Development and Evaluation of In - Situ Nasal Gel of Candesartan Cilexetil

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Abstract

Now a day's Nasal route is successfully used for the systemic delivery of various drugs like antidiabetic, antidepressants, vitamins and insulin delivery as it avoids first pass metabolism, gastrointestinal irritation, increases the bioavailability and residence time. The large surface area of the nasal mucosa offers a rapid onset of therapeutic effect, potential for direct-to-central nervous system delivery, no first-pass metabolism, and non-invasiveness; all of which may maximize patient convenience, comfort, and compliance. In the present investigation, an attempt was made to develop the pH sensitive nasal in-situ gel of Candesartan Cilexetil (2.5%) for controlling the drug release in the nasal tissues. Two polymers have been used i.e. Carbopol 940 and Xanthan gum. Carbopol 940 is the pH sensitive Mucoadhesive polymer. Xanthan gum has better effect on viscosity when exposed to the pH range 4.5-6.5. These conditions resemble the physiological conditions of the nose. The pH sensitive in-situ nasal gel so prepared were characterized for its clarity, pH, viscosity, gel strength, Mucoadhesive strength, drug content, in-*vitro* drug release and *in-vitro* permeation. This optimized formulation offers an alternative to conventional oral formulations and addresses food interaction issues.

Keywords: Gastrointestinal irritation, Bioavailability, first-pass metabolism, Mucoadhesive

Introduction

Hypertension puts strain on the heart, leading to hypertensive heart disease and coronary artery disease if not treated. Hypertension is also a major risk factor for stroke, aneurysms of the arteries (e.g. aortic aneurysm), and peripheral arterial disease and is a cause of chronic kidney disease[1]. A moderately high arterial blood pressure is associated with a shortened life expectancy while mild elevation is not. Dietary and lifestyle changes can improve blood pressure control and decrease the risk of health complications, although drug treatment is still often necessary in people for whom lifestyle changes are not enough or not effective[2].

Candesartan Cilexetil, an antihypertensive drug competes with angiotensin II for binding at the AT1 receptor subtype. As angiotensin II is a vasoconstrictor which also stimulates the synthesis and release of aldosterone, blockage of its effects results in a decrease in systemic vascular resistance[3]. It hydrolyses to Candesartan after absorption from the gastrointestinal tract and could be a Class-II drug with anti-hypertensive characteristics and low solubility and high permeability[4]. Angiotensin II binding to AT1 is selectively blocked by Candesartan Cilexetil in a number of tissues. Angiotensin II's vasoconstrictor and

associated aldosterone-secreting activities are thereby inhibited, resulting in a general decrease in force per unit area. Candesartan Cilexetil is well absorbed from the GI tract, but undergoes substantial first-pass-metabolism, leading to an oral bioavailability of only 15%. Plasma half-life of Candesartan Cilexetil is 9 h and molecular weight is 440.46.[5-6]

Nasal drug delivery is a useful delivery method for drugs that are active in low doses and show minimal or no oral bioavailability. The nasal route circumvents hepatic first pass elimination associated with the oral delivery; it is easily accessible and suitable for self-medication[7]. Currently, two classes of nasally delivered therapeutic agents are on the market. The first one comprises low molecular weight and hydrophobic drugs for the treatment of the nasal mucosa and sinus, including decongestants, topical steroids, antibiotics and other (OTC) products[8]. The second class encompasses a few drugs, which have sufficient nasal absorption for displaying systemic effects. Important candidates are the compounds, generally administered by injection and hardly absorbed after oral administration, due to their instability in the gastrointestinal tract, poor absorption properties, and their rapid and extensive biotransformation[9].

Materials and Methods

Materials

Candesartan Cilexetil was obtained as gift sample from JCPL Pharma, Jalgaon. Carbopol 940 was obtaied from Loba Chemie Pvt. Ltd. Xanthan gum was purchased from Signet Chemicals Polyethylene glycol 400, Triethanol amine, Disodium hydrogen phosphate ect was purchased from Research-Lab Fine Chem. Industry, Mumbai.

Methods

Preformulation studies
Organoleptic properties

The sample of Candesartan Cilexetil was studied for organoleptic characteristics such as colour, odour and appearance.

Melting point [10-11]

The melting point of Candesartan Cilexetil was determined by using melting point apparatus and capillary method. For determination of melting point, drug was taken in a glass capillary whose one end was sealed by flame. The capillary containing drug was dipped in liquid paraffin inside the melting point apparatus (Analab scientific industries India.) And temperature was increased gradually. Melting point was then determined and reported. (n=3)

Determination of solubility [12]

Solubility of Candesartan Cilexetil was determined by Higuchi and Connors method. An excess amount of Candesartan Cilexetil was added to distilled water, pH6.8 phosphate buffer, respectively in different screw-capped bottles. The bottles were placed in orbital shaker and shaken at room temperature (26 ± 2^0C) and shaken for 72 hrs. The samples were filtered through the whatman filter paper. The filtrate was diluted suitably and analysed using UV spectrophotometer.

