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Research Article

DEVELOPMENT AND EVALUATION OF INTRA NASAL *IN SITU* GEL OF VERAPAMIL HYDROCHLORIDE

Yogyata Sandeep Pathare [1]*, Vishakha Samir Hastak [2], Pranay Anil Kamble [3]

[1]Assistant Professor, Department of Pharmaceutics, STES's Sinhgad College of Pharmacy, Vadgaon (BK), Pune, Savitribai Phule Pune University, Maharashtra, India

[2]Assistant Professor, Department of Pharmaceutics, Shree Chanakya Education Society's Indira college of Pharmacy, Tathawade, Pune, Savitribai Phule Pune University, Maharashtra, India

[3]PG Student, Department of Pharmaceutics, Shree Chanakya Education Society's Indira college of Pharmacy, Tathawade, Pune, Savitribai Phule Pune University, Maharashtra, India

*Corresponding Author Email: yogyataspathare@gmail.com

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ABSTRACT

Nasal drug delivery has now been recognized as a very promising route for delivery of therapeutic compounds. *In situ* gel administration as sprays or drops and may be designed such that they undergo a sol–gel transition at the site of deposition with the implication that the increased viscosity and rheological synergy of the resulting mucus/Mucoadhesive system effects prolonged residence at the site of action[2]. The purpose of this research was to formulate the intra nasal *In-situ* gel of verapamil hydrochloride. Verapamil Hydrochloride is the calcium channel blocker; the half-life of drug is 5 to 7hrs. The intra nasal *In-situ* gel was prepared to prolong the nasal residence time and to increase its bioavailability. Bioavailability of drug is less i.e. 20 to 35 %. The *In-situ* gel was prepared by cold technique by using polymers poloxamer 407, poloxamer 188 and HPMC K_4M. *In-situ* gel were evaluated for pH measurement, gelling temperature, drug content, viscosity measurement, determination of mucoadhesive strength, gel strength determination, *in vitro* drug release, *ex vivo* permeation study. The formulations were found to be clear, having good *in situ* gelling capacity, having drug content 96-97%, showed controlled drug release over 24 hours period, the release kinetic study showed that the formulation followed first order controlled diffusion and fickian release mechanism. From the results obtained we can conclude that it is possible to formulate intra nasal *In-situ* gels of verapamil hydrochloride using poloxamer 407 and poloxamer 188.

Keywords: Intra nasal *In-situ* gel, Mucoadhesion, Verapamil Hydrochloride, Poloxamer, HPMC.

INTRODUCTION

In-situ gel formation have recently attracted the attention of many investigators for practical biomedical or pharmaceutical applications[1]. Such systems lend themselves to administration as sprays or drops and may be designed such that they undergo a sol–gel transition at the site of deposition with the implication that the increased viscosity and rheological synergy of the resulting mucus/Mucoadhesive system effects prolonged residence at the site of action[2].

Nasal mucosal membrane is a potential site for delivery of proteins and peptides because of 1. Patient acceptance 2. Avoidance of first pass metabolism. There are drug-delivery challenges that need to be overcome if intranasal drug delivery is to become the method of choice for the delivery of therapeutic agents.[2] Recent trends clearly show that intranasal therapeutics is steadily gaining momentum, since it provides an opportunity to deliver drugs both for local application and for systemic applications. The present day advances in formulation technology and choices of novel polymers have immensely contributed for effectively delivering a number of drugs via intranasal cavity[3].

Nasals mucoadhesive *In-situ* gel study due to some points such as ease of administration, accuracy of dosing, prolonged nasal residence, improve drug bioavailability[4]. Aqueous polymer solutions that are transformed into gels by changes in environmental conditions, such as temperature and pH, thus resulting in *In-situ* hydrogel formation with intranasal delivery, a drug is absorbed directly into the systemic circulation, bypassing the problems that occur with oral administration, including fast onset of therapeutic effect without the discomfort and inconvenience of an injection[4]. From a pharmacokinetic standpoint, intranasal administration circumvents first-pass elimination and drug absorption is rapid due to the existence of a rich vasculature and a highly permeable structure within the nasal membranes. Nasal delivery of drug offers many advantages over other delivery to improve the nasal absorption of drug, it is necessary to increase the nasal residence time[5]. One method to lengthen nasal residence time has been to include a bioadhesive in the formulation.

