Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

pH Triggered Nasal *In Situ* Gelling System for Ampicillin Sodium for Sinusitis

A GEETHALAKSHMI[1]*, ASHWINI B[2], ROSHAN M JAIN[1]

[1] Department of Pharmaceutics, R. R. College of Pharmacy, Bangalore, Karnataka, India.

[2] The Oxford College of Pharmacy, Begur road, Bangalore, Karnataka, India.

ARTICLE DETAILS

Article history:
Received on 27 June 2020
Modified on 15 January 2021
Accepted on 26 January 2021

Keywords:
Ampicillin Sodium,
β-lactum Antibiotic,
In Situ Gel,
Carbopol 934,
HPMC K4M
pH Triggered Method.

ABSTRACT

The bioavailability of conventional nasal solutions is very poor due to mucocilliary clearance, enzyme activity present in nasal mucosal membrane and drainage which remove rapidly various foreign substances including drug from the surface of the nasal. Frequent instillation of drug solution is necessary to maintain a therapeutic drug level in the nasal or at the site of action but the frequent use of highly concentrated solution may induce toxic side effects due to highly vascularized systemic absorption of drug. The recent trends in nasal drug delivery are *in situ* gelling systems. Ampicillin sodium is a β-lactum antibiotic belonging to amino penicillin family. In this investigation pH triggered nasal *in situ* gelling system was formulated by using carbopol 934 and HPMC K4M , it was evaluated for several parameters like drug-polymer interaction, appearance, clarity, pH measurement, drug content (%), gelling capacity, gelation time, viscosity, *in vitro* drug release studies, comparative diffusion studies and short term stability studies. In the developed selected formulation which exhibits 7th h 65.2% release with non-irritating, sterile and stable properties thus increasing residence time of drug with better patient compliance. Comparative diffusion studies showed to sustain release of drug for 7h than marketed nasal drops for 2h, so it was concluded that Ampicillin sodium *in situ* gel is a viable alternative to conventional system.

INTRODUCTION

Nasal drug delivery has been recognized as potential route from ancient days and nowadays it becomes an important tool in the treatment of various disorders. In recent years many drugs have been shown to achieve better systemic bioavailability through nasal route than by oral administration. Nasal drug delivery has been practiced for thousands of years have been given a new lease of life. It is useful delivery method for drugs that are active in low doses and show no minimal oral bioavailability such as proteins and peptides [1]. In humans and other animal species the major functions of the nasal cavity are breathing and olfaction. It also affords an important protective activity once it filters, heat and humidity by the inhaled air before reaching the lowest – airways [2]. In humans cytochrome P450 enzyme iso forms have been identified and they are CYP1A, CYP2A and CYP2E. Other enzymes also include carboxylesterases and glutathione S- transeferases [3-5]. Intranasal drug delivery system several approaches are considered viz, depends on the nature of pathologic condition [acute or chronic] and intended effects of drug treatment [local, systemic or at CNS] [2].

Sinusitis [6] is an inflammation, or swelling, of the tissue lining the post nasal. Normally, sinuses are filled with air, but when sinuses become blocked and filled with fluid, germs (bacteria, viruses, and fungi) can grow and cause an infection. Population based studies have estimated 134 million Indians suffer from chronic sinusitis, the symptoms of which include but are not limited to debilitating headaches, fever and nasal congestion and obstruction. Among Indians this disease is more widespread than diabetes, asthma, coronary heart disease. One in eight Indians suffer from chronic sinusitis caused by the inflammation of the nasal and throat lining, which results in the accumulation of mucus in the sinus cavity, and pressure build-up in the face, eyes and brain. Conditions that can cause

Author for Correspondence:
Email: geeshwar@gmail.com

sinus blockage include the common cold, allergic rhinitis (swelling of the lining of the nose), nasal polyps (small growths in the lining of the nose), or a deviated septum (a shift in the nasal cavity).

In situ gelation is a process of gel formation at the site of action after the formulation has been applied as solution at the site. *In situ* gel phenomenon based upon liquid solution of drug formulation and converted into semisolid muco-adhesive key depot. It permits the drug to be delivered in a solution form [7]. The principle involved is that the nasal formulations imbibe in the nasal fluid after administration and forms gel into the nasal cavity through the two phases of propulsion and preparatory phase. After the formation of the gel, dissolution occurs and or the mucociliary removal towards the nasopharynx occurs [8].

MATERIALS AND METHOD

Material

Ampicillin trihydrate was obtained as a gift sample from Yarro pharma ltd (Mumbai, India). HPMC K4M, carbopol 934, benzalkonium chloride, sodium chloride, sodium hydroxide was obtained from SD Fine chemical pvt ltd (Boisar). All other chemicals and reagents used in the study were of analytical grade.

Methods

Preformulation Studies

Fourier Transform-Infra Red Spectroscopy Studies (FT-IR)

The interaction studies between the drug, carbopol 934 and HPMC K4M were studied on FT-IR spectroscopy. Spectra of ampicillin sodium, carbopol 934, HPMC K4M and physical mixture were compared at 400-5000.

Solubility Studies

Solubility of Ampicillin sodium was checked with different solvents and distilled water at room temperature.

