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Formulation development of in-situ nasal drug delivery system of poorly water soluble drug (Indomethacin) using mixed solvency concept and their evaluation

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ABSTRACT

The present study was aimed to develop a mucoadhesive *in situ* nasal gel containing indomethacin with enhanced drug loading and transnasal permeation properties, which were achieved by improving drug solubility using the concept of mixed solvency. Poloxamer 407 was used as thermosensitive polymer and carbopol 934P as mucoadhesive polymer. Initially solubility of indomethacin was enhanced in aqueous solution by using various solubilizers like sodium benzoate (SB), niacinamide (NM), sodium citrate (SC), urea (UR), propylene glycol (PG) etc, individually and as a combination of three and four solvents, respectively. Maximum solubility of indomethacin was achieved in 30% w/v mixed solvent system containing Sodium benzoate (12% w/w) + Niacinamide (12% w/w) + Sodium citrate (3% w/w) + Propylene Glycol (3% w/w), enhancing solubility of indomethacin by 176.26 times as compared to its solubility in water. *In situ* gel was prepared by cold technique. Evaluation of the prepared gel was carried out, including properties like phase transition temperature, viscosity, *in vitro* drug release, drug content, transnasal permeation and stability studies. *In vitro* drug release studies of aqueous solution of mixed blend were performed and permeability coefficient was found to be 5.2×10^{-02} cm/hr and flux was found to be 0.40 mg/cm^2 hr. Similarly *in vitro* studies for *in situ* nasal gel were performed and percent cumulative drug release was 70.13±0.57% in 6 h. Transnasal drug permeation studies results in flux value of 124.00 µg/cm^2 hr and percent cumulative drug permeated across the membrane as 40.56±0.992%. The results from stability studies revealed that the prepared thermogel showed no significant decrease in drug content and no physicochemical change was observed upon storage in different temperature conditions resulting as a stable formulation.

Keywords: Indomethacin, in situ, mixed solvency, nasal gel, poloxamer 407, sodium benzoate, niacinamide, propylene glycol.

INTRODUCTION

The administration of drugs via nose is not a novel approach for drug delivery. In ancient days, nasal drug delivery was used for the systemic administration of psychotherapeutic compounds and other similar substances. But, in modern pharmaceutics, nasal delivery is considered as a route of choice for local effect rather than systemic effect. Delivery of drugs via nose for maintenance therapy of nasal allergy, sinusitis, nasal congestion, and nasal infections is a routine practice. Drugs

for administration via the nasal route have specific formulation requirements which affect the bioavailability of the drug administrated. Low volume and high concentration is the essential condition required for nasal drug formulation to be administered. [3] Too large volume and too weak concentration may lead to failure because the drug cannot be absorbed in high enough quantity to be effective. Ideal volume for nasal delivery is ¼ to ½ ml per nostril. [4],[5] Volume over 1 ml per nostril is too large and may result in runoff out of the nostril. Therefore nasal formulation must have a high drug loading in low volume. There are some solubility enhancing techniques out of which mixed solvency is a novel concept which enhances the solubility of the drug in the solvent medium with the aid of some solutes (solids, liquid, or gases) in combination. The concept of mixed solvency states that all substances whether liquids, solids or gases may enhance the solubility of poorly water soluble drugs. By use of these agents in combination one can enhance the solubility by synergistic effect, in addition to the additive effect. Solubility of ibuprofen, a poorly water soluble drug, has been enhanced by 47.46 folds in a 40% w/v mixed blend of PEG 400 + PEG 4000 + Urea + Sodium citrate. [6] Similarly, solubility of salicylic acid was also enhanced by 71.14 folds in a mixed blend of PEG 300 + PEG 400 + Urea + Sodium citrate, in 40% w/v concentration. [7]

The poorly water soluble drug indomethacin was taken as a model drug for research work. It is an indole derivative with prominent anti-inflammatory and analgesic-antipyretic properties. This drug is practically insoluble in water and it also exhibits a pH dependent solubility. The pH of the nasal cavity is around 5.5−6.5 and at this pH indomethacin is very slightly soluble in water.