Partition coefficient [13]

The partition coefficient of the drugs was determined by taking equal volumes of n-octanol and aqueous phases in a separating funnel. 20 mg of drug was added to n-Octanol: water (20:20) and was taken in a separating funnel and shaken for 10 minutes and allowed to stand for 2 hr. The aqueous phase was separated from organic phase. The amount of drug in aqueous phase and amount of drug partitioning in organic

phase was calculated from titrimetric method.

Loss on drying [11]

Dried the substance in the hot air oven at 80^0 C for 3 hrs. And after allowed it to cool. Weighed the contents and the bottle and calculated the difference in the initial and final weight of the substance.

Preparation of calibration curve in distilled water

Candesartan Cilexetil (10 mg) was accurately weighed and transferred to 100 ml volumetric flask. It was then dissolved and diluted up to 100 ml with distilled water. The above made solution was further diluted to obtain concentration ranging from 1-24 µg/ml. The absorbance's of the resulting solutions were recorded at 254 nm using UV-visible spectrophotometer.

Preparation of calibration curve in simulated nasal fluid

Candesartan Cilexetil (10 mg) was accurately weighed and transferred to 100 ml volumetric flask. It was then dissolved and diluted up to 100 ml with simulated nasal fluid. The above made solution was further diluted to obtain concentration ranging from 2-12µg/ml. The absorbance of the resulting solutions were recorded at 254 nm using UV-visible spectrophotometer.

Validation of analytical method

Linearity (Calibration curve): the developed method was validated as per ICH guidelines. The plot of absorbance verses concentration was plotted. It can be seen that plots are linear in the concentration range of 5-25 µg/ml.

Precision (repeatability): Intraday and interday precision was determined by measurement of the absorbance for three times on same day and on three different days.

Accuracy (recovery study): Recovery studies were carried out by adding a known quantity of pure drug to the preanalyzed formulations and the proposed method was followed. From the amount of drug found, percentage recovery was calculated as per ICH guidelines.

Sensitivity: Sensitivity studies were carried out where limit of detection (LOD) and limit of quantification (LOQ) were determined using following equation.

Compatibility study

Infra-Red spectroscopy [14, 15]

Compatibility study of drug and polymer was carried out by using Infra-Red Spectrophotometer [8400S Shimadzu. Japan]. The sample of pure drug, physical mixture of drug and polymer was prepared and samples kept for 1 month at 40^0C. The Infrared spectrum of Candesartan Cilexetil and physical mixture was recorded with KBr disc over the wave number of 4000 to 400 cm$^{-1.}$

Differential scanning calorimetric studies

The sample of pure drug, physical mixture of drug and polymer were weighed and heated at a scanning rate of 10°C/min between 40 and 300°C and 40 ml/min of nitrogen flow.

Method for Preparation of pH sensitive in-situ gel [16]

The formulations were prepared by cold method (Reported by Shmolka). The drug containing PEG, pH sensitive polymer and mucoadhesive polymers were hydrated separately in calculated amount of distilled water at room temperature, cooled and stored at 4^0C. Both polymeric solutions were mixed slowly on ice bath; Preservative was added slowly with continuous stirring in polymer solution. Both solutions (drug and polymer) were

mixed with each other by gentle stirring. The final dispersion was then stored in a

refrigerator until clear solution was obtained.

Ingredients used in Formulation

Table No.1: Composition of Formulation

Compositionon → Formulationn code	Candes artan cilexetil (%w/v)	Carbopol 940 (%w/v)	Xanthan gum (%w/v)	PEG400 (%v/v)	Methyl parabens (%v/v)	Distilled water Up to 100 (ml)
F1	2.5	0.3	0.15	10	0.033	100
F2	2.5	0.4	0.15	10	0.033	100
F3	2.5	0.5	0.15	10	0.033	100
F4	2.5	0.3	0.20	10	0.033	100
F5	2.5	0.4	0.20	10	0.033	100
F6	2.5	0.5	0.20	10	0.033	100
F7	2.5	0.3	0.25	10	0.033	100
F8	2.5	0.4	0.25	10	0.033	100
F9	2.5	0.5	0.25	10	0.033	100

carbopol 940, xanthan gum are independent variables used in the formulations. They are mucoadhesive polymers to increase the residence time of formulation in the nasal cavity and to show their effect on gel strength, viscosity, drug content and *In-vitro* drug release. *In- vitro* drug release, mucoadhesive strength and viscosity data wasoptimized.

Formulation of Nasal Mucoadhesive *In-situ* Gel

Different formulae of gel were prepared by using ingredients mentioned in table 7.3. In this formulation concentration of carbopol 940 was ranged between 0.3 to 0.5 %, concentration of xanthan gum in between 0.15 to 0.25%. Drug was dissolved in mixture of distilled water and PEG; both the polymers were hydrated separately. Preservative was added in polymeric solution. Mixing of drug and polymeric

solution was done at cold condition. Kept solutions at 4^0C until clear gel is obtained.

