Research Article

FORMULATION AND EVALUATION NASAL IN SITU GEL OF RIZATRIPTAN

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ABSTRACT
Rizatraptan, a 5-HT antagonist is an anti migraine drug. Rizatriptan undergoes hepatic first pass metabolism, hence it shows poor bioavailability. In this study attempt has been done to improve bioavailability by formulating nasal *in-situ* gel. Formulation was developed to reduce the mucociliary clearance by using mucoadhesive polymer in gel, thereby increasing the contact of formulation with nasal mucosa and hence improving the absorption of drug. The *in situ* gel was formulated by using 2 factor (i.e.% of carbopol 934P and % of poloxamer 407) 3 level center composite design. All the formulated design point formulations exhibit thermo reversible gelation property. Gels were characterized by permeation studies, pH, % drug content, mucoadhesive force, gel strength, *in vitro* diffusion, *ex vivo* diffusion, stability study. Rheological study of gel formulation indicated that increase in polymer concentration increases the viscosity, gel strength was found in range of 110-130 sec., Spectral study revealed no interaction between drug and polymer. Various responses like T50%(Y1), T(80%Y2), 'n' of peppa'a equation(Y3), 'k' of first order and 'n' of higuchi (Y5) were analyzed to obtain optimized formulation. Optimized formulation(OF) followed first order drug diffusion and theoretically obtained drug release profile for 8 Hr. on the base of dose calculation Stability study indicates that there was no significant change in the Rizatriptan benzoate. Rizatriptan benzoate formulated as bioadhesive solution for nasal administration could have potential to avoid first pass effect than oral route, thus improve bio availability of drug and as a safe and sustained release nasal delivery system to control migraine.

Key Words: Nasal *in-situ* gel, Box Wilson Design

INTRODUCTION[2,1]

The nasal mucosa has been considered as a potential administration route to achieve faster and higher levels of drug absorption.Thus when a bioadhesive excipients are used in a formulation it helps us to overcome the mucociliary clearance mechanism by adhering to mucous membrane and there prolonging the residence time in the nasal cavity, thereby improving the nasal drug absorption and bioavailability of the drug which undergo rapid first pass metabolism. The volume of each cavity is approximately 7.5 ml and has a surface area around 75 cm2. Nasal drug delivery can also provide a route of entry to the brain that circumvents the BBB because the olfactory receptor cells are in direct contact with the CNS. Penetration enhancers such as surfactants, bile salts, fusidate derivatives, and phospholipids have been used to improve the drug absorption through nasal mucosa, but toxicity tests proved that they were limited application for clinical use because of their irreversible damage to nasal mucosa accompanied with their absorption-enhancing effects. The pH of the mucosal secretions ranges from 5.5 to 6.5 in adults and 5.0 to 6.7 in children.[2] Nasal *in situ* gel (*in vivo* gel or environment sensitive gel) is a novel drug delivery system which has been instilled in nasal cavity. Compared to liquid formulations, nasal *in situ* gel is instilled as low viscosity solutions into the nasal cavity, but also release drug slowly and continuously, hence, it is especially useful for those drugs used chronically. The mechanism of gelation involves the formation of double helical segements to form a 3-D network by complexation with cations and hydrogen bonding with water. Since the nasal mucosa is covered with approximately 0.1 ml mucus, which contains sodium, potassium and calcium ions, a solution –gel phase transition can be expected. The aim of the present work is to develop a nasal *in situ* gel of rizatriptan benzoate using gellan gum along with mucoadhesive polymer like carbopole, polaxamer and Na CMC.[1]

Materials and Methods

Materials

Rizatriptan benzoate(A.R.)(Unimark remedies,Ahemdabad),Carbopol 934P (L.R.)(Chemdyes corporation,Ahemdabad), Hydroxyl propyl methyl cellulose K 4 M(L.R.)(Chemdyes corporation ,Ahemdabad), Polaxamer 407 (L.R.)(Chemdyes corporation,Ahemdabad), Benzalkonium Chloride Pure (L.R.)(Chemdyes corporation,Ahemdabad), Potassium dihydrogen orthophosphate (L.R.)(Finar chemicals limited,Ahemdabad), Sodium Hydroxide (L.R.)(Chemdyes corporation ,Ahemdabad), Sodium Lauryl Sulphate(SLS) (L.R.)(Chemdyes corporation ,Ahemdabad

Methods:[3,4.6.7,8,9]

Reformulation Study

Any formulation development work has to be preceded by preformulation studies. This

preformulation study includes drug-polymer compatibility study and analytical investigation of drug

Drug Polymer Compatibility Studies

Drug polymer compatibility studies were carried out using FTIR. Rizatriptan benzoate HPMC K4M, Carbopol 934P, Polaxamer 437 were dried at 40 °C for 2 h, and their FT-IR transmission spectra were obtained using a NICOLET iS10 spectrophotometer (Thermo scientific, MA). spectrophotometer (Thermo scientific, MA). Each powder sample was scanned over a wave number region of 400–4000 cm-1 using Omnic software (ver. 8.1.210). The characteristic peaks were recorded for different samples.

Development of Standard Curve for Rizatriptan benzoate in the phosphate buffer 7.4 at 226 nm

Primary Stock Solution

Rizatriptan benzoate 20 mg was weighed and transferred to a 100 ml volumetric flask and dissolved in borate buffer 4 pH. The flask was shaken and volume was made up to the mark with phosphate buffer 7.4 pH to give a solution containing 1000 µg / ml.

From this primary stock solution, pipette out 10 ml and placed into 100 ml volumetric flask. The volume was made up to mark with phosphate buffer 7.4 pH to give a solution containing 100 µg / ml..

Secondary Stock Solution

From the primary stock solution (100 µg / ml), pipette out 10 ml and placed into 100 ml volumetric flask. The volume was made up to mark with phosphate buffer 7.4 pH to give a solution containing 10 µg / ml..

Standard Calibration curve of rizatriptan

Appropriate volume of aliquots from standard Rizatriptan benzoate stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with phosphate buffer 7.4 pH.to obtain concentrations of 1, 2, 3, 4 and 5µg / ml. Absorbance spectra of each solution against phosphate buffer 7.4 pH.as blank were measured at 226 nm

Preliminary Study

Preliminary study was done for the screening of polymers. In the starting various polymer like sodium alginate, polaxamer 407, carbopol 934 were taken for the study. Viscosity study was done on the polymer for checking out the state like sol or gel. Viscosity study was done at various pH for carbopol 934 a pH dependent polymer. Viscosity study was done at 5°C and 37°C for polaxaer 407 a temperature dependent polymer. Viscosity was done at various rotating speed from 5 to 100. As the rpm increasing viscosity decreases. From preliminary study polymers are screened out.

Methods for preparation of formulations

Rizatriptan benzoate nasal *in situ* gel is prepared by cold technique. Weighed quantities of rizatriptan, benzalkonium chloride(preservative) were dissolved in distilled water under aseptic conditions. Then HPMC K4M was added to hydrate. To this polaxamer is added in the concentration range 16 % to 18% ranges. This solution was then stirred until Polaxamer 407 get dissolve completely in it.. Then carbopole is added in 0.5%-1.5% w/v concentration ranges. These resulting solutions were then kept at 40C overnight until clear solution is formed. Various polaxamer 407 and carbopol 937 concentration used in formulations are given in table.

