

DEVELOPMENT OF ZOLMITRIPTAN GEL FOR NASAL ADMINISTRATION

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ABSTRACT

Development of thermoreversible zolmitriptan nasal gel were aimed to improve absorption and patient compliance. In the present research work, mixture of pluronic F-127 (Poloxamer 407) and pluronic F-68 (Poloxamer 188) were used to confer temperature – sensitive gelation property. To modulate the gel strength and bioadhesive force for zolmitriptan nasal gel, bioadhesive polymers such as sodium alginate, sodium carboxymethyl cellulose and polyvinyl pyrrolidone (PVP K-25) were investigated. Incorporation of 2.5 % w/w zolmitriptan in the nasal gel showed no effect on the gelation temperature of the pluronic mixtures, while addition of the bioadhesive polymers had showed increase in gelation temperature. On the other hand these polymers reinforced the gel strength and the bioadhesive force of the prepared nasal gel formulation. The effect was most pronounced with sodium alginate. Increasing the concentration of bioadhesive polymers retarded the release of sumatriptan from the pluronic gel. PVP had less effect on the drug release. Histopathological examination of sheep nasal mucosa with control and optimized formulation did not show any histological damage to the nasal tissue.

Keywords: Nasal, zolmitriptan, gel, pluronic,

INTRODUCTION

Administration of large number of drugs such as nafarelin acetate, gentamicin ergotamine tartarate etc. by nasal route results in blood levels comparable to intravenous route^{1, 2}. However, short nasal residence time of drug formulation result in interindividual variability in absorption profile. Nasal route of administration would also circumvent the presystemic metabolism thus increasing the bioavailability of the drugs. In addition, due to the presence of microvilli and high vasculature, the absorption can be expected to be faster compared to oral route. Attempts have been made to improve nasal residence time and bioavailability by designing bioadhesive swelling microspheres, liposomes and erythrocytes – based systems³.

Zolmitriptan, 4S-4-({3-[2-(dimethylamino) ethyl]-1H-indol-5-yl} methyl)-1, 3-oxazolidin -2-one, is a second – generation triptan prescribed for patients with migraine attacks, with or without an aura, and cluster headaches. It has a selective action on serotonin (5-HT_{1B/1D}) receptors and is very effective in reducing migraine symptoms, including pain, nausea, and photo- or phonophobia⁴. It is currently available as a conventional tablet, an oral disintegrating tablet and nasal spray (2.5 mg and 5.0 mg per dose). The nasal dose is claimed to be absorbed rapidly, with detectable plasma zolmitriptan concentration within 2 min after administration. In contrast, plasma zolmitriptan concentrations are generally not detected until 15-20 min after administration of a tablet formulation (Yates et al., 2002). Patients with migraine generally suffer from nausea and vomiting; oral treatment can therefore be inconvenient or could fail (Aurora et al., 2006). The absolute bioavailability of zolmitriptan is up to 40% for both oral and nasal dosage forms (Rapoport et al., 2004). The faster clearance of the drug from the nasal cavity could explain the low bioavailability for nasal formulation.

Pluronic block copolymers which are also termed as poloxamers or synperonic consist of ethylene oxide (EO) and propylene oxide (PO) blocks arranged in a triblock structure. PEO-PPO-PEO block copolymers have been widely used in medical, pharmaceutical formulation and cosmetic system because of their ability to self – aggregate thereby forming micelles and liquid crystalline phases^{5, 6}. The core of the micelles is hydrophobic consisting of polypropylene oxide, while a hydrophilic corona consists of ethylene oxide. Self assembly is temperature dependent and at a given polymer concentration a critical micellization temperature (cmt) exists⁷. Pluronics are available in wide range of molecular hydrophilic regions. The high solubilizing capacity and non-toxic properties of pluronics make it suitable for drug delivery. Pluronic is more soluble in cold water than hot water. The hot solution process has been

