RESEARCH ARTICLE

Formulation development and evaluation of *in situ* nasal gel of poorly water soluble drug using mixed solvency concept

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The present study was aimed to develop a mucoadhesive *in situ* nasal gel containing domperidone 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 domperidone was enhanced in aqueous solution by using various solubilizers like sodium citrate (SC), urea (UR), polyvinyl pyrrolidone (PVP), polyethylene glycol (PEG), propylene glycol (PG) etc, individually and as a combination of two, three, and four solvents, respectively. Maximum solubility of domperidone was achieved at 30% w/w solvent concentration, containing mixed blend of PVP K30 (7.5% w/w) + PEG 400 (7.5% w/w) + PEG 600 (7.5% w/w) + Propylene Glycol (7.5% w/w), enhancing solubility of domperidone by 172.20 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 1.576×10^{-02} cm/hr and flux was found to be 8.64 µg/cm^{2}hr**.** Similarly *in vitro* studies for *in situ* nasal gel were performed and percent cumulative drug release was 73.05±0.57% in 6 h. Transnasal drug permeation studies results in flux value of 7.04 µg/cm^{2}hr and percent cumulative drug permeated across the membrane as 86.62±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.

Key words: *Domperidone, in situ, mixed solvency, nasal gel, poloxamer 407*

INTRODUCTION

Drugs have been administered nasally for therapeutic and recreational purposes since ancient times. From last few decades, much interest has been given to the exploitation of the nasal route for delivery of drugs to the brain via a specific site, the olfactory region.[1,2] 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.[6-9]

The concept of mixed solvency states that all substances whether liquids, solids or gases may enhance the

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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 taken for research is domperidone. It is an anti emetic, dopamine antagonist; rapidly absorbed after oral administration but bioavailability is only 15% due to first pass hepatic metabolism. So, nasal drug delivery can be a good option for by passing hepatic metabolism and targeting dopamine receptors, crossing blood brain barrier. 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 domperidone 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 (18−35%) 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.

EXPERIMENTAL

Material

Domperidone was obtained sample from Shagun Pharmaceuticals, Indore, India. Poloxamer 407 was obtained as a gift sample from BASF Corporation Mumbai, India. Benzalkonium Chloride 50%, Polyethylene Glycol (Mol wt 200, 300, 400, 600, 4000, and 6000), Sodium Citrate (SC), Urea (UR) and Propylene Glycol were purchased from Merck Chemicals, Mumbai, India. Polyvinyl Pyrolidone (K25 and K30) were purchased from Buronyl Chemicals, Mumbai, India and Carbopol 934P was purchased from Sigma Chemicals, Mumbai, India. All other substance used were of analytical grade and used without further purification.

Preparation of solutions

The solutions (w/w) containing different solubilizers as individual and as a mixed blend, in combination of two, three and four solubilizers, were prepared. The solubilizers as hydrotropes (sodium citrate, urea etc), co-solvents (propylene glycol, PEG 400 etc) and water soluble solids (PEG 4000 and 6000) were used for solubility enhancement of domperidone. 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 domperidone 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 284 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 distilled water

In vitro release of domperidone 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 methanolic buffer (Acetate buffer at pH 5.5 + methanol) was used as the receptor medium as it maintains sink condition. Dialysis membrane (Hi-Media Laboratories Pvt. Ltd. Mumbai, India), having a molecular weight cut-off between 12000-14000 Dalton and pore size 2.5 nanometers was used. 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 dialysis membrane was determined spectrophotometrically at 284 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 with 0.002% w/v benzalkonium chloride, as preservative 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°C. 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 domperidone

Formulation of domperidone in situ gel

In situ nasal gel of domperidone 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 with 0.002% w/v benzalkonium chloride. The preparation was kept at 4°C until a clear solution was obtained. Then the selected aqueous mixed solvent blend containing domperidone 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 domperidone 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 200 μL of formulation in 50 ml methanol and volume was made up to 100 ml with de-mineralized water in a 100 ml volumetric flask and was estimated spectrophotometrically using double beam UV-Visible spectrophotometer (Shimadzu® 1700) at 284 nm against reagent blank.

