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Formulation and evaluation of *In-Situ* gel of Diltiazem hydrochloride for nasal delivery

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ABSTRACT

The objective of present investigation was to develop a mucoadhesive in-situ gel; formulation was developed to have a controlled kinetic drug release and to minimize the toxic effects of diltiazem Hydrochloride (DTZ). DTZ was incorporated into the, blends of thermoreversible, bio adhesive polymers such as poloxamer (PLX) and Hydroxy Propyl Methyl cellulose (HPMC) in the form of in-situ gel by cold technique to reduce mucociliary clearance, and thereby it will increase the contact of formulation with nasal mucosa and hence improving the absorption of drug. The results revealed that as the increase of bio adhesive polymer HPMC concentration, decrease in the gelation temperature (T1) and increase in gel melting temperature (T2). pH of all the formulations were found to be within the range between 5.4 – 6.2 and the nasal mucosa can tolerate the above mentioned pH of the formulations. The drug content for all the prepared formulations was found to be 97% - 100%. The mucoadhesion test indicates that the level of HPMC increases, the mucoadhesive strength also increases. The developed formulations had optimum viscosity, and it was observed that an influence of diffusion on drug particles with increase in the concentration of HPMC. The optimized formulation shows the controlled drug release (86.32%) than aqueous drug solution (93.81%). The drug release performance was greatly affected by bio polymers used and their compositions in the in situ gels preparation, which allows absorption in nasal mucosa.

Keywords: ***In situ*** gel, Diltiazem Hydrochloride (DTZ), HPMC, Poloxamer407, Nasal delivery.

INTRODUCTION

The nasal mucosa has been considered as a potential administration route to achieve faster and higher level of drug absorption. This is due to the large surface area, porous endothelial

membrane, and high total blood flow. Also, pharmacokinetics shows, intranasal administration circumvent first-pass elimination [1]. Recently, focus has been made on the nasal mucosa as an alternate route to achieve faster and higher drug absorption [2]. The nasal cavity offers a number of unique advantages such as easy accessibility, good permeability especially for lipophilic, low molecular weight drugs, avoidance of harsh environmental conditions and hepatic first pass metabolism, potential direct delivery to the brain [3-4].

HPMC is semi synthetic, inert, viscoelastic polymer which is nonionic, a good carrier material for pharmaceutical application, can accommodate high levels of drug loading, non toxic and exhibits high swelling capacity. When the solution heats up to a critical temperature, the solution congeals into a non-flowable but semi-flexible mass. Typically, this critical (congealing) temperature is inversely related to both the solution concentration of HPMC and the concentration of the methoxy group within the HPMC molecule which in turn depends on both the degree of substitution of the methoxy group and the molar substitution. When HPMC contact with water hydrates rapidly, leading to a transition from the glassy to the rubbery state, this results in the formation of a gel layer with a significant effect on release kinetics of the incorporated drug. Moreover, they are surface active and hence provide excellent film formation [5, 6].

Poloxamer 407(PLX) is the reversible triblock copolymer used which forms clear thermo reversible gel at high concentrations. The concentrated solutions are transformed to low viscosity transparent solutions at 5^0 C to solid gel on heating to body temperature [7]. PLX is a thermoreversible gel [8]. This characteristic has allowed PXL to be used as a carrier for most routes of administration including oral, topical [9], intranasal [10], vaginal, rectal [11], ocular [12], and parenteral routes [13] .The potential use of PXL as an artificial skin has also been reported [14].

DTZ is a calcium ion cellular influx inhibitor (slow channel blocker or calcium antagonist). DTZ produces its antihypertensive effect primarily by relaxation of vascular smooth muscle and the resultant decrease in peripheral vascular resistance. DTZ is subjected to an extensive first-pass effect, giving an absolute bioavailability of about 40%. Intranasal administration allows transport of drugs to the brain circumventing Blood Brain Barrier, thus providing unique features and better option to target drugs to brain. The objective of this work is to improve nasal bioavailability of DTZ by increasing its nasal retention time [15-20].

