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ORIGINAL ARTICLE

Formulation Evaluation and Optimization of Atomoxetine Loaded Solid Lipid Nanoparticle Gel Targeted to Brain Via Intra-Nasal Route

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ABSTRACT
In the present research work, Solid lipid nanoparticles (SLNs) loaded with Atomoxetine (AT) were added to an in-situ gel created using the micro-emulsification process in order to improve AT absorption to the brain through the intra-nasal (i.n.) route. Particle size, polydispersibility index, zeta potential, drug entrapment effectiveness, in-vitro drug release, and stability of the optimized formulation were all assessed for the formulations. Various tested parameters were determined to fall within the permitted range. After 24 hours, it was discovered that the optimized in-situ gel formulation's in-vitro drug release was 98.0%. This data was fitted to the Higuchi model, which had an extremely high correlation coefficient (R2=0.9682). HPLC was used to determine the concentration of AT in the brain and blood plasma during pharmacokinetic investigations on albino male Wistar rats. AT-optimized-SLN's i.n., AT sol. i.n., and AT sol. oral were found to have brain/blood ratios of 2.44, 1.48, and 1.62 at 0.5 hours, respectively. This suggests that the drug was delivered straight from the nose to the brain, avoiding the blood-brain barrier in the nasal cavity's olfactory area. After intravenous (IV) administration of AT-SLN gel, the maximum concentration of drug in the brain (C max) was found to be significantly higher (178.32±21.03ng/ml, T max 4.03hr) than the maximum concentration obtained after oral (96.32±14.32ng/ml, T max 3.65hr) and intravenous (99.02±11.34ng/ml, T max 2.66hr) administration of AT sol. Compared to the other formulations, AT-SLNs had the highest drug-targeting efficiency (2.44%) and direct transport percentage (51.91%). Greater DTP (%) and DTE (%) indicate that AT-SLN gel outperformed other formulations in terms of brain targeting efficiency.
KEYWORDS: Atomoxetine, Box- Behnken Design, Solid lipid nanoparticles, Intra-nasal route, Pharmacokinetics, Brain distribution studies.

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INTRODUCTION

The primary barrier to the efficient delivery of brain-focusing drugs to their active site is the blood-brain barrier (BBB). The P-glycoprotein efflux, on the contrary hand, helps the BBB's protective function by removing the particles from the CNS. The healthcare sector, nevertheless, views the physiologic protective layer as a major obstacle because it must be overcome in order to transmit medications to the brains in a variety of CNS illnesses [1]. Since it carries the medication directly to the brain via the sense of smell and trigeminal nerve route, the nasal cerebral delivery method holds significant promise for overcoming the BBB. These neural pathways terminate in the brain and start at the olfactory neuroepithelium in the nose. For the treatment of Attention Deficit/Hyperactivity Disorder (ADHD) in adults and children older than six, atomoxetine (AT), a selective norepinephrine absorption inhibitor, is advised (Bowton) [2]. While the intestinal permeability and water-soluble content of atomoxetine (a BCS Class I medicine) are high, its bioavailability is only 63% in individuals with extensive metabolisms and 94% in those with weak metabolisms. Atomoxetine can be targeted to enter the brain by the olfactory

nerve, which is a member of the first cerebral pair [3]. The ability for direct communication between the nose cavity and brain is made possible by the integration of the olfactory nerve by the olfactory sensory neurons-axons. In addition to providing direct access to the brain, nasal drug administration increases the bioavailability of the medication by avoiding the hepatic first-pass metabolism, blood-brain barrier, and invasive drug delivery [4]. In order for a medicine molecule to cross the blood-brain barrier (BBB), it must also be soluble in lipid and have a molecular dimension of less than 400 D. Solid lipid nanoparticles (SLN's) are created out of a colloidal carrier system with a core composed of high-melting lipid and a coating of the drug and hydrophobic surfactant that are compatible with human physiological processes [5]. SLN's are distinctive in the field of nanotechnology due to their small size range (50 to 1000 nm), biological compatibility, capacity for self-assembly, capacity to traverse the barrier between the blood and the brain, and cost-effectiveness. Solid lipids with fatty acid origins, such as stearic acid, triglycerides, such as tristearin, and steroids, such as cholesterol, are frequently utilized [6].

