cps and Guar gum 6000 cps were of Himedia Laboratories, Pvt Ltd, Mumbai. All other chemicals were of analytical reagent grade.

Development of Circadian Rhythm Based Formulation/ Timed Drug Delivery Of Atomoxetine Hydrochloride

Drug- excipient Interaction

The IR spectrum of the mixture of excipients with drug recorded and compared with that of Atomoxetine Hydrochloride to confirm the physicochemical compatibility of the drug with the formulation excipients used in the study. The powdered mixture taken in a diffuse reflectance sampler and the spectra recorded by scanning in the wavelength of 400 to 4000cm-1in a FTIR spectrophotometer- 430 (Jasco, Japan) (Figure 1 and 2)

Development of core tablets

The coretablets formulated by direct compression method. As shown in Table 1 powder mixtures of atomoxetine hydrochloride, Avicel, polyvinylpyrollidone, Magnesium stearate, sucrose/ lactose and Mannitol/Sorbital were dry blended for 20 min and compressed by rotator tablet machine (Rimitek-minipress-MT),Karnavati Engineering Ltd, Ahmedabad, India with a 6mm punch and die to get the core tablet [6].

Table 1: Formulation of Atomoxetine Hydrochloride core tablets

Drug and Excipients(mg)	F1	F2	F3	F4	F5	F6	F7	F8
Atomoxetine hydrochloride	40	40	40	40	40	40	40	40
Mannitol	83.5	83.5	82.5	-	-	-	-	-
Sorbitol	-	-	-	83	83	84	84	84
PVP	4	4	4.5	4	4	4	4	4
Magnesium Stearate	2.5	2.5	2.5	2.5	2.5	2.5	-	2.5
Lactose	3.5	3.5	3.5	-	-	-	-	-
Sucrose	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Avicel	6.5	6.5	7	7.5	7.5	8	8	8

Development of Circadian Rhythm based press coated Tablets

Formulation of barrier layer granules for Circadian Rhythm Based press coated tablet of Atomoxetine Hydrochloride

A wet granulation process was used to prepare barrier layer granules. HPMC K100M and MCC were accurately weighed as per Table 2. 7.5 % PVP solution (hydro alcoholic mixture of iso-propyl alcohol and water in the ratio of 70:30) was used to wet mass the HPMC K100M and MCC. Then the wet mass was passed through a sieve of 710µm aperture size. It was dried for 2 h at 45^0C in a hot air oven. Then the dried mass was screened through a sieve number of 500 µm aperture size[7].

Preparation of press-coated tablets

The core tablets were press coated with 300mg of barrier layer granules.150mg of barrier layer granules weighed and transferred into a 8mm die then the core tablet placed manually at the centre. The remaining 150mg of the barrier layer granules added into the die and directly compressed to prepare press coated pulsatile tablets (S1/N1-S10/N10) [8].

The formulations prepared in different ratios of rupturable/erodible MCC and swellable HPMC such as 3:0,0:3,1.5:1.5, 1:2,2:1,0.75:2.25, 2.25:0.75 and 0.4:2.6 with a total polymer weight of 300mg and the above procedure repeated for the natural polymers swellable Guar gum (6000 cps) and erodible/rupturable Xanthan gum (1800 cps). The formulations are given in Table 2 and Table 3.

Table 2: Formulation of Atomoxetine Hydrochloride press coated tablets (Synthetic Polymers)

Excipients Synthetic	S1	S2	S3	S4	S5	S6	S7	S8
MCC	300	-	150	100	200	75	225	40
HPMC K100M	-	300	150	200	100	225	75	260
Hydroalcoholic 7.5% solution of PVP	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Table 3: Formulation of Atomoxetine Hydrochloride press coated tablets (Natural Polymers)

Excipients Natural	N1	N2	N3	N4	N5	N6	N7	N8
Xanthan Gum (1800 cps)	300	-	150	100	200	75	225	40
Guar Gum (6000 cps)	-	300	150	200	100	225	75	260
Hydroalcoholic 7.5% solution of PVP	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Evaluation of Pulsatile Tablets (Table 4)

The pulsatile tablets of atomoxetine hydrochloride from each formulation made of synthetic polymers are subjected to weight variation, hardness, thickness, friability and drug content uniformity tests

Weight Variation

Weight variation test was performed according to the official method as per USP.