attributed to excessive hydrogen bonding between water molecules and ethereal oxygen of the polymer. The pluronic solutions are transformed from low viscosity transparent solutions at 5°C to solid gels on heating to body temperatures. The temperature – dependent gelling process is micellar in nature, being constructed from cubic orientation of micellar subunits⁸. The micellar mode of association has been useful as drug delivery systems⁹. The aqueous solution of pluronic are known to exhibit the phenomenon of reverse thermal gelation, remaining as solution at temperature and gelation upon increasing the temperature^{10, 11}. The reversal thermal gelation exhibited by pluronic aqueous solutions has been used as drug delivery system for ophthalmic¹², parenteral¹³, rectal¹⁴, and percutaneous use¹⁵. Stratton et al. has reported the improved stability of proteins and hence their complete recovery of activity in pluronic gels matrix¹⁶. Furthermore, the bioadhesive polymers such as sodium alginate, sodium carboxymethyl cellulose and polyvinyl pyrrolidone would reinforce the drug sustained action, and may help to control the gel strength and bioadhesive force of nasal gel.

This research work is undertaken to develop the zolmitriptan nasal gel using pluronic F-127 and pluronic F-68 and bioadhesive polymers. The gelation temperature of the prepared pluronic solution were modulated so as to lie between 30°C and 37°C which was reported¹⁷ as being an acceptable range to ensure gelation at physiological temperature without leakage after administration. The gel strength, bioadhesive force were determined and the drug release from the prepared nasal gel formulation finally the nasal mucosa was examined histopathologically.

MATERIALS AND METHODS

MATERIALS

Zolmitriptan was a gift sample from Natco Labs, Hyderabad, India. Pluronic F-127 and pluronic F-68 was kindly supplied by BASF Corporation, Mumbai, India. Sodium alginate, sodium carboxymethyl cellulose and polyvinyl pyrrolidone (K-25) of extra pure grade were supplied by Emcure Research Center, Pune, India. Benzalkonium chloride was procured from Loba Chemicals, Mumbai, India. All other chemicals were of research grade.

METHODS

Preparation of nasal gel formulations

Aqueous zolmitriptan nasal gel using different concentration of pluronic F-127 and pluronic F-68 and various formulation additives as shown in Table 1 were prepared by cold method described by schomolka et al¹⁸. Briefly, the method involved slow addition of polymer, drug and other additive in cold water with continuous agitation. The formed mixtures were stored overnight at 4°C.

Table 1: Composition of nasal formulation

Ingredient (In %)	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈	F ₉	F ₁₀	F ₁₁	F ₁₂
PF-127	15	15	15	15	17	17	17	17	20	20	20	20
PF-68	---	5	7	10	---	5	7	10	---	5	7	10
*Drug	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sorbitol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Na-Meta.	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
BKC	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Dist.Water (Qs) in ml	10	10	10	10	10	10	10	10	10	10	10	10

The nasal gel formulation showing satisfactory gelation temperature (30°C - 37°C) were selected as optimized formulation. On this optimized formulation further study was carried out and additional amount of bioadhesive polymer namely sodium alginate, sodium

carboxymethyl cellulose (Na-CMC), and polyvinyl pyrrolidone (PVP) were added in the concentration 0.5, 1, and 1.5 %. Composition of nasal formulation containing bioadhesive polymers are shown in Table 2.

Table 2: Composition of nasal formulation containing bioadhesive polymer.

Ingredient	F ₁₃	F ₁₄	F ₁₅	F ₁₆	F ₁₇	F ₁₈	F ₁₉	F ₂₀	F ₂₁
Optimized (F ₈)	**	**	**	**	**	**	**	**	**
Sodium alginate	0.5	1.0	1.5	**	**	**	**	**	**
Sodium CMC	**	**	**	0.5	1.0	1.5	**	**	**
PVP K 25	**	**	**	**	**	**	0.5	1.0	1.5

Evaluation of nasal gel

Determination of gelation temperature

Gelation temperature was assessed using a modification of Miller and Donovan technique¹⁹. A 2 ml aliquot of gel was transferred to test tubes, immersed in a water bath at 4°C. The temperature of water bath was increased in increments of 1°C. 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.

