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Original Article

Formulation and Evaluation of Mucoadhesive Nasal *in-situ* Gel of Diclofenac Sodium

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ABSTRACT

Intranasal administration represents a viable option for local and systemic delivery of diverse therapeutic compounds. The Physiological range of the nasal mucosal temperature lies between 32-34°C. The aim of this work was to formulate and characterize thermosensitive gels based on Pluronic F127, a thermosensitive polymer, and carbopol 934P, a mucoadhesive polymer, intended for the nasal delivery of Diclofenac sodium. Nasal in-situ gel of Diclofenac sodium was prepared by cold method using different ratio of Pluronic F127 and carbopol 934P.The formulations were optimized based on gelation temperature, gelation time, drug release and mucoadhesive strength. The gelation temperature was found to be between 26.5 ± 1.52 ° C to 38.16 ± 1.52 ° C and pH was found to be in the range of 4.9 ± 0.814 to 6.1 ± 0.242 respectively. Pluronic F127 can be a promising in situ gelling vehicle for nasal drug delivery system. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the in -situ gel dosage forms very reliable.

1. INTRODUCTION

Intranasal administration represents a viable option for local and systemic delivery of diverse therapeutic compounds [1]. Diclofenac is used to treat pain, inflammatory disorders, and dysmenorrhoea. Its conventional dosage form such as tablet causes peptic ulcer, inflammatory bowel disease such as crohn's disease and ulcerative collitis.

In these conditions, the intranasal delivery seems to be an attractive alternative. The large surface area of the nasal mucosa affords a rapid onset of therapeutic effect, potential for direct-to-central nervous system delivery, no first-pass metabolism, and non-invasiveness; all of which may maximize patient convenience, comfort, and compliance [2]. However, the limitations of a nasal delivery include: potential local tissue irritation; rapid mucociliary clearance of the therapeutic agent from the site of deposition resulting in a short span of time available for absorption; low permeability of the nasal membrane for the larger macromolecules; presence of proteolytic enzymes that may cause degradation in the nasal cavity; limited formulation manipulation for changing drug delivery profiles; and possible presence of pathological conditions such as colds or allergies which may alter nasal bioavailability [3]. Strategies to overcome these limitations include: the use of bioadhesive polymers that increase residence time of the formulation in the nasal cavity thereby improving absorption; the use of nontoxic enhancers to improve the permeability of the nasal membrane [4, 5] Although the nasal mucosa poses a permeation barrier to high molecular-weight therapeutics such as peptides and proteins, the tight junctions that form this barrier to paracellular drug delivery can be reversibly and safely opened [2]. *In situ* forming polymeric formulations are drug delivery systems that are in sol form before administration in the body, but once administered, undergo gelation *in situ*, to form a gel [6]. The formation of gels depends on factors like temperature modulation, pH change, presence of ions and ultra violet irradiation, from which the drug gets released in a sustained and controlled manner. Mainly *in situ* gels are administered by oral, ocular, rectal, vaginal, injectables and intraperitoneal routes. The *in situ* gel forming polymeric formulations offer several advantages like sustained

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and prolonged action in comparison to conventional drug delivery systems [7]. Poloxamer 407 is a hydrophilic non-ionic surfactant of the more general class of copolymers known as poloxamers. Poloxamer 407 is a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol [8]. The objective of the present study was to develop a diclofenac sodium mucoadhesive in situ nasal gel which would enhance nasal residence time and absorption of drug across nasal mucosal.

2. MATERIAL AND METHODS

Materials

Diclofenac sodium was obtained from Hab Pharmaceuticals and Research Ltd. Dehradun as a gift sample. Carbopol (MW: 15,000) was received from SD-fine chemicals, Mumbai. Pluronic F127 was procured as a gift sample from Sigma. Polyethylene glycol was obtained from HiMedia Laboratories Ltd., Mumbai, India.

Preparation of *in-situ* nasal gel

Diclofenac sodium along with mucoadhesive polymer and PEG 6000 were dissolved in distilled water by agitation at room temperature. After cooling the solution to 4°C, PF127 was added slowly with agitation. The resulting dispersion was then kept overnight at 4°C until clear and viscous transparent solution was formed. Finally volume was adjusted by using cold distilled water. The physiological range of the nasal mucosal temperature lies between 32-34°C. PF127 vehicles with concentration varying from 16% wt/vol to 27% wt/vol were screened preliminarily to decide lowest possible concentration. Optimized concentration of PF127 was used for further study of effect of mucoadhesive polymer on gelation temperature, mucoadhesive strength and spreadability (Table 1).