A nasal mucoadhesive *in situ* gel appears very attractive since it is fluid like prior to nasal administration and can thus easily be instilled as a drop allowing accurate drug dosing. Poloxamer 407 (Pluronic F127) is a thermo-sensitive polymer with excellent water solubility, good drug release characteristics, and has compatibility with other excipients. [10]

It is an ABA triblock copolymer consisting of the hydrophilic polyethyelene oxide (PEO) and the hydrophobic poly propylene oxide (PPO) units. [10],[11] Aqueous poloxamer dispersions (18−25%) are solutions at low temperatures and are converted into semisolid gels at higher (or body) temperature. Physiochemical properties of Poloxamer gels have been evaluated for topical, rectal and ophthalmic routes. Nasal melatonin gels have been aimed to obtain the release resembling the nocturnal release. The effect of formulation variables like Poloxamer concentration, PEG (400, 15000), drug solvent (Ethanol) on thermal properties of gels and drug release have been studied. [12]

Carbopol 934P is an anionic bioadhesive polymer. It was used in the formulation of *in situ* nasal gel of sumatriptan for enhancing the residence time of the gel in the nasal cavity in a low concentration range of 0.1-1%. [13] They swell in water up to 1000 times their original volume and 10 times their original diameter to form a gel when exposed to a pH environment above 4.0 to 6.0.

Present study aims to overcome the problem of drug loading by utilization of novel concept of mixed solvency and minimizing the drug clearance by formulating *in situ* nasal gel. The study aims to combine the novel advantages of mixed solvency and the *in situ* polymeric drug delivery system.

Materials and Methods

Materials

Indomethacin was obtained as a gift sample from Ranbaxy laboratories, Dewas, India. Poloxamer 407 was obtained as a gift sample from signet corporation, Mumbai, India. Sodium benzoate, niacinamide, propylene glycol, urea and sodium citrate used as solubilizers for preparation of blends.

Preparation of solutions

The solutions (w/w) containing different solubilizers as individual and as a mixed blend, in combination of three and four solubilizers, were prepared. The solubilizers as hydrotropes (sodium benzoate, niacinamide, urea etc), co-solvents (propylene glycol, PEG 400 etc) and water soluble solids (PEG 4000 and 6000) were used for solubility enhancement of indomethacin. Total solute concentration was fixed at 30% w/w in all studies, since significant enhancement in solubility is expected at high concentration.

Solubility studies

Solubility of indomethacin was determined by taking an excess amount of drug in screw capped vials in each solvent system. Each solution was vortexed for 10 minutes followed by sonication for 10 minutes. The vials were shaken mechanically for 12 hours at room temperature in orbital flask shaker (Khera Instruments Pvt. Ltd., Delhi, India). The solutions were allowed to equilibrate for next 24 hours and then centrifuged for 10 minutes at 2000 rpm using centrifuge (Remi Instruments Limited, Mumbai,

India). The supernatants of each vials were filtered through Whatman® filter paper No 41. An aliquot of each filtrate was diluted suitably with distilled water and analyzed spectrophotometrically at 320 nm (using Shimadzu® 1700, double beam UV-visible spectrophotometer). [14]

The enhancement ratio in solubility was obtained by following formula:

Enhancement ratio = Solubility of drug in solubilizer/Solubility of drug in de-mineralised water

In vitro release of indomethacin from different solvent systems

Drug release studies were performed using Franz diffusion cell. Experiments were carried out at 37°C with a constant stirring of 50 rpm, using magnetic bead, for 6 hours. 50% v/v ethanolic buffer (Acetate buffer at pH 5.5 + ethanol) was used as the receptor medium as it maintains sink condition. Cellophane membrane was used for drug release study. One ml of saturated solution of drug in different solvent medium, that offer higher increase in solubility, was taken in donor compartment to ensure constant thermodynamic activity. One ml of receptor fluid was withdrawn at scheduled time interval and was replaced with fresh receptor fluid in order to maintain sink condition. The amount of drug diffused through cellophane membrane was determined spectrophotometrically at 320 nm (using Shimadzu® 1700, double beam UV-visible spectrophotometer) after diluting suitably with de-mineralized water.

Preparation of _in situ_ gels

In situ gels were prepared by using cold technique. [11],[15] Briefly, poloxamer 407 and carbopol 934P were weighed in screw cap vials, containing a calculated amount of double distilled water and was kept at 4°C until a clear solution was obtained. Then a blend of selected mixed solubilizer was added such that the concentration ranged from 15% w/w-25% w/w.