Hardness test

The hardness of formulated atomoxetine hydrochloride pulsatile time release tablets are measured by Pfizer hardness tester.

Thickness

The thickness of each tablet measured with the help of micrometer, which provides information about variation between tablets.

Friability test

Friability test is conducted to measure the strength of tablet and measured using Roche friabilator.

Drug content uniformity test

Ten tablets are finely powdered and quantity equivalent to 40mg of atomoxetine hydrochloride transferred to a 100ml volumetric flask and dissolved in a 100ml of 0.1N HCl. From this 10ml of the solution was withdrawn and further diluted to100ml by 0.1N HCl. The absorbance measured at 270nm by UV spectrophotometer (Shimadzu UV/Vis 160).

***In vitro* Dissolution Studies using Synthetic and Natural Polymers**

The drug release of the core tablets of formulations F1-F7 were unsatisfactory but the core tablet of F8 showed 95% of drug release within 1h upon contact with dissolution medium. The core tablet got ruptured and released the drug as given in Figure 3. All press coated tablets (S1-S8) made with different ratios of swellable HPMC K100M and rupturable/erodible MCC in simulated gastric fluid (pH 1.4 acid buffer for initial 2h) followed by simulated intestinal fluid (pH 6.8 phosphate buffer) till 13^{th} h. The samples were withdrawn at a regular interval. It is analyzed by UV spectrophotometer (Shimadzu UV/Vis 160) at 270nm to find existence of the drug. Cumulative percentage drug release was calculated using the standard curve. The above procedure repeated using natural polymers, swellable Guar gum (6000 cps) and erodible/rupturable Xanthan gum (1800 cps).(N1-N8) The results are given in Figure 4 and 5.

Experimental Statistical Design

Optimization of inner coating layer for pulsatile release tablets done using face centered central composite design (CCD). A set of 9 runs proposed by 3^2 factorial design by CCD. With help of this it was suitable to check the quadratic response surfaces and construction of second order polynomial equation by using Minitab 17software . Selection of independent variables (swellable and erodible/rupturable polymers) and dependent variables $t_{10\%}$ (lag time) and $t_{90\%}$(90% drug release)based on preliminary experiments. Table 5 summarizes the independent variables along with their levels. Response surface analysis carried out to check whether current study fits in model. The

effects of independent variables on dependent variables observed from 3D response surface and contour plots. The polynomial equation generated for each response using multiple linear regression analysis also shows effect of interaction of each factor on dependent variables. The effects of different factors on regression coefficients studied using one way analysis of variance (ANOVA) by Graph pad Prism software version 6 and the difference less than the probability level 0.05 was considered statistically significant. Thus the polymer concentration and coating level optimized using CCD[9].

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Stability studies

The stability study of the optimized batches in which the tablets monitored upto 3 months as per ICH guidelines at room temperature and relative humidity (25◦C±2◦C, RH75 ±5%). The tablets analysed for appearance, weight, thickness, hardness, drug content, drug release [10]**.**

RESULTS AND DISCUSSION

Formulation of Circadian Based press coated tablets using synthetic and natural polymers

As Circadian Rhythm sleep disorder causes day time sleepiness and inattention in most of ADHD patients, the present work was designed to improve the above conditions by releasing the drug in morning hours with a lag time of 8h as a time specific pulsatile delivery system. As a first step, drug-excipients compatibility study was carried out using IR spectroscopy and showed no possible interactions between mixture of excipients and drug.