Table 1. Optimization of Pluronic F127

Conc. of Pluronic F 127(%)	Diclofenac sodium (%)	Gelation Temp.(^{0}C)	Gelation time (Min.)
16.5	5	38	2.086
18.5	5	28	1.171
20.5	5	27	1.089
22.5	5	26	0.820
24.5	5	25	0.499
26.5	5	23	0.381

Optimization of mucoadhesive polymer concentration

Bioadhesive anionic polymer Carbopol 934P was slowly added to the solution with continuous agitation. C934P was added in concentration range of 0.1% wt/vol to 1% wt/vol to PF127 solution. Optimized Concentration of PF127 (18.5 %) alongwith Diclofenac Sodium (5%) was used for further studies (Table 2).

Table 2. Composition of *in-situ* nasal gel

Batch	Carbopol 934P (%)	PEG 6000 (%)	Water
A1	0	0	q.s.
A2	0.1	0.3	q.s.
A3	0.3	0.6	q.s.
A4	0.5	1	q.s.
A5	0	0.3	q.s.
A6	0.1	0	q.s.
A7	0.3	1	q.s.
A8	0.5	0.6	q.s.
A9	0	0.6	q.s.
A10	0.1	1	q.s.
A11	0.3	0	q.s.
A12	0.5	0.3	q.s.
A13	0	1	q.s.
A14	0.1	0.6	q.s.
A15	0.3	0.3	q.s.
A16	0.5	0	q.s.

3. RESULTS AND DISCUSSION

Gelation Temperature

A 5 ml aliquot of gel was transferred to test tubes, immersed in a water bath at 25°C and sealed with aluminium foil. The temperature of the water bath was increased in increments of 0.5°C and left the equilibrate for 1 minute at each new setting. 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. The gel melting temperature, the temperature at which a gel starts flowing upon tilting through 90 °C was recorded.

Gelation Time

For assessing gelation time, a glass slide was used. Provision was made so as to keep the slide in hot water. The goat nasal mucosa was pasted from serosal side on the slide then hot water was circulated for 15-20 minutes for equilibrating temperature of mucosa at 34±2°C .One drop of formulation was placed on the mucosa at an angle of 120°C and time taken for converting it into gel was recorded.

pH

The apparent pH of a product may alter on storage as chemical change. Changes in product pH also indicate chemical decomposition, most probably of a hydrolytic nature. From each formulation, 1 ml quantity was transferred to the 10 ml volumetric flask and diluted by using dis tilled water to make 10 ml. pH of resulting solution was determined by using pH meter.

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Viscosity

The viscosities of various formulations were measured by using cone and plate Viscometer. The results of gelation temperature, gelation time, pH, viscosity of various formulations is shown in Table 3.

Table 3. Gelation temperature, gelation time, pH, viscosity of various formulations.

Batch	Gelation Temp. (°C)	Gelation time (Min.)	pH	Viscosity (Cps)
A1	28.36 ± 0.5	1.171 ± 0.121	5.1 ± 0.204	13000 ± 0.221
A2	34.23 ± 0.5	1.933 ± 0.11	4.9 ± 0.814	8000 ± 0.432
A3	30.83 ± 0.5	1.836 ± 0.32	5.8 ± 0.428	10000 ± 0.421
A4	29.16 ± 0.763	1.804 ± 0.132	6.1 ± 0.306	12000 ± 0.22
A5	35.73 ± 0.5	1.921 ± 0.11	5.3 ± 0.265	4000 ± 0.53
A6	27.76 ± 0.5	1.086 ± 0.121	6.1 ± 0.242	13000 ± 0.2
A7	32.76 ± 0.763	1.189 ± 0.454	5.9 ± 0.227	9000 ± 0.32
A8	29.23 ± 0.5	1.769 ± 0.34	5.2 ± 0.214	12000 ± 0.321
A9	37.13 ± 0.5	2.511 ± 0.232	5.2 ± 0.205	4000 ± 0.432
A10	35.1 ±1	2.172 ± 0.12	5.1 ± 0.197	5000 ± 0.74
A11	26.8 ± 0.763	0.923 ± 0.5	5.5 ± 0.190	14000 ± 0.432
A12	28.5 ± 0.2	1.339 ± 0.2	5.4 ± 0.183	12000 ± 0.121
A13	38.16 ± 1.52	2.756 ± 0.3	5.2 ± 0.178	3000 ± 0.11
A14	34.9 ± 0.763	2.054 ± 0.211	5.4 ± 0.173	6000 ± 0.121
A15	30.16 ± 1.52	1.805 ± 0.333	5.7 ± 0.169	11000 ± 0.2
A16	26.5 ± 1.52	0.506 ± 0.432	5.9 ± 0.165	16000 ± 0.21