Melting Point

Melting point of Ampicillin sodium was determined by taking small amount of drug separately in a capillary tube closed at one end and placed in a melting point apparatus and the temperature at which drug melts was recorded. This was performed in triplicates and average value was reported.

Conversion of Sodium Salt of Ampicillin By Ampicillin Trihydrate (By Alkalization Method)

Conversion of sodium salt of Ampicillin was carried by using 1 mole of Ampicillin trihydrate to 1 mole of Sodium hydroxide solution in the frozen state, since ampicillin sodium is hygroscopic nature in the presence of moisture [9].

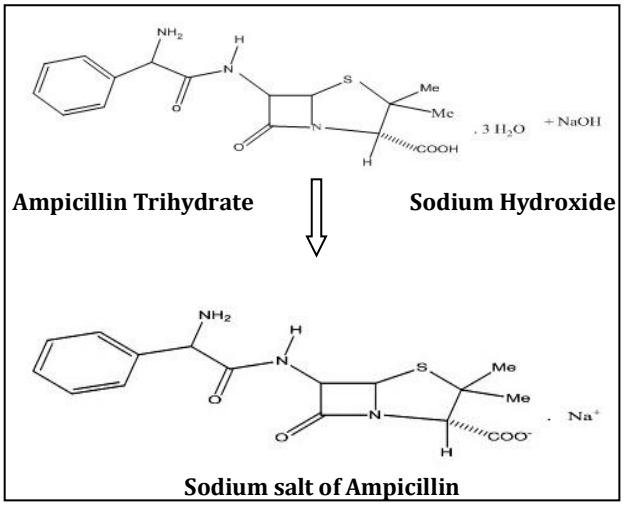


Figure 1: Conversion of Ampicillin trihydrate to sodium salt of Ampicillin

Preparation of pH Triggered *In Situ* Gelling System

The formulations were prepared by dispersing carbopol 934 in distilled water with continuous stirring until completely dissolved and allowed to hydrate overnight.

Table 1: Formulation Chart of Formulation F1-F9

Ingredients (%w/v)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ampicillin trihydrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sodium hydroxide	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Carbopol 934	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8
HPMC K4 M	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water	100	100	100	100	100	100	100	100	100

For the preparation of polymer solution first, HPMC K4 M was added in distilled water and allowed to hydrate overnight, then carbopol 934 was sprinkled over this solution. After complete hydration of polymers a separate solution of drug and sodium chloride was added to the polymeric solution. The resultant solution was thoroughly mixed. Benzalkonium chlorides were then added and mixing was confirmed until a uniform and clear solutions were formed. Final volume was made by adding required volume of distilled water [10, 11]. Terminal sterilization was carried out by autoclaving method to maintain the sterility of the nasal formulations.

Post Formulation Studies

Appearance and Clarity

The developed formulations were inspected visually for clarity, color in solution and gel form against white back ground and for particulate matter if any present [12].

pH of Formulation

pH of prepared formulations were determined by using digital pH meter [13].

Gelation Time

The gelation time is defined as the time taken for the transition of liquid phase to gel. 2ml aliquot of gel was taken in test tube and kept in an oven maintained at 37°C. The sample was examined for gelation [14].

Drug Content

Each formulation (25mg) 1ml was taken in a 100ml volumetric flask diluted to 100ml by SNF pH 6.4 & shaken to dissolve the drug. The solution was filtered through whatmann filter paper. 1ml of above filtrate was pipetted out & diluted to 10ml with SNF. The content of drug was estimated spectrometrically by UV at 272nm [15].

Gelling Capacity

The gelling capacity of the prepared formulations were determined by placing 250µl of the formulation in a test tube containing 2ml of freshly prepared SNF pH 6.4 and visually observed. The time taken for their gelling was noted [16].

Rheological Studies

The viscosity measurements were done by using Brookfield DV-II+ pro viscometer using LV-3 spindle. The developed formulations were poured into the 100ml beaker and the angular velocity was increased gradually from 10 to 100 rpm at room temperature. The angular velocity was reversed gradually. The average of the two readings was used to calculate viscosity. By adding SNF the formulations were made into gel form and viscosity was determined at room temperature as specified above using LV-3 spindle [17].

***In Vitro* Drug Diffusion Studies**

The *in vitro* diffusion of Ampicillin sodium from the formulations was studied through cellophane membrane using a franz diffusion apparatus. The diffusion medium used was freshly prepared SNF. Cellophane membrane, previously soaked overnight in the diffusion medium (SNF), was placed in between the donor and receptor compartment. 1 ml volume of the formulation was accurately instilled into the donor compartment. SNF was placed in the receptor compartment according to the capacity of it. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37±0.5°C. The magnetic bead was rotated such that it produced a vortex and touched the cellophane membrane. Aliquots, each 1 ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with SNF and analyzed by UV visible spectrophotometer at 272 nm [18]. Drug release kinetic modeling was performed.

Test for Sterility [19]

Method/ Procedure: Tests for sterility were performed for aerobic using fluid thioglycollate medium as per IP.

Sterility (Negative Control) Test: Fluid thioglycollate media was incubated at 30-35°c and soyabean casein digest medium at 20-25°C for not less than 7 days. No growth of organisms was observed.

