Original Article

Design And Optimization of Midazolam Loaded Microemulsion Using Quality by Design (Qbd) Assisted Statistical Modelling

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Abstract

Introduction: More emphasize was given in the present work is application of QbD tools and DoE approach. The existing study was aimed towards development and characterization of Midazolam (MZM) loaded microemulsion (ME) for brain delivery through nasal route.
Methods: Different QTPP and CQAs were identified initially and REM was prepared. Further D optimal design was employed with concentration of Oil (X1), concentration of Smix (X2) and concentration of water (X3) as significant independent variables (IVs). Globule size (Y1), %Transmittance (Y2) and Viscosity (Y3) were kept as important CQAs.
Results: MLR models revealed that selected IVs have great impact on Y1 and Y3, especially main and interactive effect. The results were supported by contour plots (2D and3D) and predicated Vs actual plot. % PE values for check point batches were found less than 5% indicating accuracy of evolved MLR models. Further, control space was mapped from design space of overlay plot and out of control space, one batch was optimized and revised REM was prepared. Revised REM showed low risk potential of various IVs on selected CQAs. Optimized batch was tested for zeta potential, conductivity, Polydispersity index, viscosity, refractive index, isotropic number. Moreover, *ex vivo* permeation using sheep nasal mucosa in simulated nasal fluid (SNF). The study revealed that superior permeation than Midazolam suspension which is attributed to the incorporation of MZM into ME carrier.
Conclusion: So, in a nutshell, it can be concluded that application of QbD approach and DoE concepts in MZM loaded ME yielded design space which will be useful for error free scale up plan implementation.

KEYWORDS: Midazolam, QbD, Risk assessment, D-optimal design, Control space

INTRODUCTION

The treatment of brain disorders is the greatest challenge because of a variety of formidable obstacles in effective drug delivery and maintaining therapeutic concentration in the brain. General methods that can enhance drug delivery to the brain are, therefore, of great interest. An alternative central nervous system (CNS) drug delivery strategy that has received relatively little attention is the intranasal route. CNS disorders may obstruct the social and personal life of human being. Recent advances in new chemical entities (NCEs) into health sector for CNS disorders. Despite of the fantastic progress in drug discovery, the success of NCEs is limited due to its poor bioavailability. The issues to get desired bioavailability are poor solubility, poor permeability, excessive metabolism and improper targeting.[1]

Schizophrenia is one of the most threatening, collateral and fearsome diseases of all mental disorders. Schizophrenia is the disorder that not only arouses anxiety in the patient but also makes the caretakers anxious. Schizophrenic patient may behave differently which unlike reality. Distorted thoughts, feelings of fright, hallucinations and paranoia are the common symptoms of Schizophrenia.[2, 3] If this can be sustained over an extended period, the resulting stability may enable patients

to optimize engagement in personally meaningful activities, such as employment or education. Consequently, improved patient functioning may have a significant impact upon long-term prognosis.[4]

To get good and efficient treatment of CNS disorders including schizophrenia, the drug should reach to the brain which is a major limitation of current drug and dosage forms. The major barrier in the CNS delivery is Blood Brain Barrier (BBB).[5, 6] Hence, an alternative route of administration should be preferable. Also the treatment of brain disorders is the greatest challenge because of a variety of formidable obstacles in effective drug delivery and maintaining therapeutic concentration in the brain. A practical, noninvasive and alternative route of administration for drug delivery to brain circumventing blood brain barrier (BBB) can be achieved by intranasal administration.[4]

However, the major limiting factor for nasal drug delivery is the fact that most drug molecules diffuse poorly and slowly through the nasal mucosa and thus the desired levels of the therapeutic agent cannot be achieved. Also, the nasal cavity can only accommodate limited volumes, approximately 150 μL, per nostril and excess volume will drain out into the pharynx and be swallowed.[7] Here, nose to brain delivery approach to directly target brain via olfactory and trigeminal nerve cells through nose[8] which bypasses main permeation hurdle (BBB) and enter brain directly. To achieve higher permeation, the nature of drug affects significantly. Lipophilic drugs are prone to transport across barrier. Many CNS acting drugs falling into BCS class III and IV category failed to elicit anticipated therapeutics response due to poor lipophilicity.

