ORIGINAL ARTICLE

Designing and Characterization of Polymeric Microparticles Containing Agomelatine-loaded Mesoporous Silica Nanoparticles for a Controlled Drug Delivery System

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Abstract

Introduction: Depression is a commonly occurring mental disorder distinguished by sadness, lack of interest, or pleasure in daily activities. Agomelatine is used in the treatment of depression but suffers short half-life and low oral bioavailability. The current research work aims to control the release of drug for longer duration by preparing agomelatine-loaded mesoporous silica nanoparticles incorporated into polymeric microparticles for the treatment of depression. **Materials and Methods:** Box-Behnken design was used to optimize agomelatine-loaded mesoporous silica nanoparticles prepared by sol-gel method. The drug loading was done by solvent impregnation method. The effect of independent variable such as tetraethyl orthosilicate, CTAB, and NaOH 2 M was analyzed on dependent variable. Double emulsification solvent evaporation was used to incorporate agomelatine-loaded mesoporous silica nanoparticles into ethyl cellulose polymeric microparticles. **Results and Discussion:** The optimized mesoporous silica nanoparticles showed mean particle size of 166.7 nm, 0.367 PDI, 43.3mV zeta potential, and 78.03% entrapment efficiency. Optimized mesoporous silica nanoparticles were evaluated for FE-SEM, DSC, and XRD. The polymeric microparticles were evaluated for particle size, microscopic evaluation, and *in vitro* drug release. The polymeric microparticles showed 16% initial burst release followed by a continuous release pattern for 10 h and exhibited Higuchi model with a regression coefficient(R^2) of 0.9287. **Conclusion:** The formulated polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles helped to improve the oral bioavailability of the drug and controlled its release rate, which lowers the frequency of dose and is beneficial for the long-term treatment in case of depression.

Key words: Agomelatine, Box-Behnken design, controlled release, depression, mesoporous silica nanoparticles, polymeric microparticles, sol-gel method, solvent impregnation

INTRODUCTION

Depression is a mental disorder distinguished by sadness, lack of interest, or pleasure in daily activities, disturbance in sleep, appetite, and tiredness which results in poor concentration in daily activities. Worldwide more than 0.35 billion people are affected by the depression.[1,2] The first choice of drug therapy, such as tricyclic antidepressants, selective serotonin receptor inhibitors, and monoamine oxidase inhibitors, increases the chance of serious adverse effects, which is still an unresolved problem. Agomelatine a novel antidepressant or melatonin analog licensed for use in the treatment of depression in adults. It is also used in the treatment of sessional affective disorder and anxiety disorder. It has an agonistic action on the melatonin receptor and an antagonist action on the serotonin receptor. The therapeutic dose of the drug in an adult human is 25–50 mg/day. Agomelatine belongs to a BCS Class II drug having a poor dissolution profile affects

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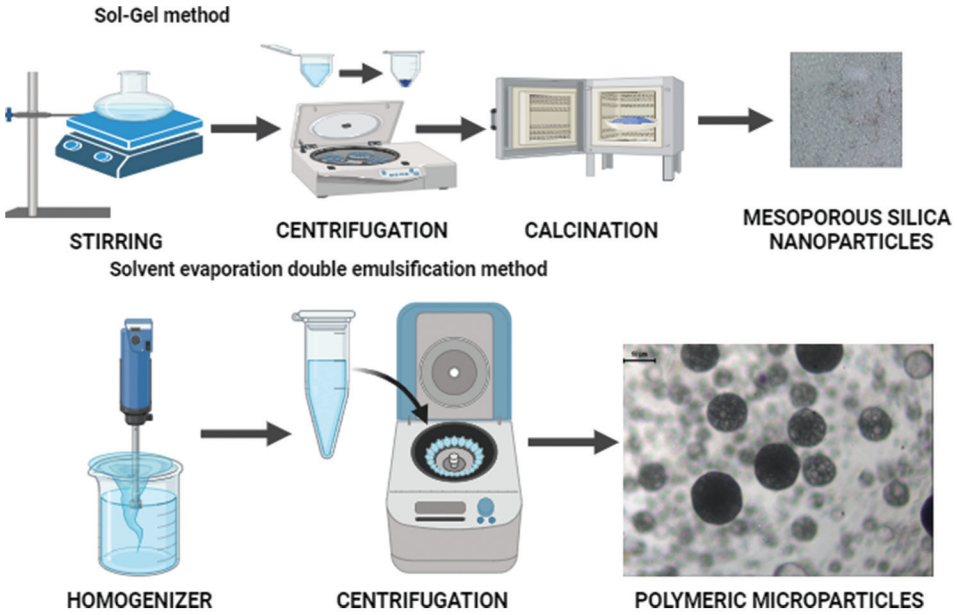
GRAPHICAL ABSTRACT



its bioavailability resulting in low therapeutic action.[3-6] Due to its low solubility and high permeability, patients need to take high doses in case of chronic disease which may lead to an increase in the chances of side effect.[7] The problem associated with the drug can be addressed by introducing a controlled release formulation, which leads to a lower dosing frequency, increased therapeutic efficacy, and decreased drug toxicity.[8]

Mesoporous silica nanoparticles are one of the most excellent drug approaches to improve the low solubility of a drug by transforming the crystalline state of a drug into an amorphous state.[9,10] Mesoporous silica is an inorganic material that has a tunable pore size in the range of 2-100 nm having ordered arrangements. It is prepared with the help of surfactants in which the polycondensation of the silica species takes place which is obtained from various silica sources such as tetraethyl orthosilicate (TEOS), TMOS, and sodium silicate.[11] Over the recent years, mesoporous silica nanoparticles have achieved great demand in the field of nanotechnology as it possesses unique features such as tunable pore size, large surface area, and exhibit resistance toward pH, heat, and chemical degradation. The large surface area of mesoporous silica nanoparticles allows the attachment of various kinds of functional groups and drug molecules.[7,12,13] Due to the amorphous nature of the mesoporous silica nanoparticles, the entrapped drugs prevent their crystallization and hence improve their dissolution.[13]

The current research work aims to improve the poor bioavailability and low dissolution profile of agomelatine using polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles by preparing a controlled release formulation. The Box-Behnken design was used to examine the influence of factors on dependent variables such as particle size, PDI, zeta potential, and entrapment efficiency using design expert software. Using the double emulsification solvent evaporation process, the optimized agomelatine-loaded mesoporous silica nanoparticles were incorporated into polymeric microparticles to control the release rate of the drug. The optimized agomelatine-loaded mesoporous silica nanoparticle was characterized by DSC, SEM, and polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles that were further evaluated by particle size and *in vitro* drug release to determine the controlled drug delivery in the treatment of depression.