MATERIAL AND METHODS

Materials: Atomoxetine was kindly supplied by the Dr. Reddy's Laboratories (Hyderabad, India). Glyceryl monostearate, Glyceryl palmitostearate, Stearic acid and polysorbate 80 were supplied by SD Fine Chemicals (Mumbai, India). Methanol of HPLC grade was purchased from Fischer Scientific (Loughborough, UK). Carbopol 940 was obtained from Acros Organics (Morris Plains, NJ). Analytical grade chemicals and solvents were utilized in all other cases. Prior to use, 0.22 µm membrane filter was employed to filter the distilled water.

Methods

Optimization of Formulation Variables

The AT-SLNs were optimised using a Box-Behnken design and Design-Expert 12 software from Stat-Ease Inc. (Minneapolis, USA). Total percent of solid lipid (X1), surfactant (X2), and sonication time (X3) were the three independent variables that were examined in turn. As dependent responses, particle size (Y1), entrapment efficiency (Y2), and PDI (Y3) were examined. 17 trial runs were displayed in the design (Table No. 1). To determine which mathematical polynomial model had the maximum prediction potential, three models were evaluated: linear (primary effects alone), 2-factors interaction (effects and interactions), and quadratic (effects, interactions, and quadratic terms). The best fitting model was used to construct the appropriate polynomial equation for each response, which was then used to evaluate the impact of the independent variables on the responses under study. A P-value of < 0.05 was designated as the statistical significance threshold. In addition, a desirability-based numerical optimisation technique was used to identify optimised SLNs with the lowest vesicular size and maximum entrapment efficiency.

Development of solid lipid nanoparticles

The technique of micro-emulsification was employed to prepare the SLNs. Ten milligrams of the medication were dissolved in two milliliters of chloroform, then soy lecithin and polysorbate 80 were mixed with water. At about 70°C, stearic acid melted. To create a transparent, yellow micro emulsion, the medication solution and surfactant system were combined isothermally while being constantly stirred. The resulting micro emulsion was then added to 40 milliliters of 0.22µ filtered HPLC grade water that had been kept at 4°C. The resulting dispersion was exposed to five minutes of probe sonication (Vibra Sonics)7. Table No. 2 displays the formulations that were created with different lipid, surfactant, and co-surfactant concentrations.

Table 1: Variables in Atomoxetine solid lipid nanoparticles (AT-SLN)

Independent variables	**Levels used actual (coded)**		
	Low (-1)	**Mid (0)**	**High (1)**
X_1: Stearic acid(mg)	250	300	350
X_2: Polysorbate 80(ml)	5	7.5	10
X_3: Sonication time(min)	3	4	5
Dependent/Response variables			
Y_1: Particle size		**Minimize**	
Y_2: Entrapment efficiency		**Maximize**	
Y_3: Poly disparity Index		**<1**	

Table 2: Composition of AT-SLN's by Box-Behnken design

Formulation code	Lipid (X1)	Surfactant (X2)	Sonication Time (X3)	Particle size	EE%	PDI
AT-SLN1	300	5	5	210	91.1	0.2
AT-SLN2	300	7.5	4	197	89.76	0.1
AT-SLN3	250	10	4	120	87.1	0.1
AT-SLN4	350	10	4	240	92.7	0.3
AT-SLN5	300	7.5	4	195	90.2	0.2
AT-SLN6	250	5	4	147	88.1	0.2
AT-SLN7	300	10	3	201	91.3	0.1
AT-SLN8	300	7.5	4	193	91.3	0.2
AT-SLN9	300	10	5	207	89.6	0.1
AT-SLN10	250	7.5	5	145	85.96	0.1
AT-SLN11	300	7.5	4	191	90	0.2
AT-SLN12	350	7.5	5	246	93.12	0.3
AT-SLN13	350	5	4	237	92.18	0.2
AT-SLN14	350	7.5	3	241	91.9	0.2
AT-SLN15	250	7.5	3	150	88.2	0.1
AT-SLN16	300	7.5	4	199	90.1	0.1
AT-SLN17	300	5	3	212	91.9	0.2