Table no 1: Formulation chart of various formulations of HC containing Rizatriptan, HPMC K4M and carbopol 934P (By Box Wilson Design) (Exhibiting different levels of independent variables)

Formulation Cod	Coded value	Rizatriptan Benzoate (mg)	HPMC K4M	Carbopol 940P (B)	Polaxamer 407 (C)
			(% w/v)	(% w/v)	(% w/v)
F1	(-1,-1)	5.45	0.5	0.5	16
F2	(0,-1)	5.45	0.5	1	16
F3	(-1,-1)	5.45	0.5	1.5	16
F4	(-1,0)	5.45	0.5	0.5	17
F5	(0,0)	5.45	0.5	1	17
F6	(1,0)	5.45	0.5	1.5	17
F7	(-1,1)	5.45	0.5	0.5	18
F8	(0,1)	5.45	0.5	1	18
F9	(-1,1)	5.45	0.5	1.5	18

Table 2: Formulation details of Rizatriptan *in-situ* gel systems prepared with Carbopol 934P, HPMC, and Polaxamer 407

API/ Excipients	Ingredient Concentration (%W/V)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Rizatriptan Benzoate*	5.45	5.45	5.45	5.45	5.45	5.45	5.45	5.45	5.45
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Polaxamer 407	16	16	16	17	17	17	18	18	18
Carbopol 934p	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Hydroxypropyl methyl cellulose K4M	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
pH 7.4 Buffer	100	100	100	100	100	100	100	100	100

Evaluation Viscosity

The rheological properties of solutions were measured using Brookfield viscometer. The developed formulation was poured into the small adaptor of viscometer and angular velocity increased gradually from 5 to 100 rpm and the measured viscosity at different rpm. The hierarchy of angular velocity was reversed. The average of two reading was used to calculate the viscosity. The formulation was then poured into an ointment jar and then the pH was raised to 6.8 by adding triethanolamine (an alkalizing agent). Then measure the viscosity of transformed gel at pH 6.8.Average of two reading was taken for calculation.

Drug Contant Uiformity Study

Pipette out 0.2 ml (7.5 mg) of 5.45% sample solution and was diluted to 10 ml with phosphate buffer (7.4 pH) in 10 ml volumetric flask. Measure the absorbance of the resulting sample solution. Repeat the above experiment for 3 times.

pH MEASUREMENT

The pH of the gel forming nasal solution was measured using pH meter.

Measuremet Of Gel Strength

Gel strength was measured by 50 g of gel was placed in a 100 ml graduated cylinder and gelled at 37°C using thermostat A weight of 35g was placed onto the gelled solution and allowed to penetrate 5 cm in the gel. Time taken by weight to sink 5cm was measured

Determination Of Mucoadhesive Strenth

Mucoadhesive strengths of gel was determined by using the modified nasal mucosal tissues, obtained from the local slaughterhouse, were carefully removed from the nasal cavity of goat and mounted on glass surface using adhesive tape while another mucosal section was fixed in inverted position to the cylinder. 50 mg of gel was placed on mucosal surface. The glass mounted mucosal surface with gel formulation and mucosal surface attached to cylinder were held in contact with each other for 2min to ensure intimate contact between them In second pan, the weights were kept rising until two mucosa get detached from each other. The nasal mucosa was changed for each measurement. The Mucoadhesive force expressed as the detachment stress in dynes/cm2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.

Mucoadhesive Strength (dynes/cm2) = mg/A---------- (1)

Where,

m = weight required for detachment in gram,

g = Acceleration due to gravity (980cm/s2)

A = Area of mucosa exposed

In –Vitro Drug Release Studies[5]

The *in-vitro* release of rizatriptan benzoate from the formulations was studied through cellophane membrane using a modified dissolution testing apparatus. The diffusion medium used was simulated with freshly prepared pH 7.4 buffer. Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 2 cm diameter). 0.2 ml volume of the formulation was accurately pipette into this assembly. The cylinder was attached to the metallic shaft and suspended in 500 ml of dissolution medium so that the membrane just touched the receptor medium surface and maintained at 37 ± 2 °C at 50 rpm

using magnetic stirrer. Aliquots, each of 5 ml volume, were withdrawn an hourly intervals till eight hours and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium and analyzed by UV spectrophotometer at 226 nm

Ex-Vitro Drug Diffusion Study[5]

The *Ex-vivo* release of rizatriptan benzoate from the formulations was studied through Nasal membrane of sheep using a modified diffusion apparatus using 6.8 Ph in the donor compartment and 7.4 pH phosphate buffer in the upper compartment. The temperature is maintained 37 °C. The assembly is put on the magnetic stirrer. *Ex-vivo* is occurred for the all designed formulations. It is done by adding SLS as a membrane permeation enhancer in the formulation. Sample has withdrawn in the interval of 1 hour. Its absorbance is checked on 226 nm in the UV spectrophotometer.

Release Kinetics Of Formulation

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing. The *in-vitro* release data were treated to different equations and kinetic models to explain the release kinetics of Amoxicillin from the floating tablets. Mathematical models used to describe drug dissolution curvesHere\Qt = Mt/M∞ = the fraction of drug released at time, t

Zero order	$Q_t = Q_0 + K_0 t$
First order	$\ln Q_t = \ln Q_0 + K_1 t$
Higuchi	$Q_t = K_H \sqrt{t}$
Korsmeyer–Peppas	$Q_t / Q_\infty = K_k t^n$

Q0 = Amount of drug present in dissolution medium at time t=0
K0 = Zero order rate constant
K1 = First order rate constant
KH = Rate constant for higuchi equation
Kk = Rate constant for Korsmeyer-Peppas equation
t = Time

For analysis of release kinetic, a zero order equations, Korsmeyar-Peppas models were used.

Zero Order Equation

The curve percentage cumulative drug release Vs. Time was plotted and the slope gives the zero order 'K' value the extent of curve fitting was decided by the R2 value

Peppas Equation[10]

The equation derived by Peppas-Korsemeyer was studied to know determine the mechanism of drug release.

$$Mt/M\infty = ktn$$

Here

Mt/M∞ : the fraction of drug released at time, t,

k: Proportionality constant which accounts for the structural and geo- metrical properties of the mat

n: Diffusion exponent indicative of the mechanism of drug release. (It depends on the polymer swelling characteristics and the relaxat rate at the swelling front.

To get the values of 'n' and 'k' the equation is converted to the log form as follows:

$$\text{Log } Mt/M\infty = \log k + n \log t$$

The values of 'k' and 'n' were determined by plotting curve of log (Mt/M∞) vs. log (t).

Slope and intercept gives the value of 'n' and log(k) respectively. This equation is a generalization of the observation that superposes two apparently independent mechanism of drug transport, Fickian diffusion and a case-II transport describes drug release from a swelling polymer. When 'n' takes the value 0.5 it indicates diffusion-controlled drug release and for the value 1.0 indicates swelling-controlled drug release. Values of 'n' between 0.5 an 1.0 can be regarded as an indicator for the both phenomena (anomalous transport). These extreme values for the exponent 'n', 0.5 and 1.0, are only valid for slab geometry. For spheres and cylinders different values have been derived. For a matrix tablets and capsules, a cylindrical geometry is considered. So, as per Ritger and Peppas, 'n' takes values in the range of 0.45–0.89 for anomalous transport.

Rational For The Selection Of Responses

In the present study, an attempt was made to formulate nasal *in situ* gel for sustained release formulation which can release rizatriptan with the sustained rate in nasal region. So, to achieve complete release in nasal region the amount of rizatriptan had to release with first order or towards first order to maintain the local concentration of rizatriptan above MIC So, in present study, to achieve sustained (first order) release, first order release rate constant, T80%, 'n' of peppas equation, 'n' of higuchi equation and first order release rate constant were selected as the dependent variable instead of zero order release rate constant. The selected

variables are optimized to find out statistically optimized formulations from the each formulation group. Responses and their maximum desirability were selected as below.