EXPERIMENT

Material and Methods

Materials

Agomelatine and TEOS were obtained as a gift sample from Mehta API and Dr. Khan Laboratory Pvt. Ltd., respectively. CTAB was purchased from Sisco Research Laboratories Pvt. Ltd., ethyl cellulose was purchased from Loba Chemie, and sodium hydroxide was purchased from otto Chemie Pvt. Ltd.

Methods

Preparation of mesoporous silica nanoparticles

The optimized batch of mesoporous silica nanoparticles was prepared using a sol-gel method with slight modification.[13-16] In the sol-gel process, hydrolysis and condensation of the silica source take place which are also known as the chemical solution deposition method.[16] In this process, CTAB was dissolved in milli-Q water and the

Table 1: Experimental plan of designing mesoporous silica nanoparticles through Box-behnken design

Independent Variables	Unit	Levels		
		Low	Medium	High
TEOS	ml	3.00	4.50	6.00
CTAB	mg	300	400	500
NaOH 2M	ml	2.00	3.50	5.00
Dependent Variable	**Constraints**			
Particle size in nm (Y1)	Minimum			
PDI (Y2)	Maximum			
Zeta potential in mV (Y3)	Maximum			
Entrapment efficiency % (Y4)	Maximum			

Table 2: Formulation batch codes and quantities of factors through Box-Behnken design

Formulation batch code	Factors		
	TEOS (ml) (X1)	CTAB (mg) (X2)	NaOH 2 M (mL) (X3)
B1	6.00	400	2.00
B2	3.00	400	4.50
B3	6.00	300	3.25
B4	4.50	300	4.50
B5	6.00	500	3.25
B6	4.50	400	3.25
B7	4.50	400	3.25
B8	4.50	500	4.50
B9	3.00	300	3.25
B10	4.50	500	2.00
B11	6.00	400	4.50
B12	3.00	400	2.00
B13	4.50	400	3.25
B14	4.50	400	3.25
B15	3.00	500	3.25
B16	4.50	400	3.25
B17	4.50	300	2.00

Table 3: Formulation batch code and quantities of excipients used in the preparation of polymeric microparticles

S. No	Formulation batch code	Quantities of excipients	
		Ethyl cellulose (mg)	0.2% PVA Solution (mL)
1.	MP01	150	200
2.	MP02	200	200
3.	MP03	250	200
4.	MP04	300	200

pH of the solution was adjusted to alkaline by NaOH 2 M solution. The solution was transferred to a round bottom flask and kept at 80°C for 30 min. The TEOS was added dropwise with continuous stirring in the above solution and stirring was continued for 2 h at 80°C to obtain nanoparticles suspension. The suspension was subjected to centrifugation at 5000 rpm for 15 min using a cooling centrifuge to remove CTAB and collecting nanoparticles. The process was repeated by redispersing nanoparticles 3 times in milli-Q water and 2 times in ethanol for the complete removal of CTAB**.** The residual powder was dried in a hot air oven and calcinated for 6 h at 550°C in a muffle furnace.

Loading of a drug into mesoporous silica nanoparticles

Loading of a drug into the pores of mesoporous silica nanoparticles was done by solvent impregnation method. Weighed quantity of 25 mg agomelatine was dissolved in 25 mL ethanol. Mesoporous silica nanoparticles were preheated in a hot air oven for 1 h at 120°C. Then, mesoporous silica nanoparticles were added to the ethanolic solution of agomelatine and kept under continuous stirring at 180 rpm. Fifty ml of distilled water was added at 1, 3, 8, 15, 24, and 72 h in the solution. The solution was filtered by vacuum filtration and washed with distilled water to get mesoporous silica nanoparticles. Further, the residue was dried in a vacuum desiccator.[17,18]

Experimental design through Box-Behnken

For optimizing the parameters of mesoporous silica nanoparticles, Box-Behnken design was used.[19] The statistical and experimental analysis was conducted through Box-Behnken design using Design Expert software. The experimental design was planned using Box-Behnken by taking. TEOS (X1), CTAB (X2), and NaOH 2 M (X3) were selected as factors while responses for these factors were particle size (Y1), PDI (Y2), zeta potential (Y3) and entrapment efficiency % (Y4) as shown in Table 1.[19] The Box-Behnken design suggested 17 batches with 5 center points per block as shown in Table 2.[19]

Preparation of the polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles

Polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles were prepared by the double emulsification solvent evaporation method. The batches of polymeric microparticles were prepared as shown in Table 3.[20,21] Ethyl cellulose was dissolved in dichloromethane to prepare a polymeric solution and agomelatine-loaded mesoporous silica nanoparticles were dispersed into the polymeric solution. The above polymeric suspension was further dispersed into PVA solution (0.2% w/v) in a dropwise manner and homogenized using a homogenizer for about 5 min and stirred overnight using a magnetic stirrer to evaporate dichloromethane. Further, centrifugation was done at 3000 rpm for about 15 min to collect the microparticles and washed 3 times with water to remove the residue of PVA and lyophilized (virtis).[20,21] The dried polymeric microparticles containing agomelatine-loaded

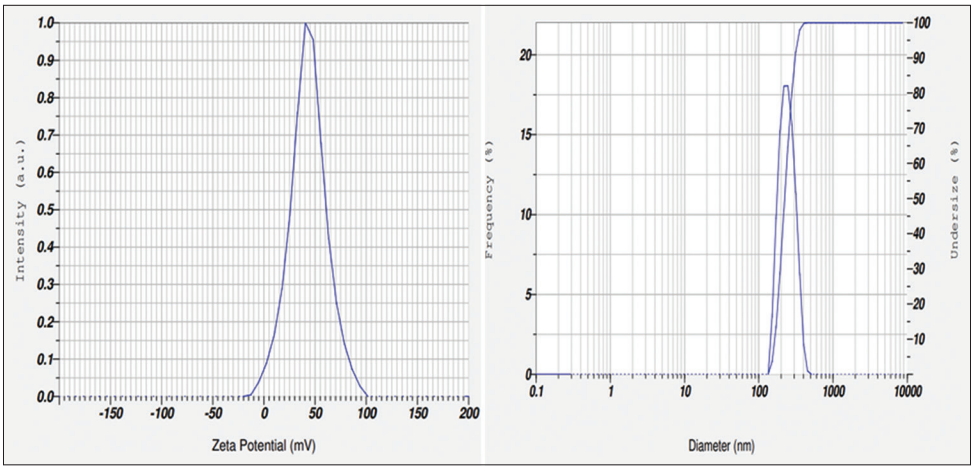


Figure 1: Particle size distribution and zeta potential of mesoporous silica nanoparticles

mesoporous silica nanoparticles were stored at 2–8°C for further studies.

Evaluation

Evaluation of mesoporous silica nanoparticles

The morphological study of optimized mesoporous silica nanoparticles was done by field emission-scanning electron microscopy and polarizing microscopy. Mean particle size, polydispersibility index, zeta potential, entrapment efficiency, DSC, and XRD analysis were done to characterize the features of optimized mesoporous silica nanoparticles.