*** 5 Centre Point**

Characterization and Evaluation of solid lipid Nanoparticles

The solid lipid nanoparticles formulated were evaluated for different evaluation parameters and were analyzed for particle size, entrapment efficiency andPDI8

Measurement of particle size and distribution: For SLN systems, particle sizes (z-average) and particle size distributions (PDI) were measured using photon correlation spectroscopy (PCS). Zetasizer Ver.7.01 was used for the measurements (Malvern Instruments Ltd., UK). A little over 1 milliliter of SLN dispersion was diluted with 10 milliliters of HPLC-grade water (Millipore, India) prior to measurements. Both the poly dispersity index (PDI) and the particle z-average diameter were measured, and each measurement was carried out in triplicate.

Determination of entrapment efficiency: The concentration of free drug (unentrapped) in the supernatant obtained after centrifugation of the SLN dispersion was used to calculate the entrapment efficiency (EE). The SLN dispersion was centrifuged at a speed of 100000 g using an ultra-centrifuge (Thermo Electron LED GmbH, D-37520 Oster ode) maintained at a temperature of 4°C. The amount of unentrapped drug was determined by analyzing the supernatant, and the entrapment efficiency was computed using the following formula:

$$EE\% = \frac{Amt\ of\ Drug\ added\ in\ Formulation - Amt\ of\ drug\ in\ Supernatant}{Amt\ of\ Drug\ added\ in\ Formulation} \times 100$$

Statistical Design analysis and Optimum desirability: Following the determination of Y1, Y2, and Y3, Design-Expert® software was used to statistically examine the data. P-values less than 0.05 were regarded as significant. Following the identification of the key variables influencing Y1, Y2, and Y3, an improved formulation of the SLN was suggested. For Y1, Y2, and Y3, the optimal formulation was developed and characterized.

Solid-state physicochemical characterizations:

Fourier transform infrared (FT-IR) spectroscopy: Using a Brucker FT-IR Spectrometer (Thermo Scientific, Inc., Waltham, MA), the FT-IR spectra of pure AT, stearic acid, polysorbate 80, propylene glycol, and the freeze-dried optimised SLN formulation were recorded in the range of 4000–400/cm.

Differential scanning calorimetry (DSC): SLN dispersion, optimised gel sample, and pure drug's thermal behavior were investigated using a Shimadzu DSC TA-50 ESI DSC apparatus (Tokyo, Japan).

Particle shape and morphological study: The shape and morphology of developed AT-SLN's was studied using Transmission electron microscopy (Hitachi H7500, Tokyo, Japan). Scanning electron microscopy was used to study the shape as well as surface characteristics of nanoparticles. These microscopic techniques also serve as tools to verify the reliability of routine particle size measurement techniques like PCS and DLS. A 1% w/v aqueous solution of phosphoric tungstic acid was used to negatively stain the samples. After that, the samples were dried on a tiny carbon-coated grid and examined under a microscope at the appropriate magnifications.

Preparation of Optimized AT-SLN Gel: An optimised SLN dispersion was identified from Design Expert software and the best formulation was incorporated in to a gel. The plain gel was prepared by dispersing

8% of Carbopol 934 in water and kept overnight and then it was neutralized by tri ethanol amine. To prepare Atomoxetine-SLN gel, optimized AT-SLN dispersion of 10ml was mixed with 5gm of plain gel under propeller homogenizer at 400 rpm.

Characterization of AT-loaded SLN Gel: The produced gel formulations were characterised for ex-vivo permeation investigations, in-vitro drug release studies, pH, viscosity, drug content, and gel strength, among other assessment criteria.