This circadian rhythm based drug formulation consisted of inner core tablet containing drug reservoir and outer coating layer with combination of rupturable/erodible microcrystalline cellulose(MCC, Avicel PH-102) and swellable hydroxy propyl methyl cellulose (HPMC K100M). MCC was chosen for its rupturable and for its wicking effect. HPMC K100M was selected for its swelling behaviour. These two polymers were individually tested for their influence over $t_{10\%}$ and $t_{90\%.}$ S1 did not show any significant lag time and revealed rupturable/erodible polymer alone cannot provide desired pulsatile release pattern. So Formulations S2 was formulated only with a swellable polymer so as to see its effect on responses. and S3-S8 were formulated with both rupturable and swellable polymers. Among these formulations, S8 was having the desired lag time and 90% drug release (Figure 4) These synthetic polymer combination was substituted by natural swellable polymer Guar gum 6000 cps and natural rupturable/erodible polymer Xanthan gum 1800 cps and found equivalent. Both synthetic and natural polymers in the ratio of 0.4:2.6 of rupturable and swellable polymer combination were considered as the optimum formulation as both had a lag time of 8h and 90% drug release in the 12^{th} hour and revealed that natural polymers are equivalent to synthetic polymers in releasing the drug in pulsatile manner.

Central Composite Design and its Statistical Validation

The dissolution study performed for all the runs and the results shown in Table 6 & Figure6.All the runs showed $t_{10\%}$or lag time ranging from 5.5 h-8h and $t_{90\%}$(90% drug release)between 9.5-12.5h. In case of synthetic polymers the desired lag time of 8h and $t_{90\%}$ in R1was 12.5 h and $t_{10\%}$ was 7.5 h and $t_{90\%}$ was 11.5h in R1 in case of natural polymers.

The slow release of the drug from HPMC is due to formation of viscous gel layer. The ability of hydrophilic polymer HPMC &Guar Gum to retard drug release was in the order of HPMC>Guar Gum and the burst release of drug sustained in the order of Xanthan Gum > MCC. MCC being a non swelling wicking agent dispersed throughout the matrix along with HPMC and creates channels for incoming aqueous fluid and thus enhances the erosion/rupture of the coating layer of the polymer.

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A statistical model incorporating interactive and polynomial terms used to evaluate the responses. using the following polynomial equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

Incorporating interactive and polynomial terms used to evaluate the responses, where Y is the dependent variable, The main effects (X1 and X2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X1 and X2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_{12} and X_{22})included to investigate nonlinearity [11].

One way ANOVA (analysis of variance) used for statistical analysis of targeted response at 5% significant level using GraphPad Prism Version 6. In above equation, b_0 is the intercept representing the arithmetic averages of all 9 runs and b_1, b_2, b_{12}, b_{11} and b_{22} are the coefficients computed from the observed experimental values of responses Y1 and Y2 and X1 and X2 stand for main response of independent variables. The terms X1X2, $X1^2$ and

$X2^2$ represent interaction and quadratic terms of independent variables respectively. In table 7 factor effects involved in CCD model and associated p-values for the responses Y1 and Y2 are given. The p-value of independent variables of synthetic and natural polymers for both the responses implies both the factors significantly affect both the responses.

After eliminating insignificant terms the last equation of the responses are given below for synthetic or natural rupturable and swellable polymers

Lag time (h) = -20.87 - 0.0417 A: MCC + 0.2477 B:HPMC (2)

90 % drug release (h) = -3.4 - 0.094 A: MCC + 0.139 B:HPMC (3)

Lag time (h) = -1.25 - 0.0018 A:Xanthan Gum + 0.0656 B:Guar Gum (4)

90 % drug release (h) = -45.6 - 0.128 A : Xanthan Gum + 0.515 B: Guar Gum (5)

Positive sign in front of the factors indicates synergistic effect and negative sign indicates antagonistic effect of the factors on responses Y1 and Y2.The value of R^2 was >0. 9 which show a highly significant and linear relationship between X1, and X2 factors and excellent goodness of fit. The relationship between the dependent and various independent variables was further elucidated using 3-D response plot and Contour plots at one time.(Figure 7-14).

Stability Study

There was no significant difference in the stability study of atomoxetine hydrochloride tablets before or after 3 months of storage either in its physical characteristics or in its dissolution profile.