The concentrated solutions (20 -30%) are transformed from low viscosity transparent solutions at 50^OC to solid gel on heating to body temperature. By modulating the gelation temperature of different PXL solutions, liquid bases for nasal use can be formulated that form a gel in nasal cavity at body temperature resulting in enhancement of residence time in the nasal cavity. The objective of the present study is to develop *in situ* gel of DTZ, characterize and study the drug release properties with favorable gelation, rheological and release property. This may give patient friendly, needle free dosage form.

MATERIALS AND METHODS

Materials

DTZ was obtained as a gift sample from Dr. Reddys, Hyderabad, India. Polaxomer 407 was kindly provided by BASF, Mumbai. HPMC (Loba Chemie Pvt. Ltd.), Potassium dihydrogen phosphate used were of AR grade.

Method

Physicochemical Studies

Drug solubility studies

The solubility of DTZ in a variety of solvents was carried. Excess amount of DTZ (100 mg) was added to 10 mL of various solvents. The dispersions were shaken in a thermostatically controlled water bath shaker at 37+0.5^{o}C until equilibrium (48 h). Afterward, samples were withdrawn, filtered through a 0.45 µm membrane filter and suitably diluted. Drug concentration was analyzed byand the solubility of the drug in different solvents after suitable dilution, absorbance of solution was measured at 236.4 nm by using UV visible spectrophotometer (Shimadzu UV-1800).

Fourier transform infrared spectroscopy (FT-IR)

FTIR spectra of pure drug, physical mixture (DTZ + PLX + HPMC) and physical mixture without drug (PLX + HPMC) were obtained using KBr pellet method (applying 6000 kg/cm ^{2}). Spectral measurements were obtained by powder diffuse reflectance on a FTIR spectrophotometer (Shimadzu, Model 8033,USA). Each spectrum was recorded in the frequency range of 4000-450 cm^{-1}.

X-ray diffractometry

The X-ray diffractograms of DTZ and Physical mixture (BHC + PLX+HPMC) were recorded by using Philips XuPert MPD (Netherland) diffractometer with tube anode Cu over the interval 4-40^{o}/2θ. The generator tension (voltage) 40kV, generator current 45 mA and scanning speed 2^{o}/min.

Preparation Of *In Situ* Gels

Pluronic gels were prepared by cold technique reported by Schmolka [21]. The weighed quantity of mucoadhesive polymer of HPMC at different ratios (0.2%, 0.4%, 0.6%, 0.8%, and 1%) and drug (10%w/v) was dissolved 10ml of distilled water. To the above formulations the weighed quantity of themosensitve polymer (HPMC), poloxamer (19%w/v used as a base), was added slowly with constant stirring and kept at 4º C overnight until to form a clear gel.

Characterization of *In Situ* Gels

Evaluation of *In Situ* Gels

Measurement of Gelation Time:

2ml aliquot of gel was taken in a test tube and kept in an oven maintained at 37^{0}C. The sample was examined for gelation.

Measurement of Gelation Temperature (T1) and Gel Melting Temperature (T2):
It was determined by using method described by Miller and Donovan technique [22]. A 2ml aliquot of gel was transferred to a test tube, immersed in a water bath. The temperature of water bath was increased slowly and left to equilibrate for 5min at each new setting. The sample was then examined for gelation, which was said to have occurred when the meniscus would no longer moves upon tilting through 90^0. After attaining the temperature T1, further heating of gel causes liquefaction of gel and form viscous liquid and it starts flowing, this temperature is noted as T2 i.e. gel melting temperature. It is a critical temperature when the gel starts flowing upon tilting test tube through 90^0.

pH of Formulation :
1ml quantity of each formulation was transferred to a beaker and diluted by using distilled water to make 25ml. pH of the resulting solution was determined using digital pH meter.

Drug Content:
1ml of formulation was taken in 10ml volumetric flask, diluted with distilled water and volume adjusted to 10ml. 1ml quantity from this solution was again diluted with 10ml of distilled water. Finally the absorbance of prepared solution was measured at 236.4 nm by using UV visible spectrophotometer (Shimadzu UV-1800).