***In-vitro* permeation study:** Franz diffusion cells, Microette Plus, Hanson Research (CA, USA) were used to test the drug's penetration from the generated SLN-gel formulations. In summary, 400µl of the SLN formulation was added to the donor cells, and an artificial diffusion membrane made of synthetic nylon with a pore size of 0.45mm was employed. The receptor compartments were filled with 7 milliliters of pH 6.8 buffer that was continuously stirred at 400 revolutions per minute at 34 degrees Celsius. Three milliliter samples were taken every twelve hours, with replacements made right away. The drug content in the samples was calculated using the previously described UV method.

***Ex-vivo* Nasal permeation study:** Using the same parameters as mentioned in the previous section, drug permeation through excised bovine nasal mucosa from SLN gel formulation loaded with either Atomoxetine or pure medication was carried out. As soon as the animal was slaughtered, a nearby abattoir provided the bovine snout, which was then kept on ice until it was brought to the lab. To reveal the septum, wall, a longitudinal incision was made along the nose's lateral wall. After the mucosa was carefully removed, it was sliced into pieces of the appropriate size and inserted into the Franz diffusion cell so that the donor compartment's anterior surface faced it.

***In-vivo* studies:** The Ethical Committee for Care and Use of Laboratory Animals' established rules were followed in the conduct and approval of all animal procedures used in this investigation. The Institutional Animal Ethics Committee authorised the protocol for the animal experiments, and project proposal no. HCOP/IAEC/PR-7/2021 was issued. Adult male albino wistar rats weighing 230–250 gm were used for in-vivo investigations of optimised drug-loaded SLN's gel. The rats were housed in regular laboratory settings and had unrestricted access to water and standard laboratory food. Three groups of rats were used: Group C was given an optimised SLN gel (NF) intranasal, Group B was given a positive control for intranasal administration (free drug/gel) (ND), and Group A was given a positive control for oral drug solution (DS) administration [9]. Based on time, each group consists of seven rats: 0.5, 1, 2, 4, 6, 12, and 24 hours. At time zero, the treatments were administered, and at the designated time, the samples were separated and examined [10]. Before the dose was administered, the rats were made unconscious by inhaling diethyl ether and were securely restrained from the back in an inclined posture. The rats were mercifully killed by inhaling an excess of diethyl ether at the prearranged intervals of time. Blood was obtained by retro-orbital ocular puncture using micro hematocrit capillary tubes. The samples were subsequently transferred into Eppendorf tubes coated with EDTA, heparin-anticoagulated, and fractionated in a centrifuge operating at 4000 rpm, 25°C, for 20 minutes. The supernatant, or plasma, was separated and kept at -21°C until HPLC was used to analyse the drug [11]. Immediately following blood collection, the animals were decapitated, the skull was opened, and the brain was carefully removed. The area was then rapidly rinsed twice with cold normal saline (4°C), blotted with filter paper to remove as much blood and macroscopic blood vessels as possible, and allowed to dry [12]. After weighing the treated brain mass, a Teflon homogenizer was used to homogenise the saline in a 2:1 ratio for a few minutes, resulting in a homogeneous, uniform homogenate. After centrifuging the brain homogenate in a cooling centrifuge for 20 minutes at 4000 rpm and 4°C, the supernatant was separated and kept at -21°C until HPLC analysis.

Using pharmacokinetic software (PK Functions for Microsoft Excel, Pharsight Corporation, Mountain View, CA, USA), the plasma concentration–time profiles of AT-SLN following intravenous and oral administration were assessed. Numerous pharmacokinetic variables were computed, including MRT, T_{max}, AUC_{0-1}, $AUMC_{0-1}$, elimination rate constant, and C_{max}. To estimate the amount of drug in the brain, the nose-to-brain direct transport percentage (DTP%) and brain targeting efficiency percentage (DTE%) were computed.

RESULTS AND DISCUSSIONS

The choice of an appropriate lipid and surfactant is crucial for the creation of AT-SLNs. The drug's solubility in the selected lipid plays a significant role in encapsulation efficiency. High lipid solubility of the medication is thought to lead to high encapsulation efficiency.

Screening of lipids:

One of the primary steps in the development of SLNs was the solubility of AT-SLNs in four distinct lipids. Stearic acid, glyceryl palmitostearate, glyceryl monostearate, and Compritol 888 ATO were found to be

excellent solubilizers of atomoxateine. When compared with other lipids, Atomoxateine showed highest solubility in stearic acid. Based on the result of solubility studies, stearic acid was selected as lipid for the formulation of AT-SLN's.