Microemulsions as potential drug delivery systems offer good solubilizing properties and the ability to enhance drug permeation through biological membranes. It is essential to localize the formulation on a mucosal layer of the nasal cavity in order to enhance drug absorption and prevent rapid nasal clearance. Addition to this, it can prevent rapid nasal clearance of drug formulations. Nasal to brain delivery approach is successfully viable for many CNS acting drugs including antimigraine drugs (Zolmitriptan and Sumatriptan) and antidepressant drugs.[9-12]

Midazolam (MZM) is 8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5-a] [1,4] benzodiazepine chemically.[13] It is a short-acting drug in the benzodiazepine class. The drug is mainly prescribed for used for acute seizures, moderate to severe insomnia, and for inducing sedation and amnesia before medical procedures. It also has potent skeletal muscle relaxant, sedative, anxiolytic, amnestic, hypnotic and anticonvulsant properties.[14]

Systematic development of drug products employing the newer paradigm of Quality by Design (QbD), which is based on the principles of Design of Experiment (DoE), provides holistic understanding of product and processes to yield the best solution. To understand the impact of risk creating factors and to minimize such factor is a main process involved in QbD approach [15]. Design of experiment helps to reduce practical trials without losing the integrity of information. So, in the present study microemulsion based system of MZM was developed systematically employing recent Quality by Design approach provides the best potential solutions by providing formulation and process understanding.

MATERIAL AND METHOD

Materials

MZM was kindly gifted by Sun Pharma Advanced Research Company (SPARC), Vadodara. Labrasol, Transcutol P were procured from Gattefosse, France. Capmul MCM Captex was purchased from Abitec corp. Olive oil, Isopropyl Myristate, sunflower oil, glyceryl oleate, Tween 80 and, Propylene carbonate, Poly ethylene glycol-400 and propylene glycol was procured from Himedia Ltd. Ultrapure water (Milli-Q® Integral system, Merck Millipore, Billerica, MA) was used throughout the study.

Methods

Application of QbD tools

Defining the QTPP & CQAs [16, 17]

Quality by Design (QbD) is a crucial part of the modern pharmaceutical industry.[18] In first phase of QbD paradigm, quality target product profile (QTPP) must be defined with succinct of important quality properties for designing ME for MZM to achieve maximum therapeutics benefits. To achieve, defined QTPP, different critical quality attributes (CQAs)

were indemnify, which affect the quality of product (i.e. Globule size, % Transmittance, Viscosity). Table 1 presents different elements of QTPP used for the development of MZM ME.

Table (1): Quality Target Product Profile (QTPP) for MZM loaded ME

QTPP	**Target**	**Justification**
Dosage form	Microemulsion (ME)	Lipophilic composite will help in permeation mucosa (Suitable for nasal to brain delivery)
Route of administration	Nasal (nasal to brain)	Recommended route for CNS drugs for better targeting
Dosage strength	5 mg	5 mg MZM was added in a single ME formulation
Ex vivo permeation	Enhanced permeation profile	Required for higher flux across membrane
Stability	At least 6 months	To maintain therapeutic integrity of API for stipulated storage period
Container closure system	System qualified as suitable for this drug product	Needed to achieve the targeted shelf life

Table 2 summarizes the justification of various variables as CQAs of the product/process affecting the performance of MZM loaded ME (s) along with apt justification (s) for each of them.