Measurement of sol-gel transition temperature ($T_{sol-gel}$)

Transition temperature is defined as the temperature at which the liquid phase makes a transition to gel phase. [10] Vial containing 5 g of poloxamer 407 solution with selected blend and a magnetic bar was stirred on magnetic stirrer and the temperature was slowly raised until the liquid in the vial becomes gel and the magnetic bar stops moving. This temperature was measured with thermometer and was determined as transition temperature. The samples were examined for gelation which was said to have occurred when the meniscus would no longer move upon titling through 90°. Measurements were done in triplicates and reported as mean±S.D. The purpose of this study was to optimize the minimum concentration of Poloxamer 407; required for sol- gel transition behavior at physiological range of nasal cavity temperature, i.e., 35−37°C. [16]

Formulation and evaluation of _in situ_ nasal gel of indomethacin

Formulation of in situ nasal gel of indomethacin was prepared by cold technique. [11],[15] Calculated amount of poloxamer 407 and carbopol 934P was weighed in a beaker, containing weighed amount of cold distilled water . The preparation was kept at 4°C until a clear solution was obtained. Then the selected aqueous mixed solvent blend containing indomethacin was added to the above solution. The preparation was mixed thoroughly on magnetic stirrer with a magnetic bar in the beaker to make the homogeneous gel and was stored at 4°C.

Physico-chemical properties of indomethacin in situ nasal gel

The _in situ_ nasal gel was evaluated for the physicochemical properties like pH, clarity spreadability, transition temperature, viscosity and drug content. The pH of the formulation was determined by using pH meter (Cyberscan® 510), which should be comparable as that of nasal cavity pH (6.0−6.5). The clarity was observed against white and black background. Transition temperature was observed in triplicate and reported as mean ± S.D. The drug content was determined by dissolving I ml of formulation in 5 ml of de-mineralised water and volume was made up to 25 ml with de-mineralized water in a 25 ml volumetric flask and was estimated spectrophotometrically using double beam UV-Visible spectrophotometer (Shimadzu® 1700) at 320 nm against water.

Rheological studies

Viscosity determination of the developed indomethacin _in situ_ gel formulation was done using Brookfield viscometer (LVT model). Viscosity was determined at two temperatures, at room temperature i.e. 25±0.5°C and at transition temperature (body temperature) i.e. 37±0.5°C. Viscosity of the sample solution was measured over a range of 0.6 to 30 rpm speed. The hierarchy of speed was reversed from 30 to 0.6 rpm. The average of the two dial readings was used to calculate the viscosity. To evaluate viscosity change at cool condition and at body temperature, rheological measurements were taken after increasing the temperature of nasal _in situ_ gel to 37±0.5°C.

In vitro drug release study of indomethacin _in situ_ nasal gel

The drug release of the indomethacin in *situ* gel was measured using Franz diffusion cell. Assembly was set and the temperature was maintained at 37±0.5°C, then 2 ml of nasal *in situ* gel of indomethacin was applied in the donor compartment, which was separated by the receptor compartment with the cellophane membrane. Three ml aliquots of samples were withdrawn at regular time intervals and replaced with an equal volume of 50% v/v ethanolic buffer as fresh receptor medium. The samples were appropriately diluted with 50% v/v ethanolic buffer and analyzed spectrophotometrically (using Shimadzu® 1700, double beam UV-visible spectrophotometer) at 320 nm.

Transnasal drug permeation study

The transnasal permeation study of developed indomethacin *in situ* nasal gel was carried out using goat's nasal epithelium membrane, in triplicate. Nasal cavity of the freshly sacrificed goat was procured from the slaughter house and safely transported to laboratory in cold condition. These nasal cavities were conscientiously dissected and nasal septum was taken out without any damage to it. Then the nasal epithelium membrane was removed carefully from the underlying bone, washed thoroughly and stored in cold saline buffer of pH 7.4. The membrane of nasal epithelium was tied efficiently to one end of the hollow cylindrical tube (7 cm long and 1 cm in diameter). The cylindrical tubes were then suspended into 15 ml of 50% v/v ethanolic buffer maintained at 37±0.5°C and adjusted to rotation at 50 rpm. Two ml of indomethacin *in situ* nasal gel was applied over the epithelium membrane. Three ml aliquots of receptor fluid were withdrawn at fixed time intervals over 5 hours and were replaced with an equal volume of fresh 50% v/v ethanolic buffer maintained at 37±0.5°C. Aliquots of withdrawn samples were diluted suitably with 50% v/v ethanolic buffer and analyzed for drug content spectrophotometrically (using Shimadzu® 1700, double beam UV-visible spectrophotometer) at 320 nm.

Stability studies

Stability studies were conducted to test the physical and chemical stability of the developed *in situ* nasal gel. A sufficient quantity of *in situ* gel, in screw capped vials was stored at different temperature condition as 4±3°C, 25±3°C, 40±3°C for one month. The physical stability, including appearance, color, pH, $T_{sol\text{-}gel}$ temperature, viscosity, and drug content was studied.

Results

Solubility studies

Solubility studies were performed in a stepwise manner. Initially, only one solubilizer was used in 30% w/w concentration for solubility enhancement then depending upon their results further combinations were prepared.

