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Quality by Design (QbD) tool to evaluate and design in situ nasal gel of Midazolam

Abstract

Background: *An antiepileptic drug Midazolam was incorporated in in situ nasal gelling system (ISNG) to administer through intanasal route. Quality by Design (QbD) tool was utilized for development of ISNG by targeting critical components and parameters.*

Methods: *Different QbD components like* ***q****uality target product profile (QTPP) and critical quality attributes (CQA) were defined and identified with risk assessment for each. Critical material attributes (CMA) vitally affected CQA of product was defined and further processed with justification as well as mitigation strategy. While initial risk assessment and ishikawa diagram concluded with two significant CMA which majorly impact on all defined CQA of ISNG.*

Result: *The effect of two independent factors (Significant CMA) concentration of gellan gum (X_1) and concentration of carbopol 934P (X_2) on viscosity at non physiological condition (Y_1), viscosity at physiological condition (Y_2), mucoadhesive strength (Y_3), % cumulative drug release in 10 min (Y_4) and in 180 min (Y_5) (CQA of ISNG) were studied by integrating design of experiment (DoE). The data were statistically analyzed using ANOVA and overlay plot was generated by defining constrains for each CQA of ISNG. Furthermore the check point batch was used to validate overlay plot by comparing predicted vs actual responses. Finally risk associated with ISNG development was nullified to low level.*

Conclusion: *QbD concept based on DoE is utilized to target product profile and corresponding selection of variable of formulation during development of novel drug delivery system such as in situ nasal gel of Midazolam.*

Key Words: Quality by Design (QbD), Design of experiment (DoE), Overlay plot, In situ nasal gel, Midazolam

Background

Quality by design (QbD) is mandatory to utilize during development of any drug formulation guidance given by the International Conference on Harmonization (ICH-Q8, Q9, Q10) and different regulatory agency [1].

The main objective of study is to draw out the optimum formula for Midazolam in situ nasal gel (ISNG) by implementing Quality by Design (QbD) concept focusing on thorough understanding of product along with risk assessment involved in each stage of development. For development of new drug delivery system (NDDS), QbD emphasizes on Quality Target Product Profile (QTPP), Critical Quality Attributes (CQA) and risk involved for each CQA [1-5]. Each risk involved in development is justified and concluded with mitigation plan [1]. Based on prior knowledge and preliminary experiments data, CMA and CQA were refined further through experimentation to determine the significance of individual variables and its levels through a combination of design of experiment (DoE), mathematical models and statistical analysis [2]. Responses were selected based on preliminary experiments which played very essential roles into whole drug delivery through formulation. Selected dependent variables are very critical for consistent formulation performance [3]. The surface plots for each response were drawn which was further utilized to architect overlay plot. For designing overlay plot, constrain with possible wide range was selected for each response which gives desired drug release. Overlay plot was validated by taking one trial which replicates the responses value predicted by model [4-6].

Methods

Materials

Midazolam hydrochloride (MDL) BP was obtained as a gift sample from Sun Pharmaceutical Industries Ltd., Vadodara, India. Gellan gum USPNF was obtained from Kelcogel F, CPKelco, USA and Carbopol 934P USPNF from Corel Pharmachem, Ahmedabad. All other ingredients and reagents were of analytical grade and were used as received.

Assessment of Quality Target Product Profile (QTPP)

Table 1 proposes assessment of QTPP by defining its elements, corresponding target and justification for selection of each element.

Exploration of Critical Quality Attributes (CQA)

Table 2 dictates CQA for each quality attributes of drug product with target and justification of criticality.

Initial Risk Assessment of Formulation

Risk assessment was divided into three types as mentioned in table 3

The materials having high and medium risk on CQA were further investigated and optimized by using Design of Experiments (DoE). The components of any drug delivery system are API and excipients which are having different level of risk on CQA of product. Table 4 acknowledges about the possible risk on CQA due to Midazolam API properties. Possible risk due to excipients (Gellan gum, Carbopol 934P and water) is described in Table 5. There was no need for further optimization of low risk factors because of non-significant effect of those factors on CQA [5, 6].

Design of experiment

The experimental design was used for further optimization of CMA concerned with high risk regards to CQA of product. The present study used a three level two factor full factorial design for optimization. Statistical experimental design was performed using software DESIGN EXPERT® version 7.0.2.8 (Stat-Ease Inc., Minneapolis, USA). Response surface graphics were used to study the factor interaction between the considered variables.