Table 4. Hardness, Thickness, Friability, Weight variation and Drug content test of press-coated tablets corresponding to F1-F8

S. No.	Formulation	Weight variation(mg) ±SD	Hardness(kg/cm^2) ±SD	Thickness(mm) ±SD	Friability(%) ±SD	Drug content(%) ±SD
1	S1	443±1.70	4.45±0.24	4.28±0.03	0.25±0.09	98.9±0.0
2	S2	443±1.20	4.43±0.25	4.23±0.04	0.28±0.08	98.4±0.07
3	S3	444±1.70	4.43±0.35	4.22±0.05	0.30±0.12	98.6±0.05
4	S4	441±1.50	4.44±0.40	4.22±0.02	0.28±0.15	98.5±0.03
5	S5	442±1.50	4.48±0.35	4.25±0.04	0.23±0.09	98.2±0.06
6	S6	442±1.70	4.40±0.25	4.24±0.05	0.20±0.12	99.2±0.03
7	S7	441±1.70	4.44±0.28	4.26±0.03	0.25±0.09	98.9±0.05
8	S8	442±1.70	4.41±0.40	4.20±0.04	0.25±0.15	99.2±0.07

n=3, Mean ±SD

Table 5 :Two factors 3 levels full factorial experimental design; factor selected and responses measure

Factors(Independent variables)	Levels			Responses(Dependent variables)
	-1	0	1	
MCC / Xanthan gum (rupturable/erodible polymer)	40	60	100	Y1=$t_{10\%}$(Lag time)
HPMC/Guar gum(swellable polymer)	200	220	260	Y2= $t_{90\%}$ (90%Release of drug)

Table6: Dissolution studies as per CCD full factorial experimental design

Run	Block	Factor1 A:Mcc (Mg)	Factor2 B:Hpmc100km (Mg)	Response1 Lag Time (h)		Response2 90% Drug Release (h)	
				Synthetic	Natural	Synthetic	Natural
1	Block1	40	260	8±0.23	7.5±0.37	12.5±0.32	11.5±0.32
2	Block1	100	220	6±0.05	6.0±0.12	10±0.27	11±0.26
3	Block1	40	200	7±0.12	6.5±0.18	10.5±0.46	10±0.03
4	Block1	60	260	7±0.35	6.5±0.18	11±0.36	10.5±0.21
5	Block1	100	200	7±0.12	6±0.25	11±0.29	9.5±0.47
6	Block1	40	220	7.5±0.35	7±0.37	12.5±0.35	12±0.22
7	Block1	60	200	6±0.25	6±0.28	10±0.08	9.5±0.41
8	Block1	60	220	7±0.35	6.5±0.42	10.5±0.21	10±0.36
9	Block1	100	260	6±0.18	5.5±0.37	11±0.33	10±0.18