Evaluation of Nasal Mucoadhesive *In-situ* Gel

Visual appearance [17]

The formulation was visually checked for clarity.

pH of formulation [17]

pH of each formulation was determined by using digital pH meter (Systronics Digital pH meter 335). The pH meter was calibrated using pH 4 and pH 7 buffer by using standard buffer tablet.

Viscosity [18]

Viscosity (Rheological Properties) of prepared gel was determined with the help of Brookfield Viscometer; type DV-II+PRO using spindle no- 62 and 63. Viscosity of formulations was determined at two different pH.

Measurement of gel strength [19-23]

A sample of 50g of the nasal gel was put in a 50 ml graduated cylinder. A weight of 5 g was placed onto the gel surface. The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm deep into the gel.

Mucoadhesive strength (detachment stress) [23]

The mucoadhesive strength of each formulation was determined by measuring the force required to detach the formulation from sheep nasal mucosal tissue by using a modified bioadhesion test apparatus that is modified physical balance. *In- vitro* mucoadhesion studies were conducted using modified bioadhesion test.

Drug content [19-23]

Drug content was determined by taking 1ml of formulation in 100 ml volumetric flask. It was dissolved in distilled water properly and final volume was made to 100 ml with distilled water. 1ml quantity from this solution was transferred into the 10ml volumetric flask and final volume was made to 10ml by using distilled water. Finally, the absorbance of prepared solution was measured at 224 nm by using UV visible spectrophotometer. By using absorbance value % drug content in the formulation was calculated.

Measurement of adhesion force

From each batch, some quantity of gel was taken and applied on the lower surface of the upper polypropylene cylinder. The beaker containing mucosal tissue secured upon lower cylinder (B), was manipulated over the base of the balance so that, the mucosal tissue is exactly below the upper cylinder (A). The exposed part of the gel was wetted with a drop of simulated nasal solution, and then a weight of 10 gm was placed above the expanded cap, left for 10 minutes. After which the gel binds with mucin. The weight was removed. Then slowly and gradually weights were added on the right-side pan till the gel separates from the mucosal surface/ membrane. The weight required for complete detachment is noted (W1) (W1-5.20G)) gives force required for detachment expressed in weight in grams. Procedure was repeated for two more times. Average was computed and recorded.

In -Vitro drug release study [23]

Preparation of simulated nasal fluid: [19-23]

Weighed accurately 0.87% NaCl, 0.31% KCl and 0.088% $CaCl_2 \cdot 2H_2O$ and dissolve in 500 ml of distilled water to produce simulated nasal fluid; finally adjusted the pH with triethanolamine to 6.4. *In- vitro* release study of the formulation was carried out using laboratory designed diffusion cell through egg membrane. 1 ml of gel was placed in donor compartment and freshly prepared simulated nasal solution in receptor compartment (100ml). Egg membrane was mounted between donor and receptor compartment. Temperature of receiver compartment was maintained at 37 ± 2^0C during experiment and content of the receiver compartment was stirred using magnetic stirrer. The position of donor compartment was adjusted so that egg membrane just touches the diffusion fluid. An aliquot of 1 ml was withdrawn from receiver compartment after 30 min, 1, 2, 3, 4, 5, 6, 7, and 8 hrs. and same volume of fresh medium was replaced. Aliquots so withdrawn were suitably diluted and analyzed using UV visible spectrophotometer at 223 nm.

Kinetics of drug release from mucoadhesive nasal gel containing

Candesartan Cilexetil: [24, 25]

To examine the drug release kinetics and mechanism from the tablets, release data was assessed using the zero-order model, first order model, Higuchi model, and Korsmeyer-Peppas model. The drug release from buccal tablets created followed zero order kinetics, according to analysis using zero order and first order kinetic models.

Ex-vivo **permeation study** [26]

In this experiment goat nasal mucosa was utilized because the respiratory area of goat is large and it is easy to get. Fresh mucosal tissue was removed from the nasal cavity of goat. The tissue was placed on the diffusion cell with permeation area $0.75cm^2$. The acceptor chamber of the diffusion cell (laboratory designed) with a volume capacity 100ml was filled with simulated nasal fluid (SNF) contained accurately 0.87% NaCl, 0.31 % KCl and 0.088% $CaCl_2 \cdot 2H_2O$ of formulation was placed in donor compartment. At predetermined time intervals of 30 min, 1,2,3,4,5,6,7, and 8 hrs 1ml of sample was withdrawn from the acceptor compartment replacing the sample removed with simulated nasal fluid after each sampling for period of 8 h. Then samples were specifically diluted and absorbance was noted at 223nm.

Stability study [27-28]

Stability studies were conducted to test the physical and chemical stability of the developed *in-situ* nasal gel. A sufficient quantity of pH sensitive *in-situ* gel, in screw capped vials was stored at different stability conditions.

Results and Discussions

Organoleptic property

It is white or almost white powder complying with the description given in the literature.

Melting point

Melting point of the drug matches with the melting point given in the literature, melting point of Candesartan Cilexetil was determined.