Determination of Mucoadhesive Strength:
The mucoadhesive strength was determined. The mucoadhesive potential of each formulation was determined by measuring a force required to detach the formulation from nasal mucosal tissue. A section of sheep nasal mucosa was fixed on each of two glass slides using thread. 50mg of gel was placed on first slide and this slide placed below the height adjustable pan. While another slide with mucosal section was fixed in inverted position to the underside of the same pan. Both the slides with gel formulation between them held in contact with each other, for 2min to ensure intimate contact between them. Then weight was kept rising in second pan until slides get detached from each other. The mucoadhesive force expressed as the detachment stress in dynes/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.

$$\text{Mucoadhesive Strength (dynes/cm 2)} = mg/A \qquad (1)$$

Where, m = weight required for detachment in gram,
g= Acceleration due to gravity (980cm/s2),
A = Area of mucosa exposed

Viscosity Measurement:
The viscosity measurements were carried out by using Brookfield programmable DV-II LV model (Brookfield Eng.Lab., Inc.USA). The gel sample was placed in small sample adapter. Temperature was increased in the range of 20^0C to 40^0C, using water circulation jacket. The temperature sensing probe was lowered in gel and temperature of gel was recorded. Viscosity at various temperatures was recorded.

***In-vitro* Release Studies:**

Drug release from gel was tested with nasal diffusion cell, using dialysis membrane (mol.wt.12,000-14,000) with permeation area of 0.785cm^2. 60ml of phosphate buffer pH 6.4 was added to the acceptor chamber. Gel containing drug equivalent to 10mg was placed in donor compartment. At predetermined time points, 1ml sample were withdrawn from the acceptor compartment, replacing the sampled volume with phosphate buffer pH6.4 after each sampling for a period of 5 hrs. The samples were suitably diluted and measured spectrophotometrically at 236.4 nm.

***In –vitro* Permeation Study:**

Fresh nasal tissue was removed from nasal cavity of sheep obtained from local slaughter house. Tissue was inserted in the nasal diffusion cell with permeation area of 0.8 cm^2. Gel containing drug equivalent to 10g was kept in donor compartment. At predetermined time point sampling was done. Blank samples (without drug) were run simultaneously throughout the experiment. Amount of drug permeated was determined by UV spectrophotometry at 236.4 nm.

RESULTS AND DISCUSSION

Physicochemical Studies

Solubility studies

The solubility of DTZ in a variety of solvents is presented in Table 1.

Table 1: Solubility of DTZ in various solvents

Solvent	Solubility (mg/ml, 30^{0}C)
Chloroform	97.5
Formic acid	97.2
Methanol	98.8
Water	98.1
Dehydrated alcohol	23.4
Benzene	1.2
Ether	0.9

Fourier Transform Infrared Spectroscopy

From the FTIR studies, the characteristic bands for important functional group of pure drug, physical mixture (without drug -formulation F2) and drug-loaded physical mixture (formulation F2) were identified as shown in Figure 1. FTIR spectra showed that the characteristics bands of DTZ were not altered after successful encapsulation without any change in their position, indicating no chemical interactions between the drug and wax used. Compared the IR spectra at 3035, 3956, 2966 cm^{-1} due to C – H stretching, 2837 cm^{-1} due to O – CH_3 stretching, 2393 cm^{-1} due to amine HCl N = C stretching, 1743cm^{-1} due to acetate C = O stretching, 1679 cm^{-1} due to lactum C = O stretching, 839 cm^{-1} due to O – substituted aromatic C- H stretching and 781 cm^{-1} due to p – substituted aromatic C- H stretching.