Selection of factors

Components denoted as critical are Midazolam (API itself), gellan gum (Ion activated in situ gelling polymer), carbopol 934P (Mucoadhesive polymer) and water (vehicle for system) [7-10]. Midazolam as such has no polymorphic form. CMA of API such as particle size distribution and solubility were controlled by specification. Deionized water was utilized for development of formulation and quantity of water made fix from preliminary study by evaluating pourability and viscosity. Thus, water was eliminated as CQA for further study. Hence, gellan gum and carbopol 934P were further investigated in DoE. Table 6 contains details of independent variables (Gellan gum and carbopol 934P) with their level coded respectively (low, medium, and high represents −1, 0 and +1 respectively).

Selection of Responses

Quality attributes of drug product such as clarity, pH and gelling time were optimized in preliminary study and its limit captured into the finished product specification of ISNG. pH of ISNG was maintained by using phosphate buffer pH 6.8. The Identification and assay were also part of the finished product specification. The only variables need to be optimized through DoE were viscosity non-physiological condition (Y_1), Viscosity at physiological condition (Y_2), mucoadhesive strength (Y_3), % cumulative drug release (% CDR) in 10 min (Y_4), and % CDR in 180 min (Y_5). Responses with their defined constrains are covered in Table 6 [11, 12].

A total of 9 experimental runs were required for analyzing the interaction of each level on formulation characters (Table 7). The response (Y_i) in each trial was measured by carrying out a multiple factorial regression analysis using the quadratic model:

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

Where, Y_i is the responses; b_0 is the arithmetic mean response of all trials; and bi is the estimated coefficient for factor Xi. The main effects, X_1 and X_2, represent the average value of changing factor one at a time; X_1X_2 represent the interaction terms and the polynomial terms X_1^2 and X_2^2 are used to assess nonlinearity.

Preparation of MDL in situ nasal gel

A weighed amount of gellan gum (0.2%, 0.5%, 0.8% w/v) and carbopol 934P (0.1%, 0.3%, 0.5% w/v) were mixed properly and dissolved in half quantity of preheated (At 100°C) deionized water [12]. Thereafter, the solution was cooled at room temperature. 5.0%w/v of MDL was dissolved in remaining one fourth quantity of deionized water. The drug solution was mixed with polymeric solution; pH 6.8 was maintained by adding few required drops of phosphate buffer pH 6.8 and make up to volume by adding required quantity of deionized water.

Overlay plot and check point batch

The overlay plot (Fig 4) of the responses generates an optimized area, as per the desired criteria defined as constrains termed for each response having upper and lower limit in Table 6. Working within the overlay plot is not considered as a change. Movement out of the design space is considered to be a change and initiate a regulatory post approval change [6].

One check-point batch was prepared on the basis of generated overlay plot of responses to validate the same. The batch detail is illustrated in table 8 and corresponding responses are captured in table 9.

Result and Discussion

Assessment of Quality Target Product Profile (QTPP) and exploration of Critical Quality Attributes (CQA)

QTPP was aimed for better patient compliance and to improve bioavailability of drug with consistent formulation for novel drug delivery system. CQA of products were the variable parameters of formulation which were most likely to be affected by any minor change in formulation or due to process and CQA must be

controlled in defined range. The concept of QbD application in development was to give scientific direction by defining QTPP and to control critical material attributes for consistent CQA.

Dosage form

A major problem with nasal solution is its rapid clearance and in nasal gel difficulty in administration due to high viscosity and dose accuracy. In situ gelling system rectifies both problems related to administration. In situ gels are liquid before administration and convert to a firm gel when instilled into the nasal cavity under influence of stimuli (Temperature, ions or pH). In situ gel prolongs the contact time between the drug and the absorptive sites in the nasal cavity as well as also release drug slowly and continuously. The convenience of intranasal administration and the social acceptability may make intranasal midazolam the preferred treatment of seizures in the prehospital setting. Therefore in situ gelling system was picked up for Midazolam delivery [7-10].

Route

Midazolam is usually given in the form of intravenous or intramuscular injections, but parenteral route has minimal patient compliance. Oral administration of midazolam is preferable, but it undergoes extensive first pass metabolism in the liver, owing to which its oral bioavailability ranges from 15% to 27% in children and from 31% to 72% in adults. In such case, administration of midazolam via transmucosal route is advantageous. Nasal route is one of the most important among them in terms of drug absorption and bioavailability of the drug for systemic effect. Intranasal midazolam delivers drug directly to the blood and cerebrospinal fluid via the nasal mucosa and provides better seizure control in pediatric status epilepticus. Hence nasal route was selected for formulation [11-18].