Figure 1: IR spectrum of pure atomoxetine HCl

Figure 2: IR spectrum of atomoxetine HCl and mixture of excipients

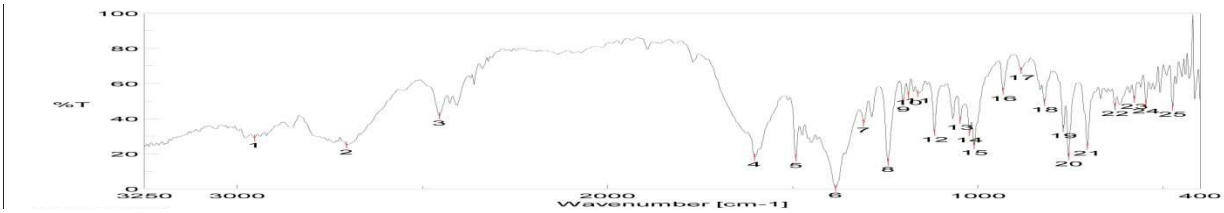


Figure 3: *Invitro* dissolution Profile of core tablet of formulation F8

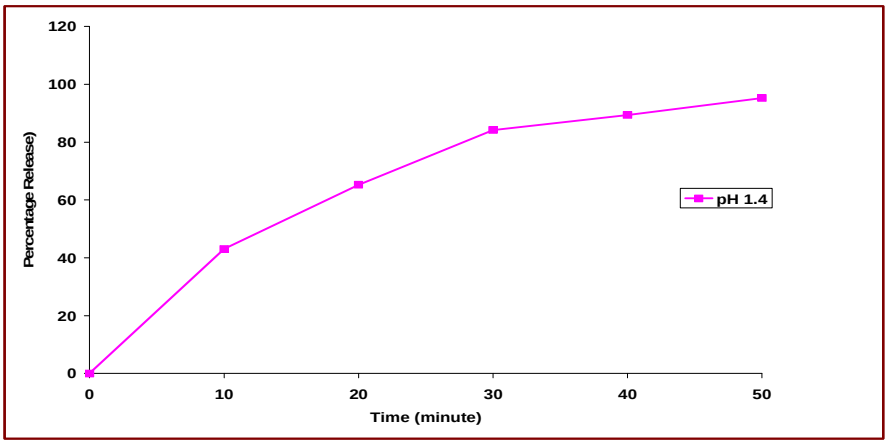


Figure 4: *Invitro* dissolution Profile of formulations S1-S8 using Synthetic polymers

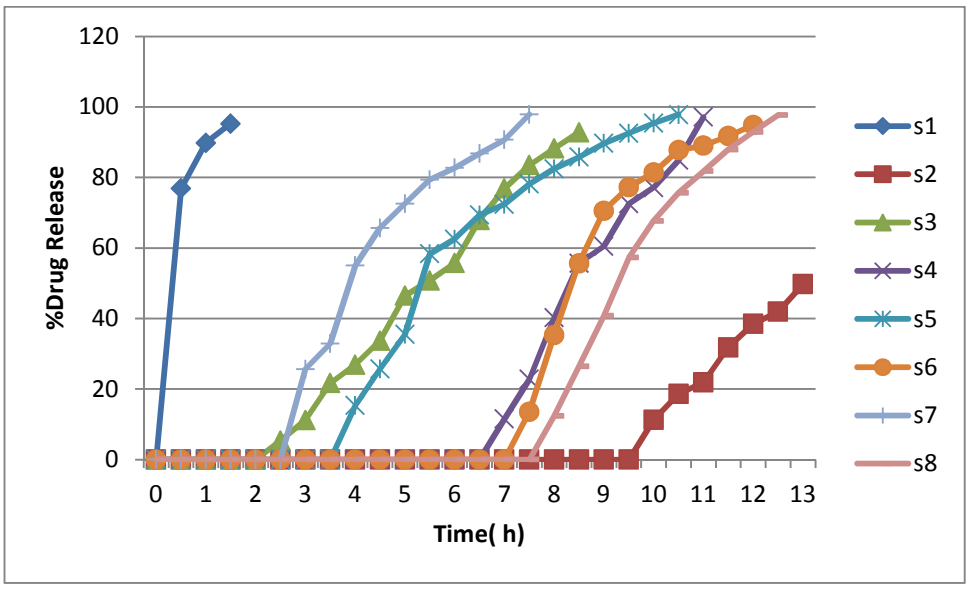


Table 7: The results of the ANOVA which was used to generate statistical models

Response model	Sum of squares	Degrees of freedom	Mean Square	F value	p value	R^2
$t_{10\%(\text{synthetic polymer combination})}$	13.09	26	1.56073	24.70	p<0.001	0.9165
$t_{90\%(\text{synthetic polymer combination})}$	22.77	26	2.72306	26.77	p<0.001	0.9225
$t_{10\%(\text{natural polymer combination})}$	10.28	26	1.17269	26.77	p<0.001	0.9430
$t_{90\%\%(\text{natural polymer combination})}$	20.30	26	2.42349	25.79	p<0.001	0.9197

Figure.5 *Invitro* dissolution profile of pulsatile tablets of formulations N1-N8 using Natural Polymers