Table 3: Parameters selected as dependent variables and their maximum desirable values

Formulation Code	T50%	T80%	K of First order equation	'n of peppas equation	'n of peppas equation
Std.Rel.-8h		5.678	5.678 0.950	0.450	34.193

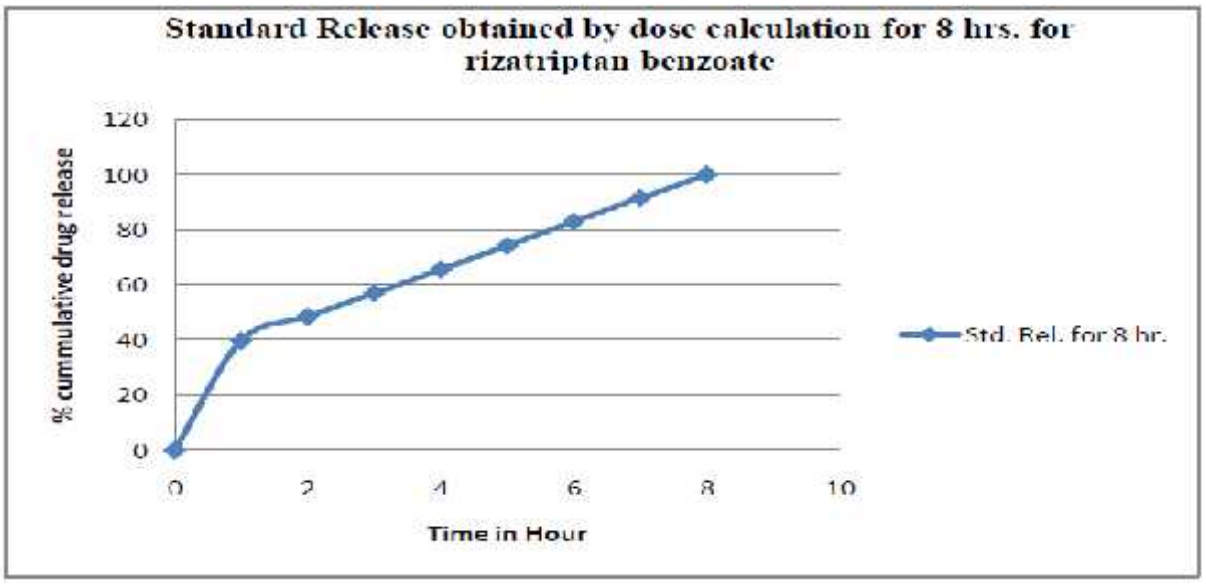

FIG 1: Theoretical *in vitro* drug release study according to dose calculation for formulation which can release the drug (sustained rate) for 8 h

Optimization Data Analysis And Model-Validation

ANOVA provision available in the software was used to establish the statistical validation of the polynomial equations generated by Design Expert®. A total of 11 runs were generated by Box-Wilson design. All the responses observed were simultaneously fitted to first order, second order and quadratic-models and were evaluated in terms of statistically significant coefficients and R2 values. Various feasibility and grid searches were conducted over the experimental domain to find the compositions of the optimized formulations. Three dimensional response surface plots were provided by the Design Expert® software, where by intensive grid search performed over the whole experimental region, one optimum checkpoint formulation was selected (from each group of formulations) to validate the chosen experimental domain and polynomial equations. The optimized checkpoint formulation was prepared and evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with that of the predicted values to calculate the percentage prediction error.

Stability Studies Of The Optimized Formulations

To assess the drug and formulation stability, stability studies were done according to ICH guidelines Q1C.The stability studies were carried out on the most satisfactory formulations as per ICH guidelines Q1C. The most satisfactory formulation sealed in aluminum packaging and kept in humidity chamber maintained at 30 ± 2 °C/60 ± 5 % RH and 40 ± 2 °C/75 ± 5 % RH for 2 months.At the end of studies, samples were analyzed for the physicochemical parameters, the drug content, *in vitro* dissolution, floating behavior and other physicochemical parameter.

Result And Discussion

In present study, rizatriptan (5-HT antagonsit) is used for treatment of migraine Nasal *in situ* gel is prepared by cold technique by using temperature dependent polymer polaxamer 407 and pH dependent polymer carbopol 937. Benzalkonium chloride is used as a preservative. SLS is used as a permeation enhancer. Nasal *in situ* gel is prepared by various concentration of polaxamer 407 from 16 % to 18% in range and carbopol 934 in 0.5% to 1.5% in range for the development of various formulations. The optimized batch is obtained after

doing optimization of all formulations by using design expert software. All the formulations are evaluated by various parameters like viscosity (at 4°C and 37°C) , pH measurement, gel strength, bioadhesive force, *in vitro* drug release for 8 hrs., *ex vivo* drug release for 8 hrs., % drug content and stability study. The comparative studies were performed between various optimized formulations for various parameters to obtain the most promising formulations which can release the drug *in vitro* for 8 h, with sustained rate (first order).

The stability studies were carried out for the most promising formulations

Result of preformulation study

Any formulation development work has to be preceded by preformulation studies. This preformulation study includes Drug- polymer compatibility study and analytical investigation of drug.

Drug-polymer/excipients compatibility studies by FT-IR spectra analysis

FT-IR study showed that there is no interaction between rizatriptan benzoate and various polymers/excipients (i.e. polaxamer 407, carbopol 934P) used in present study. So, the drug and polymers/excipients are compatible. This could be concluded by -1).verification of all the peaks at below mentioned wave numbers (cm-1) (observed in pure drug sample) in the different samples of powder mixture containing rizatriptan benzoate as a component (SD: ± 5 cm