Determination of bioadhesive force

Bioadhesion of nasal gel was determined by Hang-Gon-Choi method²⁰. The modified balance technique (Figure 1) using two-glass vials and sheep nasal mucosa was used. The 0.5 ml of gel sample was placed between the two mucosal membrane attached to the bottom of the vials. Weights were added on the other side of the balance. The minimum weight required to break the mucosal adhesion was measured.

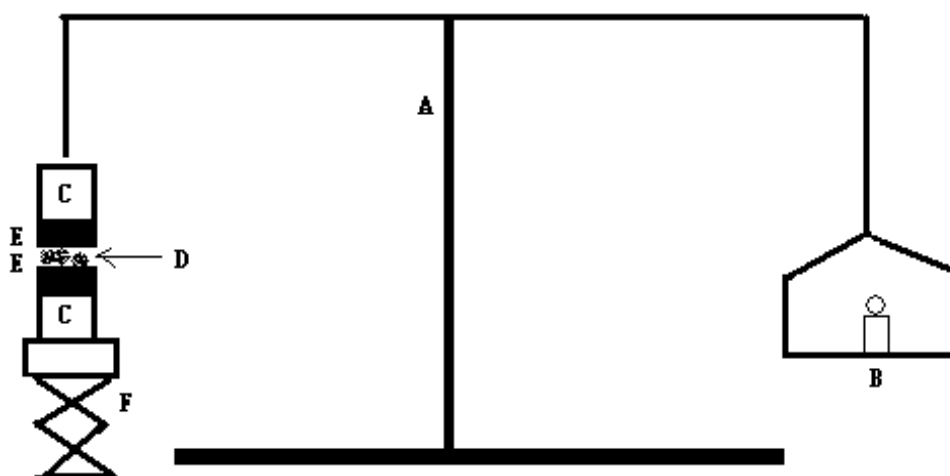


Figure 1: Bioadhesive force measuring device, (A) Modified balance (B) weights (C) glass vial (D) nasal gel formulation (E) nasal tissue (F) Height adjustable pan

Measurement of gel strength

The gel strength was determined employing the technique proposed by Choi²⁰. A sample of 50 g of the nasal gel was put in a 100-ml graduated cylinder and gelled in a thermostatically controlled water bath at 37 °C. A weight of 35 g was then placed onto the gelled solution. The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature, was determined by the time in seconds the weight took to penetrate 5 cm down through the gel.

In-vitro diffusion studies

In-vitro diffusion of gels was performed (using jacked nasal diffusion cell, sheep nasal mucosa)²¹. The recipient chamber was filled with

60 ml distilled water (37°C ± 2°C) and 0.2 ml test formulation was placed on the dorsal mucosa. Diffusion samples (1 ml) at predetermined intervals were transferred to test tubes and analyzed spectrophotometrically (Shimadzu 1700, Japan) at 228 nm.

Histopathological examination of nasal mucosa

Two nasal mucosa pieces (3 cm²) were mounted on nasal diffusion cells. One mucosa was used as control (0.6 ml water) and the other was processed with 0.6 ml optimized nasal gel formulation (condition similar to *in vitro* diffusion). The mucosa tissues were stained with hematoxylin and eosin. The sections under light microscope were photographed²².

Stability study

Sumatriptan nasal gel was tested for stability under actual condition of storage (refrigeration condition) in clean, dry, airtight, moistureproof bottles, stored away from light. The gel samples were withdrawn at 15, 30, 45, days and evaluated for sumatriptan content, viscosity and pH. The viscosity of the gel was determined using Brookfield digital RVDV + Pro viscometer (Brookfield Engineering Laboratories). The pH of gel was determined by using calibrated pH meter (Equip Tronics).