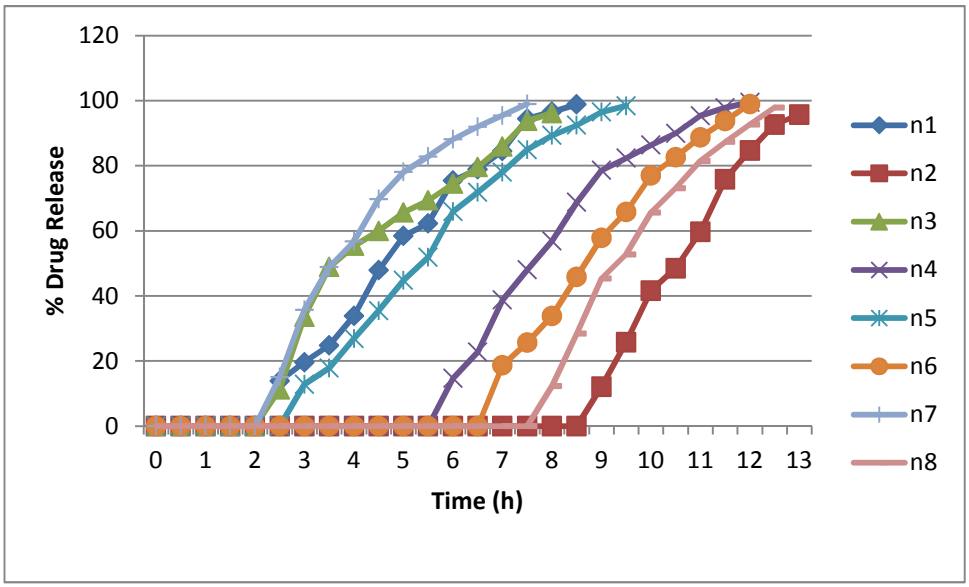
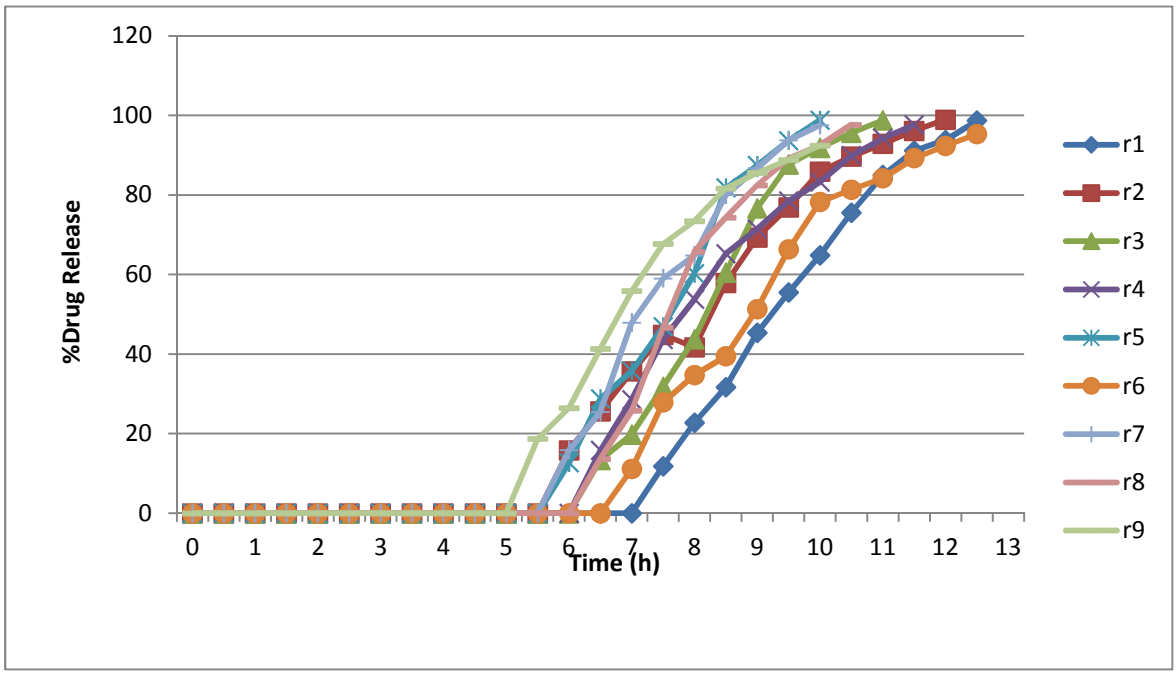