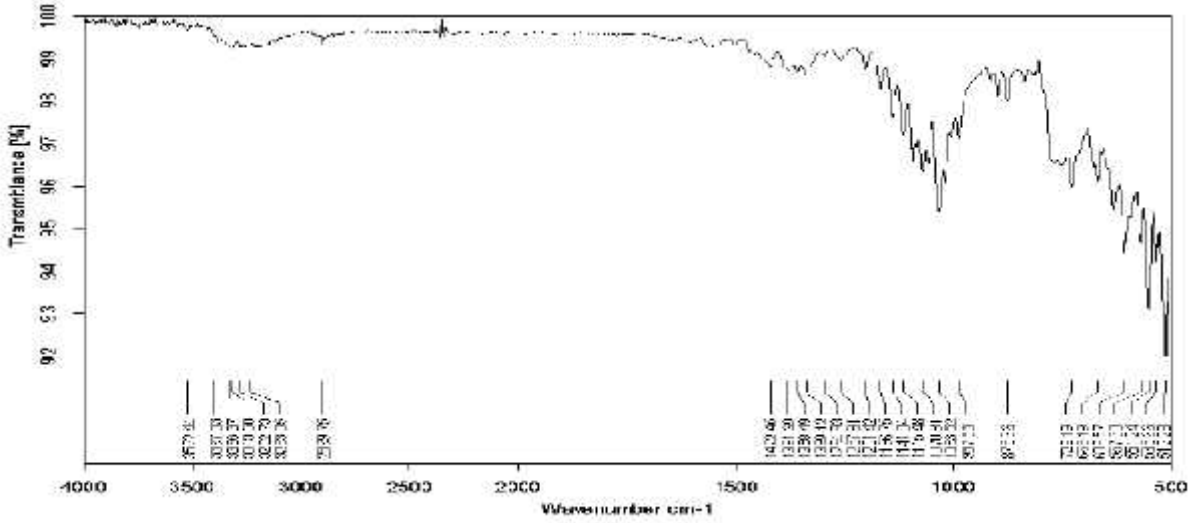

FIG 2: FTIR of rizatriptan Benzoate

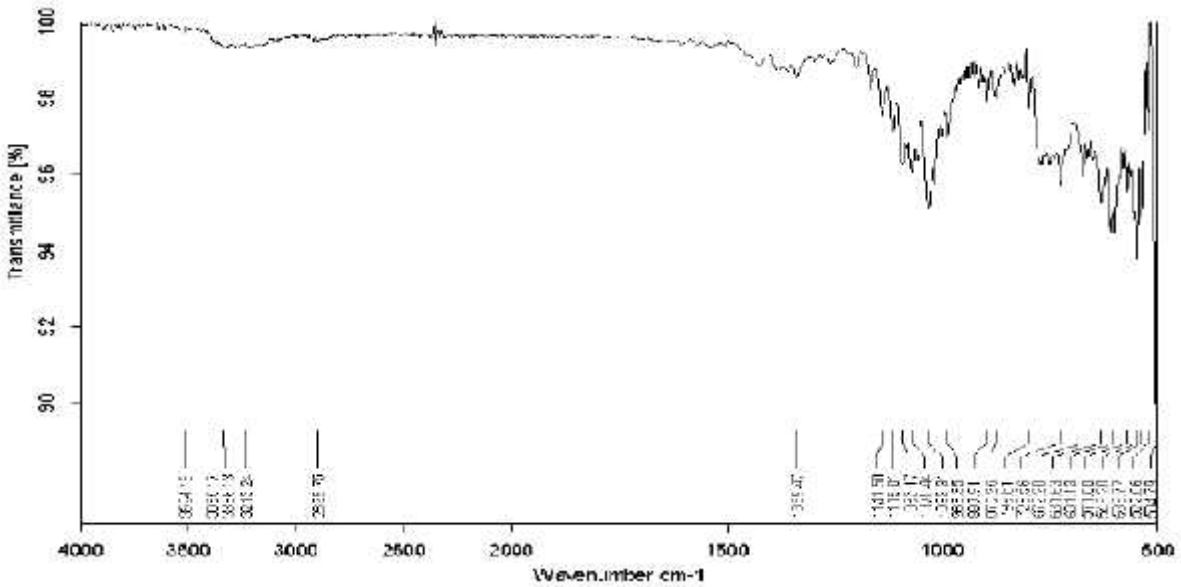

FIG 3: Rizatriptan benzoate + Polaxamer 470FIG 4: Rizatriptan benzoate + Carbopol

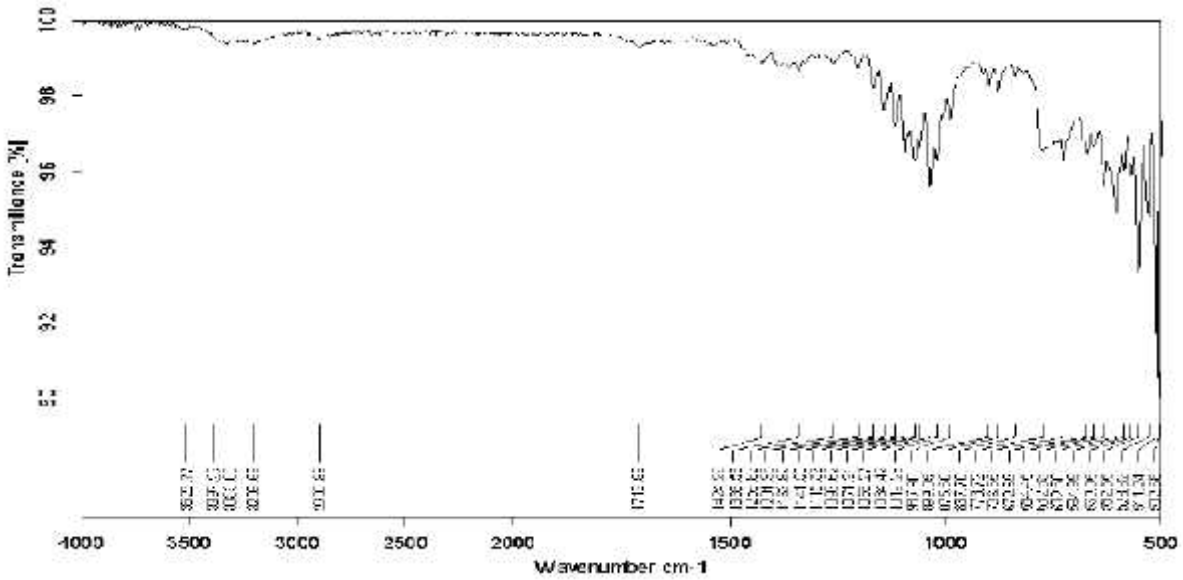

FIG 4: Rizatriptan benzoate + Carbopol 934P

Table 4: Standard calibration curve of rizatriptan in phosphate buffer (7.4 pH)

Sr. No.	Concentration (µg/ml	Absorbance				Variance1
		Trial-1	Trial-2	Trial-3	Mean±SD	
1	1	0.193	0.196	0.196	0.1952	0.0000030
2	2	0.398	0.393	0.394	0.3951	0.0000070
3	3	0.576	0.578	0.58	0.5784	0.0000040
4	4	0.791	0.788	0.788	0.7892	0.0000030
5	5	0.975	0.976	0.974	0.9752	0.0000010

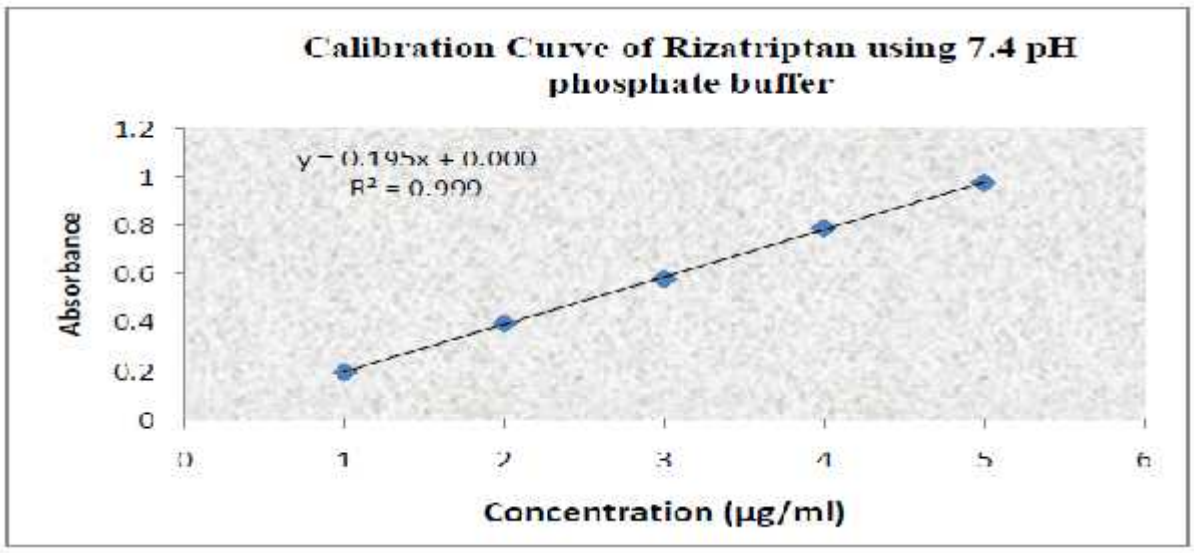

Fig 5: Standard Calibration Curve of Rizatriptan using 7.4 phphosphate buffer

Data is expressed as mean of three readings

Estimation of rizatriptan benzoate was carried out by SHIMADZU UV-1700 PharmaSpec, UV spectrophotometer at λmax 226 nm in phosphate buffer pH 7.4 The slope of the standard calibration curve were 0.195 and correlation coefficient R2 was found to be closer to 1.By using the regression co-efficient (slope) of the equation the % drug diffused and % cumulative drug release were calculated.