RESULT AND DISCUSSION

Formulation development

With nose drops and sprays, it is difficult to hold the medicaments in the nasal cavity for a long time, hence is not useful for slow releasing preparations. An approach to improve nasal drug absorption is to increase the duration of formulation residence within the nasal

cavity. This can be achieved by the use of bioadhesive polymers. The formulation on application is wetted with the intranasal secretion and gradually swells and causes the released medicaments to be absorbed through the nasal mucosa. Besides releasing the medicaments slowly, this formulation also achieves safe administration of medicaments because of the direct absorption of the drug through the mucosa of the nasal cavity. In the present study zolmitriptan gel has been formulated and evaluated. Polyhydric alcohol like sorbitol and sodium metabisulphite was used in the formulation as an humectant and antioxidant respectively and they are likely to affect drug absorption.

Evaluation of nasal gel

Gelation temperature

As it is important that nasal gel must have a gelation temperature in the range of 30-37°C so as to be in liquid form at room temperature and to form a gel phase instantaneously in the nose. The surface – active poloxamer (pluronic) consisted of water insoluble polyoxypropylene portion sandwiched between two polyoxyethylene chains and are known to exhibit the thermoreversible gelation, depending on the polymer grade, concentration and other included formulation components. The thermoreversibility of pluronics could be explained on the basis that they are more soluble in cold than hot water with an increased solvation and hydrogen bonding at low temperatures. Being surfactants in nature, the temperature-dependent gelation of pluronic solutions could be explained as being a change in their micellar properties by temperature increase. Temperature played an important role in the micelle formation process through hydration of the ethylene oxide units. At low temperature water was a good solvent for polyoxyethylene and a good solvent for polyoxypropylene also.

Ultrasonic velocity, light scattering and small angle neutron scattering studies have reported gelation of PF127 due to body – center-cubic packing of spherical micelles^{15, 23}. The ¹³C NMR studies have concluded that increased temperatures produced conformational changes in the methyl group of the

polyoxypropylene within the hydrophobic micellar region, and in the motion of the hydrophilic end chains. The subsequent dehydration increased chain friction and caused gelation^{24, 25}. According to a thermodynamic model, there existed a local higher order of water molecules around the hydrophobic unit of the polymer in solution²⁶. As gelation occurred, the interaction between the hydrophobic units of polymer molecules squeezed out these ordered water molecules into the bulk solution of lower order. This resulted in an overall disordered, which was the driving force for hydrophobic association.

At higher temperature the solubility of polyoxypropylene was reduced and micelle formation occurred. Raising the temperature of pluronic solution will be accompanied by an increase in the micellar aggregation number and a decrease of critical micelle concentration, allowing the formation of a more closely packed and a more viscous gel. Moreover, configurational changes in the orientation of the methyl groups in the side chains of poly (oxypropylene) polymer chains, constituting the core of micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation temperature^{24, 27}. These fundamental physicochemical approaches reported above can be used to illustrate and explain the results presented.

Table 3 shows that the nasal gel of less than 20 % w/w PF-127 did not gel over the temperature range tested (up to 50°C) and that increasing PF-127 concentration, by an increments of 2-3% decreased, the gelation temperature of its solution. On the other hand PF-68 in the tested range of gelation temperature, where all the recorded temperature values were >50°C (result not shown). This means that increasing the PF-68 concentration decreases the gelation temperature. The previous finding indicates that neither PF-127 alone could provide gelation at the physiological temperature. A modulation of the gelation temperature to reach the desired range (30-37°C) could be achieved through the use of a combination of the pluronic grades²⁸. The w/w percentage ratios of PF-127/PF-68 with gelation temperature (33.5°C) in the range of 30 -36 °C were 17/10 %. Among all the formulation, the formulation F₈ of PF- 127/PF-68 mixtures were selected as an optimized formulation for further study. It is to be noted that zolmitriptan nasal gel composed of 17 % of PF-127 alone showed intermediate gelation temperature (38°C) so this is the good formulation for gelation temperature modulation using PF-68. The nasal gel prepared with other concentration (15 % and 20 %) of PF-127 exhibited either high (43-48°C) or low (26-29°C) gelation temperatures compared to the range (30-37°C). In addition, the concentration of PF-127 (17 %), it was found that the gradual increase in PF-68 concentration was accompanied by a concomitant decrease in the gelation temperature of the prepared nasal gel. It was also noticed that, with all the concentration of pluronic F-68, mixtures containing lower percentage of pluronic F-127 (17 %) gelled at a higher temperature than those containing the higher percentage (20 %). This revealed that pluronic F-127 is the main polymer determining the gelation temperature of the solution and might be explained on the basis of its higher molecular weight (average molecular weight 12,500) compared to that of PF-68 (8,600) and its higher amount in formulation.