Dose strength

Available parenteral formulations of MDL are having 5mg and 10mg of strength. To make dosage form comparable with market formulation and for safety purpose lower strength was selected for further development.

Description

Formulation should be transparent clear sol form to meet patient compliance and quality (Free from particulate matter and undissolved material). Clarity of sol directly met to description requirement. Clarity did not affect the efficacy of product [19].

Drug product quality attributes

Identification

Identification was done by UV spectrometry as Midazolam gives UV maxima at specific wavelength 218 nm from literature [9]. This attribute is a critical for safety and efficacy. But formulation and process variables do not impact identity; this can be effectively controlled by the quality management system.

Assay

Assay limit for formulation was kept 90%-110% based on market requirement. Assay of formulation is critical because it may be varying due to formula composition and process.

pH

pH of ISNG should be pH 6.8 same as physiological condition of nasal to avoid irritation during instillation. Alteration in pH might lead pregelling during storage and transport. pH control by using buffering agent is advisable in such case [18].

Gelling time

Based on preliminary study, sol should be converte to gel form to eliminate rapid clearance problem from nasal cavity and to get desirable initial drug release from sol form [12].

Viscosity at Non-physiological condition

Preliminary study regarding pourability, syringibility and injectability suggested maintaining viscosity of sol form not more than 240 cps. Hence the amount of polymers used in formulation should be taken to get defined viscosity of sol form [20].

Viscosity at physiological condition

Preliminary study to decide the concentration range of polymer which released drug in predefined amount after administration concluded keeping viscosity at physiological condition not more than 1750 cps [21].

Mucoadhesive strength

To avoid the rapid clearance from nasal cavity and enhance residence time of drug on surface helping ultimately in penetration of drug into systemic circulation, requirement for mucoadhesive strength was set not more than 20g [9].

Percentage cumulative drug release (%CDR) in 10 min

% CDR in 10min should not be more than 50% to administer loading dose of MDL and to achieve rapid therapeutic effect initially to control emergency condition of status epilepticus [10].

Percentage cumulative drug release (%CDR) in 180 min

% CDR in 180min should not be less than 80% to maintain minimum therapeutic level of drug after administration.

Initial Risk Assessment of Formulation

Active Pharmaceutical Ingredients (API)

API plays the important role in any formulation which is having very strong impact of CQA of formulation. Different physicochemical properties of API work specifically to design new drug delivery system and alter the CQA of formulation. In this case, particle size distribution and solubility of API possess high risk in drug release from formulation. Both variable of API were controlled by specification. Absence of polymorphs in MDL had no impact on CQA.

Excipients

Risk related to excipients are examined and determined by mainly exploring the mechanism of action of excipients in formulation and percentage of excipients in product. The CQA impacted by the in situ nasal gel was prepared using gellan gum as an ion activated in situ gel forming polymer and carbopol 934P as a mucoadhesive agent. Both excipients were very critical with regards to CQA of product. The percentage used of each excipient in ISNG imposed high risk on CQA.

Gellan gum

Gellan gum is an anionic polysaccharide which undergoes a sol–gel transition under the influence of cations. The gelation of this polymer increases proportionally to the amount of cations present in nasal fluid as well as its concentration. The drug release from formulation was governed by gel matrix formed due to

polymer conversion from sol in presence of different cations present in nasal cavity. Hence concentration of gellan gum attended high risk on viscosity at P, % CDR at 10 min and %CDR at 180 min while medium risk on viscosity at NP and no risk on mucoadhesive strength of ISNG.

Carbopol 934P

The mucoadhesive properties of carbopol 934P result mainly due to hydrogen bonding (between –COOH groups of carbopol 934P and sialic acid –COOH groups of the mucin glycoprotein) [23]. Being very hydrophilic polymer, carbopol 934P imparted viscosity to ISNG which ultimately governed drug release. The concentration of carbopol 934P got high risk regards to viscosity at NP, viscosity at P and mucoadhesive strength while medium risk related to %CDR at 10min and 180min.

Water

The deionized water was used to avoid risk associated with pregelling of gellan gum into the sol form (before administration). Based on preliminary study, 5%w/v of MDL ISNG was prepared for efficient pourability as well as passable viscosity during storage of ISNG.