Priliminary Study

Table 5: Measurement of pH and viscosity at different rpm

Sr. No	Polymer	Conc (% w/v)	pH	Viscosity(cps)					Result
				5 rpm	10 rpm	20 rpm	50 rpm	100 rpm	
1	Sodium Alginate*	2	6.7	189	167	102	90	64	Sol
		3	6.6	278	253	198	162	154	Sol
		4	6.8	539	480	450	395	336	Gel
2	Sodium Alginate**	2	6.6	198	157	124	101	90	Sol
		3	6.7	312	267	187	152	132	Sol
		4	6.7	470	423	368	319	289	Gel
3	Polaxamer 407	14	6.7	3.1	2.7	2.5	2.3	2.1	Sol
		16	6.7	3.3	3.1	2.9	2.4	2.2	Sol
	(At 5°C)	18	6.8	3.7	3.2	3.2	3.1	3	Sol
		20	6.6	4.3	4.0	3.9	3.7	3.5	Sol
4	Polaxamer 407 (At 37°C)	14	6.8	169	156	149	132	129	Sol
		16	6.7	179	161	159	143	137	Gel
		18	6.7	203	192	185	183	157	Gel
		20	6.7	213	201	198	187	175	Gel
5	Carbopol 934 (At 4 pH)	0.5	4.1	657	534	389	298	172	Sol
		1	4.2	891	757	534	412	323	Sol
		1.5	4.2	1082	789	757	367	167	Sol
6	Carbopol 934 (At 5 pH)	0.5	5.1	1987	1684	1290	1018	785	Gel
		1	4.9	2798	2108	1978	1274	897	Gel
		1.5	5.1	3178	2967	2175	1967	1189	Gel
7	Carbopol 934 (At 6 .8pH)	0.5	6.7	3359	2100	1530	875.8	551.9	Gel
		1	6.9	5879	4799	3239	1956	1326	Gel
		1.5	6.8	6119	3959	2639	1560	1062	Gel

EVALUATION OF NASAL *IN -SITU* GEL

4.3.2. % Drug content of various formulations

Table 6: Measurement of viscosity values of various formulations

Formulation Code	Carbopol-934p (%)	Polaxamer-407 (%)	HPMC (%)	Viscocity (cps) At 370c at 5 rpm
F1	0.5	16	0.5	144
F2	1	16	0.5	1236
F3	1.5	16	0.5	164000
F4	0.5	17	0.5	309.5
F5	1	17	0.5	947
F6	1.5	17	0.5	356000
F7	0.5	18	0.5	287.9
F8	1	18	0.5	2879
F9	1.5	18	0.5	185000

Note : 1. In 0.5 % CaCl2 containing phosphate buffer of pH 6.8
2.In distill water containing phosphate buffer of pH 6.8

Table 7: 4.3.2. % Drug content of various formulations

Sr. No.	Formulation code	% drug content	Visual appearance
1	F1	98.22	Hazzy
2	F2	98.00	Hazzy
3	F3	87.24	Hazzy
4	F4	100.02	Hazzy
5	F5	99.2	Hazzy
6	F6	99.15	Hazzy
7	F7	98.16	Hazzy
8	F8	98.43	Hazzy
9	F9	98.3	Hazzy

Table 8 : Measurement pH for various formulations

Sr. No.	Formulation code	pH
1	F1	4.12
2	F2	4.27
3	F3	4.08
4	F4	4.17
5	F5	4.11
6	F6	4.13
7	F7	4.52
8	F8	4.27
9	F9	4.17

Table 9: Measurement of gel strength and bioadhesive force

Sr. No	Formulation	Gel strength (Seconds)	Bioadhesive Force (dynes/cm2)
12	F1	110	2496.815±0.1
2	F2	117	4989.469±0.13
3	F3	120	5629.393±0.115
4	F4	115	2755.744±0.125
5	F5	117	4369.426±0.13
6	F6	125	8771.219±0.115
7	F7	118	2519.933±0.115
8	F8	120	3754.470±0.152
9	F9	130	8105.402±0.115

Table 10: *In vitro* diffusion study of various formulations containing rizatriptan

Formulation code	% Cumulative Drug diffused								
	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
F1	46.64	79.47	90.95	96.1	99.46	99.36	99.67	99.54	99.12
F2	97.15	60.65	76.12	85.65	89.56	92.21	99.56	99.47	99.63
F3	32.5	46.08	61.92	70.81	79.37	86.28	89.18	99.36	99.81
F4	39.68	66.89	82.44	89.3	92.29	99.62	99.56	99.57	99.46
F5	30.81 ± 1.68	49.80 ± 2.71	64.76 ± 1.98	74.84 ± 2.38	82.73 ± 2.98	87.47 ± 1.75	90.78 ± 2.48	99.71 ± 1.78	99.63 ± 1.23
F6	27.44	36.76	49.22	59.44	67.51	72.25	79.62	84.45	88.31
F7	28.49	45.26	56.84	67.83	75.21	81.74	86.95	89.73	99.92
F8	23.43	32.98	47.59	56.69	66.34	70.84	76.48	83.1	85.66
F9	19.63	28.37	41.71	51.19	59.34	63.76	69.33	74.61	78.33

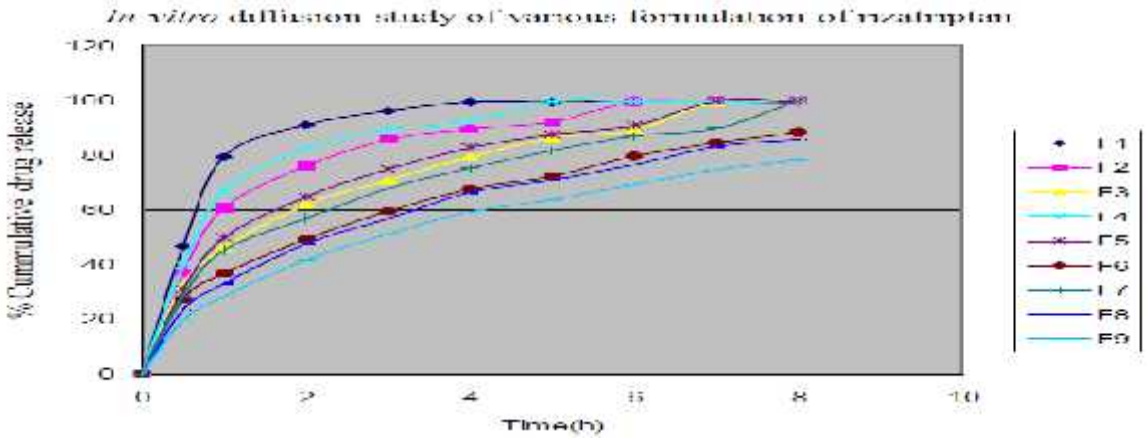

Fig 6: *In-vitro* drug diffusion study of various formulations

Table 11: Ex-vivo diffusion study of optimized formulation by adding SLS

Time (Hrs	% Cumulative Drug diffused			
	0% SLS	0.25%SLS	0.5%SLS	0.75%SLS
0	0	*0*	*0*	*0*
1	5.343 ± 1.767	7.233± 1.867	8.333 ±1.988	19.123 ±1.673
2	10.223 ± 1.896	15.435 ±1.689	16.666 ±1.862	33.454 ±1.892
3	16.112 ±1.893	22.564 ±1.743	24.999 ±1.789	46.443 ±1.498
4	23.115 ± 1.721	27.657 ±1.712	33.332 ±1.813	59.981 ±1.457
5	25.987 ± 1.789	36.546 ±1.689	41.665 ±1.856	70.656 ±1.275
6	32.553 ± 1.678	43.546 ±1.256	49.998 ±1.512	81.124 ±0.987
7	35.435 ± 1.621	49.435 ±1.467	58.331 ±1.123	91.476 ±0.789
8	42.665 ±1.478	56.645 ±1.115	66.664 ±1.107	99.367 ±0.256
9	46.443 ± 1.567	62.546 ±1.098	74.997 ±0.964	
10	50.645 ± 1.238	71.756 ±0.987	83.339 ±0.745	
11	55.956 ± 1.489	77.815 ±0.796	91.663 ±0.389	
12	62.546 ± 1.378	84.876 ±0.685	99.996 ±0.112	
13	65.654 ±1.219	91.657 ±0.562		
14	72.657 ±1.313	98.239 ±0.123		
15	76.546 ±1.201			
16	81.647 ±1.112			
17	86.435 ±0.981			
18	92.675 ±0.391			
19	97.378 ±0.089			
20	99.123 ±0.003			