Solubility studies in aqueous solutions of solid solubilizers

From [Table 1], it is evident that there was improvement in the solubility of indomethacin in all solutions containing individual solubilizers. When a single aqueous solution of solubilizers are used in 30% w/w concentration maximum increase in solubility was obtained in sodium benzoate (Solubility enhancement ratio 520.80) followed by niacinamide (Solubility enhancement ratio 73.30) and Urea (Solubility enhancement ratio 50.80).

Solubility studies in aqueous solutions of co-solvent type solubilizers

Again, solubility was observed in 30% w/w solution of single solubilizer (co-solvents). [Table 2] illustrates the solubility enhancement of indomethacin in co solvent type solubilizers. Highest increase in solubility was observed in propylene glycol (Solubility enhancement ratio 100.14) followed by PEG 400 (Solubility enhancement ratio 14.61) and PEG 600 (Solubility enhancement ratio 12.84).

Solubility studies in aqueous solutions of mixed solubilizers

Based on the results of above two studies, the combinations were prepared keeping the concentration constant that is 30% w/w. [Table 3] illustrates the advantages of making blends of solubilizers. Solubility in a solvent medium of four and five solubilizers were studied. Combinations of four solubilizers were studied and maximum (176.26) folds enhancement in solubility was observed in case of (NM_{12} + SB_{12} + PG_3 + SC_3).

Release studies

Release studies from aqueous solutions of solubilizers

Franz diffusion cell was used for determination of drug release from the solutions containing individual solubilizers and as mixed blend containing four and five solubilizers. Sink conditions were maintained at 37±0.5°C resembling the conditions of nasal mucosa, for 6 h. Scrutinizing the results of release studies of individual solubilizers and their combinations, the mixed blend which resulted in highest permeability coefficient with a lesser individual concentration of solubilizer used, was found to be (PEG 600 $_{7.5}$ + PEG 400 $_{7.5}$ + Propylene Glycol $_{7.5}$ + PVP K30 $_{7.5}$) which was then investigated for release profile. The release profile of the mixed blend showed a cumulative

release of 78.80%, flux value of 8.64 μ g/cm^2 hr and a permeability coefficient of 1.576×10^{-02} cm/hr. [Table 4], [Table 5]

Table 1: Results of solubility studies of indomethacin in 30% w/v aqueous solutions of various solubilizers (solid solibilizers)

S.No.	Hydrotropic solution (30% w/v)	Concentration (mcg/ml)	Solubility enhancement ratio
1.	NM	3940.7	73.3
2.	SB	27950.6	520.8
3.	PVP K30	807.4	15.0
4.	PEG 6000	468.1	8.7
5.	PEG 4000	549.1	10.2
6.	UR	2960.5	50.8
7.	SC	60.9	1.1

Table 2: Results of solubility studies of indomethacin in 30% w/v aqueous solutions of various solubilizers (Liquid solubilizers)

S. No.	Aqueous solution of solubilizers (30% w/v)	Solubility (mcg/ml)	Solubility enhancement ratio
1.	PEG 600	690.00	12.84
2.	PEG 600	785.77	14.62
3.	Propylene Glycol	5378.57	100.14

Table 3: Results of solubility studies of indomethacin in 30% w/v mixed blends of various solubilizers

S. No.	Blend	Mixed Solvent System (30% w/v)	Solubility (mg/ml)	Solubility Enhancement ratio
1.	A	$NM_{7.5} + SB_{7.5} + SC_{7.5} + PG_{7.5}$	7.245	134.69
2.	B	$NM_{7.5} + SB_{7.5} + UR_{7.5} + PG_{7.5}$	3.728	70.33
3.	C	$NM_{10} + SB_{10} + SC_5 + UR_5$	8.295	156.50
4.	D	$NM_{12} + SB_{12} + SC_3 + UR_3$	8.447	159.37
5.	E	$NM_{12} + SB_{12} + PG_3 + UR_3$	5.161	97.37
6.	F	$NM_{7.5} + SB_{7.5} + SC_5 + UR_5 + PG_5$	6.128	115.62
7.	G	$NM_7 + SB_{15} + PG_3 + PVP\ K\text{-}30_5$	6.580	124.15
8.	H	$NM_{15} + SB_7 + PG_3 + PVP\ K\text{-}30_5$	4.438	83.73
9.	I	$NM_{12} + SB_{12} + PG_3 + SC_3$	9.342	176.26
10.	J	$NM_{7.5} + SB_{7.5} + PEG\text{-}600_{7.5} + PG_{7.5}$	4.410	82.10