Determination of particle size, PDI, and zeta potential of mesoporous silica nanoparticles

The hydrodynamic particle size, polydispersibility index, and zeta potential of the optimized batches of agomelatine-loaded mesoporous silica nanoparticles were analyzed by the Horiba SZ-100 particle size analyzer. The particle size was measured by the phenomenon of dynamic light scattering method using the scattering angle 90°at 25°C. The temperature of the holder was 25°C and the electrode voltage was 3.3. The zeta potential of the optimized runs of agomelatine-loaded mesoporous silica nanoparticles was carried out to determine the stability of the nanoparticle suspension. The graphical representation of particle size and optimized mesoporous silica nanoparticles is shown in Figure 1 and observations are recorded in Table 4.

Morphology of mesoporous silica nanoparticles

Microscopic evaluation of optimized mesoporous silica nanoparticles was done under a polarizing microscope and FE-SEM to determine their morphological characteristics. The evaluation was done on a polarizing microscope (Leica) under an oil immersion lens at 100× magnification and FE-SEM was done using Supra 55 ZEISS. For FE-SEM studies, optimized mesoporous silica nanoparticles were placed into the aluminum pin stub and stick with the help of carbon conductive double-sided adhesive carbon tape. The sample was analyzed at 16 kV accelerated voltage and 58.7 K X magnification after coating it with gold vapors. The microscopic and FE-SEM images are shown in Figures 2 and 3, respectively.

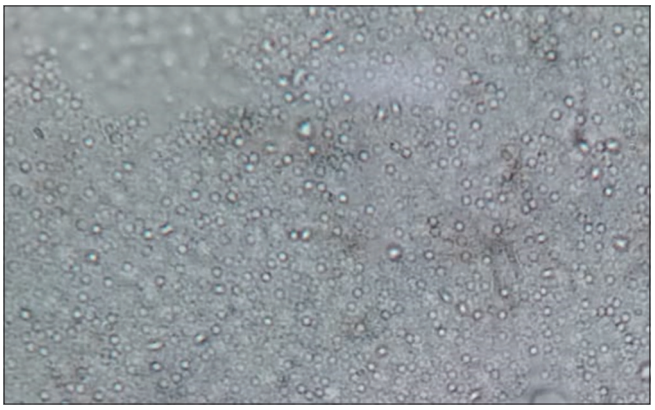


Figure 2: Microscopic image of optimized mesoporous silica nanoparticles

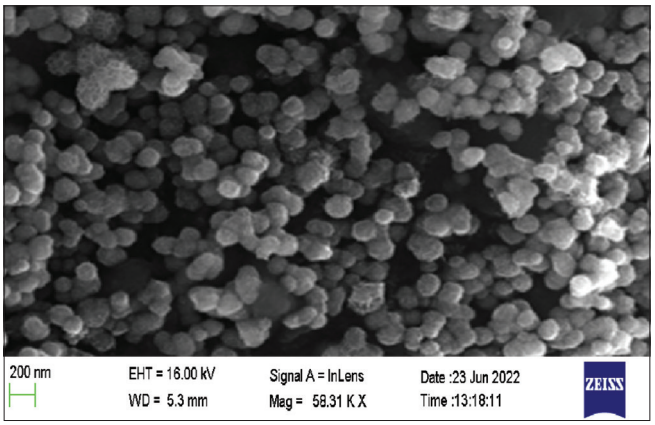


Figure 3: FE-SEM image of optimized mesoporous silica nanoparticles

DSC analysis of mesoporous silica nanoparticles

DSC experiments were performed using differential scanning calorimetry (Perkin Elmer DSC 6000) calibrated by indium. The analysis of pure drug, CTAB, physical mixture (CTAB+ agomelatine), optimized mesoporous silica nanoparticles, and the agomelatine-loaded mesoporous silica nanoparticles was performed. Approximately weighed amount 3–5 mg of the sample was placed in a standard-grade aluminum pan and

crimped. The thermogram analysis of the samples was carried out in the temperature range of 50°C–300°C at 20°C/min scanning rate and the thermogram is shown in Figure 4.

X-ray diffraction studies

XRD studies of agomelatine, mesoporous silica nanoparticles, and optimized agomelatine-loaded mesoporous silica nanoparticles were done on X-ray diffractometer (Bruker D8 Advance) to determine the crystalline or amorphous nature of drug present in the mesoporous silica nanoparticles. The X-rays were produced by sealed tube and the wavelength was 0.154 nm (Cu K-alpha) with the help of Bruker Lynx Eye detector. The XRD diffractogram is shown in Figure 5.

Entrapment efficiency % and drug loading

Entrapment efficiency and drug loading into the pores of mesoporous silica nanoparticles were determined by dispersing the sample into 5 ml ethanol and keeping it for 24 h. Further, a dispersed solution was centrifuged and the supernatant was analyzed in a UV spectrophotometer (Shimadzu 1700) at a wavelength of 229 nm. The entrapment efficiency of the drug was calculated by the equation mentioned below.

Entrapment efficiency was calculated by the formula:

$$Entrapment\ efficiency\ \% = \frac{Actual\ drug\ content}{Theoritical\ drug\ content} \times 100$$

The entrapment efficiency % of optimized batches of mesoporous silica nanoparticles is shown in Table 4.

Evaluation of polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles

Determination of particle size of prepared polymeric microparticles

The particle size of prepared polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles was done by the Malvern Mastersizer. Suitable dispersion of microparticles was prepared using milli-Q water to analyze the particle size of polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles.

Morphological evaluation

The morphological evaluation of the polymeric microparticles was done using a polarizing microscope (Leica) under the oil immersion lens at 100×. The image shows that the polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles have a smooth surface and do not have any cracks and holes. The graphical representation of polymeric microparticles was shown in Figure 6.