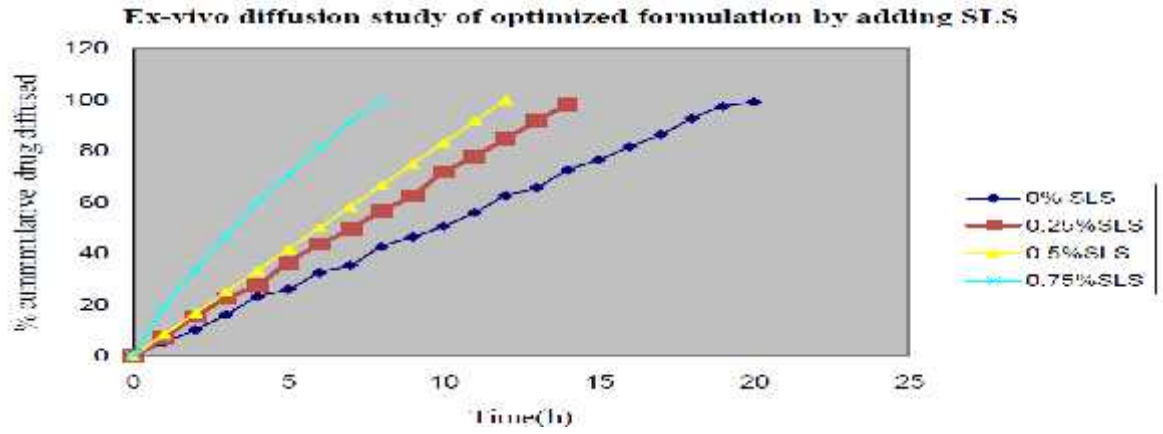

Fig 7*: Ex-vivo* diffusion study of optimized formulation by adding SLS

4.3.7.Optimization of various formulations of nasal *in situ* gel

4.3.7.1Optimization of various parameters for various formulations of rizatriptan and concentration of carbopol 934p, polaxamer 407,by using Box-Wilson design.

Table 12

Study Type	Response Surface
Design Type	Central Composite
Design Model	Quadratic
Runs	11

Table 13

Factor Code	Name	Units	Type	Subtype	Mini.	Max.
					-1	1
A	% of carbopol 934P	%w/v	Numeric	Continuous	0.5	1.5
B	% of polaxamer 407	%w/v	Numeric	Continuous	16	18

4.3.7.2. Analysis of T50%

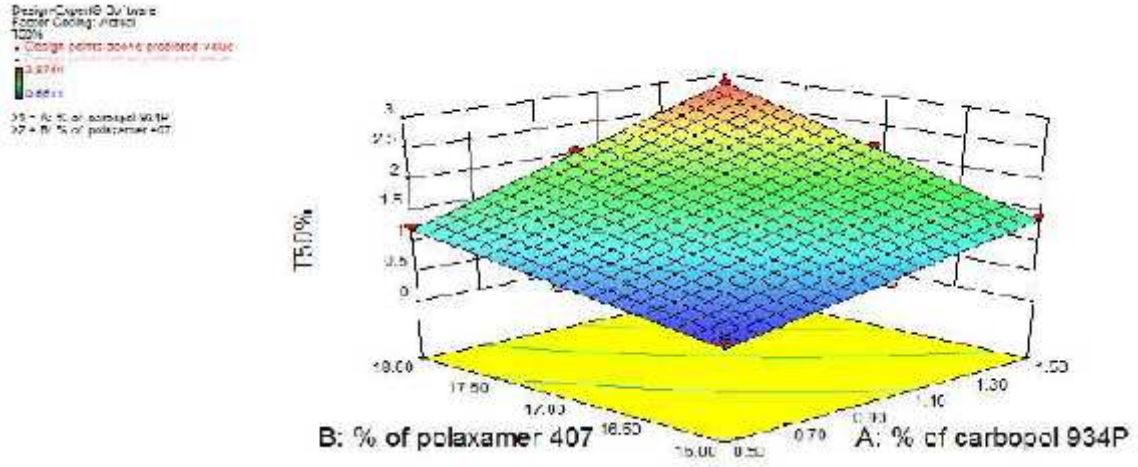

Fig 8: Analysis graph (a) Plot of Predicted vs. Actual (b) Effect of Individual Factors (c) contour plot and (d) 3D surface plot for T50%:

Final Equation in terms of coded factors:

T50% = +1.47+0.65* A+0.56 * B +0.19 * A * B

Final Equation in terms of Actual Factors

T50% =+1.46975+0.64610 * % of carbopol 934P +0.56443 * % of polaxamer 407+ 0.19220 * % of carbopol 934P * % of polaxamer 407

4.3.7.3. Analysis of T80%

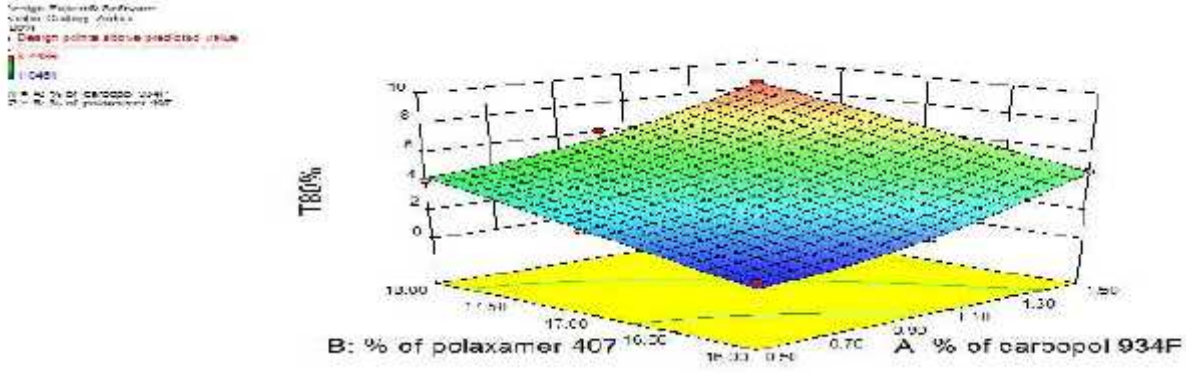

Fig 9 : Analysis graph: (a) Plot of Predicted vs. Actual (b) Effect of Individual Factors (c) contour plot and (d) 3D surface plot forT80%

Final Equation in Terms of Coded Factors:
T80% = +3.68+2.03 * A +1.83 * B +0.17 * A * B +0.72 * +0.21 * B2

Final Equation in terms of Actual Factors
T80% =+3.68244 + 2.02830 *% of carbopol 934P +1.83262 * % of polaxamer 407 + 0.16760 *% of carbopol 934P * % of polaxamer 407 +0.71976 * % of carbopol 934P2 +0.21131 * % of polaxamer 407

