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DEVELOPMENT OF NASAL DRUG DELIVERY SYSTEM OF ATORVASTATIN CALCIUM FOR ENHANCING BIOAVAILABILITY

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Keywords:

Atorvastatin calcium, propyleneglycol, Nasal solution, *in-vitro* and *ex-vivo* permeation studies

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ABSTRACT: Atorvastatin calcium is a synthetic lipid-lowering agent used in hyperlipidemia. An oral dose of Atorvastatin calcium ranges from 10mg to 80mg per day. Atorvastatin calcium belongs to BC's class II drug having low solubility and high permeability. The absolute bioavailability of Atorvastatin calcium is approximately 12%, and the systemic availability of HMG-CoA reductase inhibitory activity is approximately 30%. The low systemic availability attributed to presystemic clearance when given through oral route several approaches have been tried to improve the solubility of a drug by preparing oral formulation such as tablets, nanosuspension, nanoemulsions, and sustained release dosage forms, there was no article on nasal administration of Atorvastatin calcium. The objective of the present research study was to formulate and evaluate nasal solution containing Atorvastatin calcium 10 mg per 2 drops. As from saturation solubility studies of the drug in pure form, solid dispersions, and β-cyclodextrin inclusion complex of a drug, it was observed that the drug in a pure form showing greater solubility in propylene glycol. A nasal formulation containing Atorvastatin calcium in propylene glycol was prepared. To improve permeation, permeation enhancers such as SLS (1%), Tween 80 (1%), Chitosan (1% and 0.5%), EDTA (0.1%) were added. Formulations were evaluated for Appearance, color, clarity, PH, Drug content, Osmalarity, *in-vitro*, and *ex-vivo* permeation studies. Final optimized formulations showed good *in-vitro* and *ex-vivo* permeation and maintaining average flux value of 0.006mg/cm^2 for *in-vitro* and 0.017mg/cm^2 *ex-vivo* permeation. The best formulations were selected were selected for conducting further stability studies.

INTRODUCTION: Atorvastatin calcium is a poorly water soluble3-hydroxy3-methylglutarylcoA (HMG-CoA) Reductase inhibitor, a potent lipid-lowering agent, and is used as hypolipidemic agent.

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Following oral administration, Atorvastatin calcium (prodrug), is extensively metabolized to ortho and parahydroxylated derivatives and various beta-oxidation products [1-9]. It is available as tablets, nanosuspensions, nanoemulsions for reconstitution before use at a recommended dose of 10, 20, 40, 80mg once daily.

Atorvastatin calcium is water-insoluble, a bitter drug with poor bioavailability of 14% [10-21]. Most of the works that appeared in the literature were carried out to improve its solubility and enhance

dissolution rate, and as there is no nasal dosage form available, the aim of the research work was to develop nasal drops of Atorvastatin calcium for enhancing bioavailability [22-26].

MATERIALS AND METHODS:

Materials: Atorvastatin calcium was a gift sample from NatcoPharma Pvt ltd, Kothur, Hyderabad. India. Reagents used potassium dihydrogen orthophosphate, sodium hydroxide, disodium hydrogen phosphate, propylene glycol (SD Fine chem. Ltd), and distilled water. The materials used were of pharmaceutical grade.

Method:

Analytical Method: A UV Spectrophotometric method based on measurement of absorbance at 246nm in different media (distilled water, pH-6.8 phosphate buffer, pH-5.5 phosphate buffer, propylene glycol) was used for the estimation of atorvastatin calcium. From 1mg/ml methanolic stock solution of the drug, series of dilutions containing 2, 4, 6, 8, and 10 µg/ml of Atorvastatin Calcium was each prepared in the respective media listed above. The absorbance of these solutions was measured against the respective media as blank at 246nm using U.V-Visible double beam spectrometer Elico SL191 (n=3). The method was validated for linearity, accuracy, and precision **Table 1**.

TABLE 1: CHARACTERISTICS OF STANDARD CURVE OF ATORVASTATIN CALCIUM IN VARIOUS MEDIA AT 246 nm UV SPECTROPHOTOMETRICALLY

Medium	r	% CV range
Distilled water	0.999	0.74-0.21
pH6.8 phosphate buffer	0.998	6.89-2.91
pH5.5 phosphate buffer	0.995	1.36-1.09
propylene glycol	0.996	1.52-3.98

Preformulation Studies:

Physical Appearance: The drug sample was noted for its organoleptic properties.

Melting Point: The melting point of Atorvastatin calcium was determined using scientific melting point apparatus. Few crystals of the compound are placed in a thin-walled capillary tube 10-15 cm long, about 1mm in inside diameter, and closed at one end. The capillary, which contains the sample, and a thermometer are then suspended so they can be heated slowly and evenly. The temperature ranges over which the sample is observed to melt are taken as the melting point.

Fourier Transform Infrared (FTIR) Analysis: The FTIR spectra of pure Atorvastatin were performed on FTIR (Perkin Elmer, Spectrum one, UK) spectrophotometer. About 3 mg of sample and 100 mg of potassium bromide were mixed, compressed into a pellet, and scanned from wave number 400-4000 cm^{-1}.