Table (2): Critical Quality Attributes (CQAs) for MZM loaded ME with justification

Quality attributes of the drug products	**Target**	**Is this a CQA?**	**Justification**
Physical attributes • Color • Odor • appearance	Transparent No unpleasant odor Acceptable to patients	No	They are not directly associated to efficacy and safety

Assay and content uniformity	100%	No	MEs are thermodynamically stable and monophasic system so moderately critical
Globule size	Less than 200 nm	Yes	Smaller size will help in permeation across membrane
Permeation	High	Yes	Higher permeation leads higher availability of drug at site of action in brain
Viscosity	Moderate	Yes	Less viscous ME will drain from nose and High viscosity may hinder permeation across mucosa
% Transmittance	High	Yes	Transparency of ME is based on high transmittance

Risk assessment studies

A fishbone diagram (Figure 1) was drawn for better understanding the impact of different possible variable on quality of product (CQAs) considering all criteria of proposed MZM based ME. A risk estimation matrix was prepared to select high risk factors associated with each CQA of MZM loaded ME by giving high, medium and low values to each studied factor. High risk factors were further evaluated in experimental design.

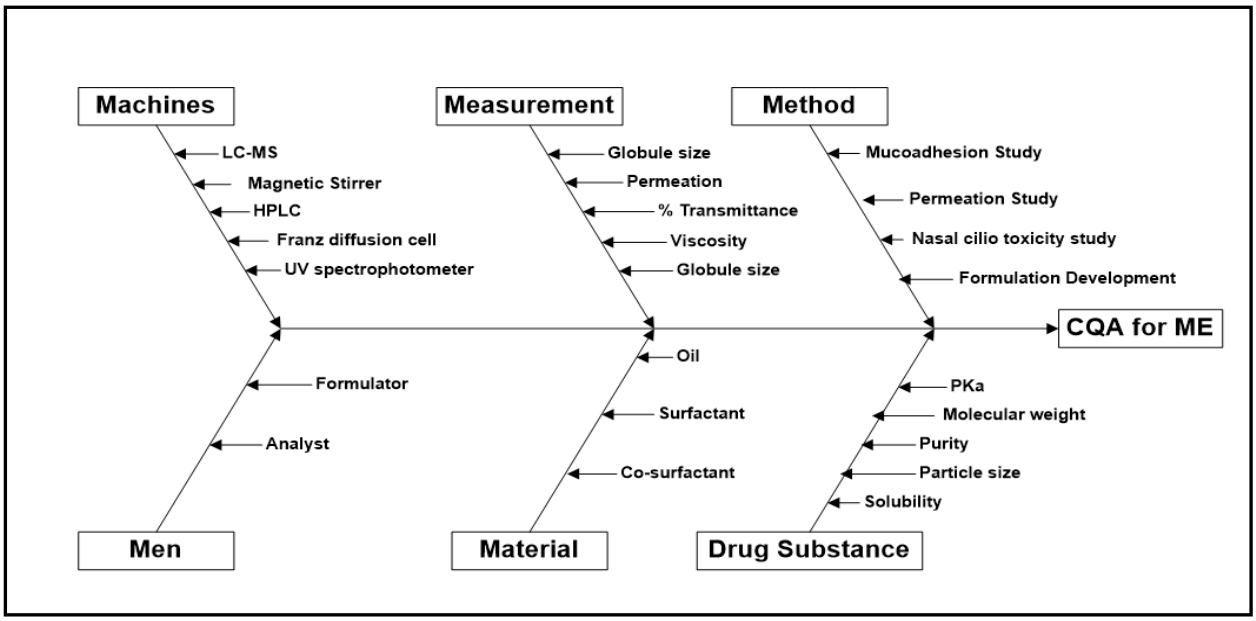

Figure (1): Fishbone diagram of MZM loaded ME

Application of D-Optimal design [19, 20]

Based on the detail study of REM, significant IVs were further studied in detail applying constrain mixture design i.e. D-optimal design. The batch layout of D-optimal design is shown in Table 3 with actual values in % v/v. MZM loaded ME was prepared using Capmul MCM (oil), Tween 80: PEG-400 (Smix) and water by titration method.