4.3.7.4. Analysis by 'n' of Peppa's equation

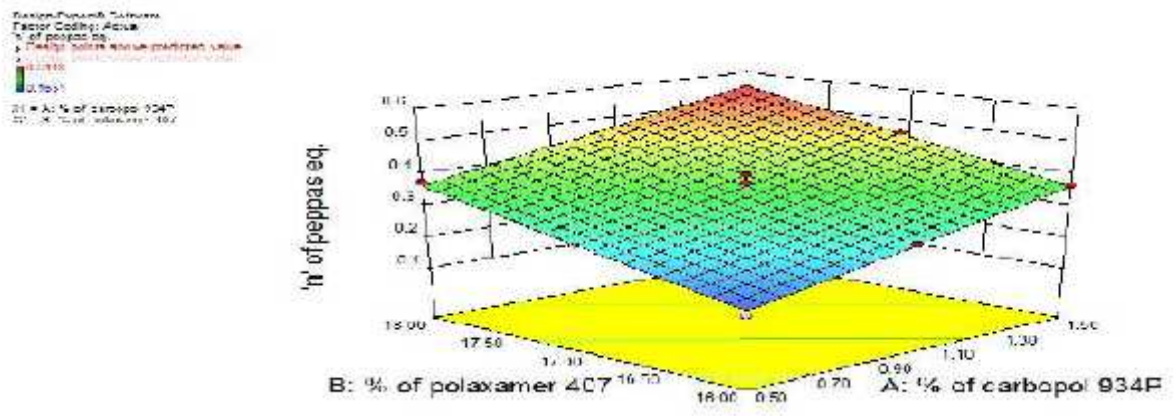

Fig 10: Analysis graph (a) Plot of Predicted vs. Actual (b) Effect of Individual Factors (c) contour plot and (d) 3D surface plot for 'n' of peppas eq

Final Equation in Terms of Coded Factors:
'n' of peppas eq. = +0.36+0.096 * A + 0.096 * B
Final Equation in Terms of Actual Factors:
'n' of peppas eq. = +0.36226 +0.095617 * % of carbopol 934P + 0.096367* % of polaxamer 407

4.3.7.5. Analysis by 'k' of first order

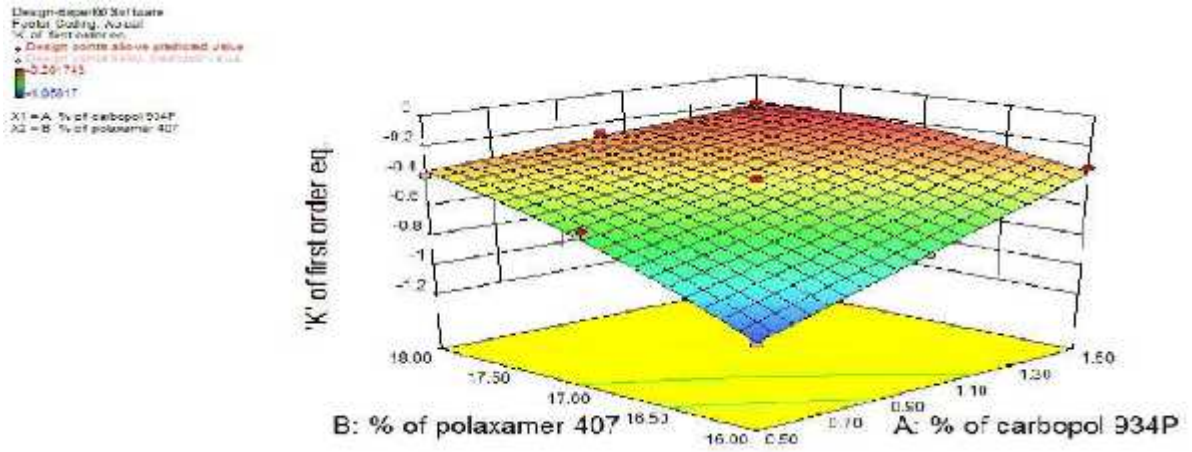

Fig 11:Analysis graph for (a) Plot of Predicted vs. Actual (b) Effect of Individual Factors (c) contour plot and (d) 3D surface plot for 'K' of first order eq

Final Equation in Terms of Coded Factors:
'K' of first order eq. =- 0.18+0.088* A +0.090 * B-0.056 * A * -0.033 * B2
Final Equation in terms of Actual Factors
'K' of first order eq.=-13.37793 + 2.08132* % of carbopol 934P +1.32956 * % of polaxamer 407 - 0.11205 * % of carbopol 934P * % of polaxamer 407 -0.033160 * % of polaxamer 4072

4.3.7.6. Analysis by 'n' of higuchi

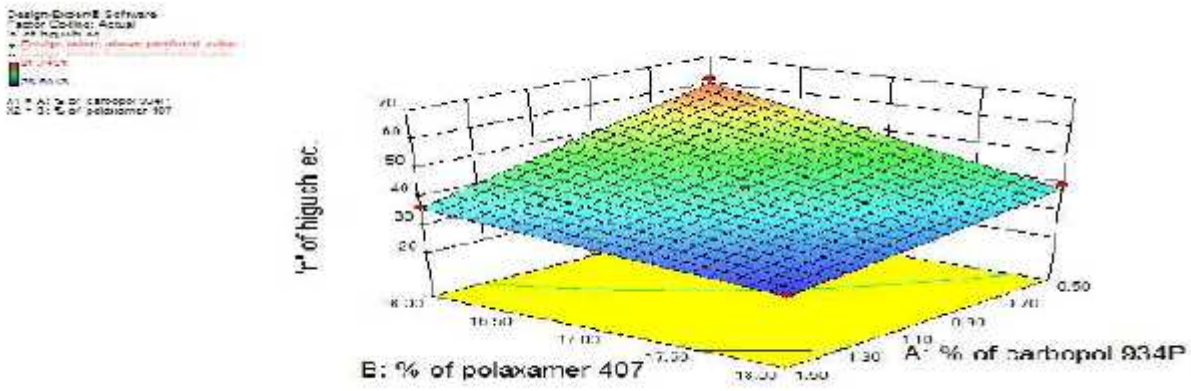

Fig 12: Analysis graph for (a) Plot of Predicted vs. Actual (b) Effect of Individual Factors (c) contour plot and (d) 3D surface plot 'n' of higuchi eq

Final Equation in Terms of Coded Factors:
'n' of higuchi eq. =+40.58-8.45* A -7.64 * B+3.56 * A * B
Final Equation in terms of Actual Factors
'n' of higuchi eq. = +40.57661 -8.45080 * % of carbopol 934P -7.63967 * % of polaxamer 407 +3.55675 * % of carbopol 934P * % of polaxamer 407

4.7.3.7. Result of Optimized Formulation:

Table 14: Optimized Fromulation

Number	% of carbopol 934P	% of polaxamer 407	T80%	'n' of peppas eq.	'K' of first order eq.	'n' of higuchi eq.	Desirability
1	1.0	17.84	5.298	0.44	0.299	34.194	0.936

Table 15: Optimized formulation chart

Sr. No	API/ Excipients	Ingredient Concentration (%W/V)
1	Rizatriptan Benzoate*	5.45
2	Polaxamer 407	17.84
3	Carbopol 934p	1
4	Hydroxypropyl methyl cellulose K4M	0.5
5	Benzalkonium chloride	0.01
6	pH 7.4 Buffer	100

Optimized formulation evaluation

Table 16: Viscosity values of optimized formulation

Sr. No.	Formulation Code	Viscocity(cp) At 370c	Viscocity(cp) At 50c
1	OF	969	567

Table 17: Measurement of pH of optimized formulation

Sr. No.	Formulation Code	Gel strength (Seconds)	Bioadhesive Force* (dynes/cm2)
1	OF	113	4123.289±0.11