Nasal drug delivery has now been recognized as a very promising route for delivery of therapeutic compounds including biopharmaceuticals. It has been demonstrated that low absorption of drugs can be countered by using absorption enhancers or increasing the drug residence time in the nasal cavity and that some mucoadhesive polymers can serve both functions[6]. The formation of *In-situ* gels depends on factors like temperature modulation, pH change, presence of ions and ultraviolet irradiation, from which the drug gets released in a sustained and controlled manner[7]

MATERIAL AND METHODS

Verapamil hydrochloride obtained as a gift sample from Watson pharmaceuticals, Goa. Poloxamer 407 and poloxamer 188 purchase from BASF, Mumbai. HPMC K_4M from S.D. fine chemicals, Mumbai. All the chemicals and reagents required for the present experimental work are of analytical grade.

Formulation of *In-situ* gels of verapamil hydrochloride

Verapamil Hydrochloride nasal gel was prepared using different concentration of poloxamer 407 and poloxamer 188 and HPMC K_4M as shown in Table 1 by cold method. For the present work 2^2 factorial designs was selected. Briefly the method involved slow addition of polymer, drug and other additive in cold water with continuous stirring. The formed sol was stored overnight at 4°C. After overnight storage it was evaluated for all the tests[8].

Evaluation of *In-situ* gel

Visual appearance and clarity

Visual appearance and clarity were checked under fluorescent light against a white and black back ground for presence of any particulate matter[8].

pH

The pH of the prepared *in situ* gelling system after addition of all the ingredients was measured using pH meter[9].

Drug content

The drug content of verapamil hydrochloride gel was determined by UV spectrophotometric method, at λ_{max} 278 nm. 0.5 g of each formulation was taken in a 50 mL volumetric flask, diluted with PBS, pH 7.4 and shaken to dissolve the drug. The solution was filtered through Whatman filter paper no 41; 1 mL of above filtrate was diluted to 10 mL with PBS, pH 7.4. The content of the drug was estimated spectrophotometrically by noting absorbance at 278 nm[10].

Gelation temperature

Gelation temperature was noted using a modification of Miller and Donovan technique. 2 mL aliquot of mixture was transferred to test tubes, which was immersed in a water bath (4°C). The temperature of water bath was increased at the rate of 1°C/min. The samples were then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting through 90°C[11].

Gel strength

The gel strength was determined employing the technique proposed by Choi *et al*. A sample of 50 g of the nasal sol was taken in a 100 mL graduated cylinder and was allowing forming gel in a thermostatically controlled water bath at 37°C. A weight of 35 g was then placed on the gel. The gel strength, which is an indication of the viscosity of the nasal gel at physiological temperature, was determined by noting time required in seconds for the weight to penetrate 5 cm down through the gel[12].

Mucoadhesive strength

The mucoadhesive strength was determined by using the modified method. The mucoadhesive potential of each formulation was determined by measuring a force required to detach the formulation from nasal mucosal tissue. A section of sheep nasal mucosa was fixed on each of two glass vials using thread. 50 mg of gel was placed on first vial and this vial placed below the height adjustable pan. While another vial with mucosal section was fixed in inverted position to the underside of the same pan. Both the vials with gel formulation between them held in contact with each other, for 2 min to ensure intimate contact between them. Then weight was added in the second pan until vials get detached from each other. The mucoadhesive force expressed as the detachment stress in dyne/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation[13].

Viscosity measurement

Viscosity determination of the prepared *In-situ* gels as well as sols was carried out by using Brookfield synchro electric viscometer. 10 mL of the sols and 8.75 g of the developed *In-situ* gelled formulations were transferred into the beaker; temperature was maintained at 37 ± 0.5°C for all the formulations throughout the experiment. Angular viscosity was increased gradually from 5 to 100 rpm and the viscosity was measured at different rpm with spindle no. 51. The viscosity measurement was carried out in triplicate and average readings were taken for calculation[14].

In vitro drug release study

In vitro release study of the formulated *In-situ* gel was carried out in two-chamber diffusion cell through dialysis membrane-70 (Hi media) with molecular weight cut off 1200-1400 KDa. Diffusion cell (Fabricated by Sai Enterprises, Pune) of diameter 1.2 cm and 20 mL capacity consisted of upper cylindrical compartment open from above and diffusion membrane at its base. This piece of dialysis membrane was soaked in PBS, pH 7.4 for 24 hrs before mounting on diffusion cell. Dialysis membrane was mounted in a two chamber cells. *In-situ* gel was placed in the donor compartment. 20 mL of PBS, pH 7.4 was placed in the receptor compartment. The temperature of receiver compartment was maintained at the $37^0C \pm 1^0C$ during experiment and the content of the receiver compartment was stirred using magnetic stirrer. The position of the donor compartment was adjusted so that dialysis membrane just touches the diffusion medium. An aliquot of 1 mL was withdrawn from receiver compartment initially after 5, 10, 15 and 30 min and then at 1 hrs interval and replaced with same amount of fresh medium. Aliquot so withdrawn was suitably diluted and analyzed using UV spectrophotometer at 278 nm for drug. *In vitro* drug release study was carried out for 24 hrs[15].