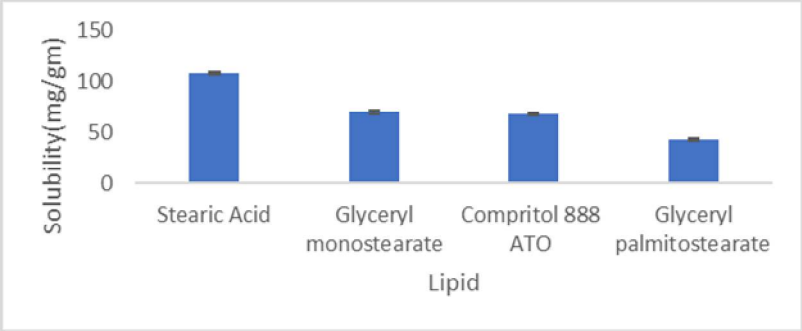


Figure 1: Solubility of drug in various lipids

Characterization of the SLN's formulation:

Table 2 displayed the particle size and entrapment efficiency results for each of the 17 created SLN formulations. All formulations had particle sizes between 120 and 241 nm, and their entrapment efficiencies ranged from 85.96% to 93.12%. Low values were obtained for the polydispersity index (PDI), a numerical measure of the produced nanoparticles' homogeneity that ranges from 0.1 to 0.3. The optimised formulation's zeta potential was discovered to be -19.2 mv, indicating that the formed AT-SLN dispersion was determined to be stable.

Effect of independent variables on particle size (Y1)

Given that the surface area accessible for medication absorption is inversely correlated with particle size, particle size is an important variable to consider when evaluating the produced formulations. The vesicular size of the prepared SLNs varied noticeably. Because of its lowest predicted residual sum of squares (PRESS) and greatest adjusted and predicted correlation coefficients (R2), which indicate model fit for the data, a linear model was chosen for the investigation of particle size. The three-dimensional surface plot displayed in Fig. 2a illustrates how the important parameters affect particle size. Stearic has a positive effect on particle size, as indicated by the positive sign of the coefficient of the linear term X2. It was discovered that when the lipid concentration increased, the produced SLNs' particle size increased [13–14]. Stearic acid was chosen as the lipid based on the drug's solubility and drug lipid compatibility studies. The surfactant was chosen based on its hydrophilic–lipophilic balance (HLB) value, which can emulsify the lipid and generate a stable micro emulsion within an acceptable concentration range. As the surfactant, polysorbate 80 with an HLB of 15 was chosen.