Table (3): Formulation layout of D optimal design batches

Formulation Batch code	Independent variable (Actual value)		
	Oil (X1)	Smix (X2)	Water (X3)
M1	37.5	22.5	40
M2	25	25	50
M3	45	15	40
M4	25	25	50
M5	28.125	26.25	45.625
M6	45	15	40
M7	35	15	50
M8	33.3333	25	41.6667
M9	25	15	60
M10	35	15	50
M11	38.125	18.75	43.125
M12	28.125	18.75	53.125
M13	30	30	40
M14	25	30	45
M15	25	15	60
M16	37.5	22.5	40
CKP1	34.33	24.59	41.07
CKP2	25.63	27.28	47.08

Validation of model

The D-optimal design was further validated by standard error graph (SEG). The later is a contour plot presenting standard error of expectation for areas in the design space (DS). If, the SE is less than 1, the design can be considered for acceptable measure.

Confirmation of model

Further, the derived multiple linear regression equations (models) were confirmed by preparing check point batches. The composition of check points batches are given in Table 3. The results of predicted and observed values were compared. Percentage error (%PE) was measured by following formula.

Percentage error (%PE) = [(Experimental value-Predicted value)/Experimental value]*100

Ex vivo permeation study of optimized MZM loaded ME [21]

Freshly excised sheep nasal mucosa was obtained from local slaughter house and dipped immediately in simulated nasal fluid (SNF of pH 6.5) was composed of 7.45 mg/ml NaCl, 1.29 mg/ml KCl and 0.32 mg/ml $CaCl_2.2H_2O$. *Ex-vivo* drug diffusion study was performed using a Franz-type diffusion cell (FDC; 10 mm D, mucosa thickness; 0.20 mm). The tissue was stabilized in SNF. The receptor compartment was filled with 10 ml SNF to maintain sink condition. MZM loaded ME was poured in donor compartment. Constant stirring was maintained to simulate blood flow. Similarly, e*x-vivo* diffusion of pure drug (MZM suspension) was conducted. Aliquots were sampled from the receptor compartment at periodic time intervals, filtered (0.45 mm nylon filter) analysed for drug content. Aliquots were replaced by an equal volume of SNF. The cumulative amount of MZM diffused through the mucosa was determined.

RESULT AND DISCUSSION

Application of QbD tools

Risk assessment

Table 4 exemplifies the REM for MZM loaded MEs. Based on impact of different materials and process variables on CQAs, different grades were assigned. Based on study, it was found that concentration of Oil, Smix and water impacting remarkably on selected CQAs with high risk. Low and medium risk factors were ignored in further study.

Table (4): Initial risk assessment for MZM loaded ME

Drug product CQAs	Independent variables						
	Conc. of Oil	Conc. of Surfactant	Conc. of Co surfactant	Amount of water	Stirring speed	Temperature	Stirring Time
Globule size	High	High	High	High	Medium	Low	Low
Permeation	High	High	High	Medium	Low	Low	Low
Drug loading	High	High	Medium	Medium	Low	Low	Low
Viscosity	High	High	High	Medium	Low	Medium	Low
Transmittance	High	High	High	High	Low	Low	Low

Application of D-Optimal design

Validation of model

In D-optimal design, the standard error as depicted by SEG was less than 1, which confirms the suitability of the design for given variables set. The SEG for applied D-optimal design is presented in Figure 2.