Ishikawa diagram representing risk assessment

Fig 1 is representing Ishikawa diagram (Fishbone diagram) for initial risk assessment specifically related to drug release from ISNG formulation because % drug release from any dosage form is the most crucial parameter to optimize. Fishbone covers problem statement (% Drug release from ISNG), cause categories (Midazolam, gellan gum, carbopol 934P, water used in preparation of formulation), specific causes (Different CMA for each material). Here to obtain desired drug release from formulation, one should only play with high risk CMA and optimize those CMA. Same way, viscosity and mucoadhesive strength of ISNG are represented in Fig 2 and 3 respectively. Indeed, ishikawa diagram simplifies the problems with all possible effective solution.

Experimental design analysis and validation of overlay plot

A 3^2 full factorial design was used to investigate effect of gellan gum conc. and carbopol 934P conc. on responses. The factorial design was carried out using the software DESIGN EXPERT®. Table 7 shows the data obtained for the experimental trails in the factorial design.

Analysis of variance (ANOVA) was applied for estimation of significance of the model. A model was considered significant if the $p<0.05$.

The highlighted with yellow color (Fig 2) termed as overlay plot indicates an optimized area as per desired criteria. From the results of check point batch (Table 9), it was found that the experimental values of response were nearer to the predicted values. Hence from this we can conclude that the proposed check point batch validated the overlay plot drawn by DoE. The prediction power of model generated by applying experimental design is good and one has flexibility to move anywhere in overlay plot to evolve new formula with desired responses.

Risk assessment after optimization of ISNG of Midazolam is illustrated in table 10. After detailing QTPP, CQA, CMA and optimization through DoE, the risk associated with CQA fell down to its lowest.

Conclusion

QbD concept based on DoE is utilized to target product profile and corresponding selection of variable of formulation during development of any new drug delivery system. For novel drug delivery system such as in situ nasal gel of Midazolam can be smoothly developed and achieved to the target profile with validated model.

Competing interests

The authors declare that they have no competing interests.

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Table 1 Assessment of Quality Target Product Profile (QTPP) with targets and justification

Assessment of Quality Target Product Profile (QTPP)			
QTPP Elements		**Targets**	**Justification**
Drug product quality attributes	**Viscosity at P**	NMT 1750 cps	To achieve desire drug release from gel matrix.
	Mucoadhesive strength	NMT 20 g	To avoid the rapid clearance from nasal cavity.
	% CDR in 10 min	NMT 50%	To achieve rapid therapeutic effect initially to control emergency condition of status epilepticus.
	% CDR in 180 min	NLT 80%	To maintain minimum therapeutic level of drug throughout time span.
			and bioavailability of the drug.
Dose strengths		5 %w/v	To prove comparison with parenteral route which are available in market.
Description		Clear transparent sol form	Patient compliance
Drug product quality attributes	**Identification**	By UV spectrometry	Midazolam gives UV maxima at specific wavelength 218 nm.
	Assay	90.0% - 110.0% of label claim	Based on Market requirements.
	Clarity	Clear transparent	Patient compliance.
	pH	pH 6.8	To achieve physiological pH of nasal and to avoid irritation after administration.
	Gelling time	Within 3 min	To avoid drainage after administration and to achieve initial drug release from formulation.
	Viscosity at NP	NMT 240 cps	Efficient administration and patient compliance.

[P= Physiological condition, NP= Non physiological condition, % CDR= % Cumulative drug release, NMT= Not more than, NLT= Not less than]

Assessment of Critical Quality attribute (CQA)				
Quality Attributes of the Drug Product		**Target**	**Is this CQA?**	**Justification**
Physicochemical Attributes	**Clarity**	Clear transparent	**No**	Clarity of sol is only for patient compliance. It does not affect the efficacy of product.
	pH	pH 6.8	**Yes**	Change in pH may lead pregelling during storage and irritation in nasal during administration. pH may varying if not controlled by buffer.
	Gelling time	Within 3 min	**Yes**	Gelling time is responsible for adherence of gel to mucosa and for initial release of drug from sol form. Gelling time is altered by percentage of polymer used in formulation.
Identification		By UV spectrometry	**Yes**	It is a critical for safety and efficacy. But formulation and process variables do not impact identity; this can be effectively controlled by the quality management system.
Assay		90.0% - 110.0% of label claim	**Yes**	Formulation and Process variables may affect the assay of the drug product