Rheological studies

Viscosity determination of the developed domperidone *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 0.3 to 30 rpm speed. The hierarchy of speed was reversed from 30 to 0.3 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 domperidone *in situ* nasal gel

The drug release of the domperidone *in situ* gel was measured using Franz diffusion cell. Assembly was set and the temperature was maintained at 37±0.5°C, then 1 ml of nasal *in situ* gel of domperidone was applied in the donor compartment, which was separated by the receptor compartment with the dialysis membrane (Hi Media dialysis membrane). Two ml aliquots of samples were withdrawn at regular time intervals and replaced with an equal volume of 50% v/v methanolic buffer as fresh receptor medium. The samples were appropriately diluted with de-mineralized water and analyzed spectrophotometrically (using Shimadzu® 1700, double beam UV-visible spectrophotometer) at 284 nm.

Transnasal drug permeation study

The transnasal permeation study of developed domperidone *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 25 ml of 50% v/v methanolic buffer maintained at 37±0.5°C and adjusted to rotation at 50 rpm. One ml of domperidone *in situ* nasal gel was applied over the epithelium membrane. Three ml aliquots of receptor fluid were withdrawn at fixed time intervals over 8 h and were replaced with an equal volume of fresh 50% v/v methanolic buffer maintained at

37±0.5°C. Aliquots of withdrawn samples were diluted suitably with de-mineralized water and analyzed for drug content spectrophotometrically (using Shimadzu® 1700, double beam UV-visible spectrophotometer) at 284 nm.

Table 1: Results of solubility studies of domperidone in 30% w/w aqueous solutions of solid solubilizers

Aqueous solution of solubilizer (30% w/w)	pH of the solution	Solubility (µg/ml)	Enhancement ratio
SC	8.30	33.84	2.18
UR	8.09	29.75	1.92
PVP K25	4.40	4333.33	279.84
PVP K30	4.67	4688.88	302.80
PVP K40	6.98	1090.25	70.40
PEG 1540	6.63	124.92	8.07
PEG 4000	7.64	159.59	10.31
PEG 6000	7.42	142.81	9.22

SC: Sodium citrate, UR: Urea, PVP: Polyvinyl pyrrolidone, PEG: Polyethylene glycol

Table 2: Results of solubility studies of domperidone in 30% w/w aqueous solutions of co-solvent type solubilizers

Aqueous solution of solubilizers (30% w/w)	pH of the solution	Solubility (µg / ml)	Enhancement ratio
PEG 200	6.30	146.82	9.48
PEG 300	5.89	145.73	9.41
PEG 400	5.69	557.40	35.99
PEG 600	5.28	725.92	46.87
Propylene Glycol	4.56	194.07	12.53
Glycerin	5.05	34.05	2.19

PEG: Polyethylene glycol

Table 3: Results of solubility studies of domperidone in 30% w/w mixed blends of two solubilizers (solid + liquid solubilizers)

Mixed solvent system (30% w/w)	Solubility (µg /ml)	Enhancement ratio
PVP $K30_{15}$ + PEG 200_{15}	2401.89	155.11
PVP $K30_{15}$ + PEG 300_{15}	2336.86	150.91
PVP $K30_{15}$ + PEG 400_{15}	2562.93	165.51
PVP $K30_{15}$ + PEG 600_{15}	2748.15	177.47
PVP $K30_{15}$ + Propylene $glycol_{15}$	2618.52	169.10
PVP $K30_{15}$ + PEG 4000_{15}	2318.62	149.73
PVP $K30_{15}$ + PEG 6000_{15}	2369.71	153.03

Values in subscript represent the individual concentration of solubilizer, PVP: Polyvinyl pyrrolidone, PEG: Polyethylene glycol

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 solvent 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 domperidone 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 PVP K30 solution (Solubility enhancement ratio 302.80) followed by PVP K25 (Solubility enhancement ratio 279.84) and PVP K40 (Solubility enhancement ratio 70.40). Very small increase in solubility is found in solutions of urea and sodium citrate.