Table No.2: Melting point of Candesartan Cilexetil against reported value

Melting Point (°C)	
Literature	**Practical**
164-165°C	164-166°C

Solubility

Candesartan Cilexetil was found to be very soluble in water, methanol, pH 6.8 phosphate buffer, and in simulated nasal fluid.

Table No.3: Solubility of Candesartan Cilexetil in different solvents

Solvent	Solubility(mg/ml)
Water	0.0005 mg/mL
0.1 N HCl	2.405 mg/mL
Simulated nasal fluid	3.205 mg/mL
pH 6.8 phosphate buffer,	1.245 mg/mL
Methanol	205 mg/mL

Loss on drying and partition coefficient

Table No.4: LOD and partition coefficient of Candesartan Cilexetil againstreported value

Parameter	Reported value	Observed value
Loss on drying	NMT 0.5%w/w	0.01 % w/v
Partition coefficient	6.0	6.1

Calibration curve of Candesartan Cilexetil in Distilled water

The calibration curve was found to be linear in the concentration range of 1- 24 µg/ml

Calibration curve of Candesartan Cilexetil in simulated nasal fluid.

The calibration curve was found to be linear in the concentration range of 2- 12 µg/ml having coefficient of regression value R^2 =0.996 and Slope y = 0.0464x+0.0127.

Table No.5: Absorbance of different concentrations of Candesartan Cilexetil insimulated Nasal fluid.

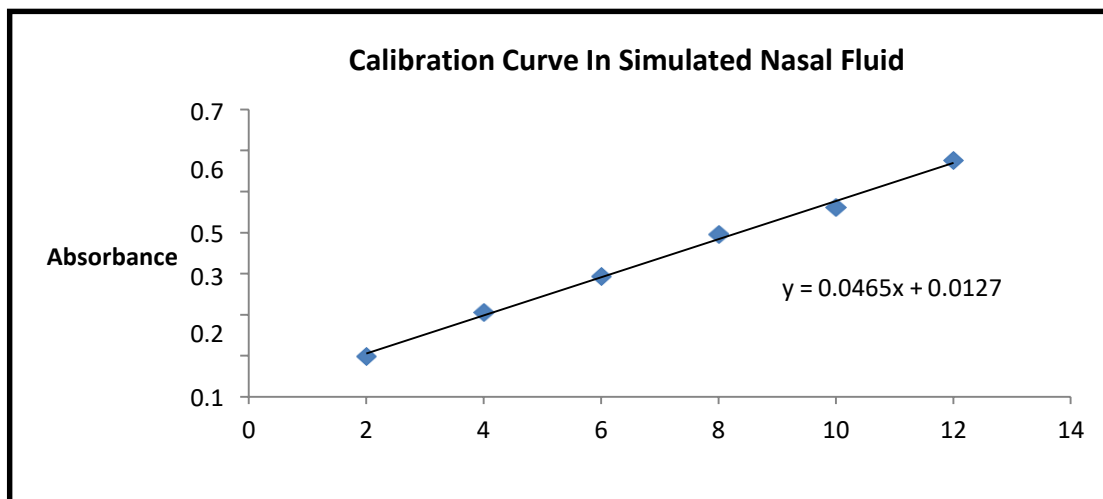| Sr.No.	Concentration (µg/ml)	Absorbance
1	2	0.0991
2	4	0.2046
3	6	0.2927
4	8	0.3956
5	10	0.4603
6	12	0.5756

Figure No.1: Calibration curve of Candesartan Cilexetil in simulated nasal fluid.

Calibration curve of Candesartan Cilexetil in 0.1N NaOH

The calibration curve (Fig.8.6) was found to be linear in the concentration range of 5- 25 µg/ml (Table 8.6.) having coefficient of regression value R^2 =0.996 and Slope y = 0.0344x+0.0218.

Table No. 6: Absorbance of different concentrations of Candesartan Cilexetil in0.1N NaOH

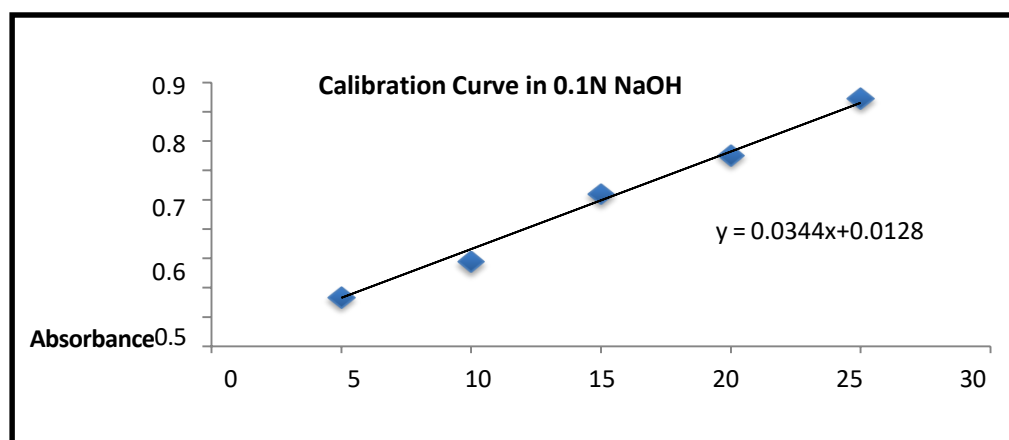| Concentration (µg/ml)	5	10	15	20	25
Absorbance	0.1670	0.2883	0.5200	0.6515	0.8460