The drug purity was identified by IR spectroscopy, and characteristic peaks obtained were compared with standard spectra of a pure drug reported in the official monograph. The IR spectrum of the drug sample was in agreement with the standard IR spectra of pure Atorvastatin, see **Fig. 1**.

Excipient-Compatability Studies by Fourier Transform-Infrared Spectrophotometer (FT-IR): FT-IR Spectra of Atorvastatin calcium along with solvents such as propylene glycol and excipients such as 1% SLS were performed on FT-IR spectrophotometer scanned from wave number 400-4000 cm^{-1}, see **Fig. 2, 3, 4**.

Estimation of Saturation Solubility of Pure Drug in Various Solvents by Shake Flask Method: Solubility studies of Atorvastatin calcium (Pure drug) were carried out in pH 6.8 phosphate buffer, pH 5.5 phosphate buffer, Propylene glycol, Glycerol, Sorbitol according to the method described by Higuchi and Connors. The Saturation solubility studies were determined by adding an excess of the drug to 2 ml of respective solvent and were kept in an orbital shaker for 24 h at 37 ºC. The solutions were filtered through a 0.45-micron filter, suitably diluted, and UV-Spectrophotometer determined their concentration at 246nm.

Preparation of Solid Dispersions of Atorvastatin calcium with PEG 4000: Physical mixtures of ATC at three different mass ratios with PEG (1:1, 1:2, and 1:3) were prepared in a glass mortar by light trituration for 5 minutes. The mixtures were passed through a sieve (60). The physical mixtures were then estimated for saturation solubility studies in distilled water, pH 6.8 phosphate buffer, pH 5.5 phosphate buffer, and the estimated results were tabulated.

Evaluation of Solid Dispersion for Estimation of Saturation Solubility of Atorvastatin by Shake Flask Method: Solubility studies of Atorvastatin calcium solid dispersions(1:1, 1:2, 1:3) was carried

out in distilled water, pH 6.8 phosphate buffer, pH 5.5 phosphate buffer according to the method described by Higuchi and Connors.

The Saturation solubility studies were determined by adding an excess of solid dispersion to 2 ml of respective solvent and were kept in an orbital shaker for 24 h at 37 ºC. The solutions were filtered through a 0.45-micron filter, suitably diluted, and their concentration was determined by UV-Spectrophotometer at 246nm.

Preparation of Inclusion Complexes of Atorvastatin Calcium with β-cyclodextrin: by kneading method: Atorvastatin calcium and β-cyclodextrin (β-CD) in the proportion of 1:1 molar concentrations were mixed in a mortar for one hour with small quantities of methanol; distilled water was added intermittently to get slurry-like consistency. The paste was dried in the oven at the temperature of 45 °C for 24 h. The dried complex was pulverized into a fine powder and sifted with sieve# 80.