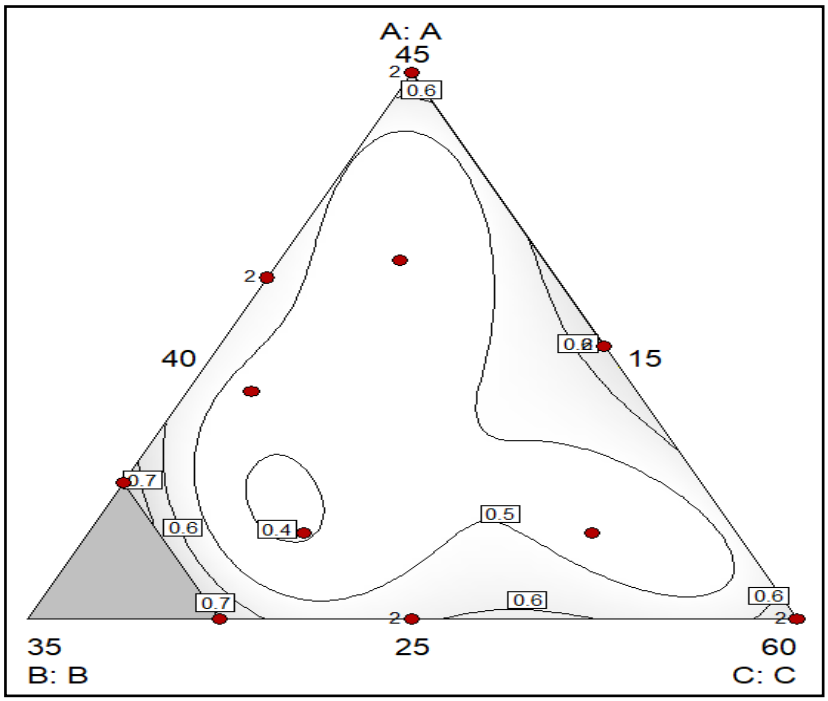

Figure (2): SEG plot of applied D optimal design

Optimization by D optimal design:

The results of CQAs of mixture design (D optimal) batches are shown in Table 5.

Table (5): Results of CQAs of D optimal batches

Batch	X1	X2	X3	Globule size Y1	Zeta potential Y2	%T Y3
M1	37.5	22.5	40	123.4	-23.64	98.32
M2	25	25	50	100.24	-22.36	99.31
M3	45	15	40	145.32	-21.87	95.34
M4	25	25	50	96.16	-19.52	99.12
M5	28.125	26.25	45.625	120.14	-16.74	99.74
M6	45	15	40	163.21	-27.34	94.32
M7	35	15	50	145.27	-29.41	95.36
M8	33.33	25	41.66	105.34	-22.35	99.31
M9	25	15	60	121.45	-21.24	99.84
M10	35	15	50	130.45	-33.24	96.41
M11	38.125	18.75	43.125	140.32	-34.21	91.24
M12	28.125	18.75	53.125	150.34	-42.15	90.14
M13	30	30	40	94.21	-20.31	99.84
M14	25	30	45	95.14	-22.34	99.34
M15	25	15	60	124.36	-24.18	98.96
M16	37.5	22.5	40	122.41	-19.24	99.31

CKP1	34.33	24.59	41.07	116.34	-19.86	98.69
CKP2	25.63	27.28	47.08	105.42	-20.45	99.12

The result of ANOVA analysis depicting relation and significance of independent variables on selected responses are shown in following Table 6. The results indicate that the applied model is significant and can be used further. The best suited model was confirmed by R^2 value from linear, 2FI and Quadratic for each selected response (Y_1-Y_3). Additionally, non-significant terms were omitted (NS; P>0.05) to strength the predictive power of model.

Table (6): ANOVA analysis (IVs Vs CQAs)

Source	**Y1**			**Y2**			**Y3**		
	Sum of Squares	**F Value**	**P value**	**Sum of Squares**	**F Value**	**P value**	**Sum of Squares**	**F Value**	**P value**
Model	6514.3	15.75	0.0008	616.2	9.54	0.0038	145.45	63.06	< 0.0001
Linear Mixture	5330.45	51.54	< 0.0001	152	9.41	0.0104	47.65	82.64	< 0.0001
AB	47.76	0.92	0.3685	2.2	0.27	0.6181	3.27	11.35	0.0119
AC	0.92	0.018	0.8977	78.41	9.71	0.0169	2.03	7.03	0.0328
BC	11.4	0.22	0.6529	0.91	0.11	0.7463	4.79E-04	1.66E-03	0.9686
A^2BC	1.73	0.034	0.86	65.82	8.15	0.0245	21	72.83	< 0.0001
AB^2C	6.27	0.12	0.7379	165.87	20.54	0.0027	27.91	96.79	< 0.0001
ABC^2	828.22	16.02	0.0052	206.11	25.53	0.0015	47.87	166.01	< 0.0001
Residual	361.95			56.52			2.02		
Lack of Fit	79.06	0.7	0.54	16.19	1	0.4301	0.052	0.066	0.9374
Pure Error	282.89			40.33			1.97		
Cor total							147.46		