Figure No.2: Calibration curve of Candesartan Cilexetil in 0.1N NaOH

Analytical method validation

Intraday precision study

Table No.7: Intraday precision study of Candesartan Cilexetil

Conc. µg/ml	Absorbance			Mean	S.D [±]	% RSD
	Trial 1	Trial 2	Trial3			
5	0.1013	0.1015	0.1020	0.1016	0.00036	0.35
10	0.1651	0.1670	0.1681	0.1667	0.0015	0.89
15	0.2247	0.2267	0.2272	0.2262	0.0013	0.5747
20	0.3301	0.3314	0.3318	0.3311	0.00086	0.2597
25	0.4520	0.4525	0.4523	0.4522	0.00026	0.057

Interday precision study

Table No.8.: Interday precision study of Candesartan Cilexetil

Conc. µg/ml	Absorbance			Mean	S.D[±]	% RSD
	Trial 1	Trial 2	Trial3			
5	0.1612	0.1620	0.1630	0.1620	0.00089	0.5493
10	0.2234	0.2240	0.2245	0.2239	0.00055	0.2456
15	0.3510	0.3540	0.3550	0.3553	0.0017	0.4811
20	0.4101	0.4120	0.4130	0.4117	0.0014	0.3400
25	0.4820	0.4831	0.4837	0.4829	0.00083	0.1718

Intraday and interday precision was determined by measurement of the absorbance for three times on same day and on three different days. The relative standard deviation for replicates of sample solutions was less than 2 % which meet the acceptance criteria for established method.

Accuracy study (Recovery study)

Table No.9: Accuracy study of Candesartan Cilexetil

Sr no	Amount of drug taken from tablets. (mg)	Amount of pure drug added(mg)	Total amount recovered (mg)	% recovery	S.D [±]	% RSD
1	10	5	14.70	98	0.0025	0.7921
2	10	10	19.76	98.8	0.00017	0.3253
3	10	15	24.54	98.16	0.00007	0.0083

Recovery studies were carried out by adding a known quantity of pure drug to the pre-analysed formulations and the proposed method was followed. From the amount of drug found, percentage recovery was calculated

Sensitivity study of Candesartan Cilexetil:

Table No.10: Sensitivity study of Candesartan Cilexetil:

Sr. No.	Solvent	Limit of detection	Limit of quantification
1	0.1N NaOH	0.9587	0.2905

Compatibility study

The FTIR spectra of drug and its polymer mixtures were identical. In the IR spectral analysis of Candesartan Cilexetil exhibits all characteristic peaks. The characteristic absorption peaks of drug Candesartan Cilexetil was remained unchanged in drug-polymer admixture which indicates that there is no prominent chemical reaction between drug and polymer mixture, proving compatibility of drugs with selected excipients for the study.
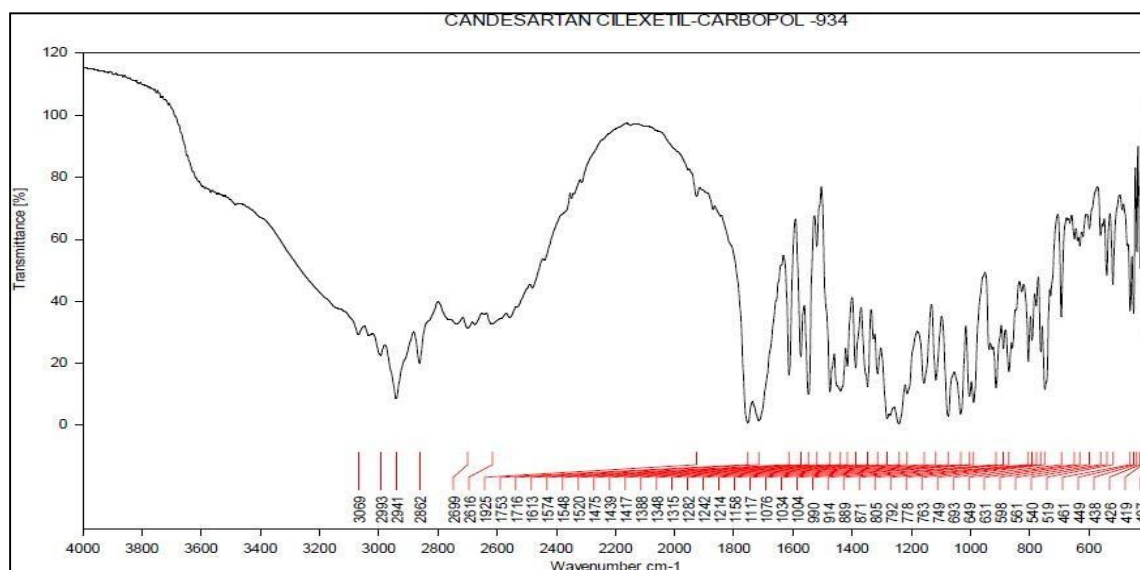