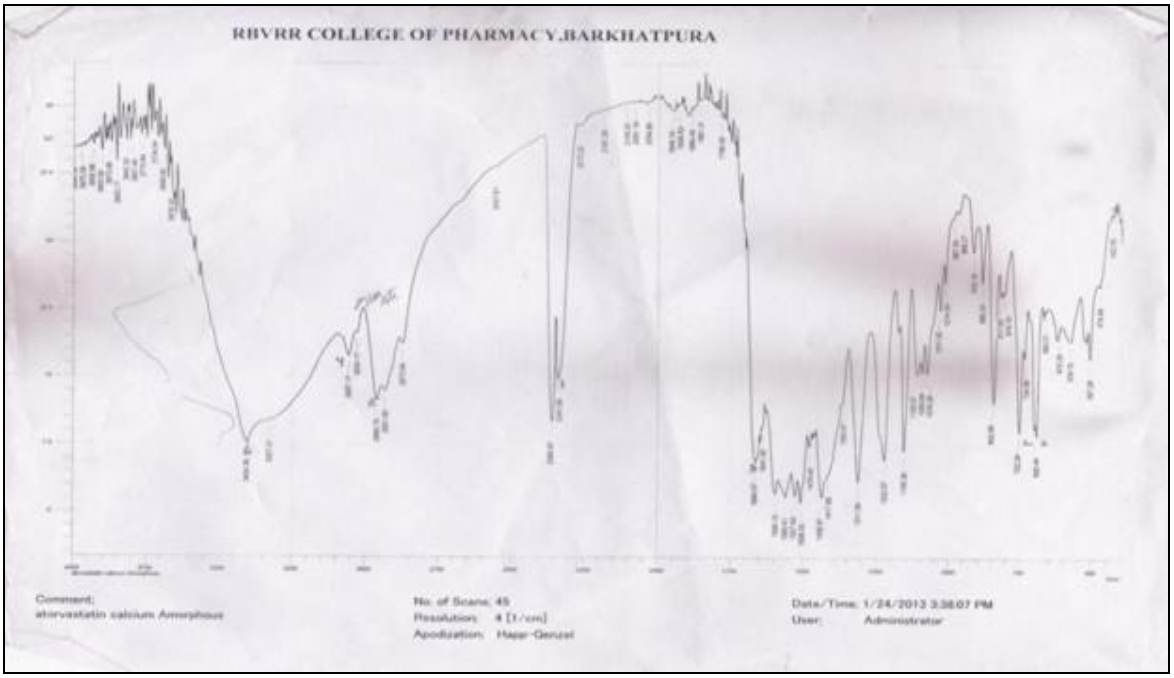


FIG. 1: FT-IR STUDIES FOR PURE DRUG

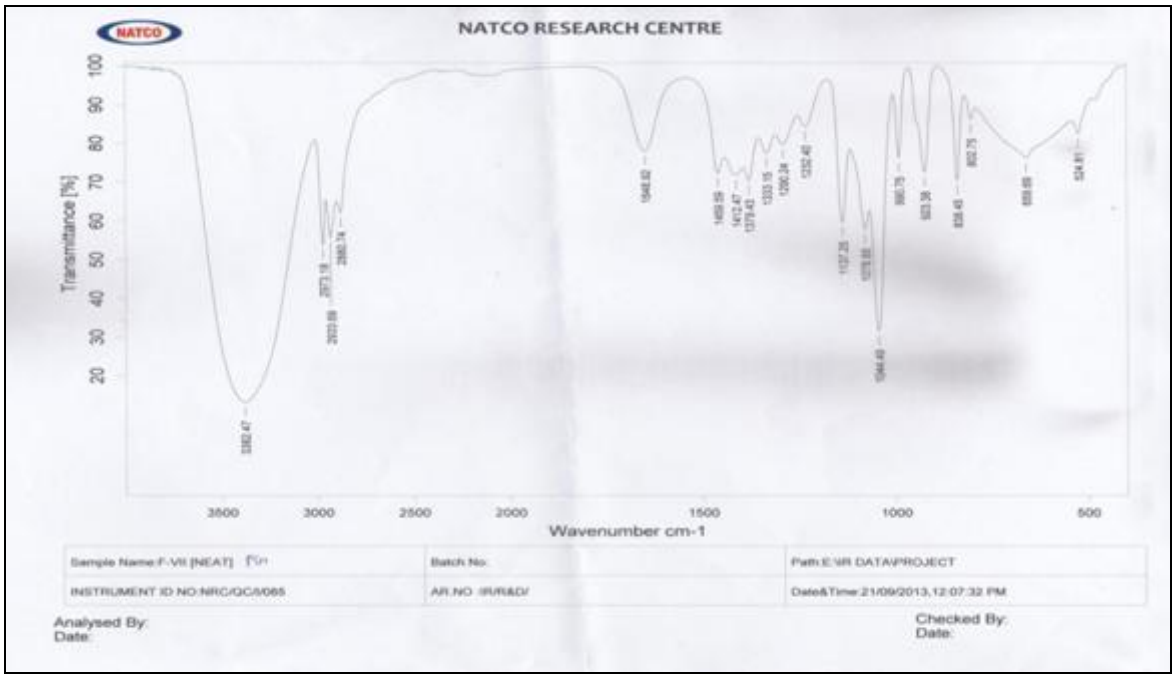


FIG. 2: IR SPECTRUM OF PROPYLENE GLYCOL (EXCIPIENT)

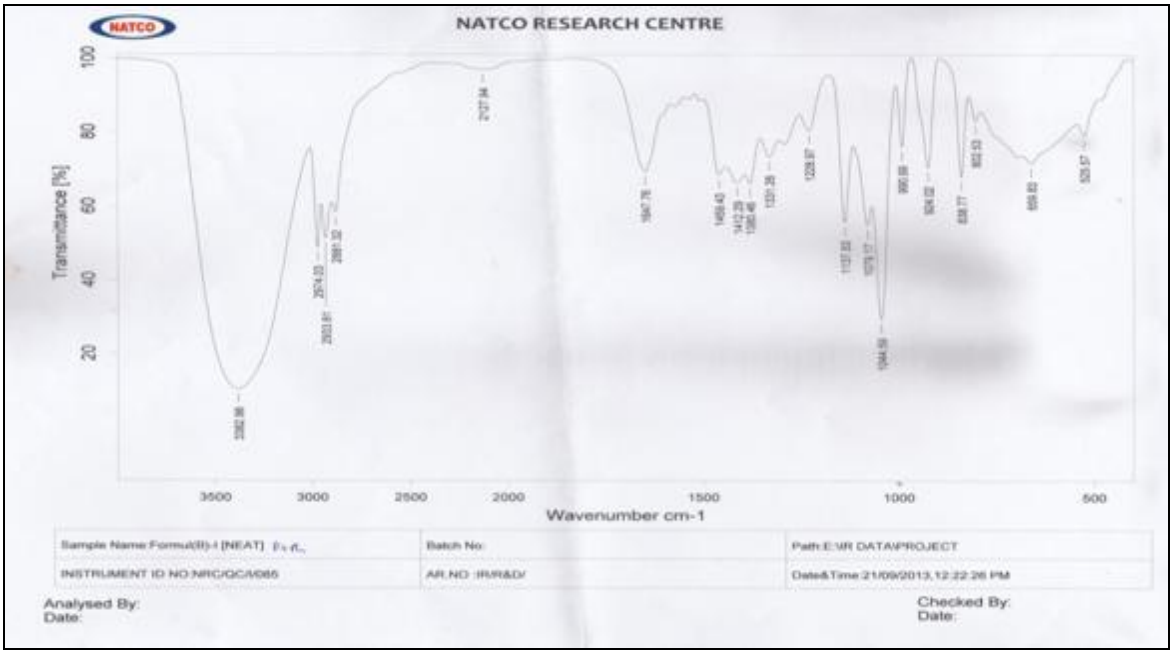


FIG. 3: IR SPECTRUM OF FORMULATION NF1 (PURE DRUG + PROPYLENE GLYCOL)