From different models derived from ANOVA analysis, it can be stated that all responses are affected by main effects of independent variables. Moreover, the predicted Vs Actual plots (Figure 3-5) of selected responses (Y1-Y3) evidenced that there is a strong variation in results of Y1 and Y3, as the data are uniformly scattered on line. CQA, Globules size (Y1) is also affected by interaction of selected IVs. So, incorporation of Oil, Water and Smix into single system shows remarkable impact on Y1. This can be proven by MLR equation of Y1, contour plot (2D and 3D) and predicted Vs Actual plot. Contour plot (3D) shows curvature which indicates interaction of all terms on Y1. As the proportion of oil increases, the globule size increases.

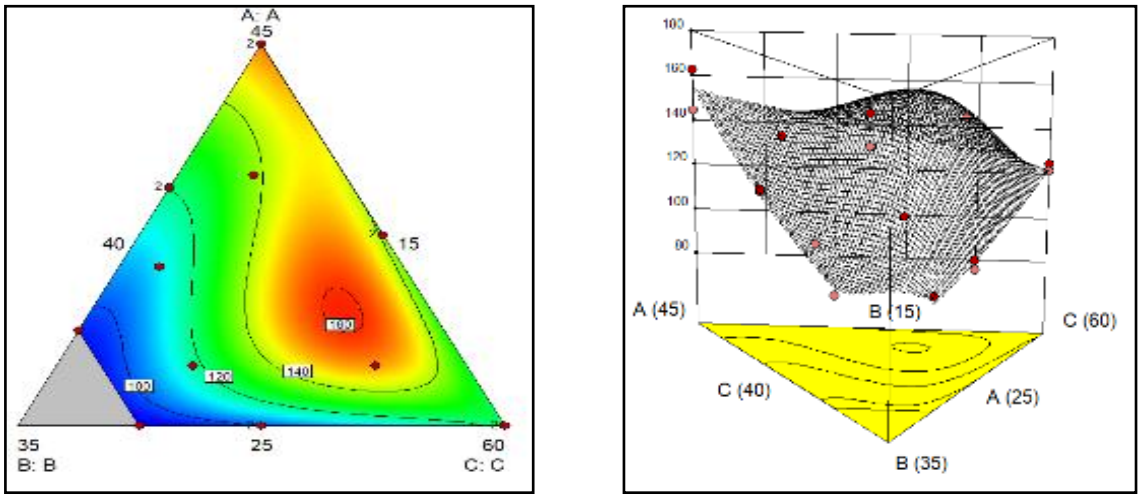

Figure (3): Contour (2D and 3D) and predicted Vs actual plot relating Y1

Other CQA, %Transmittance (Y2) was affected remarkably by set of selected excipients. The data set show negligible variation amongst all, which indicates majority of batches show monophasic system (microemulsion).