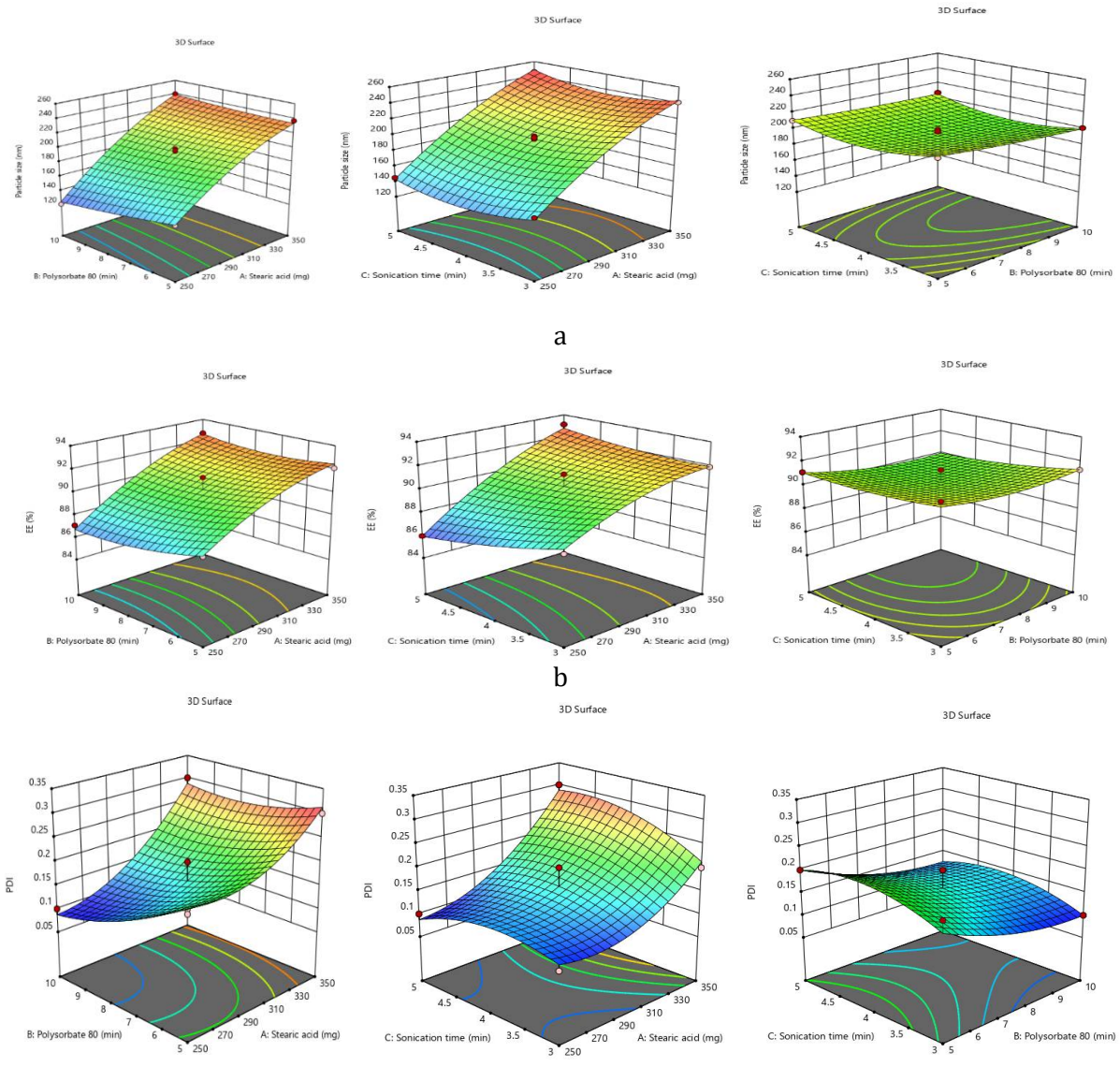
Effect of independent variables on entrapment efficiency (Y2):

As seen in Fig. 2b, the produced SLNs displayed an EE ranging from 85.96% to 93.12%. It was discovered that the quadratic model was the most appropriate polynomial model for EE. The model's statistical significance was discovered via analysis of variance (ANOVA) ($p = 0.0003$). It is implied that the model was significant by the Model F-value of 21.21. The likelihood of an F-value being that high owing to noise was about 0.03%.

Effect of Independent variables on PDI

As seen in Fig. 2c, the prepared SLNs had a PDI that ranged from 0.1 to 0.3. It was discovered that the quadratic model was the most suitable polynomial model for PDI. The model's statistical significance was discovered via analysis of variance (ANOVA) ($p = 0.0002$). It is implied that the model was significant by the Model F-value of 11.37. The likelihood of an F-value being that high owing to noise was about 0.03%.

Optimization: Design-Expert® software V.12 was used to study the desirability function and produce the ideal formulation. In order to verify the viability of the optimisation process, a fresh batch of optimised AT-SLN with the anticipated levels of formulation parameters was created. The ideal formulation was determined by focusing on the predetermined criteria of maximum entrapment efficiency and smallest particle size. With a formulation factor of 348.36 mg of stearic acid, 6.47 ml of polysorbate 80, and 4.22 minutes of sonication duration, respectively, the optimal formulation was accomplished. The optimised formulation's particle size, entrapment efficiency, and PDI were 237 nm, 92.18%, and 0.28, respectively, and these figures demonstrated good agreement with the projected values. For the optimised formulation, the predicted values for particle size, %EE, and PDI were 234 nm, 92.07%, and 0.21, respectively.