Viscosity at NP	NMT 240 cps	**Yes**	Porability of sol form is directly affected due to change in viscosity of formulation. Viscosity is varying with concentration of polymer used.
Viscosity at P	NMT 1750 cps	**Yes**	Failure to meet viscosity at physiological condition changes drug release from the matrix form after administration in nasal. Viscosity at physiological condition is directly governed by percentage of polymer used.
Mucoadhesive strength	NMT 20 g	**Yes**	Adherence to mucosa affects the penetration of drug through nasal mucosa.
In vitro drug release in 10 min	NMT 50%	**Yes**	Failure to meet the dissolution specification can impact bioavailability. Both formulation and process variables affect the dissolution profile.
In vitro drug release in 180 min	NLT 80%	**Yes**	Failure to meet the dissolution specification can impact bioavailability. Both formulation and process variables affect the dissolution profile.

Table 2 Assessment of Critical Quality Attributes (CQA) with justification

Table 3 Levels of risk assessment

Risk Assessment	
High	Further investigation needed
Medium	Further investigation may be needed
Low	No further investigation needed

Table 4 Initial Risk Assessment of Formulation components (API)

Components	Critical Quality Attributes (CQA)						Justification/ Discussion	Proposed action
	Material Attributes	Viscosity at NP	Viscosity at P	Mucoadhesive strength	% CDR at 10 min	% CDR at 180 min		
Midazolam(API)	PSD	Low	Low	Low	High	High	Dissolution, Impurities and CU can be affected by the PSD of API. Smaller particle size may affect flowability, but larger surface area can improve solubility. There may be chance of more impurity generation due to larger surface area.	Manufacturing of Trial Batches with different PSD of API with suitable manufacturing process.
	Solubility	Low	Low	Low	High	High	The API has very low water solubility, so the impact on dissolution is evaluated as high.	API particle size should be optimized in trial batches
	Polymorph	Low	Low	Low	Low	Low	No polymorph is found in Midazolam.	No polymorph is found in Midazolam.

[CU= Content uniformity, PSD= Particle size distribution]

Table 5 Initial Risk Assessment of Formulation components (Excipients)

Components	Critical Quality Attributes (CQA)					Justification/ Discussion	Proposed action
	Viscosity at NP	Viscosity at P	Mucoadhesive strength	% CDR at 10 min	% CDR at 180 min		
Gellan gum (in situ gelling polymer)	Medium	High	Low	High	High	Amount of Gellan gum potentially affect drug release profile as well as viscosity at physiological condition. Concentration of gellan gum mediumly affects the viscosity at non physiological condition. As the gellan gum having gelling property at nasal physiological condition.	Different level of Gellan gum to be check to achieve desired dissolution profile as well as viscosity at physiological condition.
Carbopol 934P (Mucoadhesive polymer)	High	High	High	Medium	Medium	Amount of Carbopol 934P potentially affect viscosity and mucoadhesive strength of formulation. Concentration of Carbopol 934P mediumly affects the drug release from formulation. As the carbopol 934P also have some tendency for gelling at mild acidic condition.	Different level and method of use of Carbopol 934P to be check to match dissolution profile. Generate Excipient compatibility data and product stability data to understand the product.

Components	Critical Quality Attributes (CQA)					Justification/ Discussion	Proposed action
	Viscosity at NP	Viscosity at P	Mucoadhesive strength	% CDR at 10 min	% CDR at 180 min		
Water (Vehicle)	High	High	Low	Medium	Low	Amount of water and type of water used potentially affect viscosity at physiological and non-physiological condition. Concentration of water also play role in case of cumulative drug release at 10 min because water amount controls the drainage of sol from nasal until gel is formed. Deionized water should be used. Because in presence of cations, gellan gum has tendency to convert into gel from sol form.	Only deionized water should be used. Optimized %w/w of water should be used in formulation.