Spreadability

As evident from the theory of mucoadhesion, a mucoadhesive formulation that is having high spreadability and high surface tension will adhere strongly to the mucus membrane. For assessing spreadability, a glass slide was used. Provision was made so as to keep the slide in hot water. The goat nasal mucosa was pasted from serosal side on the slide then hot water was circulated for 15-20 minutes for equilibrating temperature of mucosa at 34±2°C .One drop of formulation was placed on the mucosa at an angle of 120°C and the distance traveled by drop before it gets converted into gel was recorded.

Drug content

Drug must be uniformly distributed throughout the sample. This is important in relation to batch to batch uniformity and thus efficacy of the preparation. For determining drug content, samples from different sites of the container are analyzed for the presence of drug. From formulation, 1 ml was taken in 100 ml volumetric flask, 50 ml of distilled water was added with gentle shaking and final volume was adjusted to 100 ml. From this solution, 1 ml quantity was transferred into the 100 ml volumetric flask and final volume was made to 100 ml by using distilled water to get 10 mg/ml. Finally the absorbance of prepared solution was measured at 278.8 nm by using UV visible spectrophotometer. The results of mucoadhesive strength, spreadability and drug content of various formulations are shown in Table 4.

Table 4. Mucoadhesive strength, spreadability, drug content of various formulations

Batch	Mucoadhesive strength(gms)	Spreadability (cm)	Drug Content (%)
A1	14.4 ± 0.251	1.8 ± 0.251	95.33 ± 1.06
A2	12.38 ± 0.450	2.2 ± 0.152	95.62 ± 1.45
A3	13.09 ± 0.472	2.0 ± 0.3	96.9 ± 1.57
A4	13.41 ± 0.251	1.9 ± 0.360	95.75 ± 2.29
A5	11.01 ± 0.264	2.8 ± 0.305	96.13 ± 1
A6	16.39 ± 0.416	1.6 ± 0.351	96.3 ± 1.01
A7	12.63 ± 0.5	2.1 ± 0.208	97.17 ± 0.947
A8	13.54 ± 0.2	1.9 ± 0.152	94.2 ± 1.07
A9	10.77 ± 0.305	2.9 ± 0.305	95.87 ± 0.775
A10	12.25 ± 0.251	2.3 ± 0.351	95.31 ± 0.810
A11	17.13 ± 0.5	1.5 ± 0.152	94.5 ± 1.14
A12	13.54 ± 0.763	1.9 ± 0.083	95.04 ± 1.20
A13	10.19 ± 0.655	3.0 ± 0.305	94.22 ± 2.71
A14	12.31 ± 0.2	2.3 ± 0.152	96.4 ± 1.10
A15	13.25 ± 0.2	2.0 ± 0.3	95.22 ± 0.978
A16	18.25 ± 0.2	1.5 ± 0.378	94.98 ± 1.58

Ex-vivo permeation study

Ex- vivo studies can help in investigating mechanisms of skin permeation of the drug. Also, permeation data of drugs such as lag time, flux, permeability coefficient can be obtained from *in-vitro* permeation experiments.

The most common technique used to gather this type of data involves diffusion cells. Diffusion cells generally comprise two compartments, one containing the active component (donor vehicle) and the other containing receptor solution, separated by a piece of excised skin or other membrane. Although many variations of diffusion cells exist, there are two basic designs; the static or non-flowing cell, and the flow through cell.