In vitro drug release

In vitro drug release of polymeric microparticles was done with the help of a low-pressure ultrafiltration method using stirred cell ultrafiltration unit by taking polyethersulfone

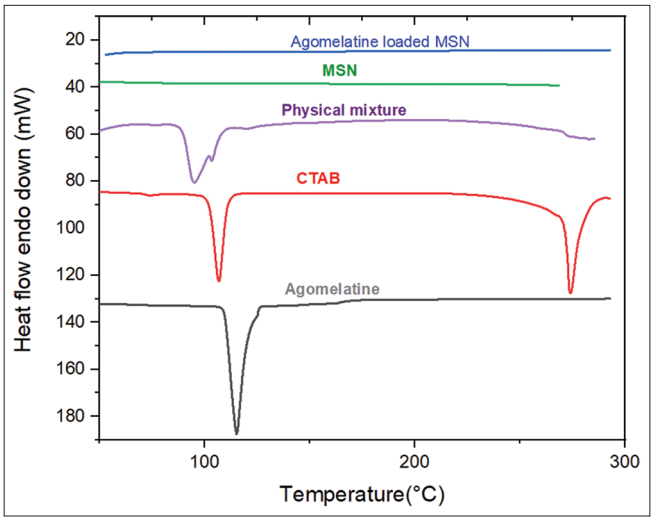


Figure 4: DSC thermogram of drug-loaded MSN, MSN, CTAB, and agomelatine

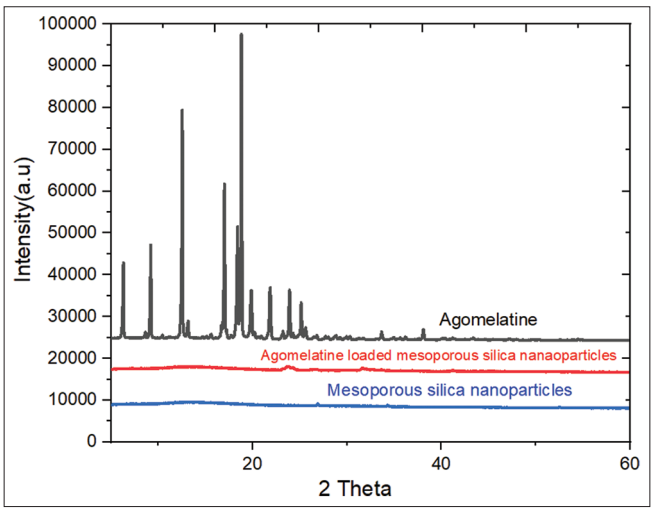


Figure 5: XRD pattern of agomelatine, mesoporous silica nanoparticles, and agomelatine-loaded mesoporous silica nanoparticles

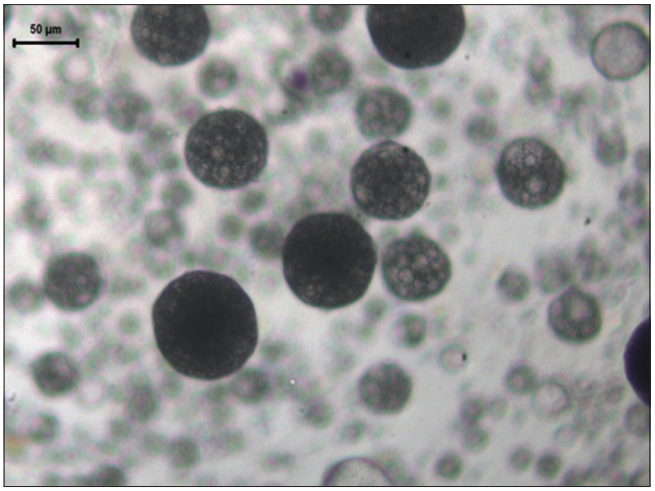


Figure 6: Microscopic image of polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles

ultrafiltration membrane (MWCO 100 Kda) on its base plate. Weighed quantity of polymeric microparticles was added in stirred cell having release media and kept at 100 rpm. Sampling was done by applying low-pressure nitrogen on stirred cell at pre-determined time interval. 5 ml of aliquots were withdrawn and the same quantity of media was replaced by fresh media to maintain sink condition. The sample was analyzed at 229 nm in UV spectrophotometer (Shimadzu 1700) to determine the drug release.[22]

RESULTS AND DISCUSSION

Optimization of mesoporous silica nanoparticles

The data obtained through experiments were statistically analyzed as shown in Table 5[19] by design expert software and the desired characteristic criteria of the final formulation were set. The optimization of the independent variables such

Table 4: Measured response of Box-Behnken design of optimized agomelatine-loaded mesoporous silica nanoparticles

Formulation batch code	Response			
	Particle size (Y1)	PDI (Y2)	Zeta potential (Y3)	Entrapment efficiency % (Y4)
B1	471.3±0.95	0.532	44.40	43.06±0.16
B2	247.1±0.62	0.350	41.00	75.00±0.07
B3	552.3±0.70	0.427	35.70	41.86±0.30
B4	207.5±1.30	0.365	40.80	54.41±0.37
B5	336.2±0.75	0.362	44.60	64.10±0.13
B6	378.6±0.36	0.314	42.70	64.20±0.10
B7	378.6±0.36	0.314	41.00	64.20±0.10
B8	308.4±0.50	0.365	47.30	47.59±0.18
B9	108.5±1.22	0.290	37.00	58.16±0.03
B10	239.1±0.26	0.212	48.20	63.03±0.21
B11	222.2±1.63	0.282	41.00	52.25±0.22
B12	284.5±0.26	0.389	44.00	57.95±0.20
B13	378.6±0.36	0.314	41.00	64.20±0.10
B14	378.6±0.36	0.314	42.20	64.20±0.10
B15	152.6±0.26	0.238	45.60	66.96±0.26
B16	378.6±0.36	0.314	41.00	64.20±0.10
B17	177.0±0.60	0.321	33.60	44.62±0.06

Particle size and entrapment efficiency data are expressed as mean±S.D (*n*=3)

Table 5: Summary of model fitting and statistical summary

Response	Suggested Model	Suggested P-Value	Suggested F- Value	Lack of Fit	R^2	Adjusted R^2	Predicted R^2	Adequate precision
Particle size (Y1)	Linear	2.60	0.0971	-	0.3746	0.2302	–0.1930	4.8941
Zeta Potential (Y2)	Linear	0.0001	15.85	0.0295	0.7853	0.7358	0.5626	10.5345
PDI (Y3)	Linear	0.2663	1.48	-	0.2544	0.0824	–0.5344	4.1556
Entrapment efficiency% (Y4)	Quadratic	0.0809	3.44	-	0.8493	0.6554	3473.57	4.1411

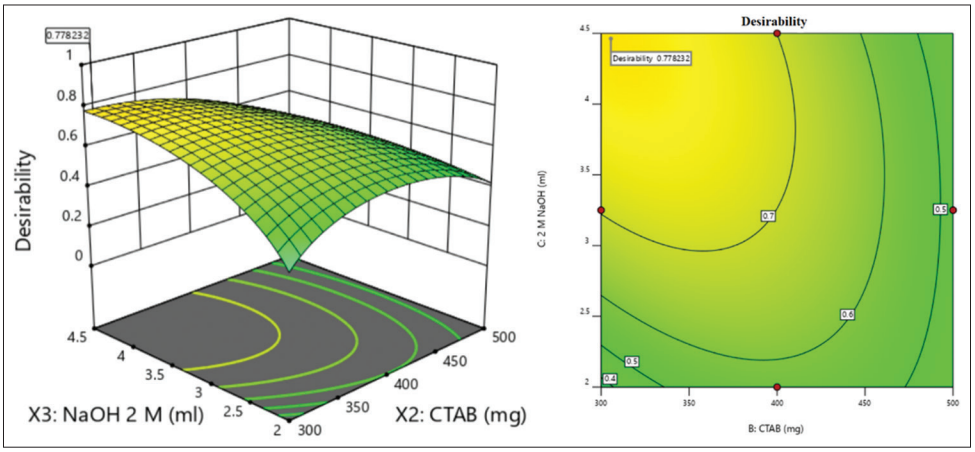


Figure 7: Response surface and contour plot of maximum desirability of mesoporous silica nanoparticles