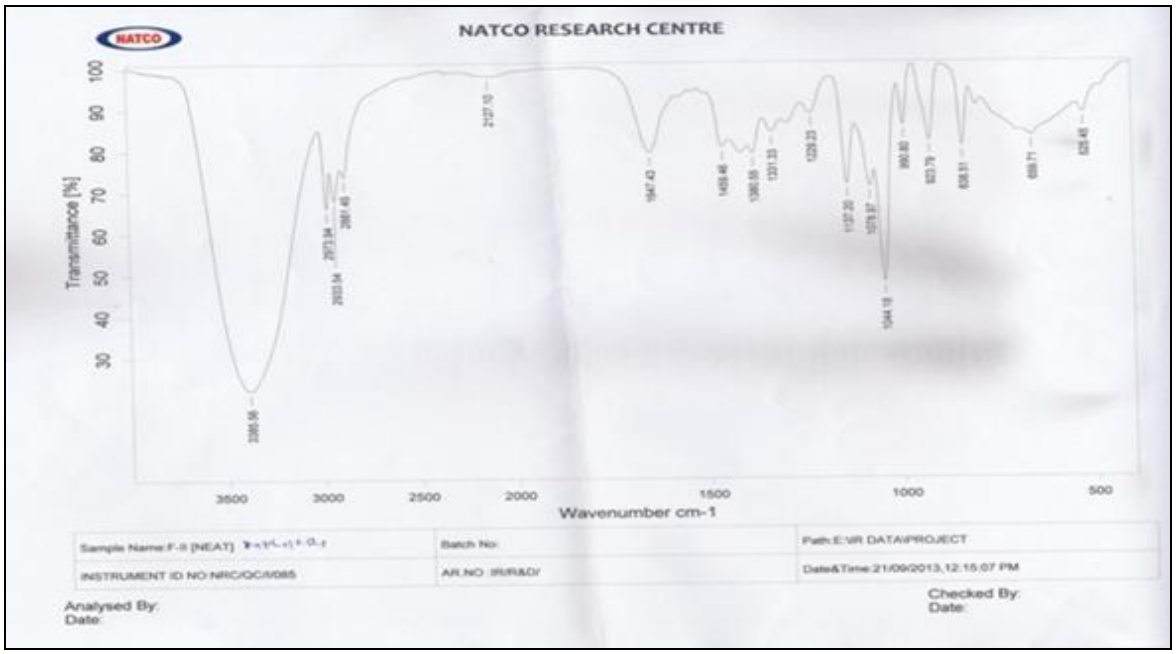


FIG. 4: IR SPECTRUM OF FORMULATION NF2 (PURE DRUG + PROPYLENE GLYCOL + 1% SLS)

Evaluation of β-cyclodextrin Complex of Atorvastatin Calcium for Saturation Solubility in Non-aqueous Media: Solubility studies of β-Cyclodextrin complex (1:4) were carried out in 2ml of a mixture of propylene glycol and water, propylene glycol and glycerol, Propylene glycol and sorbitol, and propylene glycol. Saturation solutions prepared in vehicles were kept in an orbital shaker for 24 h at 37 ºC. The solutions were filtered through a 0.45-micron filter, suitably diluted, and UV-spectrophotometer determined their concentration at 246nm **Table 2**.

TABLE 2: EVALUATION OF β-CYCLODEXTRIN COMPLEX OF ATORVASTATIN CALCIUM FOR SATURATION SOLUBILITY IN NON-AQUEOUS MEDIA

Formulation code	β-CD	Solvents	Saturation solubility (mg/ml)
NBF1	1:4	propylene glycol+water	0.123
NBF2	1:4	propylene glycol+glycerol	0.356
NBF3	1:4	glycerol+water	0.116
NBF4	1:4	propylene glycol+sorbitol	0.212
NBF5	1:4	propylene glycol	0.40

Preparation of Nasal Atorvastatin Calcium Formulations: The nasal Atorvastatin solution (NF1) formulation was prepared by dissolving the pure drug (Atorvastatin calcium) in propylene glycol and then sonicated for about 15 min and then filtered through a 0.45µm membrane filter. Quantity sufficient flavoring agent was added until it masks the odor and the final formulation taken in 5ml container and kept aside for further use. The same procedure is carried out for nasal formulations prepared by using permeation enhancers. The permeation enhancers used in the concentration range of 50mg (1% SLS) in NF2, 50mg (1% tween) in NF3, 50mg (1% SLS) and 5mg (0.1% EDTA), 25mg (0.5% chitosan), 50mg (1% hitosan).

Evaluation of Nasal formulations: The prepared nasal solutions were evaluated for Appearance, color and clarity, pH, Osmolality, Drop volume, Drug content, in-vitro, and ex-vivo permeation studies.

Appearance, Color, and Clarity: The final formulations were checked for Appearance, color, clarity with the naked eye. The prepared formulations must be clear and free from particulate matter or any turbidity.

pH: The nasal formulations' pH was determined by using a Digital pH meter.