Figure No.3: Fourier Transform Infra-red spectrum of Drug and Polymer mixture
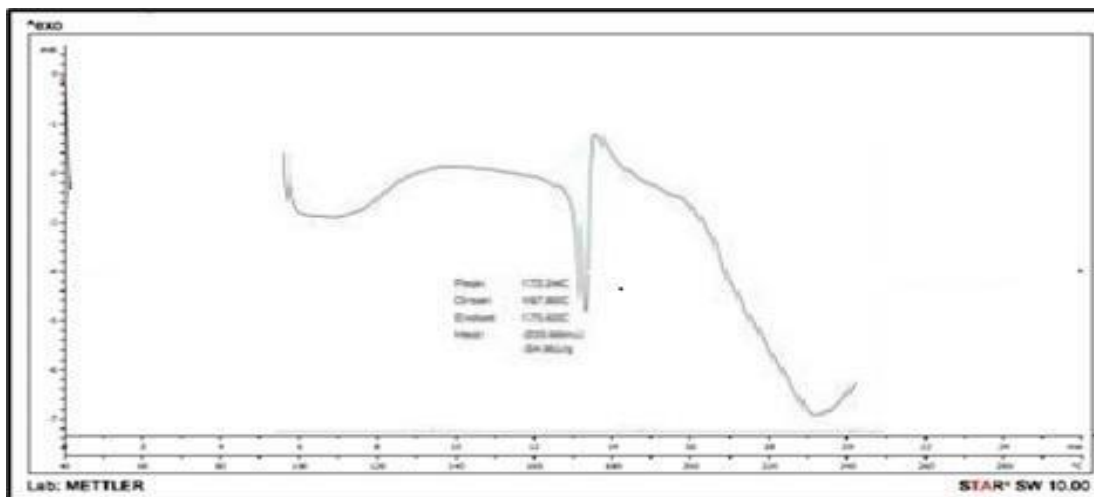
Differential scanning calorimeter

Figure No.4: DSC thermogram of drug and polymers [A= physical mixture. B = Drug, C = xanthan gum and D= carbopol 940.]

The thermal behavior of drug and physical mixture of drug with polymers (Candesartan Cilexetil + Carbopol 940 + xanthan gum) was studied by using DSC thermogram. DSC thermogram of drug exhibited characteristic peak at 163.09^0C and physical mixture exhibited characteristic peak at 161.24^0C. From the results it can be concluded that there is no interaction between drug and polymers.

Evaluation of nasal gel formulation

Physical parameter

Table No.11: pH values of formulations. (n=3)

Sr. No	Formulation code	Observed pH (±S.D.)
1	F1	5.66±0.01
2	F2	5.47±0.015
3	F3	5.22±0.026
4	F4	5.51±0.022
5	F5	5.36±0.022
6	F6	5.45±0.017
7	F7	5.68±0.012
8	F8	5.81±0.01
9	F9	5.73±0.014

Ideally, the nasal solutions should possess pH in the range of 4-7, so as to minimize

discomfort or irritation due to acidic pH and microbial growth due to basic pH.

Rheological study

Viscosity

Table No.12: Viscosity of formulations at respective pH

Rpm	Viscosity (cp) at respective pH								
	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
25	45.39	90.81	272.76	118.78	172.78	362.39	308.58	386.78	470.38
50	30.59	63.39	127.33	89.79	126.99	248.59	163.59	267.19	317.58
75	23.99	50.51	109.20	78.39	98.19	140.39	149.49	169.19	279.78
100	18.00	42.99	94.09	69.99	82.39	129.69	92.79	102.99	169.59

Measurement of the gel strength

The gel strength was found to be affected by concentrations of gelling and mucoadhesive polymers.

Table No.13: Gel strength of formulations. (n=3)

Sr. No	Formulation code	Gel strength (sec) (± S.D.)
1	F1	1.05±0.015
2	F2	1.14±0.012
3	F3	1.22±0.02
4	F4	1.22±0.01
5	F5	1.24±0.021
6	F6	1.27±0.022
7	F7	1.38±0.021
8	F8	1.42±0.031
9	F9	1.66±0.026

Table No.14: Mucoadhesive strength of formulations. (n=3)

Formulation Code	Detachment stress (gm) (±S.D.)
F1	22.4 ±0.14
F2	27.57±0.036
F3	43.93±0.025
F4	59.66±0.05
F5	70.15±0.026
F6	96.87±0.021
F7	69.97±0.032
F8	107.73±0
F9	145.02±0.025

The mucoadhesive strength was determined for nasal gels. Results of this test indicate that the variable xanthan gum and Carbopol 940 both are having effect on mucoadhesive strength. It shows that mucoadhesive force was increased with the increasing concentration of the xanthan gum or carbopol 940.