a

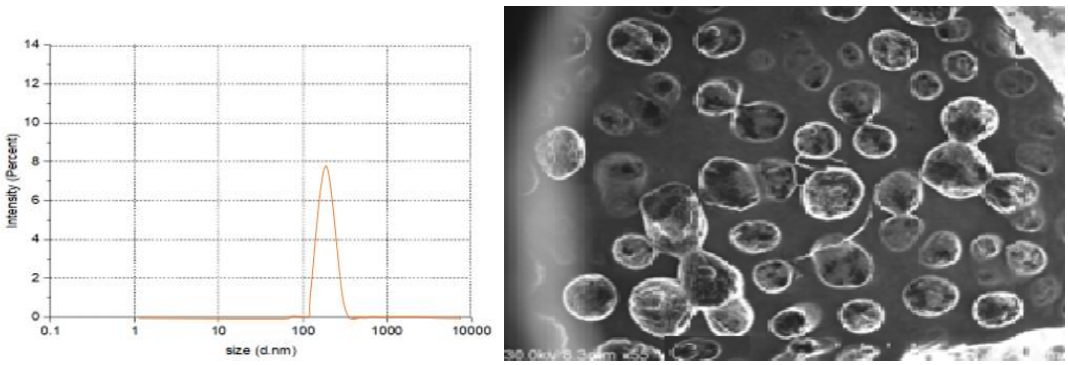
b

c

Figure 2: 3D plots a) Particle size b) Entrapment Efficiency% c) PDI

Particle size and Surface morphological study

The optimized Atomoxetine loaded SLN showed the particle size 237nm(Fig:3a) and the spherical shape (Figure 3b) was confirmed from SEM. The SEM images also showed there is no aggregation between particles

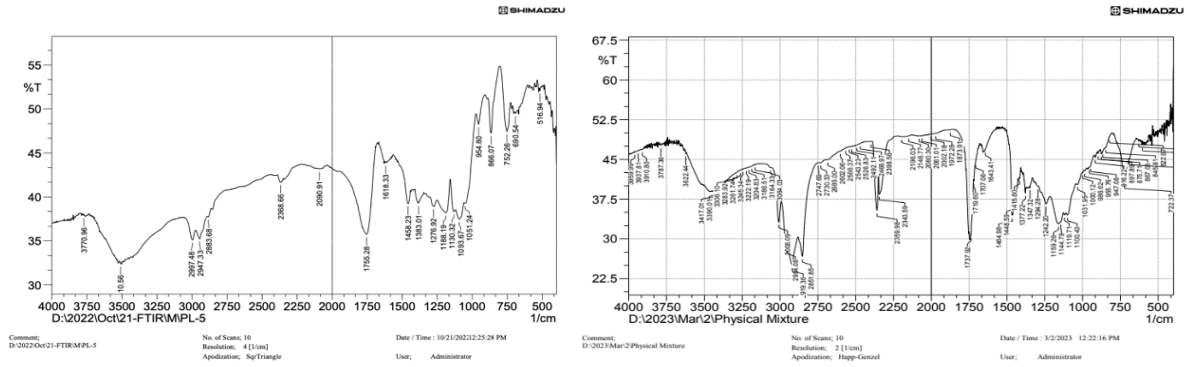


A **B**

Figure 3: a) Particle size of Optimized Atomoxetine SLN b) SEM image of optimised AT-SLN