Osmolality: Osmolality was found to be in the range of 200- 700m Osmol/kg for marketed formulations Osmolality was determined by using Model 3250 Osmometer from Natco Pharma Pvt. Ltd, Kothur, Hyderabad. The 3250 Osmometer works via measuring the freezing point of the solution tested.

Drop Volume: Drop volume was determined for the nasal formulation solution (5ml) by counting the number of drops for 5ml by using a dropper. The number of drops for each formulation counted, and for each drop, drop volume in ml was calculated.

Drug Content (Assay): Nasal drops are assayed by spectrophotometric analysis. Each formulation (2 drops) was taken in a 100 ml volumetric flask and diluted with propylene glycol. The solution was filtered through Whatman filter paper, and the filtrate was further diluted if necessary with propylene glycol. Drug content is determined using at specific wavelength on UV visible Spectrophotometer at 246 nm.

***In-vitro* Drug Release Studies:** The permeation studies were carried out by vertical diffusion cell method. The apparatus consisted of clamped preconditioned synthetic membrane (dialysis membrane) on to glass diffusion cell between donor and receptor compartments. The receptor solution was 100ml of phosphate buffer pH 6.8. The receptor solutions were magnetically stirred at 400 rpm throughout the experiment. The donor compartment was with two drops of nasal solution (10 mg/ml). The aliquots withdrawn from the receptor compartment at various intervals for 1 h were immediately analyzed for drug concentration in spectrophotometry (246nm) directly and the receptor compartment was refilled with same volume (5ml) off fresh buffer solutions. Three replicates of each experiment were performed. Synthetic membrane was first hydrated for 30min in the buffer solution (pH 6.8 phosphate buffer) at. Sink conditions were maintained in the receptor compartment during *in-vitro* permeation studies.

***Ex-vivo* Drug Permeation Studies:** Goat nasal mucosa was used for the *ex-vivo* permeation studies.

Data Treatment: The permeation of Atorvastatin from nasal preparations was investigated. The cumulative amount-time profiles were plotted. A linear profile (steady state) was observed during 1hr period and the slope of the linear portion of the curve was determined by linear regression. The effective permeability coefficients and flux values at steady state were calculated from the slope according to Eq. 1 and 2, respectively.

$$P_{eff} = V/AC0\ dc/dt \quad .....(1)$$

$$J = (dc/dt)\ V/A \quad .....(2)$$

V =Volume of the receiver compartment (ml)
Co = Initial concentration in the donor compartments (mg/ml)
P_{eff} = Effective permeability coefficient (cm/s)
J = Flux (mg/cm^2 s)
A = Permeation area (cm^2)
dc/dt = Pseudo steady-state change of concentration over time (mg/ml)

Results are expressed as the mean ± SD from at least six measurements.

Stability Studies: All the prepared nasal formulations were subjected to preliminary stability studies at 40 °C ± 2 °C/75% ± 2% RH for a period of one month. All the formulations were placed in self sealing cover and kept at above specified condition in a heating humidity chamber for about 1 month. After 3 weeks the formulations were analyzed for the appearance, color, clarity, drug content, pH, *in-vitro* permeation studies and *ex-vivo* permeation studies. TLC estimation was carried out in order to determine the degradation of the compounds [27].

RESULTS AND DISCUSSION:

Saturation Solubility Studies of Pure Drug in Various Solvents: Saturation solubility of Atorvastatin (pure drug) was found to be 0.020mg/ml in distilled water, 0.052mg/ml in pH 5.5 phosphate buffer, 0.047mg/ml in pH 6.8 phosphate buffer, 95mg/ml propylene glycol, 75 mg/ml in propylene glycol and glycerol and 2.62 mg/ml in sorbitol. From the solubility of the drug in various solvents Table 3, it was observed that Atorvastatin calcium in a pure form shows the highest solubility in propylene glycol compared to aqueous solubility in water.

TABLE 3: SOLUBILITY STUDIES OF PURE DRUG IN VARIOUS SOLVENTS

Material	Solvents	Saturation solubility (mg/ml)
Pure drug	Distilled water	0.020
Pure drug	pH-5.5 phosphate buffer	0.052
Pure drug	pH-6.8 phosphate buffer	0.047
Pure drug	Propylene glycol	95
Pure drug	Propylene glycol and glycerol	75
Pure drug	Sorbitol	2.62

Preparation of Solid Dispersion of Atorvastatin Calcium with PEG4000 and Evaluation of Saturation Solubility in Various Solvents: To enhance aqueous solubility of Atorvastatin calcium, solid dispersions were prepared by taking various proportions of drug and PEG-4000 as a carrier (1:1, 1:2, 1:3). Saturation solubility of Atorvastatin calcium solid dispersion was determined by the shake flask method in various solvents. Saturation solubility of Solid dispersions of ATV in ratios (1:1, 1:2, 1:3) was found to be 0.025 mg/ml, 0.042 mg/ml, 0.051 mg/ml for (1:1, 1:2, 1:3) in distilled water, 0.051 mg/ml, 0.068 mg/ml, 0.079 mg/ml for (1:1, 1:2, 1:3) in pH 5.5 phosphate buffer, 0.054 mg/ml, 0.062 mg/ml, 0.072 mg/ml of (1:1, 1:2, 1:3) in pH 6.8 phosphate buffer. From the solubility data, it was observed that the solubility of ATC solid dispersions showing improved aqueous solubility compares to pure form. The enhanced solubility in various aqueous media is not appreciable **Table 4**.