Table 3: Gelation temperatures of pluronic solutions.

Pluronic	Concentration (% w/w)	Gelation temperature (°C) ± S.D.
Pluronic F-127	15	>50
	17	41.5 ± 0.67
	20	27 ± 1.2
PF-127/PF-68	15/5	50 ± 0.7
	15/7	48 ± 1
	15/10	45 ± 0.58
	17/5	40 ± 0.7
	17/7	38.5 ± 0.1
	17/10	33.5 ± 1
	20/5	27 ± 1.2
	20/7	26.5 ± 1.1
	20/10	21 ± 0.7

Table 4: Effect of bioadhesive polymers on the gelation temperature.

Code	Bioadhesive polymer	Conc. (In %)	Gelation Temp.(°C) ± S.D
F ₁₃	Na- alginate	0.5	31.5 ± 1.01
F ₁₄		1.0	29 ± 0.81
F ₁₅		1.5	26 ± 1.73
F ₁₆	Na-CMC	0.5	32 ± 1.69
F ₁₇		1.0	30.5 ± 0.28
F ₁₈		1.5	29 ± 1.54
F ₁₉	PVP (K-25)	0.5	33 ± 1.09
F ₂₀		1.0	32.5 ± 1.14
F ₂₁		1.5	32 ± 2.02

Bioadhesive force

The different bioadhesive polymers used in this study are either swellable or water soluble but differ in their nature and charge, like sodium alginate, sodium carboxy methyl cellulose, polyvinyl pyrrolidone (K-25). Table 4 shows that the addition of any of the used bioadhesive polymers lowered the gelation temperature of nasal gel. The impact of bioadhesive polymers on the gelation temperature was found to depend on their nature and concentrations. Increasing the concentration of any of the used bioadhesive polymer from 0.5 % to 1.5 % produced a gradual decrease in the gelation temperature of nasal gel.

In comparison of formulation containing bioadhesives the mean average decrease in the gelation temperature noticed with all the bioadhesives used can be arrange in the following order,