The Keshary-Chien (K-C) diffusion cell is the modified Franz diffusion cell. The K-C diffusion cell is one of the most widely used static designs for studying in-vitro permeation. This cell has a static receptor solution reservoir with a side arm sampling port. A thermal jacket is positioned around the receptor compartment and is heated with an external circulating bath. Other designs of diffusion cells that are in existence include: Valia-Chein (V-C) cell, Ghannam-Chein (G-C) cell, Jhawer-Lord (J-L) rotating disc system, etc. Fresh nasal mucosa was carefully removed from the nasal cavity of goat obtained from the local slaughterhouse. The mucosa was stored in normal saline with few drops of gentamycin sulphate injection to avoid bacterial growth. After the removal of blood and bony cartilage from the mucosal membrane it becomes ready for use. Modified K-C diffusion cell was used to study in vitro drug diffusion profile. 100 ml of simulated nasal electrolyte

solution (SNES) pH 6.4 at 34°C was added to the acceptor chamber. The temperature within the chamber was maintained at 34°C by circulating hot water. After a preincubation time of 20 minutes, formulation equivalent to 40 mg of Domperidone was placed in donor chamber. At predetermined time intervals, 1ml of sample was withdrawn from the acceptor compartment and replaced the sample volume with SNES pH 6.4 after each sampling, for a period of 12 hours. The samples withdrawn were diluted to 10 ml by SNES, filtered and used for analysis. The amount of permeated drug was determined using UV-visible spectrophotometer (λ max =278.8 nm). In vitro drug permeation study was carried out in triplicate.

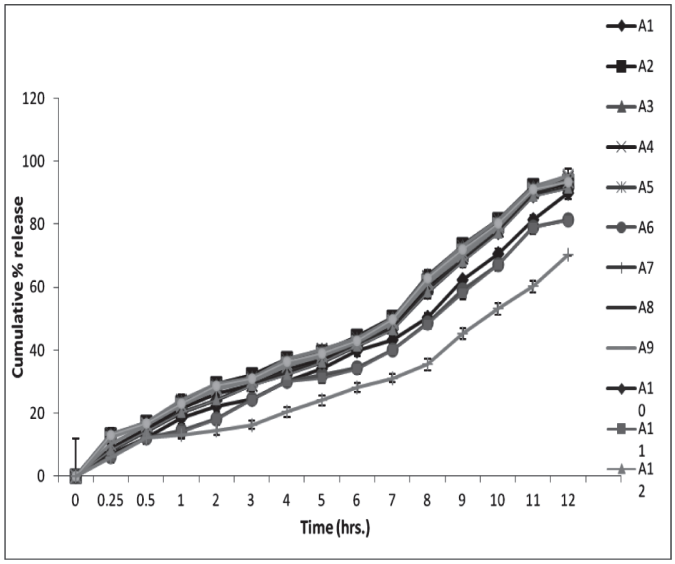

Fig. 1. *Ex-vivo* nasal permeation study of various formulations

Table 5. Permeability data of Diclofenac sodium

Batch	Jss (mg cm^{-2} h^{-1})	Kp (cm h^{-1})	Er
A1	0.98	0.024	1
A2	1.19	0.029	1.214
A3	1.004	0.025	1.024
A4	0.96	0.024	0.979
A5	1.21	0.030	1.234
A6	0.58	0.014	0.591
A7	1.19	0.029	1.214
A8	0.92	0.023	0.938
A9	1.38	0.034	1.408
A10	1.20	0.03	1.224
A11	0.57	0.014	0.590
A12	0.89	0.022	0.908
A13	1.23	0.030	1.537
A14	1.20	0.03	1.224
A15	0.97	0.024	0.989
A16	0.53	0.013	0.540

Table 6. Drug release kinetics of Diclofenac sodium from optimized formulation

Batch	Zero Order		1st order equation		Peppas Korsmeyer's equation		Higuchi Equation	
	K	R^2	k	R^2	n	R^2	K	R^2
A7	6.41	0.971	0.091	0.699	0.108	0.852	0.242	0.974

Nasal *in-situ* gel of Diclofenac sodium was prepared by cold method using different ratio of Pluronic F127 and Carbopol 934P. The formulations were optimized based on gelation temperature, gelation time, drug release and mucoadhesive strength. The optimized formulation was A7.