Table 6 CMA with levels and CQA with constrains for 3^2 full factorial design

CMA		
Coded value	Actual values	
	Gellan gum conc. X_1 (% w/v)	Carbopol 934P conc. X_2 (% w/v)
Low (-1)	0.2	0.1
Medium (0)	0.5	0.3
High (+1)	0.8	0.5
Dependent variables		
CQA	**Constraints**	
Viscosity at non physiological condition (Y_1)	180 to 200 cps	
Viscosity at physiological condition (Y_2)	1000 to 1200 cps	
Mucoadhesive strength (Y_3)	14 to 18 g	
Drug release in 10min (Y_4)	40 to 50%	
Drug release in 180min (Y_5)	85 to 100 %	

All batches contained 5.0%w/v of MDL

Table 7 3^2 Full factorial design and response of in situ nasal drug delivery system

Batch	Independent variables (CMA) in coded value		Dependent variables (CQA)				
	X_1	X_2	Y_1 (cps)	Y_2 (cps)	Y_3 (g)	Y_4 (%)	Y_5 (%)
G1	-1	-1	149 ± 0.58	889 ± 0.69	14.60 ± 0.59	46.13 ± 1.50	94.94 ± 2.98
G2	0	-1	189 ± 0.29	1076 ± 0.09	15.20 ± 0.08	44.42 ± 1.10	91.97 ± 1.65
G3	+1	-1	199 ± 0.69	1534 ± 1.09	15.45 ± 0.57	32.42 ± 0.25	88.36 ± 0.69
G4	-1	0	194 ± 1.25	1128 ± 1.25	15.80 ± 0.21	40.28 ± 2.58	90.48 ± 0.45
G5	0	0	208 ± 0.09	1298 ± 0.98	16.32 ± 0.36	38.27 ± 1.25	85.73 ± 0.98
G6	+1	0	229 ± 0.97	1611 ± 0.87	17.35 ± 0.12	31.51 ± 2.57	79.74 ± 2.58
G7	-1	+1	257 ± 0.36	1697 ± 0.24	18.80 ± 0.45	41.27 ± 0.58	87.25 ± 2.08
G8	0	+1	302 ± 0.12	1703 ± 0.69	19.05 ± 0.22	34.30 ± 1.58	75.56 ± 1.28
G9	+1	+1	326 ± 0.58	1796 ± 0.58	19.11 ± 0.19	25.58 ± 0.69	77.18 ± 1.08

n=3, Mean±SD

Table 8 Formula and responses of check point batch

Formula	
Ingredients	**Concentration in Batch G10**
Midazolam (g)	5.0
Gellan gum (g)	0.36
Carbopol 934P (g)	0.21
Deionised water (ml)	100

Table 9 Responses of check point batch

Response	Check point batch	
	Experimental value± SD	**Predicted value**
Viscosity at NP (cps)	189.28± 3.14	181.152
Viscosity at P (cps)	1168.78± 5.24	1154.965
Mucoadhesive strength (g)	17.67± 4.47	15.453
% CDR in 10 min. (%)	39.730 ± 1.46	42.678
% CDR in 180 min. (%)	89.315 ± 4.77	90.608

Table 10 Risk Assessment after optimization

Components	Critical Quality Attributes (CQA)					Justification/Discussion*
	Viscosity at NP	**Viscosity at P**	**Mucoadhesive strength**	**% CDR at 10 min**	**% CDR at 180 min**	
Gellan gum	**Low**	**Low**	**Low**	**Low**	**Low**	Concentration of Gellan gum is optimized in range of 0.2 to 0.8% w/v.
Carbopol 934P	**Low**	**Low**	**Low**	**Low**	**Low**	Concentration of Carbopol 934P is optimized in range of 0.1 to 0.5% w/w.
Water	**Low**	**Low**	**Low**	**Low**	**Low**	Only deionized water is used for formulation. Specific quantity of Midazolam is dissolved in water to produce 5%w/v of solution.