TABLE 4: PREPARATION OF SOLID DISPERSION OF ATORVASTATIN CALCIUM WITH peg 4000 AND EVALUATION OF SATURATION SOLUBILITY IN VARIOUS SOLVENTS

Formulation code	Ratio	Solvents	Saturation solubility (mg/ml)
F1	1:1	Distilled water	0.025
F1	1:1	pH 6.8 phosphate buffer	0.054
F1	1:1	pH 5.5 phosphate buffer	0.051
F2	1:2	Distilled water	0.042
F2	1:2	pH 6.8 phosphate buffer	0.062
F2	1:2	pH 5.5 phosphate buffer	0.068
F3	1:3	Distilled water	0.051
F3	1:3	pH 6.8 phosphate buffer	0.072
F3	1:3	pH 5.5 phosphate buffer	0.079

Preparation of Inclusion Complex of Atorvastatin Calcium with β-CD and Evaluation of Saturation Solubility in Various Solvents: A part from solid dispersions, for solubility enhance-ment of pure drug inclusion complexes using β-CD were prepared in various proportions of drug and carrier by kneading method they were also evaluated for solubility studies by shake flask method in various aqueous and non-aqueous media. The water solubility of Atorvastatin calcium in inclusion complex was improved with increasing ratio of carrier (1:4) by kneading method that was

found to be 0.074 mg/ml. Saturation solubility β-cyclodextrin inclusion complex of ATC in ratios (1:1, 1:2, 1:3, 1:4) was found to be 0.045 mg/ml, 0.054 mg/ml, 0.064 mg/ml, 0.074 mg/ml for (1:1, 1:2, 1:3, 1:4) in distilled water, 0.074 mg/ml, 0.089 mg/ml, 0.018 mg/ml, 0.221 mg/ml for (1:1, 1:2, 1:3, 1:4) in pH 5.5 phosphate buffer, 0.067 mg/ml, 0.084 mg/ml, 0.015 mg/ml, 0.192 mg/ml of (1:1, 1:2, 1:3, 1:4) in pH 6.8 phosphate buffer. The aqueous solubility of a β-CD complex of the drug was enhanced compared to the pure drug. But solubility was not appreciable. Hence the solubility of β-CD complex of the drug was evaluated in non-aqueous media. The solubility of the β-CD complex in propylene glycol was found to be 0.40 mg/ml showing enhanced solubility compare to aqueous media. The β-CD complex of the drug in a mixture of propylene glycol and water values, propylene glycol and glycerol values, glycerol and water values, propylene glycol and sorbitol showed reduced solubility **Table 5**.

From the solubility data β-CD complex of the drug, it was various aqueous and non-aqueous media it was concluded that there was no appreciable solubility enhancement.

TABLE 5: PREPARATION OF INCLUSION COMPLEX OF ATORVASTATIN CALCIUM WITH β-CD AND EVALUATION OF SATURATION SOLUBILITY IN VARIOUS SOLVENTS

Formulation code	Ratio	Solvents	Saturation solubility (mg/ml)
F1	1:1	Distilled water	0.045
F1	1:1	pH 6.8 phosphate buffer	0.067
F1	1:1	pH 5.5 phosphate buffer	0.074
F2	1:2	Distilled water	0.054
F2	1:2	pH 6.8 phosphate buffer	0.084
F2	1:2	pH 5.5 phosphate buffer	0.089
F3	1:3	Distilled water	0.064
F3	1:3	pH 6.8 phosphate buffer	0.015
F3	1:3	pH 5.5 phosphate buffer	0.018
F4	1:4	Distilled water	0.074
F4	1:4	pH 6.8 phosphate buffer	0.192
F4	1:4	pH 5.5 phosphate buffer	0.221

Formulation Development: Based on solubility study of pure drug, SD, and β-CD complex in various aqueous and non-aqueous media, Atorvastatin calcium showing highest solubility in propylene glycol in pure form, so final formulation were tried with propylene glycol as non-aqueous media with pure drug. Permeation enhancers such as SLS (1%), tween 80 (1%), EDTA (0.1%), chitosan (1%) were added to improve permeation of drug through the membrane. Final non-aqueous solutions were prepared. The prepared nasal solutions were evaluated for pH, Drug content, Osmolality, drop volume, *in-vitro*, and *ex-vivo* permeation studies **Table 6**.

TABLE 6: FORMULATION OF ATOVASTATIN CALCIUM NASAL SOLUTION

Ingredients	NF1	NF2	NF3	NF4	NF5	NF6
Atorvastatin calcium (mg) (Pure drug)	543	543	543	543	543	543
Propyleneglycol (ml) (Non-aqueous solution)	5ml	5ml	5ml	5ml	5ml	5ml
SLS (mg)	-	50(1%)	_	50(1%)	-	-
Tween (mg)	-	-	50(1%)	_	-	-
EDTA (mg)	-	-	-	50(1%)	-	-
Chitosan (mg)	-	-	-	-	25(0.5%)	-
Chitosan (mg)	-	-	-	-	_	50(1%)
Vanilla essence	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

SLS-Sodium Lauryl Sulphate, EDTA-Ethylene diamine tetraacetic acid, Q.S-Quantity sufficient

pH: The prepared six nasal solution formulations pH was determined. NF1 to NF6 formulations are maintaining pH within the range of 5.5-6.5 as per the nasal requirements see **Table 7**.