The gelation temperature and gelation time of different formulations are reported in Table 3. The gelation temperature was found to be between 26.5 ±1.52 °C to 38.16 ± 1.52 °C. Values were significant, ($p<.05$) (compared to control group) by using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Gelation temperature of the optimized formulation A7 was found to be 32.76 ± 0.763 °C. The gelation time of the nasal in-situ gel varies from 0.506 to 2.756 min. Gelation time of the optimized formulation A7 was found to be 1.189 min. Tsol-gel lowering effect of mucoadhesive polymer could be explained by its ability to bind to PEO chains present in the PF127 molecules promoting dehydration and causing an increase in entanglement of adjacent molecules with more extensive intermolecular hydrogen bonding. The increase in Tsol-gel observed upon addition of PEG 6000 chains. The concentration of PEG 6000 was adjusted in such a manner that could be attributed to its interference with the process of micellar association of PF127 formulations may form gel at nasal physiological temperature. The pH was found to be in the range of 4.9 ±.814 to 6.1 ±.242. The pH for all formulations was well within range of nasal pH and did not cause irritation in the nose.

The spreadability was found to be in the range of 1.5 ± 0.378 to 3.0 ± 0.305 cm. Values were significant, ($p<.05$) (compared to control group) by using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Spreadability of the optimized formulation A7 was found to be 2.1± 0.208 cm. Assessment of spreadability in terms of distance traveled by *in-situ* nasal gels reveals that spreadability may be related to the viscosity of *in-situ* nasal gels. Increase in concentration of mucoadhesive polymer decreases the distance traveled by in situ nasal gels, since mucoadhesive polymer increase the viscosity of *in-situ* nasal gels.

Mucoadhesion may be defined as the adhesion between a polymer and mucus. In general, mucoadhesion is considered to occur in 3 major stages: wetting, interpenetration, and mechanical interlocking between mucus and polymer. The strength of mucoadhesion is affected by various factors such as molecular mass of polymers, contact time with mucus, swelling rate of the polymer and the biological membrane used in the study. Mucoadhesive strength of formulation was found to be between 10.19 ±0.655 to 18.25 ±0.2 gms. Values were significant, ($p<.05$) (compared to control group) by using

one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Mucoadhesive Strength of optimized formulation A7 was found to be 12.63±.5 gms. The addition of PEG 6000 to the formulations decreases the mucoadhesive strength in concentration dependent manner; this might be related to the decrease in viscosity caused by PEG 6000. Viscosity of optimized formulation A7 was found to be between 9000 (Cps).

In *ex-vivo* drug release study, the formulations A13 and A9 composed of Polyethylene Glycol gave extended drug release as compared to other formulations. Formulation A13 releases 95.34%, A9 releases 95.32% and A16 releases 70.26% in 12 hrs. Optimized formulation A7 released 93.54±.568 % in 12 hrs. It was found that the in vitro drug release of A7 was best explained by Higuchi's equation, as the plots showed the highest linearity ($r^2 = 0.974$), followed by zero order ($r^2 = 0.971$) and first order ($r^2 = 0.699$).Values were not significant, ($p<.05$) (Compared to Control Group) by using one way analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. The retardation of Diclofenac sodium release with the Mucoadhesive polymer could be explained by their ability to increase the overall gel product viscosity. Also the molecular interactions between Diclofenac sodium and Mucoadhesive polymer appeared to be involved in the release retarding effect of the Mucoadhesive polymer. Also the release enhancing effect of PEG 6000 might be due to its higher water solubility and its viscosity lowering effect. Permeability coefficient increases with increasing concentration of the PEG 6000, which proves its release enhancing effect. The enhancement ratio of Diclofenac sodium from A13 was found to be significantly larger than other formulations (A1-A16). Hence it was assumed that permeation of A13 from optimized batch was highest.

4. CONCULSION

The formulation and characterization of thermosensitive gels based on Pluronic F127, a thermosensitive polymer, and carbopol 934P, a mucoadhesive polymer, intended for the nasal delivery of Diclofenac sodium was performed. Nasal in-situ gel of Diclofenac sodium was prepared by cold method using different ratio of Pluronic F127 and carbopol 934P. Characterization data shows that Pluronic F127 can be a promising in situ gelling vehicle for nasal drug delivery system. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the in -situ gel dosage forms very reliable.

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