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 domperidone in co solvent type solubilizers. Highest increase in solubility was observed in PEG 600 (Solubility enhancement ratio 46.87) followed by PEG 400 (Solubility enhancement ratio 35.99) and Propylene Glycol (Solubility enhancement ratio 12.53).

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. Tables 3 and 4 illustrates the advantages of

Table 4: Results of solubility studies of domperidone in 30% w/w mixed blends of three-four solubilizers

Mixed solvent system (30% w/w)	Solubility (µg/ml)	Enhancement ratio
PVP $K30_{10}$ + PEG 400_{10} + Propylene $Glycol_{10}$	1844.44	119.11
PVP $K30_{10}$ + PEG 600_{10} + Propylene $Glycol_{10}$	2000.06	129.16
PVP $K30_{10}$ + PEG 400_{10} + PEG 600_{10}	1985.19	128.20
PVP $K30_{10}$ + PEG 600_{10} + $Glycerine_{10}$	2014.81	130.11
PVP $K30_{10}$ + Propylene $Glycol_{10}$ + $Glycerine_{10}$	1866.05	120.50
PVP $K30_{7.5}$ + PEG $400_{7.5}$ + PEG $600_{7.5}$ + Propylene $Glycol_{7.5}$	2666.66	172.20

Values in subscript represent the individual concentration of solubilizer, PVP: Polyvinyl pyrrolidone, PEG: Polyethylene glycol

making blends of solubilizers Solubility in a solvent medium of two; three and four solubilizers were studied. In the blend of two solubilizers, highest increase in solubility was observed in case of (PVP K30 + PEG 600) that is 177.47 folds enhancement [Figure 1]. Combinations of three solubilizers were studied and 130 folds enhancement in solubility was observed in case of (PVP K30 + PEG 600 + Glycerin). A satisfactory increase in solubility was obtained in a 30% w/w mixed blend, ($PVPK30_{7.5}$ + $PEG\ 400_{7.5}$ + $PEG\ 600_{7.5}$ + $Propylene\ Glycol_{7.5}$), Solubility enhancement ratio is 172.20 times more as compared to solubility of domperidone in water. Also, by utilizing the blend of four solubilizers, the individual concentration is reduced minimizing the chances of toxicity and irritation.

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 two, three and four solubilizers. Sink conditions were maintained at 37±0.5°C resembling the conditions of nasal mucosa, for 6 h. Drug release profiles for individual solubilizer showed maximum cumulative drug release of 89.12% for Propylene glycol (30% w/w) [Table 5]. Mixed blend of solubilizers when subjected to release studies showed 75.91% cumulative drug release with $Propylene\ Glycol_{15\%}$ + $PVP\ K30_{15}$ [Table 6], 76.83% with $Propylene\ Glycol_{10}$ +$PEG\ 400_{10}$+ $PVP\ K\ 30_{10}$ [Table 7]. 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 μg /cm^{2}hr and a permeability coefficient of 1.576×10^{-02} cm/hr. [Table 7].

Measurement of Sol-Gel transition temperature

Transition studies showed that loading of domperidone into mixed blend of solubilizers formulated with Poloxamer 407 solutions increased the $T_{sol\text{-}gel}$. Using the

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

Aqueous solvent system (30% w/w)	% Cumulative drug release	Cumulative drug released per cm² (µg)	Flux (µg/cm²hr)	Permeability coefficient (cm/hr)
PVP K25	46.65	321.93	5.92	3.588×10^{-03}
PVP K30	47.97	358.14	6.27	3.409×10^{-03}
Propylene glycol	89.12	33.05	1.47	1.275×10^{-01}
PEG 400	80.01	64.17	6.78	5.29×10^{-02}
PEG 600	84.34	97.49	7.95	3.872×10^{-02}