Drug Content: 2 drops of nasal solution were assayed for drug content. NF1, NF2, NF3 formulations showed good drug content values of about 9.822 mg/ml, 9.654 mg/ml, 9.000 mg/ml per 2 drops. NF4, NF5, and NF6 showed decreased drug content values indicating drug degradation **Table 7**.

Drop Volume: 5ml of the nasal formulation was counted for a number of drops. For the 5ml formulation, 107 drops were estimated. Each drop

having 0.046 ml drop volume for all the six formulations see **Table 7**.

Osmolality: Osmolality was found to be within the specified range of 200-700m Osmol/kg. For NF6 formulation, the Osmolality was deviated from the specified range see **Table 7**.

***In-vitro* Permeation Studies:** *In-vitro* permeation studies were conducted on final nasal formulations. The experimental results of *in-vitro* data showed 95% drug release for about 60mins from NF1 to NF2. 85% drug release for about 6.min from NF3 to NF4, 82% for NF5, 72% for NF6 formulation. And the profiles of *in-vitro* permeation of Atorvastatin from non-aqueous nasal solution through the synthetic membrane were given in a linear relationship were obtained for all the six non-aqueous nasal formulations. Additionally, the data is treated for calculating *the in-vitro flux and permeation rate. T*he treated data showed the *in-vitro* flux of the drug from the formulations found to be smooth and continuous for during one hour period. The average flux values for NF1, NF2 found to be 0.067 and 0.0117 **Table 8**.

***Ex-vivo* Permeation Studies:** *Ex-vivo* permeation studies were performed for final formulations using goat mucosa. The *ex-vivo* permeation data found to be similar compared to *in-vitro* permeation. The profiles of *ex-vivo* permeation of Atorvastatin from non-aqueous nasal solution formulation through the goat nasal mucosa. Initially, only pure Atorvastatin non-aqueous solution showed slow permeation through the membrane, but the permeation rates were enhanced with the formulations containing penetration enhancers. Effective flux and permeability coefficients were calculated for NF1, NF2. The average flux values were found to be 0.0067 and 0.0121 **Table 9**.

TABLE 7: EVALUATION PARAMETERS

Formulation	Color	pH	Drug content (2 drops/mg)	Drop volume per 1 drop µl	Osmolality (mOsm)
NF1	Pale yellow	5.4	9.822	46	511
NF2	Pale yellow	5.7	9.654	46	235
NF3	Pale yellow	5.3	9.000	46	292
NF4	Pale yellow	5.1	9.012	46	400
NF5	Pale yellow	50	8.012	46	339
NF6	Pale yellow	5.4	8.124	46	768

TABLE 8: *IN-VIVO* PERMEATION STUDIES

Time	Cumulative % release (N=3)					
	NF1	NF2	NF3	NF4	NF5	NF6
5	4.911	4.951	4.917	5.155	5.654	5.610
10	9.997	10.33	9..900	10.44	11.38	11.29
15	15.61	16.75	14.93	15.79	17.17	17.04
20	22.53	23.76	20.11	21.49	23.12	22.95
25	38.74	31.22	25.33	27.48	29.13	28.90
30	38.74	38.64	30.91	33.96	35.54	35.49
35	47.55	46.77	36.78	40.71	42.29	42.14
40	56.57	55.38	44.15	47.80	49.58	49.18
45	65.96	64.39	52.82	55.69	57.17	56.43
50	75.50	73.99	61.84	64.36	65.15	64.03
55	85.45	83.98	72.34	73.55	73.38	71.72
60	95.47	94.20	83.45	83.27	81.96	79.61