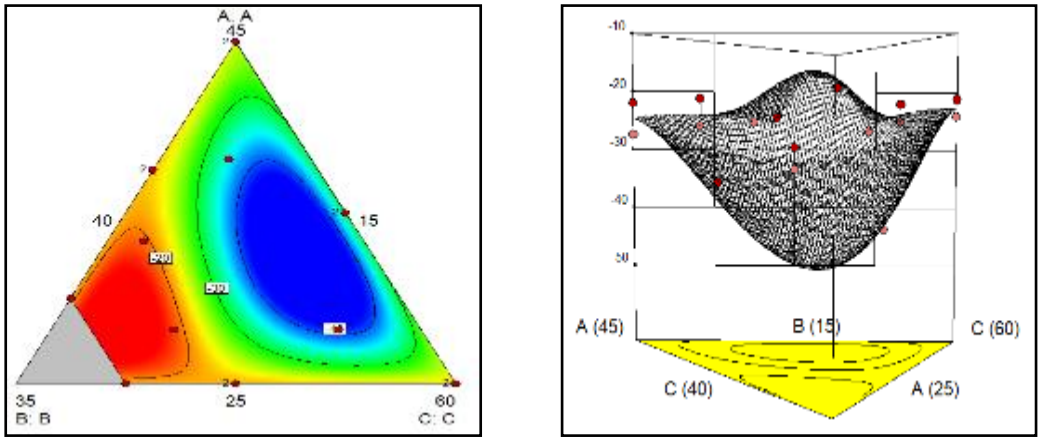

Figure (4): Contour (2D and 3D) and predicted Vs actual plot relating Y2

Other CQA, Viscosity (Y3) was greatly affected by selected independent variable. MLR equation of Y3 shows that along with main effects, the interactive terms also have significant impact on Y3. The contour plot (2D and 3D) indicates sensitivity of IVs on Y3. Considering the final application of MZM loaded ME into nasal delivery, viscosity impacts at a great extent for successful retention in nasal cavity and permeation through olfactory region.

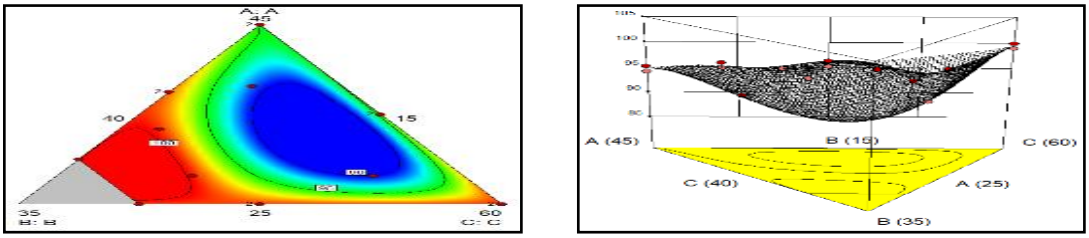

Figure (5): Contour (2D and 3D) and predicted Vs actual plot relating Y3

Based on all contour plots, one overlay plot (Figure 6) was plotted to define design space (DS) with check point batch flag and also control space (CS) was derived from the desired yellow region. The aim of deriving CS was to set robust conditions which could give risk free formulation.

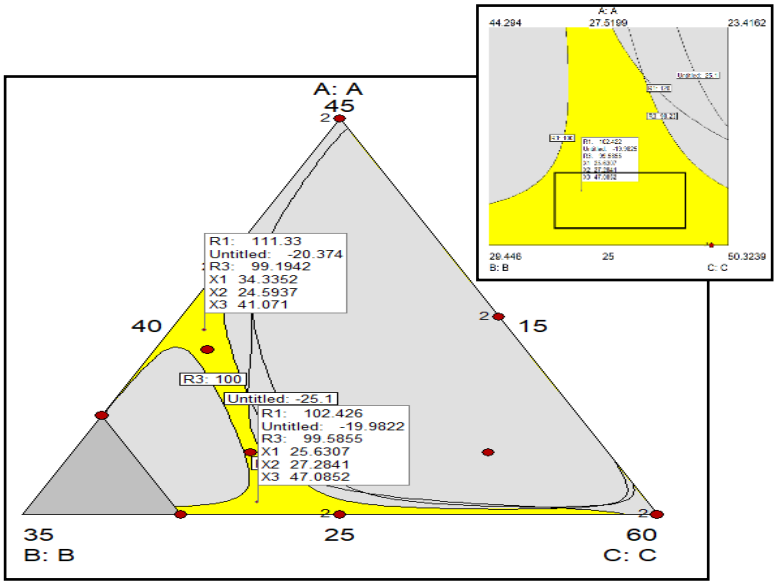

Figure (6): Overlay plot with control space

Check point batches (CKP1 and CKP2) were outlined from the extreme ends of overlaid region to check the validity of evolved models. PE in % of check point batches were calculated and were found below 5%, which indicates the authenticity of developed models. Based on control space revised risk assessment study was performed.