Values in subscript represent the individual concentration of solubilizers

Where SC= Sodium Citrate, UR= Urea, PG= Propylene Glycol, SB= Sodium Benzoate, NM= Niacinamide , PEG=Poly-ethylene Glycol

Table 4: Results of release studies of indomethacin from aqueous solutions of mixed solubilizers

S.No	Blend	Mixed solvent system	Percent cumulative drug released					
			1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour
1.	A	$NM_{7.5} + SB_{7.5} + SC_{7.5} + PG_{7.5}$	29.12	41.96	59.16	65.13	70.56	76.14
2.	B	$NM_{10} + SB_{10} + SC_5 + UR_5$	21.23	39.28	46.20	51.20	58.69	65.93
3.	C	$NM_{12} + SB_{12} + SC_3 + UR_3$	23.21	34.46	50.37	62.31	76.22	81.23
4.	D	$NM_{12} + SB_{12} + PG_3 + SC_3$	30.35	41.71	54.62	67.15	77.26	82.16
5.	E	$NM_7 + SB_{15} + PG_3 +$ PVP K-30_5	25.63	37.87	49.41	57.91	64.44	70.26

Values in subscript indicates total concentration of all soubilizers taken

Table 5: Various parameters of release studies of indomethacin from aqueous solutions of mixed solubilizers

S.No	Blend	Mixed solvent system	Cumulative drug released per cm^2 (mg)	Flux (mg/cm^2hr)	Permeability coefficient (cm/hr)
1.	A	$NM_{7.5} + SB_{7.5} + SC_{7.5} + PG_{7.5}$	1.756	0.29	4.0×10^{-02}
2.	B	$NM_{10} + SB_{10} + SC_5 + UR_5$	1.723	0.28	3.3×10^{-02}
3.	C	$NM_{12} + SB_{12} + SC_3 + UR_3$	2.185	0.36	5.2×10^{-02}
4.	D	$NM_{12} + SB_{12} + PG_3 + SC_3$	2.444	0.40	5.2×10^{-02}
5.	E	$NM_7 + SB_{15} + PG_3 +$ PVP K-30_5	1.472	0.24	5.1×10^{-02}

Values in subscript indicates total concentration of all solubilizers taken

Table 6: Composition of different concentration of poloxamer 407 solutions for *in-situ* nasal gel of indomethacin

S. No	Amount of blend (SH/BLEND/ IND) (g)	Amount of poloxamer 407 (g)	Distilled water (g)	Concentration of Poloxamer 407 in the final solution (% w/w)	Formulation Code
1.	2.0048	0.7505	2.2499	15.00	SH/IND/P407/15
2.	2.0042	0.8012	2.2938	16.00	SH/IND/P407/16
3.	2.0085	0.8502	2.1606	17.00	SH/IND/P407/17
4.	2.0077	0.8997	2.1057	18.00	SH/IND/P407/18
5.	2.0085	0.9499	2.0500	19.00	SH/IND/P407/19
6.	2.0089	1.0005	2.0135	20.00	SH/IND/P407/20
7.	2.0092	1.0509	1.9543	21.00	SH/IND/P407/21

8.	2.0074	1.0992	1.9074	22.00	SH/IND/P407/22
9.	2.0053	1.1479	1.8545	23.00	SH/IND/P407/23
10.	2.0049	1.1989	1.8067	24.00	SH/IND/P407/24
11.	2.0067	1.2459	1.7508	25.00	SH/IND/P407/25

SH/IND/P407/* where * shows the concentration of poloxamer in solution.

Table 7: Results of phase transition temperature study by visual method of poloxamer 407 solutions for *in-situ* gel of indomethacin

S.No	Formulation Code	Temperature (ºC)		Observation
		At initial (liquid) stage	At gelling stage	
1.	SH/IND/P407/15	5	-	No phase transition took place till 40ºC
2.	SH/IND/P407/16	5	36.0	Gelled at 34ºC-37ºC
3.	SH/IND/P407/17	5	35.0	Gelled at 33ºC-35ºC
4.	SH/IND/P407/18	5	33.0	Gelled at 32ºC-34ºC
5.	SH/IND/P407/19	5	32.5	Gelled at 31ºC-33ºC
6.	SH/IND/P407/20	5	30.5	Gelled at 30ºC-32ºC
7.	SH/IND/P407/21	5	27.5	Gelled at 28ºC-29ºC
8.	SH/IND/P407/22	5	26.0	Gelled at 26ºC
9.	SH/IND/P407/23	5	22.0	Gelled at 23ºC
10.	SH/IND/P407/24	5	20.5	Gelled at 20ºC-21ºC
11.	SH/IND/P407/25	5	19.0	Gelled at 18ºC-19ºC