Ex-vivo permeation study

Franz diffusion cells (FDC) were used for *ex-vivo* permeation studies. The sheep nasal membrane was mounted between the compartments of the diffusion cell with the mucosal surface facing the donor compartment. The mucosal surface of the membrane was kept in intimate contact with intranasal formulation under test. The donor compartment of FDC cell was tightly fitted with clamp to avoid any possible slip of the membrane from its position thus avoiding leakage of sample into the receptor compartment. The donor compartment contained 8 mg of verapamil hydrochloride gel and 20 mL of PBS pH 7.4 was taken in the receptor of the FDC. The whole assembly was kept at 37 ± 0.5^0C by circulating constant temperature at 38 ± 0.5^0C water through jacket of FDC and stirred at 50 rpm on a magnetic stirrer. Samples of 0.5 mL were collected through side arm of FDC every hour since the start of permeation up to the end of 24th

hour and replaced every time with an equal volume of PBS pH 7.4. *Ex vivo* permeability study was carried out on optimised formulation F1, F3, F6[16].

Model fitting

To analyse the drug release mechanism from *In-situ* gel and to interpret the best fitted model, the diffusion study data was fitted into various kinetic models such as Zero order, First order, Higuchi, Korsmeyer-Peppas and Hixon Crowell. The drug release mechanism, analysis was done with Korsmeyer equation and the release exponent (n) was calculated. The value of release exponent is an indicative of release mechanism of the drug. The values considered for n in this study were n = 0.45 (indicating Fickian diffusion-controlled drug release), n = 0.89 (case II relaxation release transport) and for 0.45 < n < 0.89 (non-Fickian or anomalous transport including both diffusion of the drug in hydrated matrix and polymer relaxation).

IR spectroscopy

IR spectrum was recorded by using Fourier transform infrared spectrophotometer (Shimadzu affinity 1-8400 S, Japan) The pellets of drug and potassium bromide were prepared by compressing the powder at 20 psi on KBr-press and the sample was scanned in the wave number range of 4000- 600 cm^{-1}. FTIR study was carried on pure drug, physical mixture of drug and polymers to confirm the compatibility of drug with other excipients used in the preparation of *In-situ* gel.

Differential scanning calorimetry

DSC study was done to check interaction amongst the drug and excipients. The DSC of verapamil hydrochloride and other excipients was recorded using S II nanotechnology (SIEKO) DSC 6220 by placing 10 mg of each, sample and alumina as a reference on aluminium pan in sample and control compartment of furnace respectively. Programmed temperature was increased from 30ºC to 300ºC (at the rate 10ºC / min.) and nitrogen gas flow rate was adjusted to 50 mL/min.

Stability Studies

Stability studies were carried out on optimized formulations according to international conference on harmonization (ICH) guidelines. A sufficient quantity of formulations in previously sterilized vials was stored in desiccators containing a saturated solution of sodium chloride, which gives a relative humidity of 75 ± 5%. The desiccators were placed in a hot air oven maintained at a temperature 40^0C ± 0.5^0C and at room temperature. Samples were withdrawn at 15 days interval for 60 Days. Percent drug remaining was calculated and plotted against time in day.

Table 1: Formulation of *In-situ* gels of verapamil hydrochloride

Formulation code	F1	F2	F3	F4	F5	F6	F7
Poloxamer 407 (%w/v)	17	17	19	19	15	17	19
Poloxamer 188 (%w/v)	7	9	7	9	----	---	---
HPMC K_4M (% w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Drug (% w/v)	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Distilled water (ml) q. s.	5	5	5	5	5	5	5