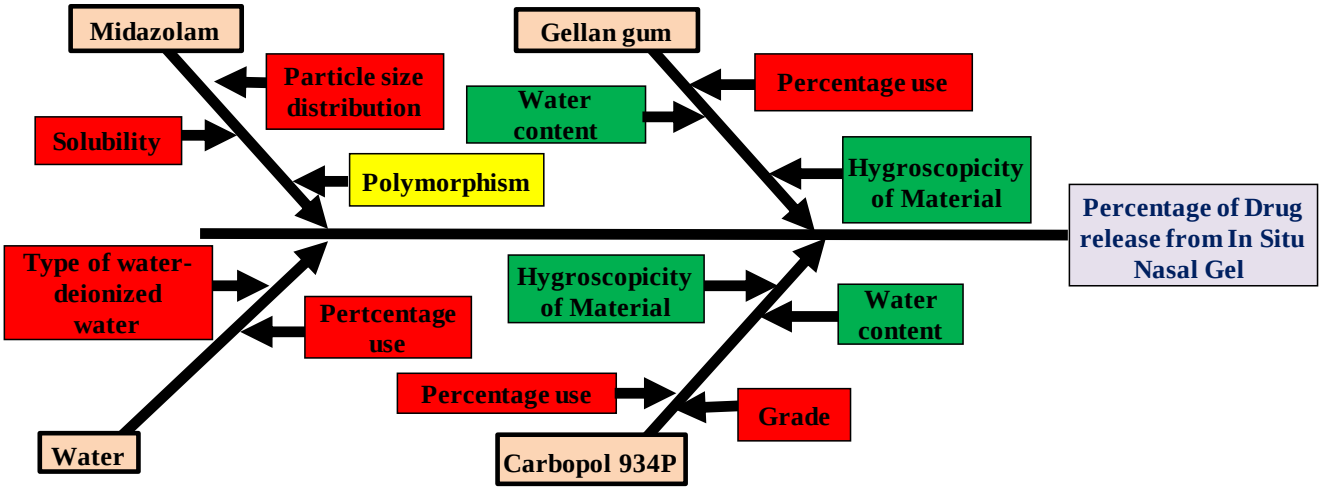

Fig 1 Fish bone diagram for % drug release of Midazloam ISNG

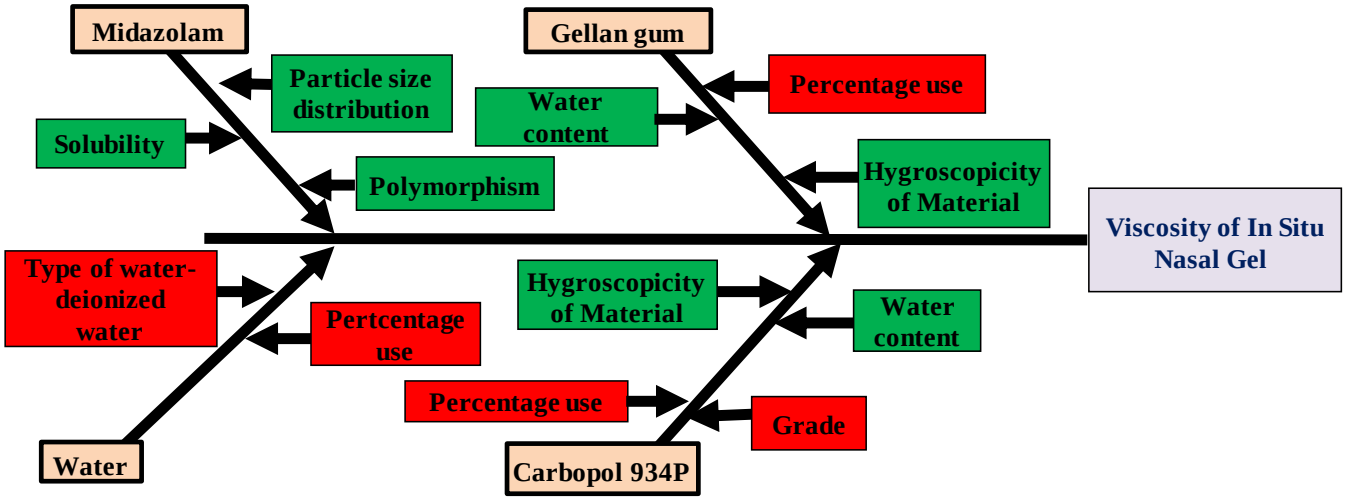

Fig 2 Fish bone diagram for viscosity of Midazloam ISNG

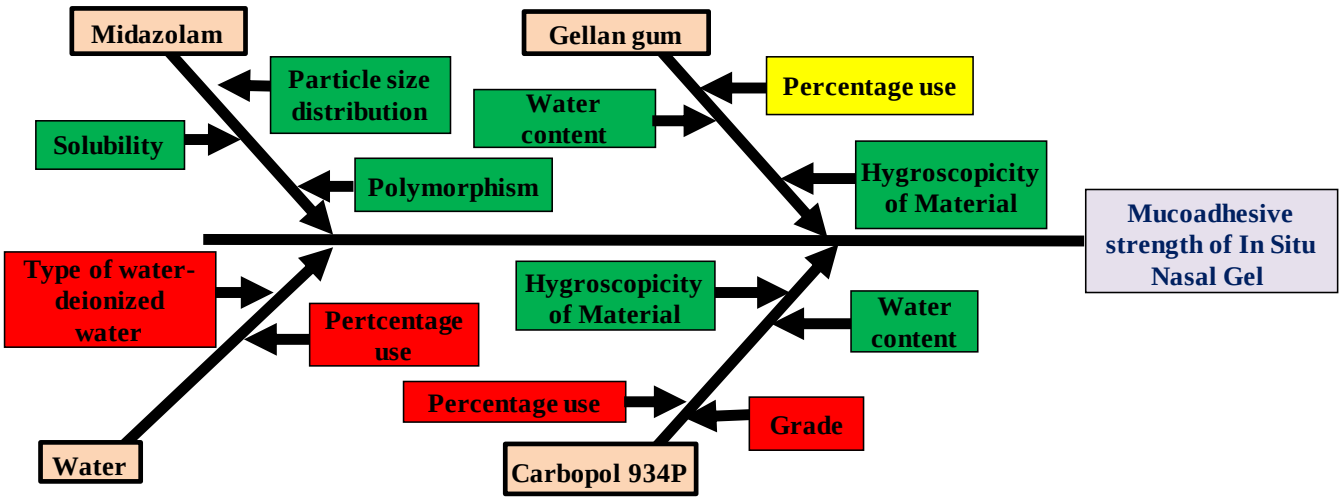