Drug content

Table No.15: Percent drug content of all formulations. (n=3)

Formulation code	Drug content(%) (±SD)
F1	99.95±0.00042
F2	99.66±0.00067
F3	100.29±0.01
F4	100.71±0.00049
F5	99.60±0.01
F6	100.56±0.00007
F7	99.65±0.00024
F8	101.13±0.00081
F9	100.46±0.0005

The percentage drug content of all prepared nasal formulations was found to be in the range of 99-101%. Therefore, uniformity of content was maintained in allformulations.

In-vitro drug release study

The In-vitro drug release study of formulation is shown in Table.

Table No.16: Cumulative drug release of all formulations. (n=3)

Time [h]	Cumulative Drug Release (%) (±S.D.)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	18.00 ± 0.0003	20.08 ± 0.0002	17.00± 0.0001	21.06± 0.0001	29.43± 0.0001	23.42± 0.0002	28.70± 0.0003	26.65± 0.0002	19.76 ± 0.00022
1	30.4 6± 0.00026	29.75 ± 0.0002	26.02± 0.00021	27.83± 0.0001	36.22± 0.00015	31.29 ±0.0002	36.13 ± 0.00021	37.17± 0.0003	28.13 ± 0.0002
2	33.2 8± 0.00024	35.46 ± 0.00023	34.89± 0.0002	34.50± 0.00015	48.14± 0.00025	43.13± 0.0002	44.04± 0.0026	44.61± 0.0001	38.91 ±0.0001
3	40.4 ± 0.0001	42.20± 0.0001	43.87± 0.0001	43.68± 0.00017	55.15± 0.0002	50.62± 0.0001	52.09± 0.0001	53.86± 0.00017	43.3± 0.00021
4	48.75± 0.00017	50.32 ±0.0002	52.82± 0.0003	55.13± 0.00036	62.22± 0.00026	57.05± 0.0002	60.61± 0.0002	62.34± 0.00012	54.57± 0.0001
5	56.47± 0.00026	57.07± 0.00023	57.25± 0.0003	60.29± 0.0001	70.14± 0.00017	64.01 ±0.0001	68.37± 0.0002	72.52± 0.00017	62.11± 0.0001
6	62.2 8± 0.00014	62.86± 0.00026	60.76± 0.00015	66.14± 0.00023	76.15± 0.00021	75.73 ±0.0003	75.15± 0.0001	78.04± 0.00021	75.37± 0.00021
7	69.07± 0.00017	72.87± 0.00017	68.62± 0.00026	74.63± 0.00023	88.68± 0.00022	82.54±0 .00023	83.56± 0.0001	86.54± 0.00021	84.34 ± 0.00022
8	74.06± 0.0002	78.25± 0.00007	73.17± 0.00022	80.00± 0.00017	96.47± 0.00029	90.47± 0.0007	89.85± 0.0001	92.20± 0.0002	90.86± 0.0001

Amongst all formulations F5 showed maximum drug release of 96.47% after 8 hrs of study and also showed better contact with biological membrane.
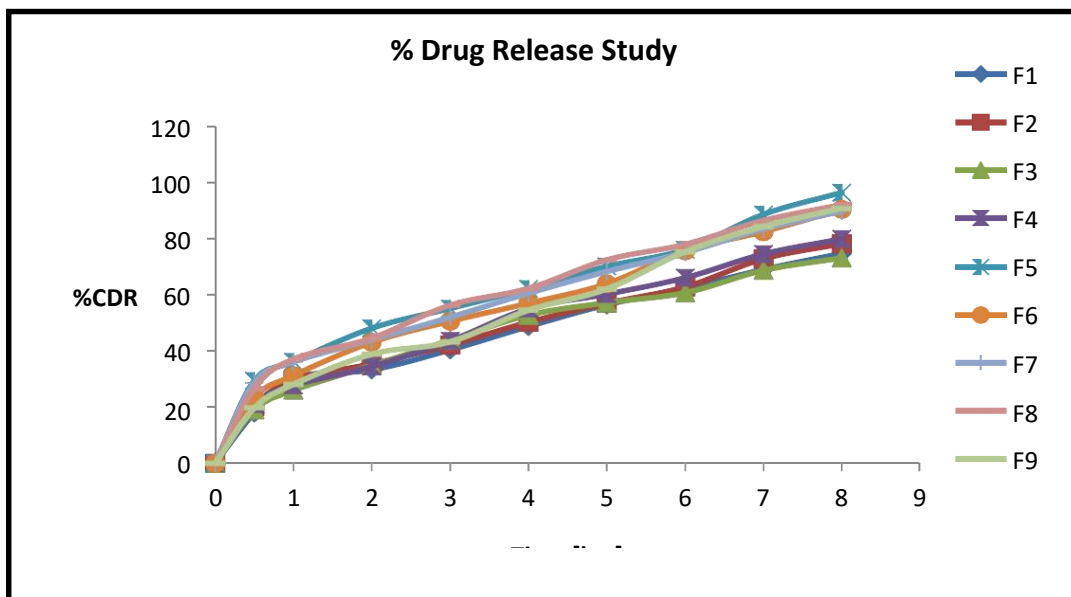
Figure No. 5: *In-vitro* **drug release profile of all formulations**