Growth Promotion (Positive Control) Test: Here, the sterile media was inoculated with about 100 viable micro-organisms and incubated according to the conditions specified. The test media were satisfactory, if clear evidence of growth appears in all media within 7 days. Nasal preparations should be sterile and must be checked for the presence of any bacteria or fungi before it is used. In each test, three sterile test tubes were used in the study and are labeled as 'negative control', 'test' and 'positive control'.

Test for Aerobic Bacteria: 20 ml each of sterile fluid thioglycollate was transferred to 3 tubes aseptically. The tube was labeled as positive control was inoculated with viable aerobic microorganism *bacillus subtilis* aseptically. 2.5 ml of the nasal preparation was added to the test tube labeled as test. Then all three test tubes were incubated at 30-35°C for not less than 7 days.

Test for Fungi: 20 ml each of sterile soyabean-casein digest medium was transferred to 3 tubes aseptically. The tube labeled as positive control was inoculated with *candida albicans* aseptically. 2.5 ml of the nasal preparation was added to the tube labeled as test. Then all the three test tubes were incubated at 20-25°C for not less than 7 days. The sterility testing of nasal drug delivery system were performed for aerobic bacteria and fungi by using fluid thioglycollate medium and soyabean casein digest medium respectively as per the IP procedure

Isotonicity Evaluation

Isotonicity is an important characteristic of the nasal drug delivery system. Isotonicity has to be maintained to prevent tissue damage. Formulations were mixed with few drops of blood and observed under microscope at 45x magnification and compared with standard marketed nasal formulation. The shape of blood cell was compared with standard marketed nasal formulation as per IP [20].

***In Vivo* Measurement of Mucociliary Transit Time (MTT)**

All the animal experiments are carried out after prior approval of the protocol by institutional animal ethics committee. Male Wistar rats (n=6), weighing 250–320 g are anesthetized using intra-peritoneal injection of urethane solution (1200 mg/kg). Using a micropipette, 5 µl of prepared gel containing a dye, xylene cyanol (3 mg/ml), is instilled 0.5 cm deep into one of the nostrils of the rats. The pharyngeal cavities of the dosed rats were swabbed with cotton tipped applicators. The swab samples are collected at a minute's interval for first 30 min and then at an interval of 5 min for next 90 min. As controls, 5µl of dye solution (prepared in normal saline) and 5µl dye loaded pH triggered gel (without mucoadhesive polymer) are administered to rats. After suitable dilution in water, dye content from each swab is estimated using a validated UV-visible spectrophotometric method [21, 22].

Comparative Diffusion Study on Marketed Product

The marketed product (Sinarest AF) and selected formulation F6 are subjected to diffusion studies. The study is preformed using 100ml of SNF pH 6.4 at temperature 37±0.5°C and at the speed of 50rpm in the franz diffusion cell. 1ml sample was withdrawn at different interval of time and replaced with 1ml SNF in the diffusion media. 1ml of sample was diluted with 10ml of SNF pH 6.4, the solution was assayed spectrophotometrically at 272nm.

Stability Studies

Stability studies were carried out on most satisfactory formulations as per ICH guidelines. Sterile gel forming nasal solution were filled in autoclavable transparent plastic bottles, closed with autoclavable rubber closures and sealed with aluminum foils. The formulations were kept in stability chamber of at 40±2°C & 75±5% RH for 6 months. Samples were evaluated for appearance, pH, clarity, drug content, gelling capacity, isotonicity and *in vitro* diffusion [23].

RESULTS AND DISCUSSION

Fourier Transform-Infra Red Spectroscopy Studies (FT-IR)

The studies were carried out using *FT-IR* spectroscopy to establish any possible interaction of polymer and excipients with the drug in the formulation. The *FT-IR* spectrum of drug alone as well as combination of drug with polymer and excipients were obtained analysed for the compatibility. *FT-IR* study showed that there is no interaction between drug and polymer because it shows the characteristic peak of drug and excipients; hence the drug and polymer are compatible. Results were shown in Fig. 2 and 3.