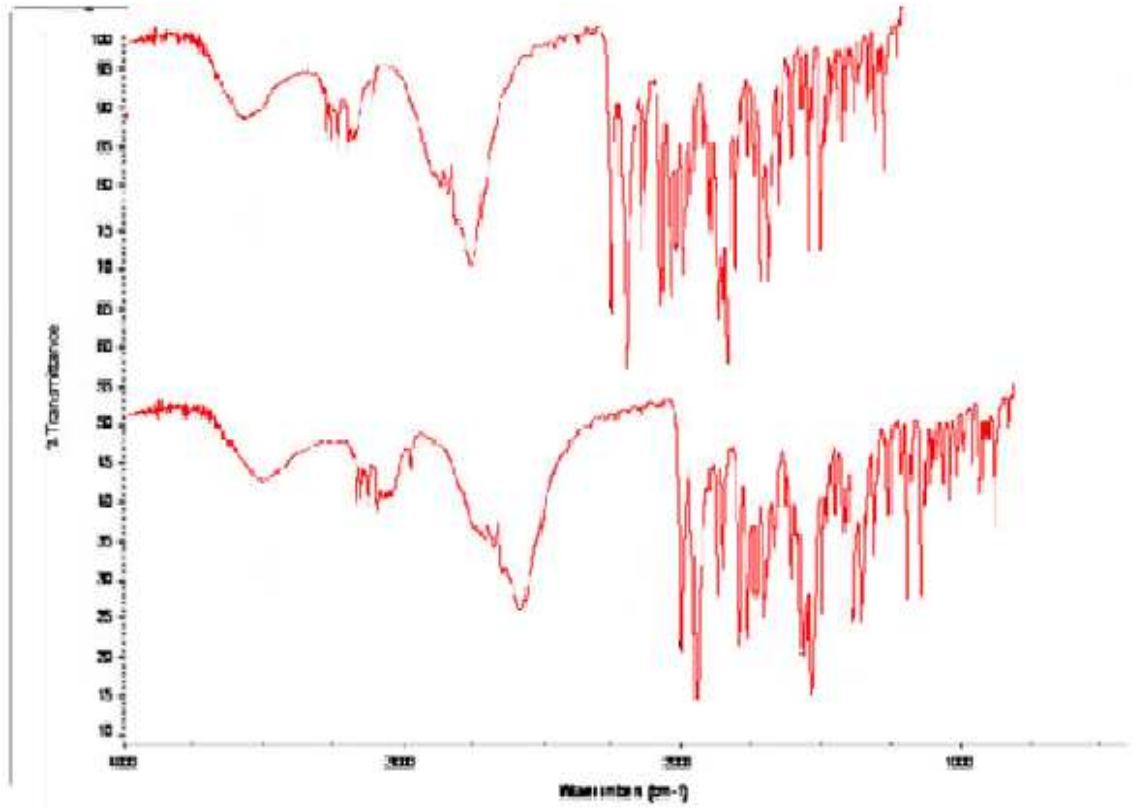

Figure 1: FT – IR spectra of BHC and physical mixture (Formulation F2)
DTZ = Diltiazem Hydrochloride

X-ray diffractograms

XRD study was performed to understand the crystalline or amorphous nature and the solubility of the drug after dispersed into a polymeric *in situ* gel formulation. For the determination of the existence of a possible interaction between DTZ and the polymers, PLX and HPMC in the formulations, we first investigated the XRD patterns of the pure drug and physical mixture (with drug - formulation F2). The X-ray diffractograms of DTZ and physical mixture (with drug - formulation F2) are presented in Figure.2. The pure DTZ showed several diffraction peaks, exhibiting a main sharp peak at 11.2, 18.3 and 28.1^{o} and secondary peaks at 8.3, 14.2, 15.3, 22.4^{o}. The diffraction patterns of physical mixture (formulation F2) exhibiting a main sharp peak at 11.8, 18.2 and 28.3^{o} and secondary peaks at 8.7, 14.7, 15.5, 22.5 ^{o}. The crystallinity of DTZ was clearly seen by its XRD pattern. The several sharp diffraction peaks from DTZ diffractogram exhibited that the drug DTZ is in crystalline form. The diffraction patterns of physical mixture (formulation F2) corresponded to the superimposition of DTZ indicated that the crystallinity of DTZ was not changed. The fine crystals of DTZ either dispersed on to the surface or cavities of PLX and HPMC.