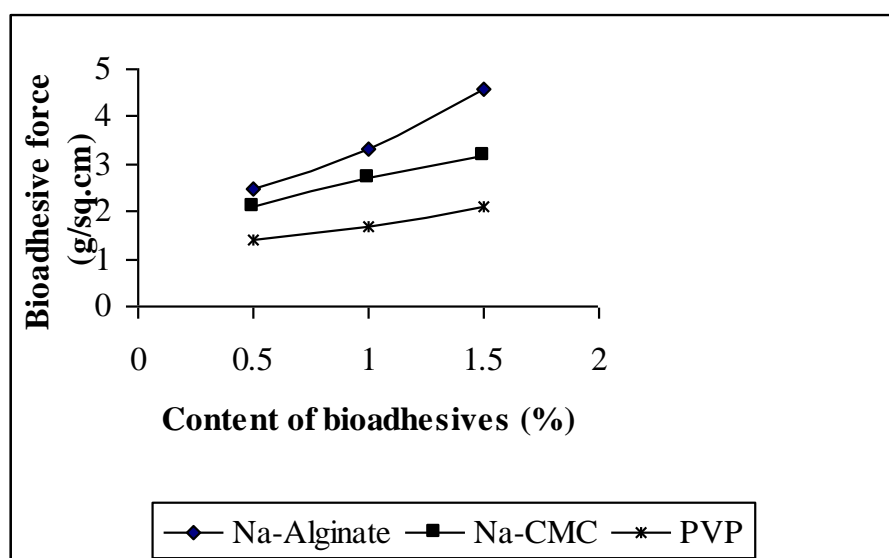
Na- alginate > Na- CMC > PVP

This order of arrangement correlated well with viscosity of all formulation containing these polymers. The direct relation between gelation temperature and viscosity of polymer solution has been reported by Jeong et al²⁹. The gelation temperature lowering effect of bioadhesive polymer could be explained by their ability to bind to the polyoxyethylene chains present in the pluronic molecules. This will promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding which will lead to gelation at lower temperature.

Bioadhesive force means the force with which nasal gel binds to nasal mucous membranes and it is an important physiological parameter for gelling the nasal gel since it prevents the gelled solution dropping out of the nose. Since the nasal mucous membrane consisted of glycoproteins, capable of interacting with diverse material²¹. Zolmitriptan nasal gel possessed moderate bioadhesive force through hydrogen bonding and chain penetration effect in mucosa. The stronger the bioadhesive force is, the more it can prevent the gelled solution coming out of the nose. But if the bioadhesive force is too excessive, the gel can damage the nasal mucous membranes. Therefore, nasal gel must have the balanced bioadhesive force. The force, with which zolmitriptan nasal gel bound to sheep nasal mucosa obtained by modified balance method. The addition of different bioadhesive polymers reinforced the bioadhesive force of nasal gel and the bioadhesive force significantly increase as the concentration of bioadhesive polymers increased over the range of 0.5 to 1.5 % are showed in Figure 2. Their bioadhesive polymers could be arrange according to their bioadhesive enhancing effect at 1.5 % concentration of nasal gel as,

Na -Alginate > Na-CMC > PVP

Our result showed that, Sodium alginate has a higher bioadhesive force than sodium CMC. The mechanism of the increase can not be explained, but might be related to hydrogen bonding between the gel and mucosal membrane (glycoproteins) via carboxyl group in the bioadhesive polymers.