* Bioadhesive Force study is replicated for 3 times here

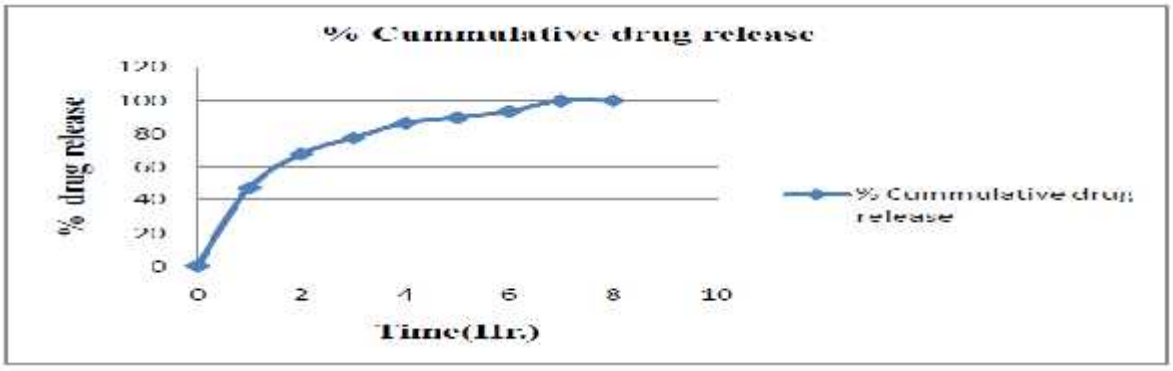

Fig 13:*In vitro* diffusion study of optimized formulation containing rizatriptan drug

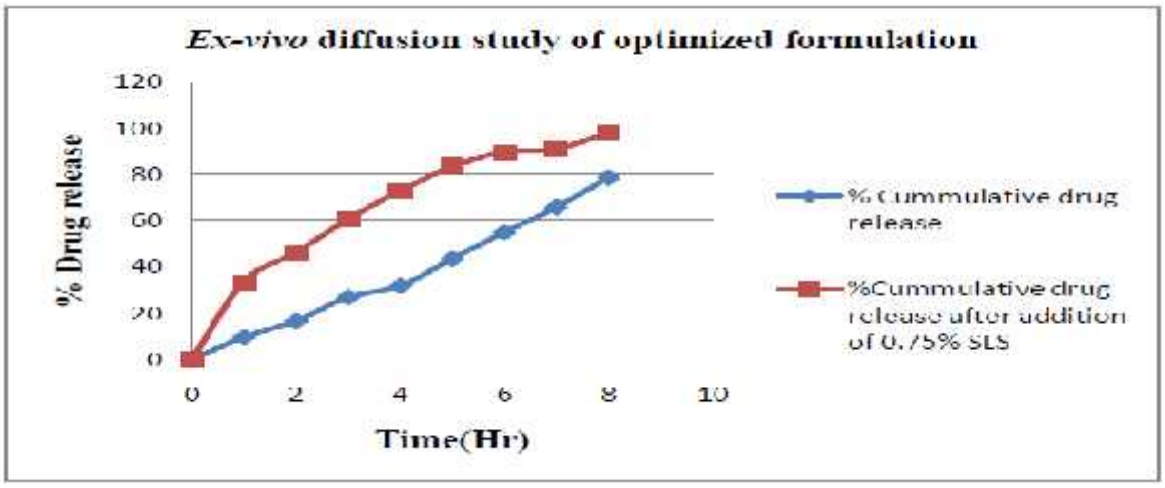

Fig 14:*Ex-vivo* diffusion study of optimized formulation containing rizatriptan drug

Table 18: Kinetic study of optimized formulation containing rizatriptan drug

Formulation code	Zero order	First order	'n' of Higuchi eq.		'n' of Peppa's eq.	
			R2	N	R2	N
OF	0.5995	0.9896	0.9722	5.8432	0.9291	0.4624

Table 19: Stability study for viscosity

Formulation Code	Viscosity(cps) at 5 rpm	
OF	4°C	567
	37°C	947
After stability studies at 30 ± 2 ºC (65 ± 5 %RH) for 1 month		
OF	1022	
After stability studies at 40 ± 2 ºC (75 ± 5 %RH) for 1 month		
OF	1012	

Table 20: Stability study for pH, bioadhesive force, gel strength

Formulation Code	pH	Bioadhesive Force (dynes/cm2)	Gel strength (Seconds)
Before stability studies			
OF	4.13	4123.289±0.11	113
After stability studies at 30 ± 2 ºC (65 ± 5 %RH) for 1 month			
OF	4.15	4234.312 ± 0.12	116
After stability studies at 40 ± 2 ºC (75 ± 5 %RH) for 1 month			
OF	4.14	4247.564 ± 0.11	117

Table 21: Stability study for *In vitro* diffusion study, *Ex-vivo* diffusion study

Formulation Code	*In vitro* diffusion study	*Ex-vivo* diffusion study
Before stability studies		
OF-8 hr	96.23± 1.76	78.64 ± 1.98
After stability studies at 30 ± 2 ºC (65 ± 5 %RH) for 1 month		
OF-8 hr	95.47±2.34	77.67± 2.12
After stability studies at 40 ± 2 ºC (75 ± 5 %RH) for 1 month		
OF-8 hr	94.52±2.12	77.89 ± 1.95

Stability study indicates that there was no significant change in the Rizatriptan benzoate after 30 days when compared with the initial value. The results indicated that the formulation did not show any change in pH, viscosity, bioadhesive force, bioadhesive force during stability testing period .*In vitro* diffusion study, *Ex-vivo* diffusion study.

Conclusion

In the present study, an effort was made to formulate *in situ* gel for nasal drug delivery system containing rizatriptan benzoate, which can release the drugs in the physiological environment of the nasal membrane with sustained rate for the desired time duration (8 h) for the treatment of migraine. Rizatriptan benzoate was selected to overcome the migraine due to its lower half-life (2-3 hrs)during conventional delivery, to improve its bioavailability, as well as to reduce its gastric adverse effects. Estimation of rizatriptan benzoate was carried out by UV spectrophotometer at λmax 226 nm in phosphate buffer (pH:7.4). The slope of the standard calibration curve was 0.195 and R2 was found to be closer to 1.FT-IR study showed that there is no interaction between rizatriptan benzoate and various polymers/excipients (i.e.polaxamer 407 and carbopol 934P) used in present study. So, the drug and polymers/excipients are compatible. Various formulations of nasal *in situ* gel containing rizatriptan benzoate were formulated by using center composite design. For the center composite design., concentration of carbopol 934P (a) and polaxamer 407(b) were selected as two dependent variables. The formulated dosage forms were evaluated for physicochemical properties (like pH, % drug content, mucoadhesive force, gel strength, *in vitro* diffusion, *ex vivo* diffusion, stability study.). pH of all the formulations was maintained between 4.The mucoadhesive force, gel strength, *in vitro* diffusion, *ex vivo* diffusion of the developed formulations was within the acceptance value. The formulated dosage forms were evaluated for *In vitro* drug release studies and analysis of release mechanism like T50%., T80%,'k' of first order equation, 'n' of Peppas equation, 'n' of Higuchi equation were carried out to obtain optimized formulation which have released the drug from the dosage form with sustained rate for desired time duration (8 h). The optimized formulations (on the bases of maximum desirability (for first order release) were formulated from the designs..Short term stability studies as per ICH Q1C guidelines at 30 ± 2 ºC (65 ± 5 %RH) and 40 ± 2 ºC (75 ± 5 %RH) were performed for the most promising optimized formulations followed by evaluation of physicochemical properties like pH, mucoadhesive force, gel strength, *in vitro* diffusion, *ex vivo* diffusion. *Ex vivo* drug diffusion study and *in vitro* diffusion study at 8 h was closely met to standard first order release and exhibited the sustained release profile within desired time duration.

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