as TEOS, CTAB, and NaOH 2 M was done in the range of 3–6 mL, 300–500 mg, and 2–5 mL, respectively. The response variables, i.e., minimum particle size, minimum PDI, required zeta potential, and entrapment efficiency were selected for evaluation. The design expert software suggested several solutions, with various desirabilities. Solution with maximum desirability, i.e., 0.778 was selected as the optimized formulation and the agomelatine-loaded mesoporous silica nanoparticles were prepared. The desirability contour and response surface plot predicting the formulation with maximum desirability are shown in Figure 7. On the above-mentioned design, it was confirmed that the entrapment efficiency followed the quadratic model while the particle size, PDI, and zeta potential followed the linear model. The polynomial equation in terms of actual factors was generated to demonstrate the relationship between the formulation variables. The polynomial equation of optimized mesoporous silica nanoparticles is shown in Table 6.

Table 6: Polynomial equation for response variables

Response variable	Polynomial equation
Particle size (Y1)	98.66A–1.12B-23.34C
PDI (Y2)	0.0420A–0.0282B–0.0115C
Zeta potential (Y3)	–0.2375A+4.83B–0.0125C
Entrapment efficiency % (Y4)	–7.10A+5.33B+2.57C+3.36AB–1.96AC–6.31BC-0.8888A^2–5.54 B^2–6.25C^2

Figures 8-15 of the response surface graph show that with increasing CTAB concentration, the particle size of mesoporous silica nanoparticles decreases whereas with increase in the concentration of TEOS, the particle size increases. CTAB prevents the agglomeration of particles and forms a layer around the surface of particles which

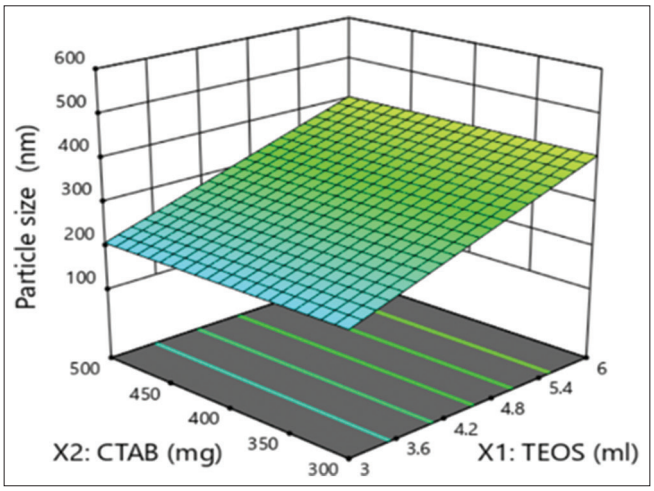


Figure 8: Response surface plot showing the effect of concentration of CTAB and TEOS on the particle size of mesoporous silica nanoparticles

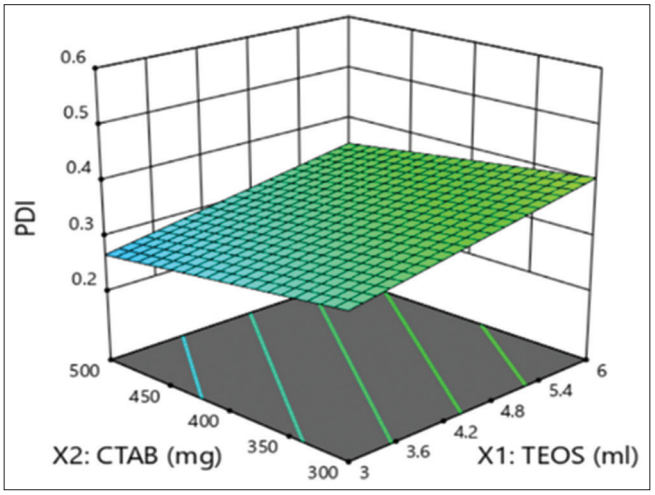


Figure 10: Response surface plot showing the effect of concentration of CTAB and TEOS on PDI of mesoporous silica nanoparticles

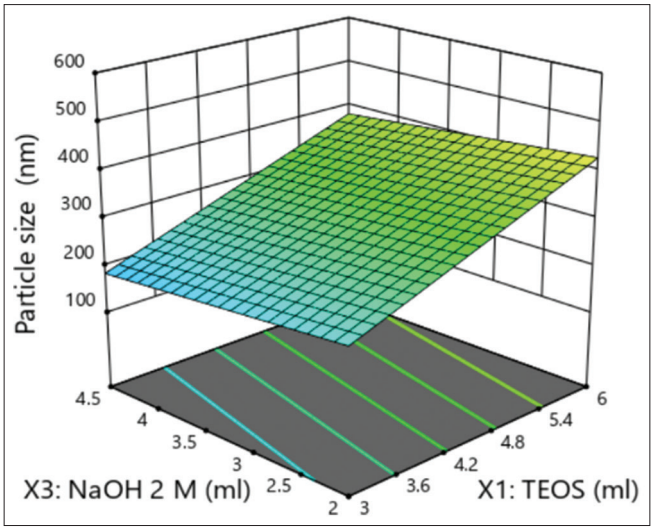


Figure 9: Response surface plot showing the effect of concentration NaOH 2 M and TEOS on the particle size of mesoporous silica nanoparticles

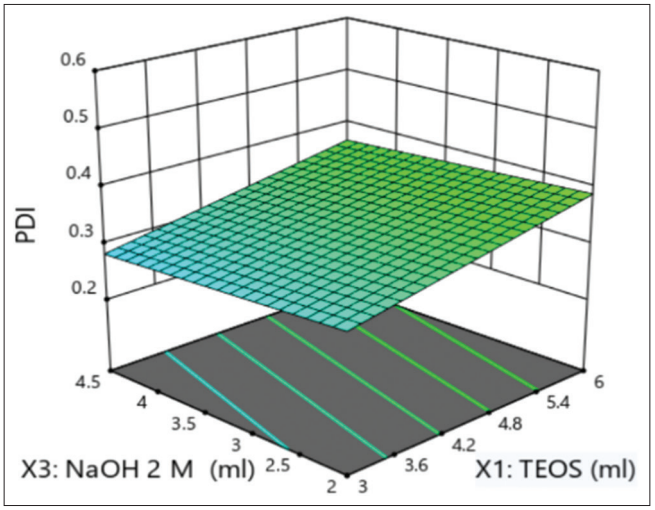


Figure 11: Response surface plot showing the effect of concentration of NaOH 2 M and TEOS on PDI of mesoporous silica nanoparticles