PVP: Polyvinyl pyrrolidone, PEG: Polyethylene glycol

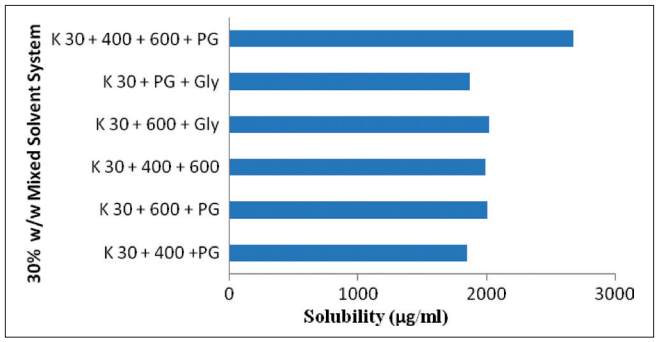

Figure 1: Graphical representation of solubility of domperidone in 30% w/w mixed blends of solubilizers

Table 6: Various parameters of release studies from aqueous solutions of mixed solubilizers

Mixed solvent system (30% w/w)	% Cumulative drug release	Cumulative drug released per cm² (µg)	Flux (µg/cm²hr)	Permeability coefficient (cm/hr)
$PEG\ 400_{15}$ + $PVP\ K30_{15}$	66.20	270.20	8.14	8.609×10^{-03}
$PEG\ 600_{15}$ + $PVP\ K30_{15}$	54.42	238.17	0.33	6.600×10^{-03}
$Propylene\ Glycol_{15}$ + $PVP\ K30_{15}$	75.91	316.52	5.36	9.662×10^{-03}

Values in subscript indicates total concentration of all solvents taken, PVP: Polyvinyl pyrrolidone, PEG: Polyethylene glycol

Table 7: Various parameters of release studies from aqueous solutions of mixed solubilizers

Mixed solvent system (30% w/w)	% cumulative drug release	Cumulative drug released per cm² (µg)	Flux (µg/cm²hr)	Permeability coefficient (cm/hr)
$Propylene\ Glycol_{10}$ + $PEG\ 400_{10}$ + $PVPK\ 30_{10}$	76.83	225.658	3.89	1.38×10^{-02}
$Propylene\ Glycol_{10}$ + $PEG\ 600_{10}$ + $PVP\ K30_{10}$	69.53	221.450	7.21	1.15×10^{-02}
$PEG\ 600_{10}$ + $PEG\ 400_{10}$ + $PVP\ K30_{10}$	61.04	192.969	7.55	1.024×10^{-02}
$Glycerine_{10}$ + $PEG\ 600_{10}$ + $PVP\ K30_{10}$	66.96	214.856	3.23	1.107×10^{-02}
$PEG\ 600_{7.5}$ + $PEG\ 400_{7.5}$ + $Propylene\ Glycol_{7.5}$ + $PVP\ K30_{7.5}$	78.80	209.143	8.64	1.576×10^{-02}

Values in subscript indicates total concentration of individual solvents taken, PVP: Polyvinyl pyrrolidone, PEG: Polyethylene glycol

Table 8: Results of phase transition temperature study by visual method of poloxamer 407 solutions

Concentration of poloxamer 407 in the final solution (% w/w)	Temperature (°C)		Observation
	At initial (liquid) stage	At gelling stage	
15	4	-	No phase transition took place till 40°C
16	4	-	No phase transition took place till 40°C
17	4	-	No phase transition took place till 40°C
18	4	-	Viscosity increases at 38°C but no phase transition took place till 40°C
19	4	37.03±0.98	Gelled at 37°C-38°C
20	4	32.70±0.46	Gelled at 32°C-33°C
21	4	28.50±1.2	Gelled at 28°C-29°C
22	4	26.00±0.31	Gelled at 26°C
23	4	23.00±0.16	Gelled at 23°C
24	4	20.80±0.45	Gelled at 20°C-21°C
25	4	17.80±0.37	Gelled at 17°C-18°C

Poloxamer concentration in the range of 15-25% w/w for determination of $T_{sol\text{-}gel}$, results showed that the 18, 19 and 20% w/w Poloxamer concentrations had $T_{sol\text{-}gel}$ value above 40°C, 37°C, and 32°C, respectively [Table 8]. The concentration of Poloxamer 407 which normally shows phase transition behavior at 37°C (body temperature) is 18% 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 19% 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 284nm and was found to be 99.12±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 6.16±0.51 which was in accordance with the nasal pH requirements. Transition temperature for the prepared formulated gel was found to be 37.08±0.77°C. Spreadability was found to be good [Table 9].