Table 2: Evaluation of formulated in situ gel of Verapamil hydrochloride

Formulation code	Appearance	Clarity	pH	Gelling capacity	Gelling temperature (ºC)	Gel strength (sec)*	Mucoadhesive strength of gel (dynes/ cm^2)*
F1	Transparent	Clear	6.4	+++	38	25.33 ± 1.52	3601.042 ± 111.37
F2	Transparent	Clear	6.3	++	37	25.66 ± 1.15	3408.133 ± 294.68
F3	Transparent	Clear	6.2	++	33	24.66 ± 1.15	3279.52 ± 192.91
F4	Transparent	Clear	6.5	++	32	26.66 ± 1.52	3215.213 ± 111.38
F5	Transparent	Clear	6.3	+	42	25.66 ± 1.52	3022.173 ± 111.26
F6	Transparent	Clear	6.3	++	38	26 ± 1 ± 1	3343.827 ± 112.38
F7	Transparent	Clear	6.5	+	34	24.33 ± 0.57	3086.61 ± 192.91

Note: *mean (n = 3)
++ Gelation immediate and remains for few hours, +++ shows gelation immediate and remains for extended period.

Table 3: Rheological studies of *In-situ* gels before gelation

Shear rate rpm	Viscosity of the formulation (cP)						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
10	1855	1635	1120	825	550	1380	535
20	1270	1190	790	640	380	860	395
50	850	655	440	420	260	485	245
100	485	320	260	240	190	280	175

Table 4: Rheological studies of *In-situ* gels after gelation

Shear rate rpm	Viscosity of the formulation (centipoise)						
	F1	F2	F3	F4	F5	F6	F7
10	4180	3280	2600	1405	1320	2020	1290
20	2270	2190	1320	1110	710	1545	695
50	1530	1080	735	680	460	890	445
100	620	560	440	405	320	500	305