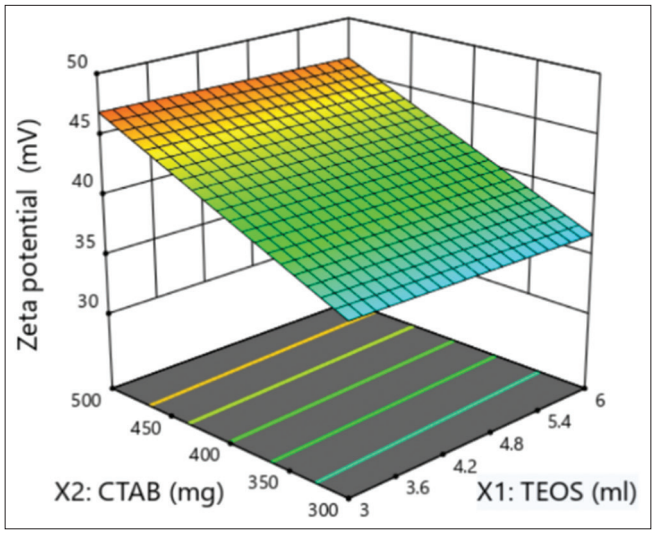


Figure 12: Response surface plot showing the concentration of CTAB and TEOS on the zeta potential of mesoporous silica nanoparticles

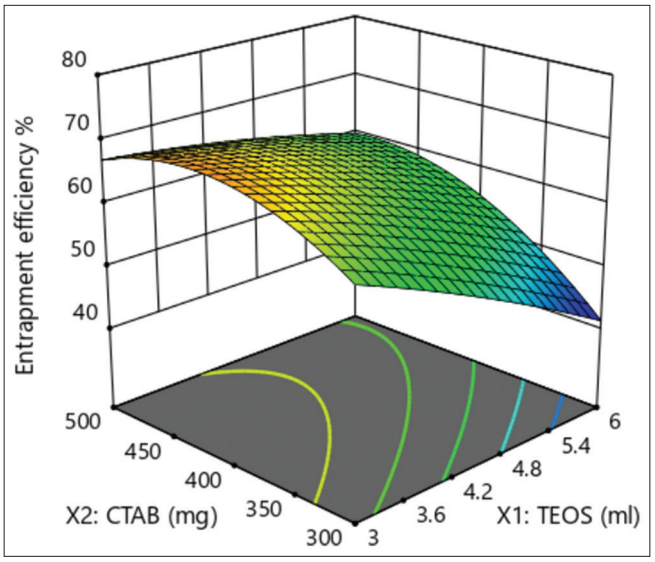


Figure 14: Response surface plot showing the effect of concentration of CTAB and TEOS on entrapment efficiency % of mesoporous silica nanoparticles

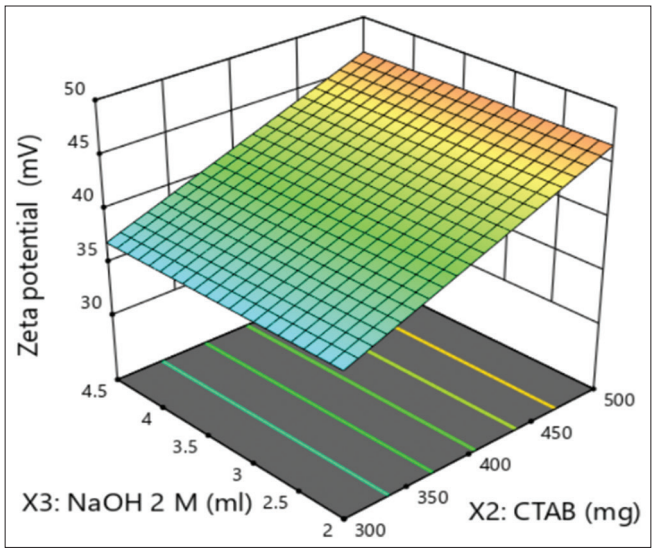


Figure 13: Response surface plot showing the effect of concentration of NaOH 2 M and CTAB on zeta potential of mesoporous silica nanoparticles

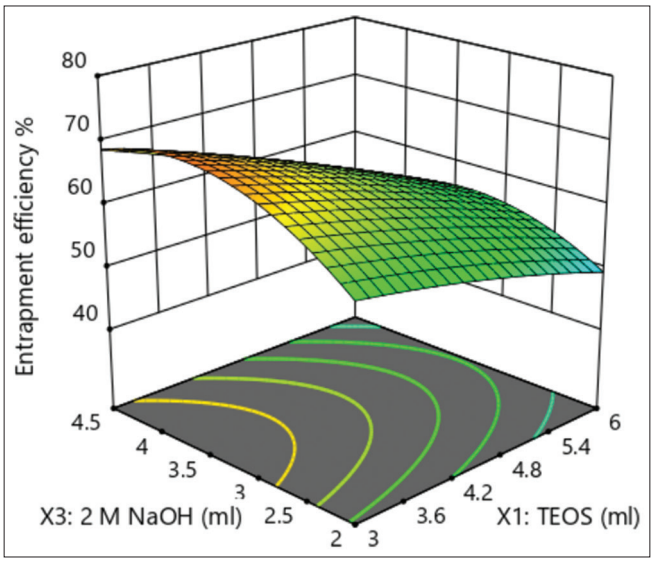


Figure 15: Response surface plot showing the effect of concentration of NaOH 2 M and TEOS on entrapment efficiency % of mesoporous silica nanoparticles

reduces the surface energy and prevents the interaction of particles which involves in particle size reduction. On increase in concentration of TEOS, the rate of nucleation of nanoparticles increases with concentration which results in the growth of particle size. Zeta potential on nanoparticle suspension is a measure of charge and corresponds to the physical stability of the suspension. When a large amount of CTAB is used, then it results in stable particle formation and distribution by forming well-dispersed micelles. The charge on the surface of CTAB is positive as it belongs to the category of quaternary ammonium surfactant, therefore, the zeta potential increase with increased concentration of CTAB decreases with increasing the concentration of TEOS and NaOH as it is negatively charge. This shows that the mesoporous silica nanoparticle suspension is stable. For loading the drug into the pores of the mesoporous silica nanoparticles, a high surface area of mesoporous silica nanoparticles would be required. A high concentration of TEOS forms thicker wall around the surface of micelles and reduces surface area which decreases the entrapment of the drug. On increasing the CTAB concentration, well-dispersed micelles are formed which have high surface area and thus increase the entrapment of the drug into the pores of mesoporous silica nanoparticles as entrapment efficiency is one of the important parameters for the effective drug delivery of agomelatine.