Table 9: Physicochemical properties of domperidone *in situ* nasal gel (n=3)

Parameter	Observation
Drug content (%)	99.12±0.61%
Clarity	Clear
pH	6.16±0.51
Transition temperature	37.08±0.77°C
Spreadability	Good

In vitro drug release study of domperidone *in situ* nasal gel

In vitro release profile of *in situ* nasal gel was carried out for 6hrs using franz diffusion cell resembling the conditions of nasal mucosa at 37±0.5°C maintaining sink conditions [Figure 2]. Percentage cumulative drug release was found to be 73.05±0.57% and flux value of 13.73 μg /cm^{2}hr at the 6th hour [Table 10].

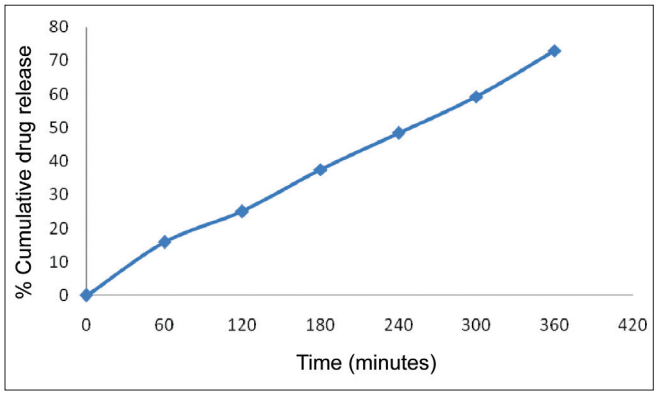

Figure 2: Graphical representation of drug release study of domperidone from *in situ* nasal gel formulation

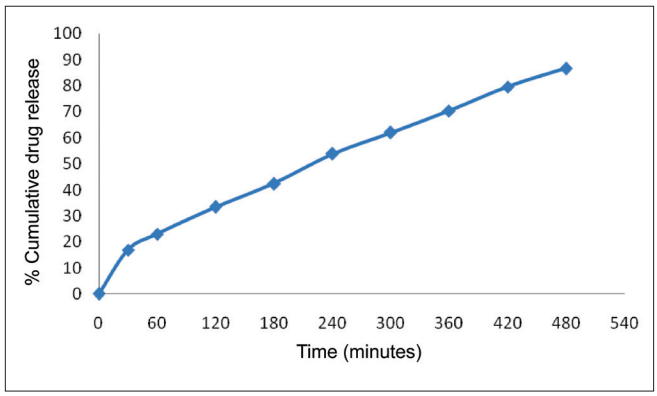

Figure 3: Graphical representation of *Ex-vivo* release studies from domperidone *in situ* nasal gel formulation

Transnasal drug permeation study

Transnasal drug permeation studies were performed on goat's nasal epithelium and drug samples were analyzed

spectrophotometrically at 284 nm [Figure 3]. 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 μg /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 μg /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 11].

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 65 cps and 850 cps, respectively, at 12 rpm.

Table 10: Results of release studies from domperidone *in situ* nasal gel formulation (*n*=3)

Time (minutes)	Cumulative drug release per cm² (µg)	Percent cumulative drug release	Flux (µg/cm²hr)
60	40.36±0.73	15.87±0.32	15.87
120	63.87±1.56	25.12±0.49	9.25
180	95.96±0.98	37.50±0.37	12.38
240	123.24±1.75	48.46±0.83	10.96
300	150.84±1.12	59.32±0.42	10.86
360	185.75±1.45	73.05±0.57	13.73

Table 11: Results of ex-vivo release studies from domperidone *in situ* nasal gel formulation, (*n*=3)