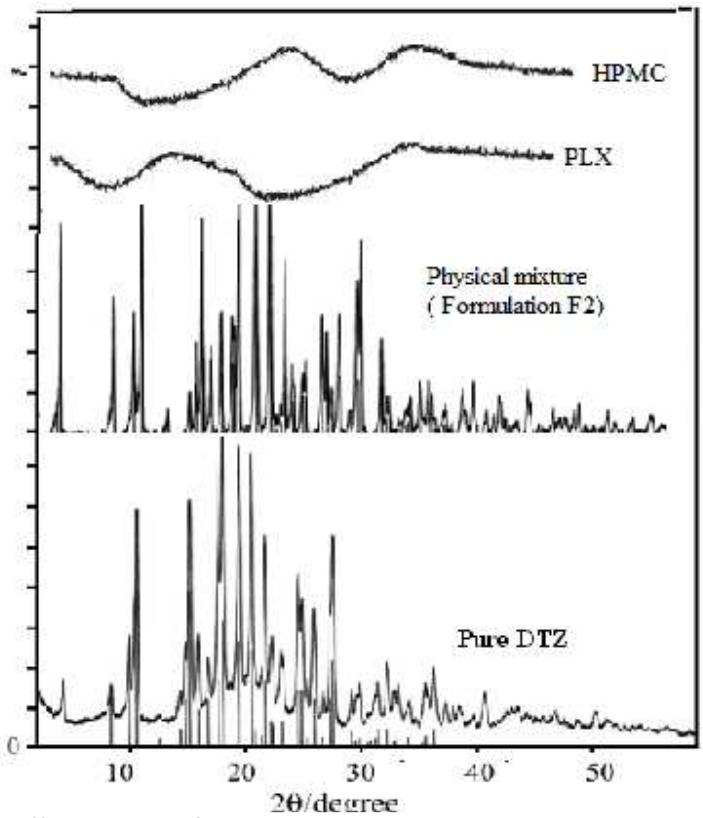

Figure 2: Ray diffractograms of DTZ, Physical mixture (Formulation F2), PLX and HPMC

DTZ = Diltiazem Hydrochloride, PLX = Poloxamer and HPMC = Hydroxy Propyl Methyl Cellulose

Evidence have shown in the recent years that bio adhesive polymers have the physical properties and behavior suitable to prepare bio compatible bio degradable in situ gels to release the drug in the nasal mucosa[19, 20]. In the present study, the modified novel cold technique was employed using bio adhesive polymers and non toxic solvents to prepare the in situ gels. The present method is quite different from that reported by Schmolka [16] because in the present study, HPMC was used as a bioadhesive polymer (0.2- 1% W/V), PLX as base (19% W/V) at various concentration to study the gelation temperature, mucoadhesive strength, viscosity and % cumulative drug release. The Solubility of DTZ in a variety of solvents presented in Table 1.

Gelation Time

The gelation time is defined as the time taken for the transition of liquid phase to a gel. In the present study, for the prepared formulations the gelation time was found to be within 2 min. PLX 19% W/V was used to produce the *in situ* gels with in 2 min.

Gelation Temperature (T1) and Gel melting Temperature (T2):

At gelation temperature, liquid phase makes transition into gel. Due to the addition of HPMC and DTZ there is a change in T1 of gel formation. Study shows that formulation F1 having gelation temperature of 31^0C (low level of HPMC-0.2%W/V) where as F2 has a T1 of 27^0C which is having high level of HPMC (0.4%).The results presented in Table 2. This indicates that the mucoadhesive polymer, HPMC has significant T1 lowering effect. The gelation temperature

lowering effect might be caused due to increased viscosity after dissolution of mucoadhesive polymer. The gel melting temperature (T2) was also found to increase with increasing concentration of HPMC from 0.2%- 1.0%W/V.