Table 7: Predicted value and the experimentally observed value of dependent variables of optimized agomelatine-loaded mesoporous silica nanoparticles

Components		Quantity	
A: TEOS		3.30 mL	
B: CTAB		305.57 mg	
C: 2 M NaOH		4.40 mL	
Evaluation parameter	**Predicted value**	**Practically observed value**	**Relative error**
Particle size	185.66	166.7±0.40	10.21
PDI	0.309	0.367	15.46
Zeta Potential	37.499	43.30	18.77
Entrapment efficiency %	67.91	78.03±0.21	14.90

Data are expressed as mean±S.D. (*n*=3)

Table 8: Formulation batch code and particle size of polymeric microparticles

S. No	Formulation batch code	Particle size (µm)
1.	MP01	33.615
2.	MP02	57.943
3.	MP03	60.518
4.	MP04	70.536

Prediction of optimized formulation of mesoporous silica nanoparticles

In the present research work, the software shows predicted maximum desirability of 0.778 containing 3.3 mL of TEOS, 305.57 mg of CTAB, and 4.4 mL of 2 M NaOH as the final optimized batch. The procured optimized batch was prepared and evaluated for particle size, PDI, zeta potential, and entrapment efficiency. The practically observed results were compared to the predicted result as shown in Table 7.

Particle size, polydispersibility index, and zeta potential

The particle size polydispersibility index and zeta potential play a major role in their interaction and adhesion during biological uptake. The particle size and PDI of optimized mesoporous silica nanoparticles were found to be 166.7 nm and 0.367, respectively. The zeta potential indicates the stability of mesoporous silica nanoparticles suspension and it was found to be 43.3 mV which confirms the good stability of optimized mesoporous silica nanoparticles.

Microscopic characteristics of optimized mesoporous silica nanoparticles

Using a polarizing microscope (Leica), the morphological characteristics of optimized mesoporous silica nanoparticles were determined in which the image showed small spherical shape particles having pores. FE-SEM image revealed that developed optimized mesoporous silica nanoparticles are mono-distributed circular structures which are the property of mesoporous silica nanoparticles and show particle size in the range of 79 nm–261 nm.

3.5 Entrapment efficiency % and loading of drug

The entrapment efficiency and loading of drug molecules in mesoporous silica nanoparticles are the desirable characteristics of mesoporous silica nanoparticles. The entrapment efficiency and drug loading of the final optimized agomelatine-loaded mesoporous silica nanoparticles were found to be 78.03% and 36.52%, respectively.

DSC analysis of optimized mesoporous silica nanoparticles

The DSC analysis of pure drug sample, CTAB, physical mixture, optimized mesoporous silica nanoparticles, and agomelatine-loaded mesoporous silica nanoparticles was performed to find their crystalline and amorphous nature. The DSC of the pure drug sample shows an endothermic peak at 107°C which represents the melting point of drug and confirms the drug powder present in the crystalline state. CTAB and physical mixture show sharp distinct peak. DSC analysis of mesoporous silica nanoparticles does not show any peak. The absence of a crystalline peak in the agomelatine-loaded mesoporous silica nanoparticles shows that the drug was entrapped into the mesoporous silica nanoparticles. Thus, the DSC thermograph showed no endothermic peak which confirms the conversion of crystalline agomelatine into an amorphous form of drug-loaded mesoporous silica nanoparticles.

3.7 X-ray diffraction studies

XRD was done for agomelatine, mesoporous silica nanoparticles, and optimized agomelatine-loaded mesoporous silica nanoparticles to determine the physical state of drug in

Table 9: Time interval (min) and cumulative % drug release of polymeric microparticles

S. No	Time (min)	Cumulative % Drug release (MP01)	Cumulative % Drug release (MP02)	Cumulative % Drug release (MP03)	Cumulative % Drug release (MP04)
1.	0	0	0	0	0
2	15	20.87±0.74	16.68±0.58	11.91±0.21	18.35±0.30
3.	30	35.31±0.65	24.16±0.21	22.83±0.42	26.75±0.39
4.	60	46.10±1.21	45.72±0.44	44.96±0.30	38.86±0.65
5.	120	47.21±0.29	48.60±0.85	57.03±0.48	41.38±0.26
6.	240	54.77±0.49	56.67±0.59	61.37±0.39	43.47±0.38
7.	360	60.34±0.21	68.34±0.46	65.33±0.53	48.38±0.35
8.	480	67.66±0.48	73.94±0.47	66.37±0.55	55.20±0.27
9.	600	83.09±0.73	79.91±0.42	68.92±0.11	58.06±0.48

Cumulative % drug release data are expressed in mean±S.D. (n = 3)

mesoporous silica nanoparticles in which agomelatine shows crystalline peak. The X-ray diffractogram of agomelatine shows that sharp distinct peak at 6.29°, 9.20°, 12.54°, 17.01°, and 18.81° on 2θ reveals the crystalline behavior of agomelatine. In diffractogram of mesoporous silica nanoparticles and optimized agomelatine-loaded mesoporous silica, there was no peak observed which is an indication that mesoporous silica nanoparticles are amorphous in nature and agomelatine is present in mesoporous silica nanoparticles in amorphous form.

Particle size and PDI of polymeric microparticles

The particle size of the polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles was done using Malvern Mastersizer. The particle size of all 4 batches was found in the range of 30–75 µm. The size of polymeric microparticles depends on the concentration of ethyl cellulose used during the preparation of polymeric microparticles. The polymeric coating layer enlarges with an increase in ethyl cellulose concentration, producing microparticles with a greater particle size. The particle size of polymeric microparticle containing agomelatine-loaded mesoporous silica nanoparticles is shown in Table 8.

Microscopic evaluation of polymeric microparticles

Using polarizing microscopy, the morphological behavior of polymeric microparticles was determined. The image of microparticles showing a smooth surface and absence of any crystals confirms that optimized agomelatine-loaded mesoporous silica nanoparticles are completely incorporated into polymeric microparticles.

In vitro drug release

In vitro drug release of polymeric microparticles was performed on phosphate buffer pH 7.4. The controlled

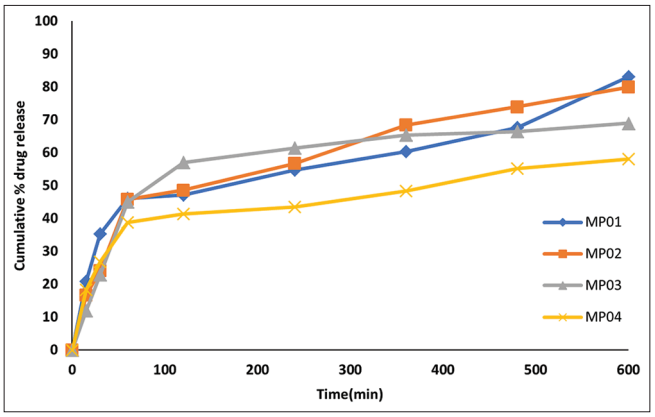


Figure 16: In vitro drug release of polymeric microparticles. All values shown in the graph are measured as mean ±S.D. (n = 3), Error bar indicates the S.D of replicate

release pattern of the polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles depended on the concentration of ethyl cellulose. On increasing the concentration of ethyl cellulose, the release rate of polymeric microparticles decreases. The % drug release of all the batches was found to be 83.09%, 79.91%, 68.92%, and 58.06%, respectively, because the concentration of ethyl cellulose from MSN-MP01 to MSN-MP04 increases; hence, release rate decreases with respect to time.