The optimized Microemulsion was further evaluated for other physicochemical characteristics are provided in the Table 7 for MEs. The result indicates that MEs are optically isotropic, clear and an oil in water in nature with uniform droplet size distribution. The transparency of MEs were proved by refractive index and transmittance values. The conductivity of optimized formulation was 137.4±4.36 µs/cm for MZM which indicates O/W nature of MEs.[22] Dye solubilization test also shows rapid incorporation of water-soluble dye, confirming O/W type of microemulsion. The pH of optimized formulation was very close to nasal pH, thus devoid of nasal irritation. Centrifugation of optimized ME at 4000 rpm does not show phase separation, confirming stable characteristics of MEs. PDI value near to zero indicated that the system has uniform particle distribution.

Table (7): Results of different testes of MZM loaded ME

No.	Tests	MZM ME
1	% Transmittance (256 nm)	99.24±0.14
2	% Assay	99.67±0.22
3	Refractive index	1.38
4	Isotropic nature	Optically isotropic
5	Globule Size (nm)	103.52 ± 3.47
6	Zeta Potential (mv)	-20.34 ± 1.25
7	pH	6.23 ± 0.4
8	Viscosity(cps)	19.37 ± 3.47
9	Conductivity(µs/cm)	137.4±4.36
10	PDI	0.124

Ex vivo permeation study of MZM loaded ME

The results of *ex vivo* permeation study of MZM loaded ME is presented in Table 8 and Figure 7. The results indicate that there is significant difference permeation profile of MZM loaded ME and MZM suspension. This variation is due to accommodation of MZM into vesicular lipidic system which favors permeation across membrane.

Table (8): Ex vivo diffusion study for MZM loaded ME and MZM suspension

Time(min)	Percentage drug diffused (%w/w)	
	MZM Suspension	MZM loaded ME
30	3.21	11.32
60	8.47	26.34
90	15.34	39.41
120	20.47	52.34
150	26.34	62.34
180	32.14	71.3
210	35.24	81.24
240	44.31	86.37
270	52.34	90.35
300	58.36	98.31

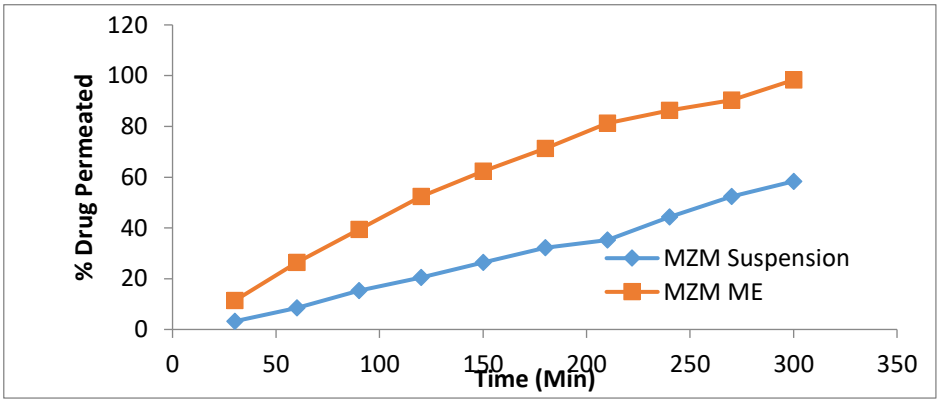

Figure (7): % Drug permeated from MZM formulations

CONCLUSION

The present study to prepare ME based nasal formulation for Midazolam, using QbD and DoE approaches have assisted greatly for judicious selection of factors. D-optimal mixture design was adopted with different trials and impact of factors IVs on CQAs and also suggested that how it would change if one factor changes slightly. REM has helped to identify high risk factors. Role of selected lipophilic components for ME based system which facilitated better permeation across mucosa. Overall, statistical modeling has derived robust control space for manufacture of MZM loaded ME. Comparative *ex vivo* permeation studies revealed that MEs based formulation showed better permeation as compare to MZM suspension.

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