IND-Indomethacin

Table 8: Physico-chemical properties of optimized formulation of diazepam *in situ* nasal gel Formulation Code (SH/DZP/RS/2013)

S.No.	Parameter	Observation
1.	Clarity	Clear
2.	pH	6.321
3.	Drug content	0.417 mg/ml
4.	Transition temperature	37ºC

Table 9: Results of Ex-vivo release studies from indomethacin *in situ* nasal gel formulation (SH/IND/RS/2014)

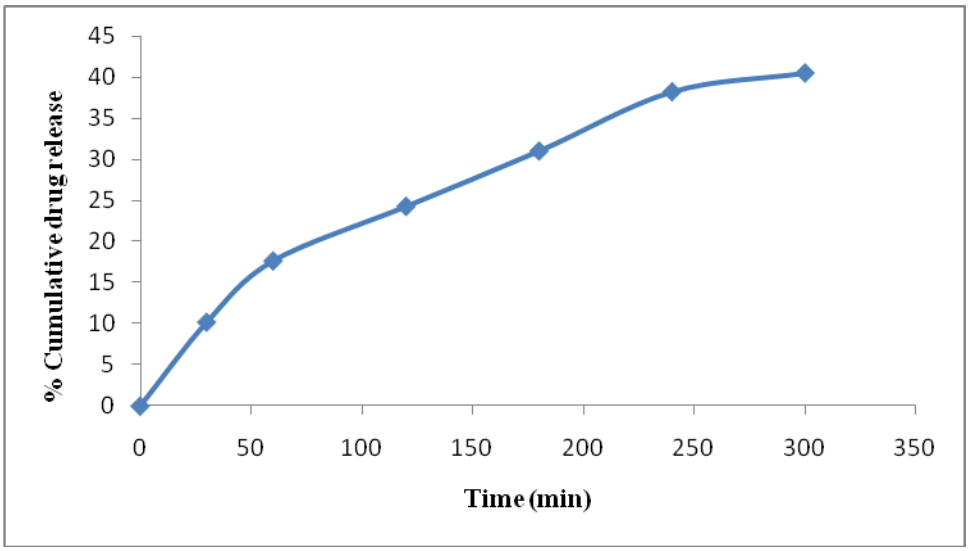
S.No.	Time (minutes)	Cumulative Drug Release per cm^2 (mcg)	Percent Cumulative Drug Release
1.	30	156.07	10.21
2.	60	270.26	17.68

3.	120	371.77	24.32
4.	180	474.80	31.06
5.	240	579.48	38.27
6.	300	620.02	40.56

Table 10: Stability data for optimized *in situ* nasal gel of indomethacin

S. No.	Time	Storage Condition	Color	Clarity	pH	Drug Content
1.	Initial	NA	Yellowish	Clear	6.32	98.80%
2.	One month	2-8˚C	Yellowish	Clear	6.32	98.53%
		RT	Yellowish	Clear	6.31	98.36%
		40˚C	Yellowish	Clear	6.28	98.05%

Fig.1: Graphical representation of Ex-vivo release studies from indomethacin *in situ* nasal gel formulation



Measurement of Sol-Gel transition temperature

Transition studies showed that loading of indomethacin into mixed blend of solubilizers formulated with Poloxamer 407 solutions increased the $T_{sol\text{-}gel}$. Using the Poloxamer concentration in the range of 15-25% w/w for determination of $T_{sol\text{-}gel}$, results showed that the 16, 17 and 18% w/w Poloxamer concentrations had $T_{sol\text{-}gel}$ value above 40°C, 37°C, and 32°C, respectively [Table 6,7]. The concentration of Poloxamer 407 which normally shows phase transition behavior at 37°C (body temperature) is 16% w/w. But in presence of selected mixed solvent blend, the concentration of Poloxamer 407 required for phase transition was more, that is 19% w/w. At this Poloxamer concentration sol-gel behavior was observed (*n*=3) at 37.03±0.68°C. Therefore for *in situ* nasal gel only 16% w/w Poloxamer 407 was selected for further studies, whereby their mucoadhesive properties could be improved by the incorporation of mucoadhesive polymer. The mucoadhesive polymer used was Carbopol 934 P.