**Figure 2: Effect of bioadhesive polymers on bioadhesive force**

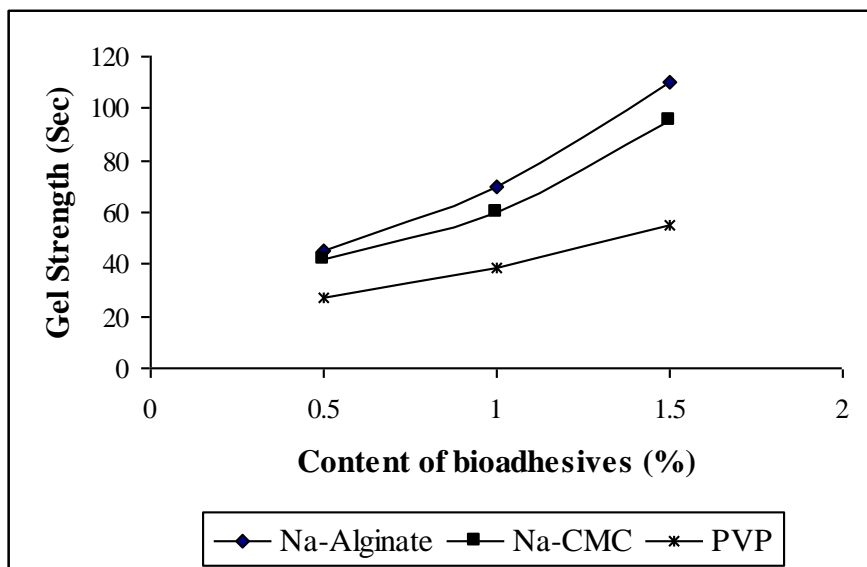


Figure 3: Effect of bioadhesive polymers on gel strength

Gel strength

In the development of zolmitriptan nasal gel, the gel strength is important in finding the condition, which allows the easy nasal administration of gel and no leakage from the nose. It is very important that the nasal gel formulation must have suitable gel strength. Figure 3 shows the effect of bioadhesive polymers on the gel strength. The gel strength of nasal gel formulation at 37°C, increased as the concentration of sodium alginate, Na-CMC, PVP increased. Among all the five bioadhesive polymers, sodium alginate exhibited the largest increase. The mechanism of the increase was hydrogen bonding between pluronic and bioadhesive polymers in the nasal gel.

In-vitro diffusion studies

Diffusion studies were carried out using the nasal diffusion cell. In this study for optimization of formulation the concentration of additives like sorbitol, sodium chloride, benzalkonium chloride, and sodium metabisulphite were held constant. The previous findings indicate that PF-127 and PF-68 alone could not provide suitable

gelation temperature and also the drug release were affected i.e. when we increased the concentration of PF-127 the drug release is get decreased. The decrease in release may be due to increase in number and size of micelles and increase in overall microviscosity of aqueous channels, because of that reason the combination of pluronic F-127 and pluronic F-68 was used. As the concentration of pluronic F-127 increased, the mixtures needed smaller amounts of pluronic F-68 to study the effective drug release.

In addition, it was obvious from Figure 4 that the release of zolmitriptan was not only affected by pluronic concentration but also by the type of bioadhesive used. So, all the bioadhesive polymer retarded the drug release from nasal gel, sodium alginate exhibited highest retardation than sodium carboxy methyl cellulose and hydroxyl methyl cellulose. The less effect of PVP may be attributed due to its low viscosity (K-25) grade used in this study and partly due to its water soluble nature which allowed more rapid penetration of dissolution fluid in to the product initiating product surface dissolution.

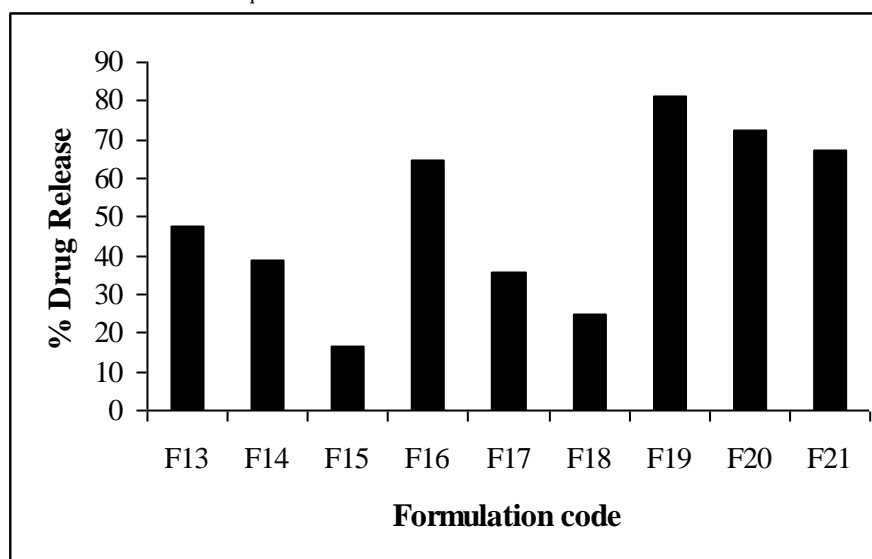


Figure 4: Effect of bioadhesive polymer on drug release

The retarding effect of the bioadhesive polymers could be attributed to their ability to increase the overall product viscosity as well their

ability to distort or squeeze the extracellular aqueous channels of pluronic micelles through which the drug diffuses thereby delaying

the release process. The bioadhesive can be arranged according to their release retarding effect as follows,

Na- alginate > Na- CMC > PVP

This order of arrangement correlated well with the viscosities and the gelation temperature lowering effect of these polymers as previously mentioned.