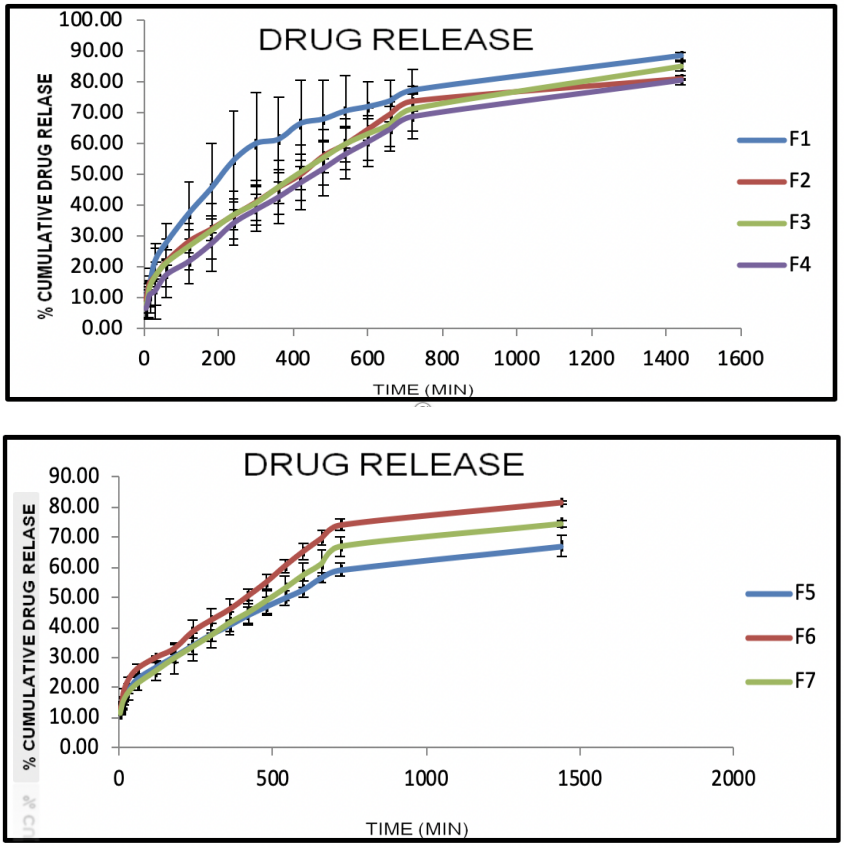

Figure 1: *In vitro* drug release of prepared formulation of *In situ* gels

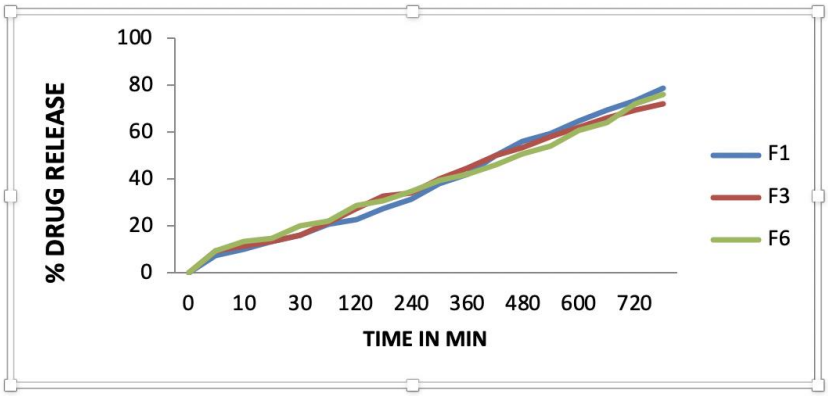

Figure 2: *Ex vivo* permeation of formulation of *In situ* gel

RESULT AND DISCUSSION

All the prepared formulations were evaluated for preliminary steps such as visual appearance, clarity, pH these formulations were transparent and clear. The pH of the formulations was found to be 6.4 to 6.5. Prepared *In-situ* gelling systems were evaluated for drug content. All the formulations gave satisfactory results. Prepared *In-situ* gelling systems were evaluated for the *in vitro* gelation temperature, gelation capacity and gel strength. Mucoadhesive strength of all the formulations was studied by using the glass vial and the nasal mucosal membrane and formulation F1 was better amongst all formulation. The results for various evaluation parameters are shown in Table 2.

Rheological Studies

The dynamic viscosities of formulations were measured as the change of shear rate before and after gelation by using Brookfield viscometer spindle no-51. The results of viscosity measurement before gelation and after gelation were shown in Table 3 and 4 respectively.

In vitro drug release study

The *in vitro* release of verapamil hydrochloride from the formulations was studied through cellophane membrane using diffusion cell. The table shows the % cumulative drug releases of the formulations. Formulation F1and F6 showing maximum drug release at 24 hrs i.e. 88.42% & 81.63 % respectively as shown in Figure 1.

***Ex-vivo* permeation study**

Permeation studies were repeated three times to confirm the result in each case (n = 3). Formulation F1 showing maximum *ex-vivo* permeation as shown in Figure 2.

Model fitting

The model formulations F1, F3 and F6 were found to follow Peppas Model kinetics which describes the drug release as a diffusion process based in Fick's law. The drug release mechanism, analysis was done with Korsmeyer equation and the release exponent (n) was calculated. The value of release exponent is an indicative of release mechanism of the drug. The values considered for n in this study were n = 0.45 (indicating Fickian diffusion-controlled drug release), n = 0.89 (case II relaxation release transport) and for 0.45 < n < 0.89 (non-Fickian or anomalous transport including both diffusion of the drug in hydrated matrix and polymer relaxation). The value of n was found to be 0.3903 for formulation F1, 0.3984 for formulation F3 and 0.3423 for formulation F6. Thus, the release mechanism suggested by R2 and n values was found to be fickian diffusion-controlled drug release/ Peppas model.

Differential Scanning Calorimetry

The thermal behaviour of verapamil hydrochloride was examined by DSC, using S II nanotechnology (SIEKO) DSC 6220. From the spectra of pure drug and the combination of drug with polymer, DSC thermograms of the pure drug and in combination with the polymers it was observed that all the characteristic peaks of verapamil hydrochloride were present in the combination thermogram, thus indicating compatibility of the drug and polymer.

IR Spectroscopy

The result of these studies revealed that there were no definite changes obtained in the bands of drug with respect to pure drug.

Stability studies

According to ICH guideline, the stability studies were carried for prepared *in situ* gelling systems. All the formulations were analyzed for visual appearance, clarity, pH and drug remaining. 60 days of stability studies reveal that there was no change in visual appearance and clarity. All the formulations showed slight changes in pH, but it was in acceptable limits (± 0.5). Study of % drug content in all the formulations reveals that there were no definite changes observed.

CONCLUSION

In this study intra nasal *In-situ* gel of verapamil hydrochloride, which is anti-hypertensive agent was prepared by using poloxamer 407, poloxamer 188 (*In-situ* gelling agent) and HPMC K_4M (mucoadhesive agent). It was found that proper ratio of poloxamer 407 and poloxamer 188 used for formulations gives controlled release formulation. Depending on the various evaluation parameters like *in vitro* drug release, mucoadhesive property, *ex-vivo* permeation rate and gelling capacity formulation F1, F3 and F6 were found to be optimized batches. Out of these formulations, F1 was found to be the best. Optimized formulations F1 (17% poloxamer 407 and 7% poloxamer 188), F3 (19% poloxamer 407 and 7% poloxamer 188), F6 (17% poloxamer 407) were liquid at room temperature. When the temperature was changed to 37^0C, they gel. The formulations were found to be clear, having good *in situ* gelling capacity, having drug content 96-97%, showed controlled drug release over 24 hours period, the release kinetic study showed that the formulation followed first order controlled diffusion and fickian release mechanism. Hence from the above results we can conclude that it is possible to formulate intra nasal *In-situ* gels of verapamil hydrochloride using poloxamer 407 and poloxamer 188.