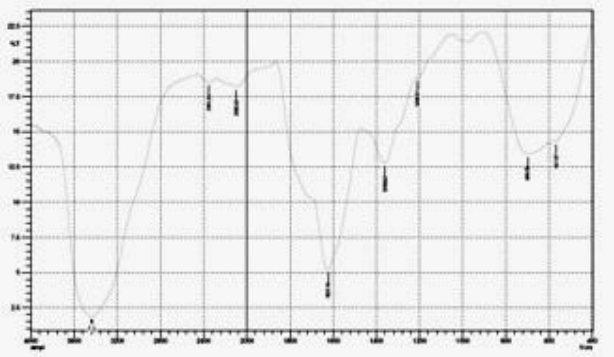


Figure 2: *FT-IR* spectrum of pure Ampicillin sodium

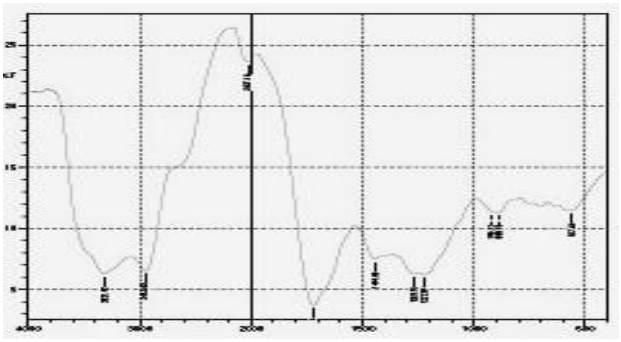


Figure 3: *FT-IR* spectrum of Ampicillin sodium+ HPMC K4M+ carbopool 934

Solubility Studies

Solubility of Ampicillin sodium shows to have higher water solubility than any other solvent proves drug is soluble in nasal fluids hence increases bioavailability as shown in Table 2.

Table 2: Solubility studies of Ampicillin sodium on different solvents

Solvent	Solubility (mg/5ml)
Water	1010±0.56
Chloroform	32±0.39
Methanol	490±0.73

Melting Point

Melting point of drug lies in standard melting range shows its identity of Ampicillin sodium and its purity as shown in Table 3.

Table 3: Melting point of Ampicillin sodium

Sl. No.	Actual Melting Point	Observed Melting Point
1	212°C-215°C	215°C
2		214°C
3		215°C

Gelation Time

Gelation time was found within 1 min due to combination of mucoadhesive polymer (HPMC K4M) and pH sensitive polymer (carbopol 934) proves to have an immediate gel formation as shown in Table 4.

Table 4: Gelation Time of F1-F9 Formulations

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Time (secs)	65	61	63	51	58	54	56	55	58

Appearance, Clarity and pH

The formulations from F1 to F9 were opaque and clear. The pHs of all the formulations were within the acceptable range i.e.6.2-6.5 and hence would not cause any irritation upon administration (Table 5).

Gelling Capacity

The two main perquisites of gelling system are viscosity and gelling capacity. The formulations should have an optimum viscosity for easy installation into the nose as a liquid which undergo *sol-gel* transition. All the formulation gelled instantaneously with a translucent matrix on exposure to SNF, this may be due to the gelling agent. Out of F1-F9 formulations, F1 and F2 formed gel are not for extended period and from F3-F9 showed its action for prolonged period of time proving to drug remain bound to nasal mucosa (Table 5).

Drug Content

The drug content of all the formulations lies in the range of 97.3-99.4%. For F1-F9 formulations, indicating the greater uniformity of the dosage in the formulations. The evaluation results are mentioned in Table 5.

Table 5: Physico Chemical Evaluation of *In Situ* Gel of F1-F9 Formulations.

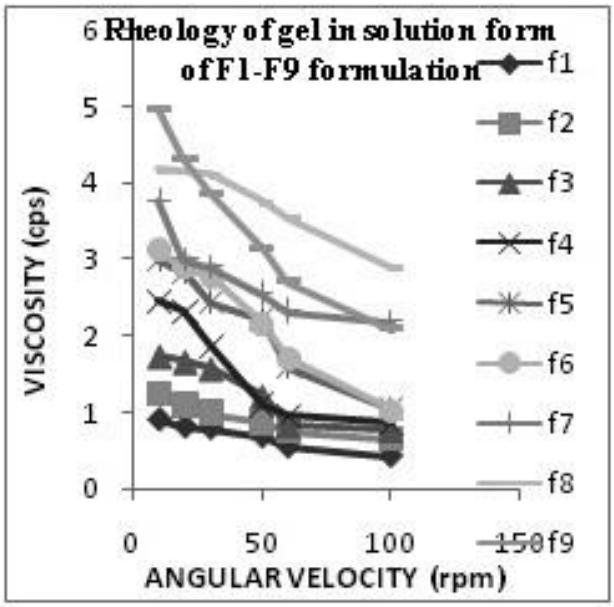
Formulation Code	Drug Content (%)	Visual appearance	Clarity	Gelling capacity	pH
F1	97.3±0.52	Opaque	Clear	+	6.22±0.68
F2	97.7±0.98	Opaque	Clear	+	6.52±0.32
F3	98.9±0.34	Opaque	Clear	++	5.94 ±0.78
F4	99.2±1.2	Opaque	Clear	++	6.23±0.11
F5	98.6±1.55	Opaque	Clear	++	6.42±0.09
F6	97.9±0.98	Opaque	Clear	+++	6.51±0.72
F7	98.3±0.88	Opaque	Clear	+ ++	6.34±0.66
F8	99.4±0.65	Opaque	Clear	+++	6.40±0.52
F9	97.2±0.21	Opaque	Clear	+++	6.50±0.32

+: Gels after few minutes, remains for up to 2-3 hour. ++: Gelation immediate remains for up to 4-6 hour. +++: Gelation immediate remains for up to 7-9 hour.