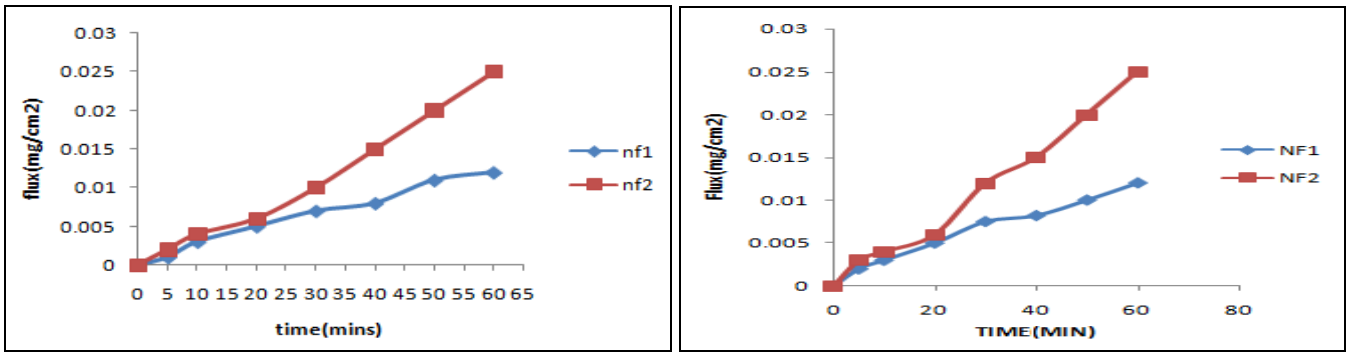
TABLE 9: *EX-VIVO* PERMEATION STUDIES

Time	Cumulative % release (N=3)					
	NF1	NF2	NF3	NF4	NF5	NF6
5	4.911	4.910	4.887	5.155	5.654	5.773
10	9.117	9.902	10.90	11.22	11.38	11.60
15	14.61	15.50	15.93	14.79	17.17	17.34
20	24.53	28.04	21.15	24.49	2312	18.74
25	32.74	35.24	24.33	27.88	29.13	24.66
30	38.74	43.24	28.91	32.66	35.55	31.35

35	44.51	51.63	34.78	41.55	42.22	38.62
40	52.75	60.30	42.15	46.08	48.88	46.40
45	61.96	69.49	50.82	54.55	57.11	54.97
50	72.50	79.01	62.84	62.66	68.55	63.76
55	81.45	88.62	71.34	72.44	78.88	72.68
60	93.47	98.34	84.45	84.66	85.96	81.90

TABLE 10: DATA TREATMENT FOR *IN-VITRO* AND *EX-VIVO* PERMEATION STUDIES

Time (min)	*In-vitro* permeation studies				*Ex-vivo* permeation studies			
	NF1 formulation		NF2 formulation		NF1 formulation		NF2 formulation	
	Flux (mg/cm^2)	Peff (cm/s)	Flux (mg/cm^2)	Peff (cm/s)	Flux (mg/cm^2)	Peff (cm/s)	Flux (mg/cm^2)	Peff (cm/s)
5	0.001	0.056	0.002	0.0186	0.002	0.002	0.003	0.028
10	0.003	0.006	0.004	0.0093	0.003	0.003	0.004	0.004
20	0.005	0.009	0.006	0.434	0.005	0.005	0.006	0.006
30	0.007	0.03	0.01	0.018	0.0075	0.0075	0.012	0.01
40	0.008	0.04	0.015	0.112	0.0082	0.0085	0.015	0.0015
50	0.011	0.009	0.02	0.046	0.01	0.01	0.02	0.02
60	0.012	0.06	0.025	0.4672	0.012	0.012	0.025	0.0025
	Average flux=0.0067	Average flux= 0.029	Average flux=0.0117	Average flux=0.156	Average flux=0.0067	Average flux=0.147	Average flux=0.012	Average flux=0.010



GRAPH 1: *IN-VITRO, EX-VIVO* DATA TREATMENT FOR FORMULATION NF1, NF2

Stability Studies: The final formulations NF1 to NF6 were subjected to stability studies (40 ºC, 75% RH) for about 3 weeks to determine temperature and humidity on drug stability. After three weeks, formulations were evaluated for pH, drug content, and *in-vitro* and *ex-vivo* permeation studies. In the case of NF3 formulation, it is maintaining the same color, physical appearance, but there is a slight change in pH value and drug content also decreased to 9.0 mg/ml, indicating drug loss during stability studies. NF4 to NF5 formulation's physical appearance was changed from solution to turbidity, and drug content values were reduced. For NF4 formulation, drug content value was reduced after the stability period. NF6 formulation initially showed an increased Osmolality value. The drug content value was decreased after the stability period. For NF1 to NF2 formulation, *in-vitro* and *ex-vivo* permeation studies showed similar drug permeation before and after the stability period. The average *in-vitro* and *ex-vivo* permeation coefficient values were found to be similar after the stability period **Table 11**.

TABLE 11: DEVELOPMENT OF TLC METHOD AFTER 3 WEEKS OF STABILITY CONDITIONS

Formulation	Colour	Physical appearance	pH	Drug content(mg/2drops)	Development of TLC method after 3 weeks of stability conditions: R_f value	
					Room temperature	40º c/75% RH
NF1	Pale yellow	Solution	5.0-6.1	9.022	0.67	0.67
NF2	Pale yellow	Solution	5.0-6.4	9.244	0.67	0.67
NF3	Pale yellow	Solution	5.2-6.2	9.102	0.67	0.55
NF4	Pale yellow	Solution	5.6-6.2	8.452	0.67	0.40
NF5	Pale yellow	Solution	5.5-6.1	8.324	0.67	0.45
NF6	Pale yellow	Solution	5.4-6.1	8.524	0.67	0.40

CONCLUSION: The present research approach is novel from work done earlier on the drug Atorvastatin calcium. ATC was satisfactorily prepared as new non-aqueous nasal solutions, each drop of nasal solution containing 4.9mg of Atorvastatin Calcium. Two drops of nasal solution give a dose of 10mg of the drug.

Finally, it was concluded that the major objective of the study was achieved. The formulation was found to be stable.

A further Investigation is required to detect blood levels of the drug from the nasal formulation. A well-controlled *in-vivo* study should be designed to determine pKa parameters of the drug from the nasal formulation.

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CONFLICTS OF INTEREST: Nil

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