Fig 3 Fish bone diagram for Mucoadhesive strength of Midazloam ISNG

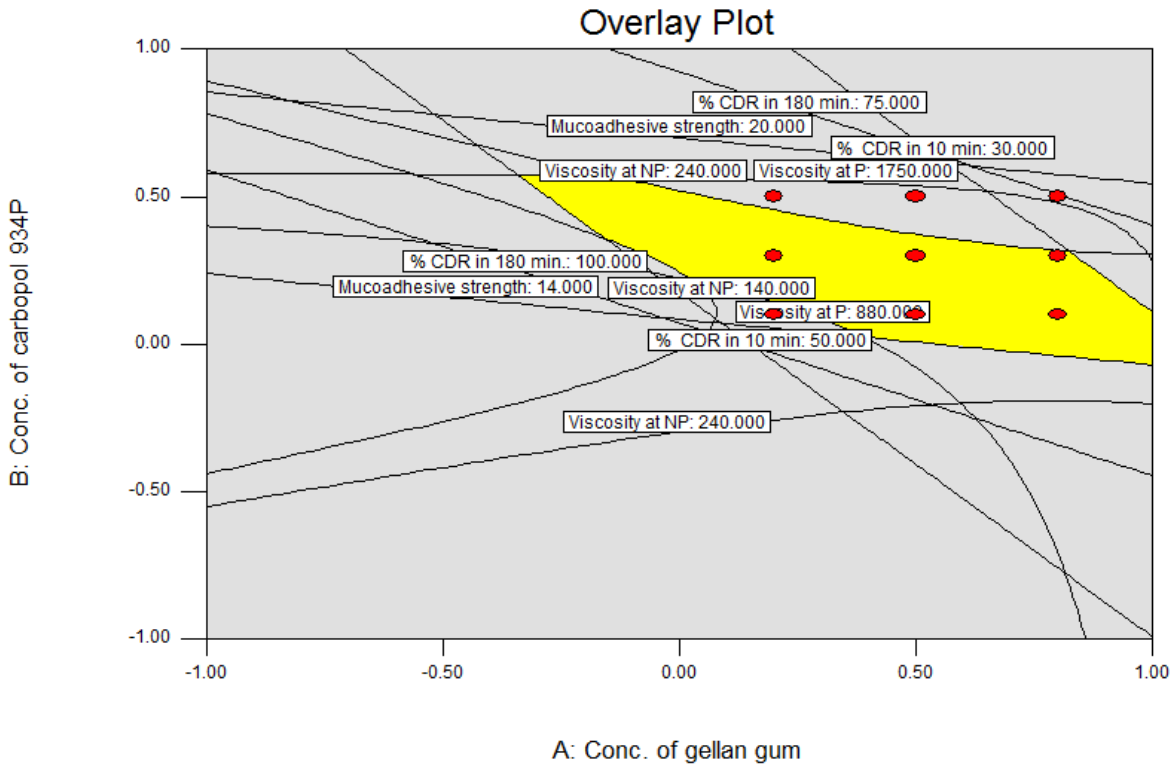

Fig 4 Overlay plot indicating design space for ISNG

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