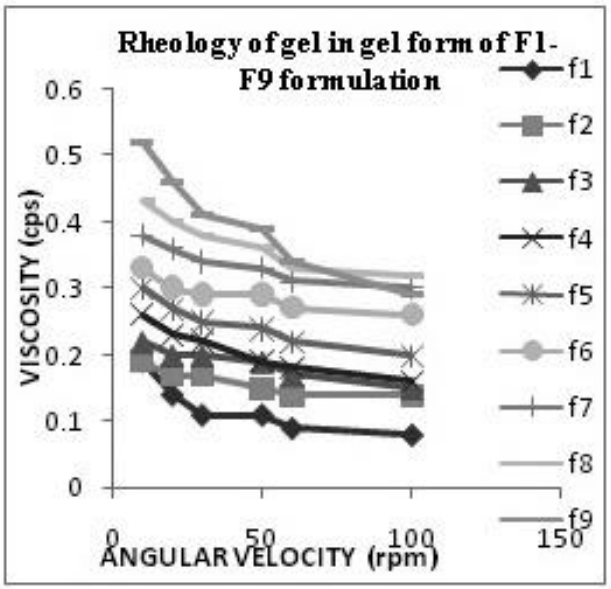
Rheological Studies

Formulations F1-F9 ranged from 0.90-2.09 cps at 22°C in solution form. All the formulations exhibited plastic rheology in solution form, i.e. an increase in viscosity with increase in angular velocity. All the formulation ranged from 0.52-0.19 cps exhibited pseudo-plastic rheology in gel form as shown by shear thinning and a decrease in the viscosity with increase in angular velocity. Among all F6, F7 gave good viscosity range and gelling capacity (Fig. 4). The order of viscosity of all the formulation:

F9(1.8%)>F8(1.6%)>F7(1.4%)>F6(1.2%)>F5(1.0)>F4(0.8%)>F3(0.6%)>F2(0.4%)>F1(0.2%)



(A)



(B)

Figure 4: Rheological studies of F1-F9 formulations (A) before gel and (B) after gel.

Isotonicity Evaluation

Isotonicity has to be maintained to prevent tissue damage or irritation of nasal. F6 formulation was subjected to isotonicity testing, since they did not show any crenation or swelling of blood cells when compared with the marketed formulation.

Test for Sterility

The formulation passed the sterility test as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 7 days at 30-35°C in case of fluid thioglycolate medium and at 20-25°C in case of soyabean casein digest medium. The overall results of the sterility test showed that, the prepared nasal formulation passed the sterility test.

***In Vivo* Measurement of Mucocilliary Transit Time Study**

The study was performed to assess and compare *in vivo* mucoadhesive properties of selected formulation of F6 and F7 based on *in vitro* drug release data. From the result plain pH triggered gel showed significant increase ($p<0.05$) in MTT value than control (aqueous dye solution). Both formulations containing mucoadhesive polymers displayed significantly ($p<0.01$) higher MTT than plain pH triggered gels without mucoadhesive. However formulation F6 showed higher MTT value than formulation F7 showing increased residence time in nasal cavity, mentioned in Fig. 5.

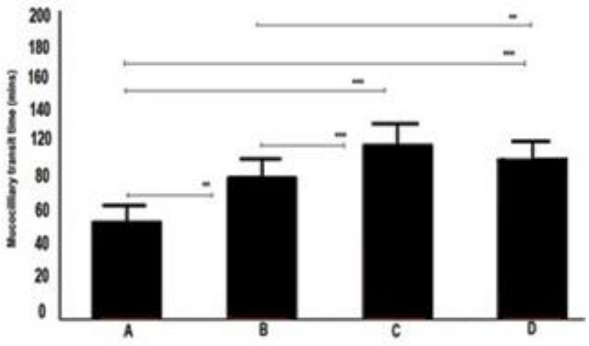


Figure 5: *In vivo* MTT of nasal *in situ* gel . *In vivo* MTT of nasal *in situ* gel \(A) Control: Aqueous dye, (B) Control 1: plane pH triggered gel without mucoadhesive, (C) F6 formulation, (D) F7 formulation.

** Indicates very significant difference in the compared values at $p<0.05$, *** Indicates extremely significant difference in the compared values at $p<0.01$