Time (minutes)	Cumulative drug release per cm² (µg)	Percent cumulative drug release	Flux (µg/m²hr)
30	42.92±1.866	16.88±0.736	18.88
60	58.50±0.962	23.00±0.378	6.12
120	84.81±1.935	33.35±0.761	8.35
180	107.73±1.312	42.36±0.516	9.01
240	136.79±1.963	53.79±0.770	11.43
300	157.38±1.532	61.97±0.965	8.18
360	185.87±1.853	70.32±0.869	8.35
420	210.73±1.789	79.58±0.451	9.26
480	242.82±1.951	86.62±0.992	7.04

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 284 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 Table12. None of the samples showed any change in color or appearance under all storage conditions for one month period. The viscosity of gel changed slightly from 64±0.98 cps at 0 month to 65±0.79 cps at the end of one month period. Considering initial drug content which was found to be 99.80% as 100%, after 30 days the drug content was found to be 99.53%, 98.46%, and 98.15%, respectively. Transition temperature was also determined initially and after 30 days, results observed that the formulations gelled at 37.1±0.97°C, 36.9±0.65°C, and 36.6±0.86°C when stored at above mentioned temperatures.

DISCUSSION

Drug loading and solubility enhancement of poorly water soluble drugs has been a challenging task for pharmacist. 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 solutes. 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 domperidone was enhanced in aqueous solution by using various solubilizers like sodium citrate, urea, polyvinyl pyrrolidone and polyethylene glycol individually and in mixed blend as a combination of two, three, and four solubilizers, respectively. From Table 1 result showed an increase in the solubility of domperidone in all solutions containing individual solid solubilizers. The greatest enhancement in solubility was observed in case of 30% w/w PVP K30 solution and least in the case of 30% w/w 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

Table 12: Stability data for *in situ* nasal gel of domperidone (*n*=3)

Time	Storage condition (°C)	Clarity	pH	Transition temperature (°C)	Drug content (%)	Viscosity (cps)	
						Sol	Gel
Initial	NA	Clear	6.32	36.8±0.84	100*	64±0.98	850±0.12
One month	4±2	Clear	6.32	37.1±0.97	99.53	65±0.53	847±0.72
	25±2	Clear	6.31	36.9±0.65	98.46	65±0.79	848±0.16
	40±2	Clear	6.28	36.6±0.86	98.15	65±0.93	848±0.98

*Initial Drug content which was found to be 99.80% was taken as 100%

observed in PEG 600 followed by PEG 400 and Propylene glycol [Table 2]. Domperidone exhibits a pH depended solubility profile, which indicates that it is very slightly soluble at a pH range from 5.5 to 7.0 and is practically insoluble above pH 7. But when single solubilizer where employed it shows a high increase in solubility approximate 302.80 folds enhancement in case of PVP K30, which has a pH of 4.67 while nearly about same pH is also exhibit by PVP K25 that is 4.40 but it gives 279.84 folds enhancement, Table 1. Moreover, PVP K40 has a pH 6.98 and shows 70.40 folds enhancement in solubility, while PEG 200 [Table 2] exhibit approximate same pH 6.30 but has a 9.48 folds enhancement in solubility. So, we can conclude that the solubility enhancement is because of the solvent action/nature of the solubilizer used not because of the change in pH. 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 of two; three and four solubilizers were studied. Tables 3 and 4 illustrate the advantages of making blend of solvents. A satisfactory increase in solubility was obtained in a 30% w/w mixed blend of PVP 7.5% w/w + PEG 400 7.5% w/w + PEG 600 7.5% w/w + Propylene Glycol 7.5% w/w, enhancing solubility of domperidone by 172.20 times as compared to its solubility in water. These results demonstrate the principle of mixed solvency concept that water-soluble substances whether hydrotropes or 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.[7-9] Therefore in developing liquid (solutions) dosage forms, blends of solubilizers can be employed to reduce the toxicities of solubilizers by reducing the individual concentration of solubilizers (instead of employing one solubilizer in higher concentration which may be toxic for same solubility enhancement). Blends of water soluble substances (hydrotropes, cosolvents, water soluble excipients etc.) can be made in safe level of concentrations of individual solubilizer to give a concentrated solution (say 25%, 30% w/v etc.) to act as solubilizing system for development of liquid (solutions) syrups or topical solutions or injections etc.[14,17]