Ex-vivo permeation study for optimized batch F5:

Table No.16: Ex-vivo permeation study for optimized batch F5. (n=3)

Sr no.	Time (hrs.)	Drug permeation rate (mg/cm/hr.) (± S.D.)	% Cumulative drug Permeation (±S.D.)
1	0	0	0
2	0.5	0.4672 ±0.026	17.53±0.094
3	1.0	0.3456±0.023	27.67±0.098
4	2.00	0.2357 ±0.032	40.22±0.11
5	3.00	0.1827±0.01	49.53±0.051
6	4.00	0.1437±0.015	55.64±0.085
7	5.00	0.1261±0.02	64.14±0.1053
8	6.00	0.1167±0.014	69.74±0.09
9	7.00	0.1020±0.026	80.4±0.15
10	8.00	0.090±0.03	86.52±0.17

Ex-vivo permeation study was performed for the optimized batch using goat nasal mucosa. The percent drug permeated after 8 h was found to be 86.52% from nasal gel formulation.
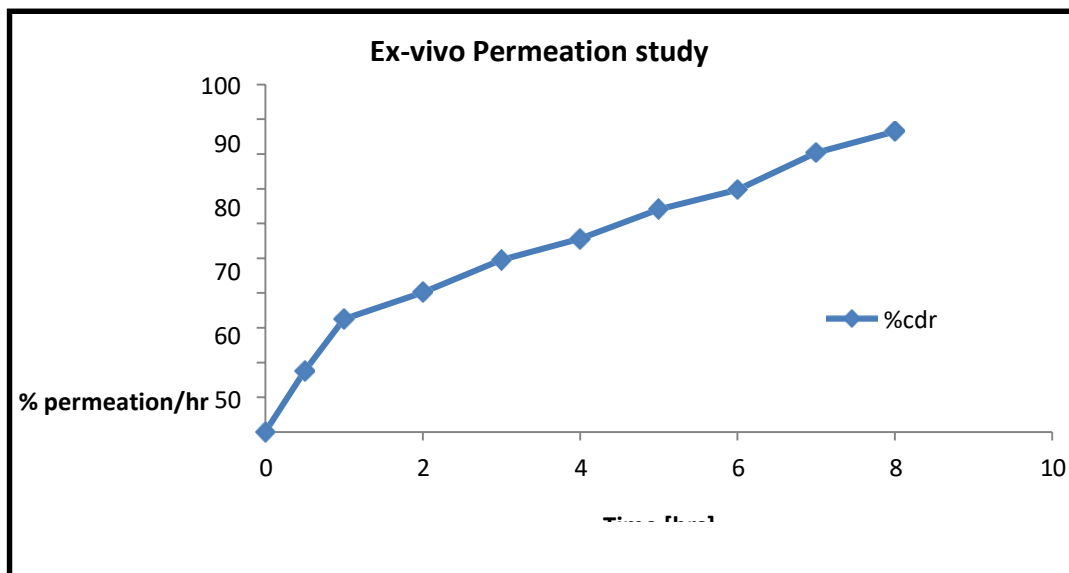
.

Figure No.6: *Ex-vivo* **permeation study for optimized batch F5**

Stability data

Table No 17: Results of stability study. (n=3)

Sr. no.	Test	Before stability testing	After stability testing		
			1 month	2 months	3 months
1	Clarity	Clear	Clear	Clear	Clear
2	Visual appearance	Transparent	Transparent	Transparent	Transparent
3	pH	5.45	5.47	5.43	5.40
4	Drug content	99.60±0.01	99.64±0.0004	99.60±0.0002	99.62±0.0002

The data of stability studies shows that there is no change in the clarity, appearance and pH and the drug content of optimized formulation F5.

Conclusion

In the present investigation, an attempt was made to develop the pH sensitive nasal in-situ gel of Candesartan cilexetil (2.5%) for controlling the drug release in the nasal tissues. Two polymers have been used i.e. Carbopol 940 and Xanthan gum. Carbopol 940 is the pH sensitive mucoadhesive polymer. Xanthan gum has better effect on viscosity when exposed to the pH range 4.5-6.5. These conditions resemble the physiological conditions of the nose. The pH

sensitive in-situ nasal gel so prepared were characterized for its clarity, pH, viscosity, gel strength, mucoadhesive strength, drug content, in-*vitro* drug release and *in-vitro* permeation. Candesartan cilexetil 2.5 % pH sensitive in- situ nasal gel formulation meets requirements for nasal use, improving viscosity and mucoadhesive properties. This optimized formulation offers an alternative to conventional oral formulations and addresses food interaction issues.

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