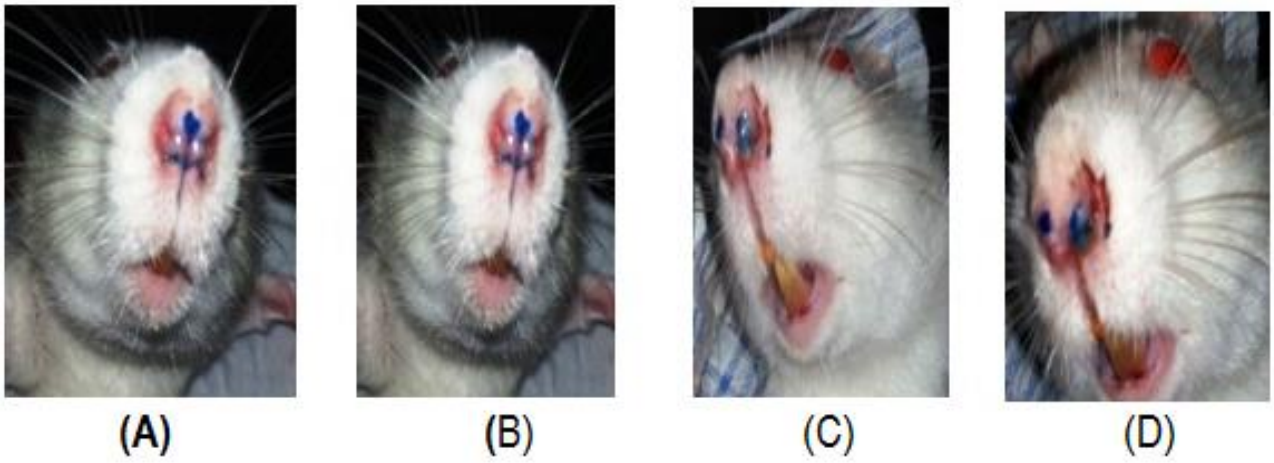


Figure 6: *In vivo* MTT of nasal *in situ* gels. (a) Control: aqueous dye. (b) control 1:plane pH triggered gel without mucoadhesive, (c) Formulation F6, (d) Formulation F7.

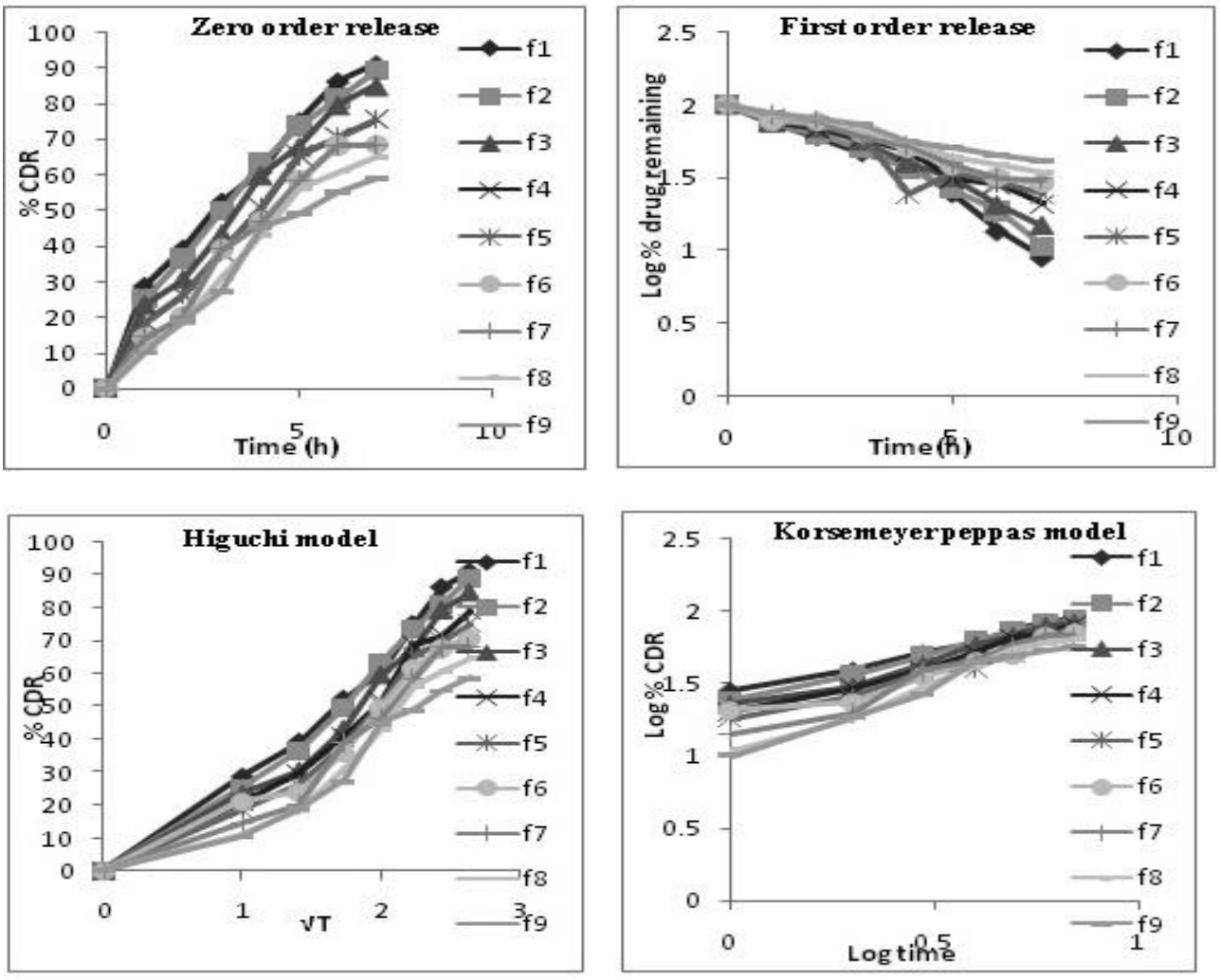


Figure 7: Kinetic release model of F6 formulation depicting Zero order release, First order release, Higuchi model and Korsemeyer peppas model of F1-F9 formulation.

Release Kinetic Study of Formulation F1-F9

All the prepared formulation was fitted in zero order release, first order release, Higuchi model and Korsemeyer peppas model based on the *in vitro* drug release data. The diffusion data was found best fitted in first order showing that the drug release is dependent on concentration of polymer, carbopol 934, results are shown in Fig. 5, Table 6.