Drug release studies were performed on aqueous solutions of solubilizers containing individual solubilizer and mixed blend of two, three and four solubilizers. Figures 4-6 show the *in vitro* release profile of domperidone. Aqueous solution (30% w/w) containing single solubilizers mainly PVP K 25, PVP K 30, Propylene Glycol, PEG 400 and PEG 600 showed cumulative percent drug release values as 46.65%, 47.97%, 89.12%, 80.01%, and 84.34%, respectively. Flux (μg/cm^{2}hr) and permeability coefficient (cm/hr) values was found to be highest with PEG 600 while cumulative drug released per cm^2 (μg) was found to be maximum with PVP K 30. From the release profiles of individual solubilizers PEG 400, PEG 600, Propylene Glycol and PVP K 30 were selected for formulating as mixed blend (30% w/w) of two solubilizers keeping individual concentration of solubilizers as 15% w/w. PEG 400 and PVP K 30, PEG 600, and PVP K 30, Propylene Glycol and PVP K 30

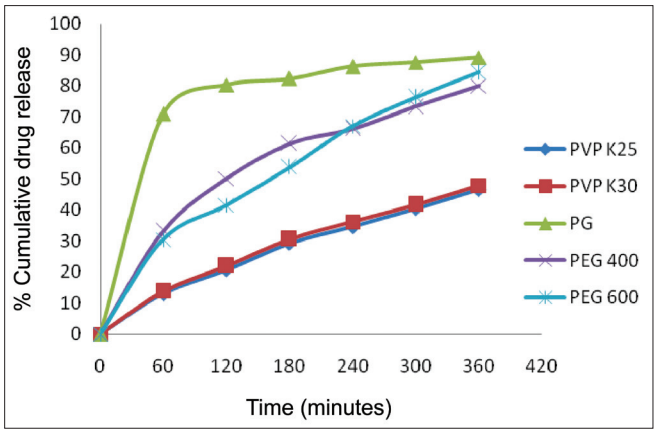

Figure 4: Graphical representation of release studies from 30% w/w aqueous solutions of solubilizers

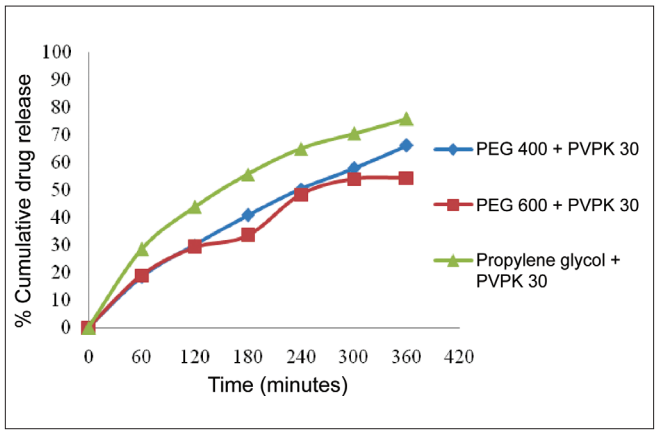

Figure 5: Graphical representation of release study from 30% w/w aqueous solutions of mixed solubilizers

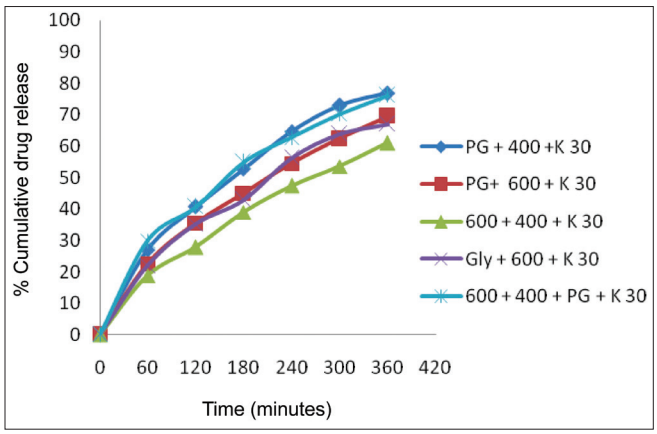

Figure 6: Graphical representation of release studies from 30% w/w aqueous solutions of mixed solubilizers