Table 5: Release and Kinetic data of zolmitriptan nasal gel formulations.

Code	Bioadhesive polymer	Conc. (In %)	% Drug Release (After 8 hrs) in %	Model fitting	
				Release Exponent (n)	Kinetic constant (K)
F ₁₃	Na- alginate	0.5	47.49 %	0.5478	0.1631
F ₁₄		1.0	39.01 %	0.6832	0.2145
F ₁₅		1.5	16.69 %	0.8281	0.4179
F ₁₆	Na-CMC	0.5	64.42 %	0.7238	0.6587
F ₁₇		1.0	35.92 %	0.8706	0.7547
F ₁₈		1.5	24.90 %	0.7615	0.9213
F ₁₉	PVP (K-25)	0.5	81.12 %	0.7426	0.9021
F ₂₀		1.0	72.55 %	0.6956	0.7937
F ₂₁		1.5	67.19 %	0.7260	0.7612

Histopathological study

The histology of nasal mucosa in control and treated with optimized nasal gel formulation after 8 hours is shown in Figure 5. The microscopic observations indicate that the optimized formulation

has no significant effect on the microscopic structure of mucosa. The surface epithelium lining and the granular cellular structure of the nasal mucosa were totally intact. No major changes in the ultrastructure of mucosa morphology could be seen and epithelial cells appeared mostly unchanged.

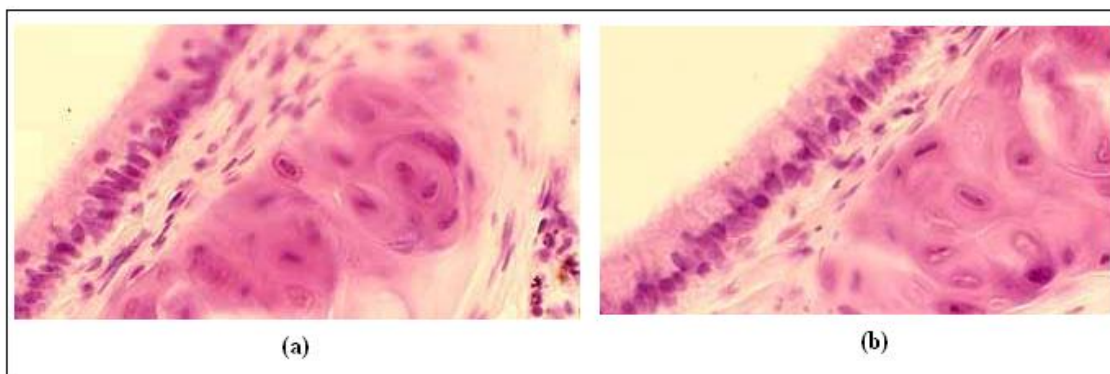


Figure 5: Histology of nasal mucosa (a) Control (b) Optimized nasal gel formulation.

Stability study

The United States Food and Drug Administration (FDA) accepts only real - time stability data for pharmaceuticals for the purpose of assessing shelf life, hence accelerated stability studies may only serve as a tool for formulation screening and stability issues related to shipping or storage at room temperature³⁰. The results of stability studies are shown in Table 6. The drug content results indicated that there was no significant change in the zolmitriptan after 45 days when compared with the initial value. One of the important parameters that have to be considered while formulating a gel formulation is the viscosity. In case of the nasal drug delivery, viscosity is also required to retain the formulation at the point of application. The results (Table 6) indicated that the formulation did not show any change in viscosity when stored under refrigeration conditions. Similarly no change in pH was observed during the stability testing period.

CONCLUSION

Study revealed that the temperature sensitive gelling system can be formulated using optimum concentration of PF-127 and PF-68 that can gel at the body temperature. Addition of bioadhesive polymers can prolong the release of zolmitriptan that may be helpful for migraine treatment.

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