Figure.6 *Invitro* Dissolution studies as per CCD full factorial experimental design



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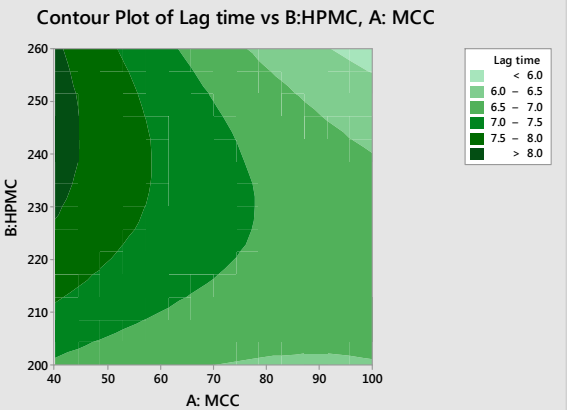

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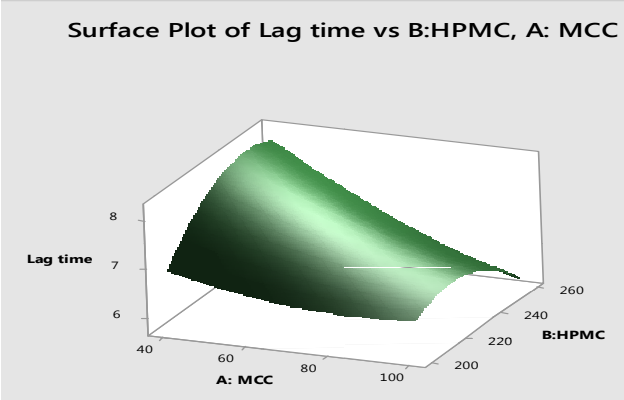

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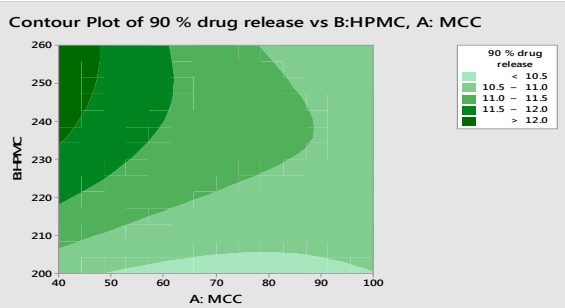

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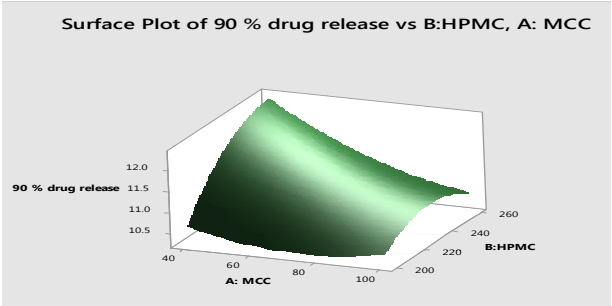


Figure 11:

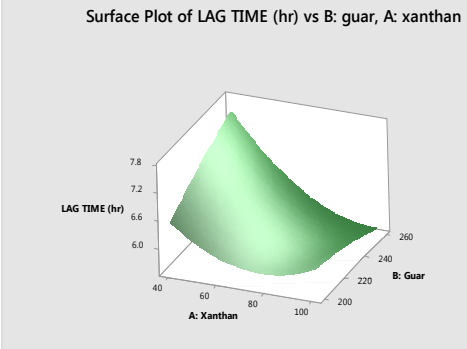


Figure 12:

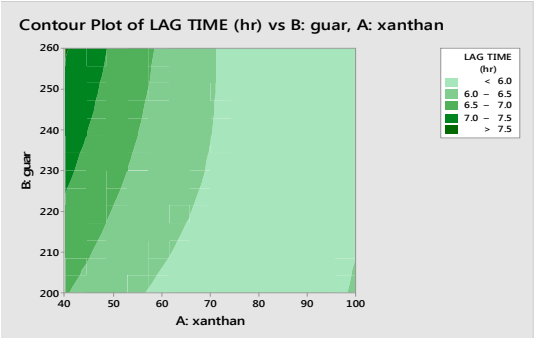


Figure 13:

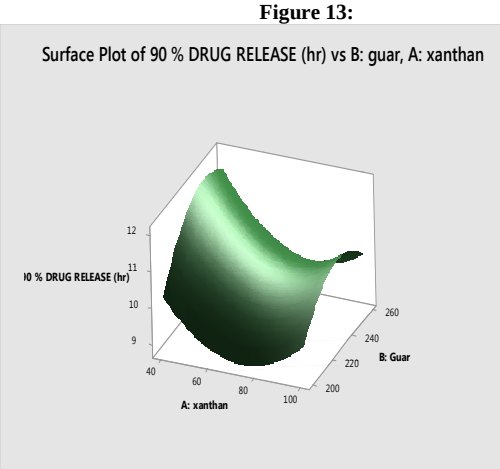
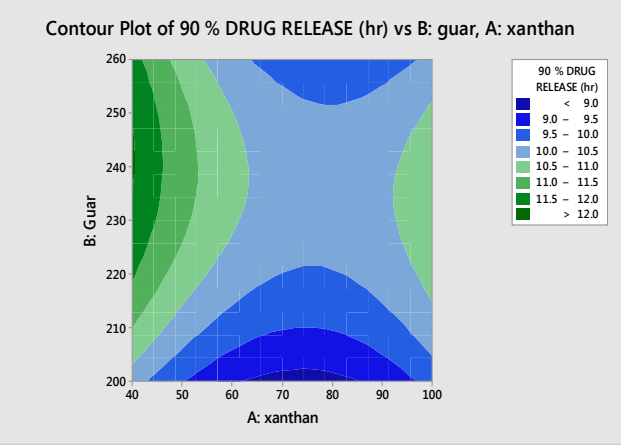


Figure 14:



CONCLUSION

Oral drug delivery is the largest, oldest, and most preferred route of drug delivery. Universally sustained and controlled-release products offer a desired therapeutic effect, but fall for diseases following biological rhythms like ADHD. As there is an evidence that ADHD might be associated with misalignment of the circadian clock with the environment. The present research work in a new dimensional approach can effectively crack this problem as it will modulate according to body's circadian clock giving release of drug after a specified lag time which surely assure a bright and promising future for ADHD patients.

The central composite design was successfully used to develop time-lagged coating formulation with both rupturable and swellable polymers. From response surface methodology, it is easy to understand the change of responses with independent variable.

From the present study it could be concluded that HPMC100KM/Guar Gum serves as a potential candidate in formulating a time controlled release or circadian rhythm based drug delivery systems with a defined lag time. Additionally, HPMC 100KM /Guar Gum shall be used along with MCC/Xanthan Gum to meet reproducible result, since HPMCK100M /Guar Gum individually can offer lag time of 10h which is a higher value than desired and there was no 90% drug release within the desired 12 h. The amount and composition of HPMC 100KM /Guar Gum and MCC/Xanthan Gum used shall be in right proportion to get preferred t10%and t90%. Polymers in 1:1 ratio may not yield the required result. Quantity of MCC/Xanthan Gum used decides the overall lag time, as more amount of MCC/Xanthan Gum might increase permeation of the press coat, thereby shortening lag time. HPMCK100M /Guar Gum forms a swellable gel layer to enhance lag time by providing necessary strength and support to the outer press coat layer.

The current research work used natural polymers and found equal to synthetic polymers and their use are attractive because they are economical, readily available, non toxic, capable of chemical changes, potentially biodegradable and biocompatible. The conclusion about the validity of circadian based formulation of Atomoxetine HCl needs to be confirmed with reproducible *invitro* and *invivo* studies.

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