Physicochemical properties

Drug content of the *in situ* nasal gel was determined spectrophotometrically at 320 nm and was found to be 98.80±0.61%. Clarity of the formulation when observed against a white and black background was found to be clear and no sign of turbidity or gelation was observed. pH of the gel was found to be 6.4±0.51 which was in accordance with the nasal pH requirements. Transition temperature for the prepared formulated gel was found to be 36.08±0.77°C. Spreadibility was found to be good [Table 8].

Transnasal drug permeation study

Transnasal drug permeation studies were performed on goat's nasal epithelium and drug samples were analyzed spectrophotometrically at 320 nm [Figure 1]. Drug content was identical (98−100%) with 2%±S.D. Presence of different solubilizer and additives did not interfered with the drug estimation. *In situ* nasal gel showed initial high flux value as 18.88 μ g /cm^2 hr. A decrease in the value of flux was observed at 2, 3 and 4 hrs resulting in flux value of 6.12, 8.35, and 9.01, respectively. However, a second peak in flux value i.e. 11.43 μ g /cm^2 hr was observed at 240 min. This indicated that poloxamer 407 gel required 240 min to absorb sufficient water from medium to break the gel structure. This released higher amount of drug and hence, the second peak Flux [Table 9].

Rheological studies

Viscosity determination was done using Brookfield viscometer (LVT model), at temperatures, Room temperature i.e. 25±0.5°C and at transition temperature (body temperature) i.e. 37±0.5°C. Viscosity of sol and gel was found to be 125 cps and 42500 cps, respectively, at 12 rpm.

Stability studies

The physical stability including appearance, color, pH, transition temperature, viscosity, and drug content of the formulation was studied under various storage conditions. The drug content of the formulation was determined using double beam UV-visible spectrophotometer (Shimadzu® 1700) at 320 nm. *In situ* gel remained as a liquid for a period of one month without occurrence of turbidity or gelation at 4±2°C, at room temperature 25±2°C and 40±2°C. The observations so recorded are presented in [Table 10]. None of the samples showed any change in color or appearance under all storage conditions for one month period. Considering initial drug content which was found to be 98.80% as 100%, after 30 days the drug content was found to be 98.53%, 98.36%, and 98.05%, respectively. Transition temperature was also determined initially and after 30 days, results observed that the formulations gelled at 36.1±0.97°C, 35.9±0.65°C, and 35.6±0.86°C when stored at above mentioned temperatures.

Discussion

Solubility enhancement of poorly water soluble drugs has been a challenging task for development of any formulation. Over the last decade various solubility enhancing techniques have been developed out of which mixed solvency, a novel concept based on hydrotrophy is studied. The solubility of the drug is enhanced in the solvent medium with the aid of solubilizers. The aim of the present research study was to explore the possibility of employing the mixed solvent system to enhance drug loading and transnasal permeation of poorly water soluble drugs and its formulation as in situ gel.

Solubility of indomethacin was enhanced in aqueous solution by using various solubilizers like sodium benzoate, urea, niacinamide and polyethylene glycol individually and in mixed blend as a combination of four and five solubilizers, respectively. From [Table 1] result showed an increase in the solubility of indomethacin in all solutions containing individual solid solubilizers. The greatest enhancement in solubility was observed in case of 30% w/v sodium benzoate solution and least in the case of 30% w/v Urea solution [Table 1]. As all solubilizers used are of solid nature, therefore they enhance the solubility up to many folds. Again solubility was observed in 30% w/w solution of single solubilizers (Co-solvents). Highest increase in solubility was observed in propylene glycol [Table 2]. Based upon the results of above two studies, the combinations were prepared keeping the concentration constant that is 30% w/w. Solubility in a solvent medium containing four and five solubilizers were studied. [Table 3] illustrate the advantages of making blend of solvents. A satisfactory increase in solubility was obtained in a 30% w/w mixed blend of Sodium benzoate 12% w/v + Niacinamide 12% w/v + sodium citrate 3% w/v + Propylene Glycol 3% w/v, enhancing solubility of indomethacin by 176.26 times as compared to its solubility in water. These results demonstrate the principle of mixed solvency concept that water-soluble substances whether hydrotropes or co-solvents or water-soluble solids (like PEG 4000, PEG 6000, etc) can be combined randomly to give a desired solubility for a poorly water-soluble drug. [7],[8],[9]

Drug release studies were performed on aqueous solutions of mixed blends containing four and five solubilizers. [Figure 4] show the in vitro release profile of indomethacin from various mixed blends. Finally, based upon the release profiles considering flux, permeability coefficient, cumulative drug released per cm^2 and cumulative percent drug release a mixed blend containing four solubilizers sodium benzoate + niacinamide + sodium citrate + Propylene glycol, was selected for formulation of in-situ nasal gel. Permeability coefficient (cm/hr) was found to be 5.2×10^{-02}. Flux (mg/cm^2hr) was found to be 0.40. Cumulative drug released per cm^2 (mg) was 2.444 and cumulative percent drug release was 82.16%.