The *in vitro* drug release of polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles is shown in Table 9 and Figure 16. The data were fitted into zero order, first order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell model equation as shown in Table 10. The graph was plotted to analyze the drug release kinetic of prepared polymeric microparticles as shown in Figure 17. The regression coefficient (R^2) and release rate constant (k) of MSN-MP02 followed the Higuchi model as it shows highest regression coefficient (R^2) 0.9287 because among all release kinetics graph as the formulation shows initial burst release followed by continuous release pattern.

Table 10: Drug release kinetic data and model fitting of optimized agomelatine-loaded mesoporous silica nanoparticle

S. No.	Drug release kinetic model	Equation	K	R^2
1.	Zero order	Q_o-Q_t=K_ot	1.28×10^{-1} mol/min	0.7837
2.	First order	log Q=log Q_o-kt/2.303	-1.1×10^{-3} mol/min	0.9094
3.	Higuchi	Q_o- Q_t=$Kt^{1/2}$	3.50×10^{-1} mol/min	0.9287
4.	Korsmeyer-peppas	log (Qo- Q_t) = log k-n log t	6.39×10^{-1} mol/min	0.9119
5.	Hixson-Crowell	$Q_o^{1/3}$- $Q^{1/3}_t$=Kt	3.1×10^{-3} mol/min	0.8725

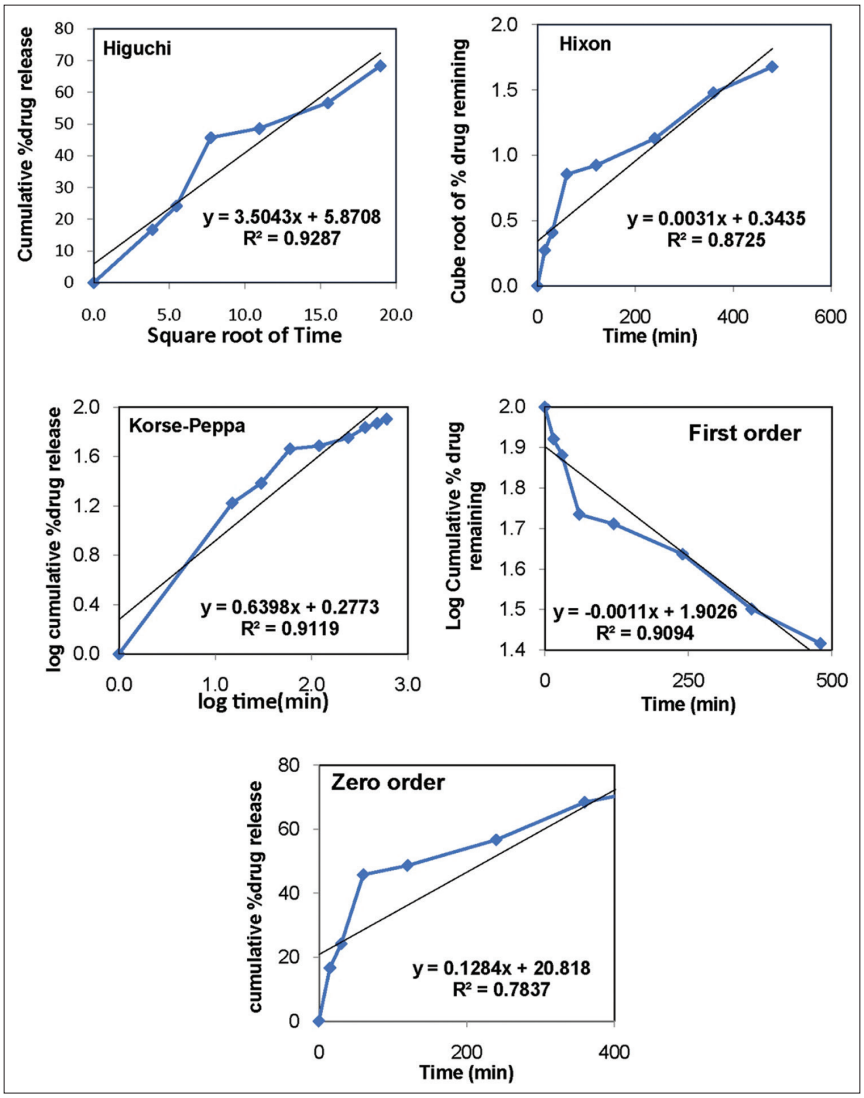


Figure 17: Kinetic modeling of polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles

CONCLUSION

Mesoporous silica nanoparticles have attracted great attention, especially in the field of nanotechnology. Polymeric microparticles containing agomelatine-loaded mesoporous silica nanoparticles were developed to improve the low bioavailability and poor dissolution profile of agomelatine. The release rate of drug was achieved by incorporating mesoporous silica nanoparticles into polymeric microparticles for the treatment of depression. Mesoporous silica nanoparticles were successfully prepared through optimization by Box-Behnken design using Design Expert software. The optimized mesoporous silica nanoparticles show a mean particle size of 166.7 nm, 0.367 PDI, 43.3mV zeta potential, and 78.03% entrapment efficiency. Optimized mesoporous silica nanoparticles were evaluated for FE-SEM DSC and XRD, in which the SEM image reveals the spherical shape particle while DSC analysis shows an amorphous peak

of optimized agomelatine mesoporous silica nanoparticles as a drug was completely encapsulated into the pores of mesoporous silica nanoparticles. Polymeric microparticles of agomelatine-loaded mesoporous silica nanoparticles were prepared using ethyl cellulose and PVA. The polymeric microparticles were evaluated for particle size, microscopic evaluation, and *in vitro* drug release. The polymeric microparticle containing agomelatine-loaded mesoporous silica nanoparticles (MP-02) shows the Higuchi model with a regression coefficient (R^2) of 0.9287. The release of polymeric microparticles shows an initial burst followed by a continuous release pattern. Hence, it can be concluded from the present research, that the oral bioavailability of agomelatine will improve by incorporating optimized agomelatine-loaded mesoporous silica nanoparticles into polymeric microparticle which results in controlled the release pattern of drug and hence reducing dosing frequency which is effective in the long-term therapy of depression.

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