Table 6: Release kinetic study data

Formulation code	Zero order	First order	Higuchi	Kosermeyer-Peppas	
	r^2	r^2	r^2	r^2	N
F1	0.944	0.988	0.002	17.4	2.77
F2	0.946	0.983	0.012	15.7	2.75
F3	0.906	0.990	0.087	14.9	2.79
F4	0.889	0.964	0.104	13.9	2.69
F5	0.918	0.973	0.107	11.3	2.65
F6	0.853	0.961	0.180	12.1	2.57
F7	0.899	0.975	0.197	10.4	2.50
F8	0.871	0.970	0.232	10.4	2.42
F9	0.917	0.974	0.231	7.90	2.37

Comparative *In-Vitro* Diffusion Studies

The selected formulation was compared with the marketed formulation (Sinarest AF) based on *in vitro* release data. F6 formulation showed 62.3% at 7th h, in case of marketed product drug release was found 91% at 2nd h which indicated that prepared formulations were of extended release for longer period, as shown in Fig. 8.

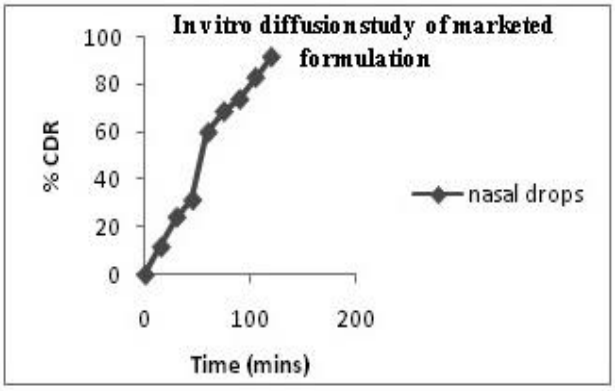


Figure 8: *In vitro* diffusion study of marketed formulation (Sinarest AF)

Stability Studies

Stability studies of the formulations were carried out as per the ICH guidelines. Appearance, pH, clarity, gelling capacity, drug content of selected formulation F6 stored at 40 ± 2°C & 75 ± 5% RH for 6 months at due to optimum results based on % CDR and viscosity to assess their long term stability as per ICH guidelines. The results showed that there was no statistically significant change in the *in vitro* drug diffusion studies of F6 formulation. Results are as shown in Fig. 9, 10 and Table 7.

The stability study readings of rheological studies of selected F6 formulation after six months at 40 ± 2°C & 75 ± 5% RH are shown in Fig. 9.

Table 7: Stability study readings of physico chemical evaluation after six months of selected F6 formulation

Formulation code	1st month	3rdmonth	6thmonth
Clarity	Clear	Clear	Clear
pH	6.47	6.45	6.44
Gelling Capacity	+++	+++	+++
Isotonicity	Isotonic	Isotonic	Isotonic
Drug content	97.9±1.35	99.9±1.38	99.4±1.09

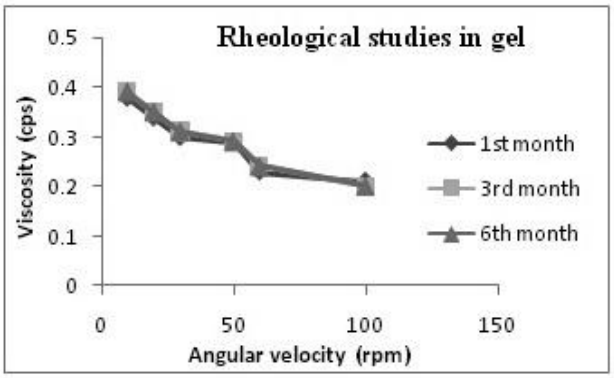


Figure 9: Rheological studies of selected F6 formulation

In vitro drug diffusion studies after six months of selected F6 formulation

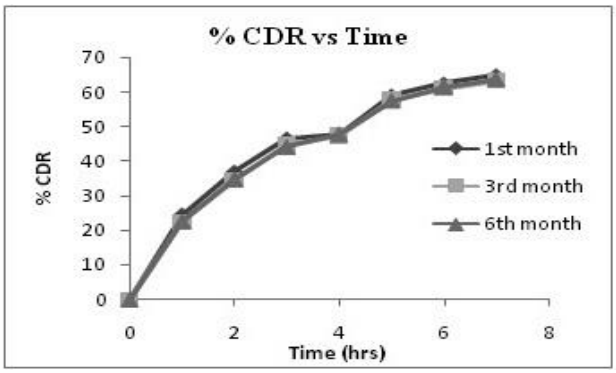


Figure 10: *In vitro* drug diffusion studies after six months of selected F6 formulation

CONCLUSION

In the present work, an *in situ* gel of Ampicillin sodium was formulated successfully which when instilled into nasal fluid the sol becomes gels, thereby it provides increased contact time, so that it improves the bioavailability results in better therapeutic effects. The nine formulations were prepared by using carbopol 934 and HPMC K4M by pH triggered method. In this F6 showed good results in the evaluation tests as pH, appearance, clarity, gelation time, *in vitro* studies. The developed *in situ* gelling system may be the alternative to conventional nasal drops as

it may provide better patient compliance through easy and decreased frequency of administration.