Formulation of in situ gels appears very attractive since it is fluid like prior to nasal administration and can thus easily be instilled as a drop allowing accurate dosing, but sets into a gel with increased residence time at body temperature. [10] Poloxamer 407 has excellent thermosensitive gelling properties, low toxicity and irritation, excellent water solubility, good release characteristics and compatibility with other excipients. Carbopol 934P was selected as mucoadhesive agent. Poloxamer is more soluble in cold water than in hot water therefore gels were prepared by cold technique. All the formulations had clear appearance and showed pH values comparable to that of nasal pH range. All the formulations were having the drug content within the limit, i.e. 98.82±0.61. The formulations had an optimum viscosity, which will allow its easy administration into nasal cavity, as a liquid, which will then undergo rapid sol to gel conversion. From the results it was found that viscosity of sol and gel form of gel was found to be 125 cps and 42500 cps, respectively. The concentration of Poloxamer 407 polymer used as thermosensitive polymer was 16% w/w, based upon the results of sol-gel transition temperature.

Transition temperature for Poloxamer gels was observed for the concentration range of 15−25% w/w. during which the polymer exhibited phase transition. The concentration of Poloxamer 407 which showed phase transition behavior at 37°C was at 18% w/w concentration [Table 8]. But in the presence of selected mixed solvent blend, the concentration of Poloxamer 407 required for phase transition was more (n=3), that is 16% w/w and the temperature of phase transition was 36.03±0.68°C. As the temperature increases, micellar entanglement is promoted, leading to gel formation and an overall increase in bulk viscosity. [12] Gelation phenomenon is a result of body centered cubic packing of spherical micelles. Temperature plays an important role in the miscelle formation through temperature dependent hydration of the ethylene oxide units. A decrease in gelation temperature was observed as the concentration of Polymer was increased. In vitro release profile of in situ nasal gels was determined using franz diffusion cell for 6 h. Percentage cumulative drug release was found to be 70.13±0.57% and flux value of 178.67 mcg/cm^2hr at the 6 th hour [Table 10].

Transnasal permeation studies are an important parameter to study permeation of drug across the nasal epithelium membrane. The percentage cumulative drug release after 1, 4, and 8 hrs was found to be 10.21±0.736%, 31.06±0.516%, and 40.56±0.992%, respectively [Table 11]. Developed indomethacin *in situ* nasal gel formulation showed a profile with 52.44% permeation of drug in 5 hr through the nasal epithelium membrane. The flux calculated was 124.00 mcg/cm^2hr and the permeability coefficient was 2.5×10^{-02} cm/hr.

Stability studies of the formulated gel were carried in different storage conditions for drug content, effect of temperature, transition temperature, pH, clarity and viscosity parameters. Three different temperatures were selected and the formulation was evaluated for one month. Storage conditions were mainly refrigerated condition (4±2°C), room temperature (25±2°C) and elevated temperature (40±2°C) [Table 12]. None of the samples showed any change in clarity or appearance under all the above mentioned conditions of storage and the formulations was found to be clear without any signs of turbidity or gelling phenomena. Drug content of the gel was determined spectrophotometrically at 320 nm, initially and after 30 days, results showed no significant decrease in the drug content. Transition temperatures were determined initially and after one month, formulation gelled at slight changes of transition temperature which are under the standard deviation ranges. pH of the formulation had marginal changes which are under acceptable limits of nasal pH range. Therefore, results based on all evaluation parameters leads to the fact that the formulation was stable and no significant physicochemical changes occur during different conditions of storage.

Conclusion

Mixed solvency concept is a promising approach towards increasing solubility and drug loading properties of poorly water soluble drugs like indomethacin. Use of mixed blend of solubilizers enhances the solubility by hundred folds at

the same time; the individual solubilizer's concentration is minimum neglecting the chances of toxicity. Research studies demonstrated enhanced solubility and drug loading as compared to mere water solubility. Poloxamer 407 gel formulation with Carbopol 934 P as a mucoadhesive polymer shows pseudo-plastic rheological properties. Gelation range broadens with polymer concentration. Transnasal permeation studies by using bovine nasal mucosa exhibited significant permeation of drug across the membrane. In conclusion, this research study demonstrates that by combining the concept of mixed solvency with intranasal in situ gels comprising thermosensitive polymers can be a very effective approach for delivery of poorly soluble drugs.

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