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REFERENCES

1. Ruchir S, Chetan B, Ankita G. A review on polymer used *In-situ* gel drug delivery system. IJPI 2011; 2(1)2: 110-11.
2. Nazar H, Fatouros DG, Merwe SM, Bouropoulo N, Avgouropoulos G, Tsibouklis J *et al*. Thermosensitive hydrogels for nasal drug delivery: The formulation and characterisation of systems based on N-trimethyl chitosan chloride. European Journal of Pharmaceutics and Bio pharmaceutics 2011; 77(2): 225–232.
3. Nuggehally RS. Recent research trends in intranasal therapeutics – Select case studies of various therapeutic strategies with pharmacokinetic pharmacodynamic attributes and drug development considerations. Asian Journal of Pharmacodynamics and Pharmacokinetics 2009; 9(3): 169-178.
4. Zakia MN, Awada GN, Mortadaa N. Enhanced bioavailability of metoclopramide HCl by intranasal administration of a mucoadhesive *in situ* gel with modulated rheological and mucociliary transport properties. European Journal of Pharmaceutical Sciences 2007; 32: 296–307.
5. Dhakar RC, Maurya SD, Tilak VK, Gupta AK, Dangi G. A review on factor affecting the design of nasal drug delivery system. IRJP 2010; 1(1): 29-42.
6. Kant A, Reddy S, Shankraiah MM. Venkatesh JS, Nagesh C. In situ gelling system an overview. Pharmacology online newsletter 2011; (2): 28-44.
7. Parmar V, Lumbhani AN. Development and evaluation of ion dependent *In-situ* nasal gelling system of metoclopramide hydrochloride as an anti-migraine model drug. International Journal of Latest Research in Science and Technology 2012; 1(2): 80-89.
8. Nandgude T, Thube R, Jaiswal N, Deshmukh P, Chatap V, Hire N. Formulation, and evaluation of pH induced *In-situ* nasal gel of salbutamol sulphate. International Journal of Pharmaceutical Science and Technology 2008; 1(2): 177-183.
9. Nisha GS, Maithil P, Charyulu RN. Formulation and development of nasal *in Situ* gels of triptans, for anti-migraine activity. International Journal of Research in Pharmaceutical and Biomedical Sciences 2012; 2(3): 861-868.
10. Gowda DV, Tanuja D, Khan M, Desai J, Shivakumar HG. Formulation and evaluation of *In-situ* gel of diltiazem hydrochloride for nasal delivery', scholars research library 2011; 3(1): 371-381.
11. Yadav DJ, Kunjwani HK., Suryawanshi SS. Formulation and evaluation of thermosensitive *in Situ* gel of salbutamol sulphate for nasal drug delivery system. International Journal of Pharmacy and Pharmaceutical Sciences 2012; (4) 4: 188-194.

12. Uttarwar S. Formulation and development of *in situ* gelling system for nasal administration for an antiemetic drug ondansetron hydrochloride by using pluronics 127p and pluronics 68. International Journal of Research in Pharmaceutical and Biomedical Sciences 2012; 3(3): 1103-1118.
13. Basu S, Bandyopadhyay AK. Development and characterization of mucoadhesive *in situ* nasal gel of midazolam prepared with *Ficus carica* mucilage. AAPS Pharm Sci Tech 2010; 11(3): 1223-1231.
14. Athare AB, Rohamare P, Bansode A, Mahale N, Chaudhari S. Formulation, and evaluation of eletriptan hydrobromide thermo reversible nasal *In-situ* gel. IJPRD 2012; 4(4): 267-275.
15. Kolsure PK, Rajkapoor B. Development of zolmitriptan gel for nasal administration. Asian Journal of Pharmaceutical and Clinical Research 2012; 5(3): 88-94.
16. Nirmal HB, Bakliwal SR, Pawar SP. *In-situ* gel: New trends in controlled and sustained drug delivery system. International Journal of Pharm Tech Research 2010; 2(2): 1398-1408.

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