were formulated and drug release profile was determined. Results showed that flux, permeability coefficient, and cumulative drug released per cm^2 was found to be maximum with Propylene Glycol and PVP K 30 combination. Further mixed blend of three solubilizers were formulated and evaluated keeping individual concentration of solubilizers

as 10% w/w. Value of flux was found to be maximum with (10% w/w PEG 600 + 10% w/w PEG 400 + 10% w/w PVP K30). But permeability coefficient was found to be maximum with 10% w/w Propylene Glycol + 10% w/w PEG 400 + 10% w/w PVP K 30. 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 PEG 400 + PEG 600 + PVP K 30 + Propylene glycol, was formulated and evaluated on above parameters. Permeability coefficient (cm/hr) was found to be 1.576×10^{-02}. Flux ($\mu g/cm^2hr$) was found to be 8.64. Cumulative drug released per cm^2 (μg) was 202.035 and cumulative percent drug release was 78.80%.

Scrutinizing the results of release studies, mixed solvent blend which showed highest permeability coefficient with a lesser individual concentration was selected. Mixed blend ($PEG\ 600_{7.5}$ +$PEG\ 400_{7.5}$ + $PEG\ 600_{7.5}$ + $Propylene\ Glycol_{7.5}$) in 30% w/w concentration was selected for *in situ* nasal gel formulation containing domperidone. Synergistic increase in solubility was observed in the mixed blend, which contains a lower concentration of individual solubilizers that is 7.5% w/w as compared to their total concentration that is 30% w/w. The lower concentration of individual solubilizers leads to formation of a non toxic and non irritant system as these are GRAS approved solubilizers and were used in much lower concentration than their $LD_{50.}$

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. 99.12±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 65 cps and 850 cps, respectively. The concentration of Poloxamer 407 polymer used as thermosensitive polymer was 19% 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 19% w/w and the temperature of phase transition was 37.03±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. Water is a good solvent for PEO as well as PPO chains of polymer at low temperatures. However, at higher temperature the solubility of PPO is reduced and micelle formation occurs.[12] PEG causes an increase in sol-gel transition temperature of poloxamer solution. The hydrophilic end chains of poloxamer comprise the same polyoxyethylene chains that are present in the PEG. It is suggested that the esters bind to these chains promoting dehydration and causing an increase in entanglement of adjacent micelles. The results are in agreement with the effect of solutes and polymers on gelation of Poloxamer. 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 73.05±0.57% and flux value of 13.73 $\mu g/cm^2hr$ 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 18.88±0.736%, 42.36±0.516%, and 86.62±0.992%, respectively [Table 11]. An initial high flux value was observed i.e.18.88 mcg/hr/sq.cm at the 30^{th} min. Results resembling previously reported studies suggest[12] that this initial high flux value may be due to formation of weak gel structure at 19% w/w concentration of Poloxamer 407 used for formulation. A gradual decrease in the flux value was obtained with respect to time in the 2^{nd}, 3^{rd}, and 4^{th} hour. But a second sharp peak was again observed as in flux value i.e. 11.43 $\mu g/cm^2hr$ at 240 min. occurrence of second peak in the values indicates that Poloxamer 407 gel required 240 min time period to absorb sufficient water from medium to break the gel structure and therefore a higher amount of drug release was observed which corresponds to the second peak flux. As already reported in the previous research works an overall increase in the drug release profiles can be achieved due to inclusion of the PEG, and it might be due to the interference of the PEG with micellar association thereby increasing gel fluidity and decreasing the retardant effect of Poloxamer 407. Role of PEG as a co-solvent also have reported to be an important factor towards drug release mechanisms.[12]

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 284 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 domperidone. 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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