Table 2: Formulations and evaluation parameters of formulations

Formulation code	HPMC : Poloxamer	Gelation Temp. (T1 ^{0}C)	Gel melting Temp. (T2 ^{0}C)
F1	0.2: 19	30	45
F2	0.4: 19	28	48
F3	0.6: 19	24	56
F4	0.8: 19	23	61
F5	1.0: 19	20	68

Values shown in the table mean percent of three batches (n= 3)

pH of The Formulation:

The normal physiological pH of the nasal mucosa ranges from 4.5-6.5. But the nasal mucosa has the capability to tolerate pH between 3-10. pH of all the formulations was found to be between 5.4-6.2 i.e., within the range which nasal mucosa can tolerate [21] and the results are presented in Table 3.

Table 3: Evaluation parameters of formulations

Formulation code	pH (mean± S.D.)	Drug Content (mean± S.D.)	Mucoadhesive strength (Dynes/cm2)
F1	5.9 ± 0.12	98.51 ± 1.22	2613 ± 0.71
F2	6.1± 0.2	97.88 ± 0.43	3593 ± 0.82
F3	5.7 ± 0.13	99.12 ± 1.42	3920 ± 0.52
F4	6.3 ± 0.05	96.05 ± 1.05	4573 ± 0.32
F5	6.0 ± 0.2	98.03 ± 0.98	4736 ± 0.39

Values shown in the table mean percent of three batches (n= 3)

Drug Content:

The percentage drug content of all the formulations was found to be in the range of 97.05-99.23% as shown in Table 3.

Mucoadhesive Strength:

Mucoadhesive strength was determined by measuring the force required to detach the formulation from mucosal surface i.e., detachment stress. Results reveal that variable HPMC is having effect on mucoadhesive strength. It shows that as level of HPMC increases, mucoadhesive strength also increases. The results are presented in Table 3. This was due to wetting and swelling of HPMC, permit intimate contact with nasal tissue, interpenetration of bioadhesive HPMC chains with mucin molecules leading to entanglement and formation of weak chemical bonds between entangled chains [22].Due to stronger mucoadhesive force, it can prevent the gelled solution coming out of the nose. But higher ratio of HPMC responsible for excessive bioadhesive force and the gel can damage the nasal mucosal membrane.

Viscosity:
Viscosity measurement of the formulations at various temperatures (20-40^0 C), shows that there was increase in viscosity with increase in the temperature. Figure 3 shows viscosity profiles of formulations at 37^{0}C and the mucoadhesive polymer HPMC had a viscosity enhancing effect.

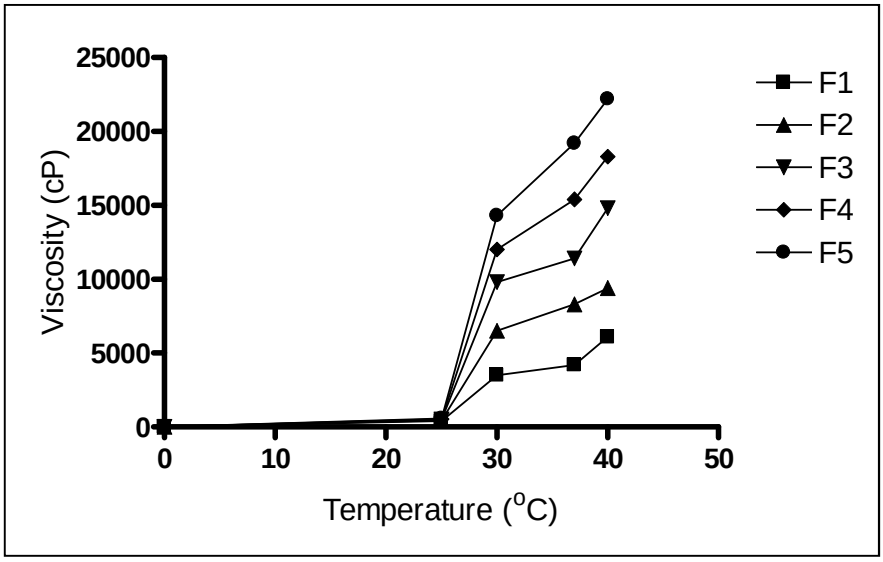

Figure 3: Viscosity measurement of formulations at various temperatures.