ACKNOWLEDGMENT

The authors would like to take the priviledge to thank The Oxford College of Pharmacy from the bottom of my heart who supported and encouraged me to complete this venture comfortably and successfully.

REFERENCES

[1] Khairnar P.S, WalkeP.S, NarkhedeM.R, Nehete J.Y. Formulation and i*n-vitro* evaluation of thermoreversible Rizatriptan benzoate nasal gel. Int J Pharm Pharm Sci. 2011;3(4):250- 256.

[2] Ramya D. D, Abhirami M, Brindha R, Gomathi S, Vedhahari B.N. In-Situ gelling system – potential tool for improving therapeutic effects of drugs. Int J Pharm Pharm Sci.2013; 5(3): 27- 30.

[3] Thornton Manning J.R, Dahl A.R.Metabolic capacity of nasal tissue interspecies comparisons of xenobiotic metabolizing enzymes. Mutat Res. 1997; 380(1-2): 43-59.

[4] Aceto A, Di Llio C, Angelucci S, Longo V, Gervasi P.G, Federici G.Glutathione transferases in human nasal mucosa. Arch Toxicol. 1989; 63: 427-431.

[5] Krishna N.S, Getchell T.V, Dhooper N, Awasthi Y.C,Getchell M.L. Age and gender-related trends in the expression of glutathione S-transferases in human nasal mucosa. Ann Otol Rhinol Laryngol. 1995; 104:47-56.

[6] Sinusitis. Available at: http://www.webmd.com/allergies/sinusitis-and-sinus-infection.

[7] Chien Y.W, Su K., Chang S.F. A text book of anatomy and physiology of the nose, in nasal systemic drug delivery: drugs and the pharmaceutical sciences. New York: Marcel Dekker, 1989; 1–26.

[8] Bajpai V. *In situ* gel nasal drug delivery system – A review. Int J Pharma Sci. 201; 577-580.

[9] Conversion to sodium salt. Available at: www.patentgenius.com/patent/4130558.html

[10] Keny R. V, Bhat S. S, Monteir V. Design and evaluation of novel carbopol based ocular sol-gel phase transition system of flucanazole in the management of fungal keratitis. Ind J Pharm Sci. 2004; July – Aug: 567-568.

[11] Geethalahshmi A, Roopa karki. pH triggered in situ gelling system for brimonidine tartrate. Int J Pharmagen. 2011; 2(2):159-164.

[12] Suh H, Jun H.W. Physicochemical and release studies of noproxenin poloxamer gels. Int J Pharm. 1996; 13-20.

[13] Patel R. P, Baria A. H, Pandya N. B, Tank H. M. Formulation, evaluation and optimization of stomach specific *in situ* gel of ranitidine hydrochloride. Int J Pharm Sci Nanotech. 2010; 3(1): 141-153.

[14] Vijay Kumar M, Aravindram A. S, Rohitash K. D. V. Gowda Parjanya K. Formulation and evaluation of *in-situ* gel of bromhexine hydrochloride for nasal delivery. Der Pharmacia Sinica. 2012**;** 3(6):699-707.

[15] Charoo A, Kohli K., Ali A. Preparation of in situ forming ophthalmic gels of ciprofloxacin hydrochloride for the treatment of bacterial conjunctivitis: *in vitro* and *in vivo* studies. J Pharm Sci. 2003; 92(2):407-413.

[16] Balasubramanium J, Kant, Pandit, J.K.. *In vitro* and *in vivo* evaluation of the gelrite gellan gum based ocular delivery system forIndomethacin. Acta Pharm.2003;53(4): 251-261.

[17] Hanan M, Laithy E, Demiana I, Nesseem, Shoukry M. Evaluation of two *in situ* gelling systems for ocular delivery of Moxifloxacin. J Chem Pharm Res.2011; 3(2):66-79.

[18] Kavitha K., Rajas N. J. Sustained ophthalmic delivery of levofloxacin hemihydrate from an ion activated *in situ* gelling system. Int J Pharm Tech Res. 2011; 3(2): 702-706.

[19] Indian Pharmacopoeia Ghaziabad: The Indian pharmacopoeia Comission. Sterility.2007; 52-55.

[20] Sautou Miranda V, Labret F, Grand-Boyer A, Gellis C, Chopineau J. Impact of deep freezing on the stability of 25mg/ml vancomycin ophthalmic solutions. Int J pharm. 2002; 234(1-2): 205-207.

[21] Lale A.M, Mason J.D, Jone N.S. Mucociliary transport and its assessment: a review. Clin Otolaryngol. 1988; 23(5): 388–96.

[22] Lewis J.L, Nikula K.J, Novak, R, Dahl A.R. Comparative localization of carboxyl esterase in F344 rat, beagle dog and human nasal tissue. Anat Rec. 1994; 239(1): 55-64.

[23] ICH guidelines. Available at: URL: http://www.tga.health.gov.au/docs/pdf/euguide/ich/273699r2en.