***In vitro* drug release:**
The release profile of DTZ from all the formulations it reveals that as the level of HPMC is increasing, the drug release is decreasing due to higher viscosity of the formulation. The retarding effect of mucoadhesive polymer, HPMC could be attributed to their ability to increase the overall product viscosity as well as their ability to distort or squeeze the extra micellar aqueous channels of PLX micelle through which the drug diffuses thereby, delaying the release process. From the result it clearly shows that only HPMC is affecting drug release. Formulation F1, F2, F3, F4 and F5 shows the 86.32, 84.12, 80.18, 76.56 and 75.32% drug release at 5h respectively. This decrease in drug release might be due to higher level of HPMC in these formulations.

***In vitro* Permeation Study:**
In vitro permeation was observed for the aqueous drug solution and formulation F3. Formulation F3 exhibited good drug release profile with favorable gelation and rheological properties. Hence, the formulation F3 was chosen as an optimized formulation, to see the permeation of drug through nasal mucosa. It was observed that permeation of drug from aqueous solution was 93.81% where as the optimized formulation F3 shows release of 84.03 % at the end of 5 h as shown in Figure 4. The release of DTZ from the gel formulation was found lower as compared to the aqueous solution, may be due to the inverse relationship between viscosity and drug release. Viscosity of the formulation influences release of DTZ from gel formulation. This shows that when viscosity is increased, the release of the incorporated drug can be prolonged.

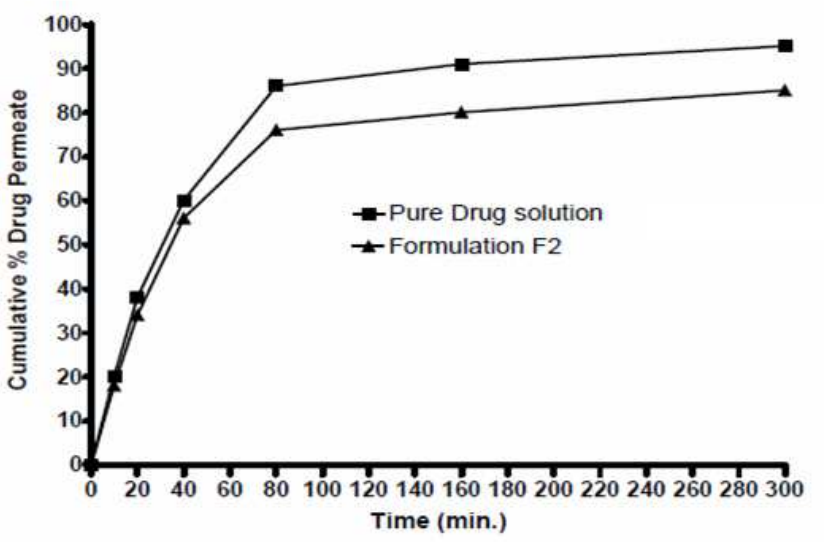

Figure 4: *In vitro* permeation of pure drug solution and formulation F2.

CONCLUSION

In the present study, PXL gel with HPMC is formulated for nasal delivery of calcium ion cellular influx inhibitor DTZ, which would enhance nasal residence time due to its increased viscosity and mucoadhesive strength.DTZ has the characteristic of presystemic metabolism of upto 80%. So, the formulation as an intra nasal gel could attribute to escaping first pass metabolism due to its administration as an *in situ* gel. Optimized formulation F2 was found out to be (composed of 1%W/V DTZ , 19% W/V PLX, 0.4%W/V HPMC) better with respect to its rheological properties, gelation time, gelation temperature, pH, mucoadhesive strength, *in vitro* drug release and *in vitro* permeation studies when compared to other formulations. These results demonstrate the potential is of PXL and HPMC for the fabrication of controlled delivery devices for many partially water soluble or hydrophilic drugs.

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