Solid state characterization

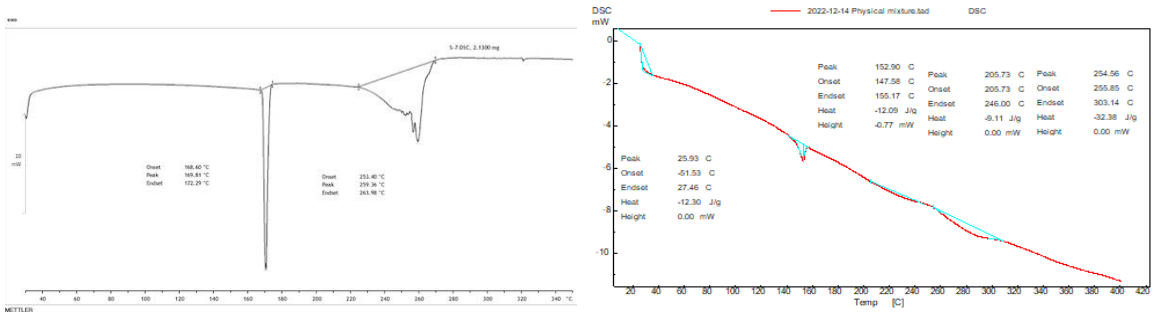
FTIR Study: Fourier transform infrared spectroscopy, was used to analyse drug-polymer compatibility and for changes in the properties of drug, as a result of its incorporation into SLN's (FTIR). Pure Atomoxetine IR spectra are displayed in Figure 4a and b. The FT-IR spectra of pure Atomoxetine revealed a characteristic drug peak at1755.28 C=O cm^{-1} for NH-stretching, 1618.33 cm^{-1} for C=C stretching, 1276.92 cm^{-1} for C-N amine bond, 516.94 cm^{-1} for halogen compound (C-Cl) link, 2997.48 (alkane compound), 2947.33, 2883.68 with O-H bond, and 1188.19 with C-N bond. It was also suggested that the same functional groups found in stearic acid were responsible for the absence or overlap of the distinctive Atomoxetine peaks with the lipids under study.



a **b**

Figure 4: FT-IR spectra of a) pure drug ATM b) Physical mixture of Atomoxetine along with excipients

DSC Study: For the pure drugs, the ATM DSC thermogram showed a single, sharp melting endothermic peak at 170°C, while for the optimised formulation, it was detected at 150°C (Fig. 5a and b). The drug's homogeneous dispersion in the lipid matrix is responsible for the optimised formulation's thermogram exhibiting the disappearance of the drug melting endotherm, a sign of the drug's transition from a crystalline to an amorphous physical state.



a **b**

Figure5: DSC thermogram for a) pure drug-Atomoxetine b) optimised AT-SLN

***In-vitro* drug release and Release kinetics:** Because of the drug's slow diffusion through the lipid core, drug release from the SLN gel formulations was observed to range from 28.0% to 98.0%. (Figure 6) Models of zero order, first order, and Higuchi kinetics were fitted using the drug release data. The order of drug release for the optimised batch was determined to have a correlation coefficient value of (R2=0.7774) for zero order models and (R2=0.9484) for first-order models. Diffusion was determined to be the drug release mechanism, with a Higuchi correlation coefficient value of (R2=0.9682).

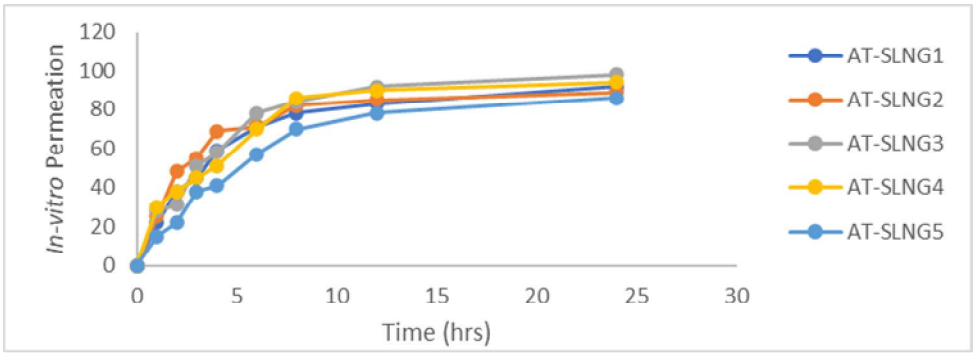


Figure 6: *In-vitro* drug release studies for SLN gel formulations and Opt.SLN gel formulation

***Ex-vivo* Nasal Permeation Study**

The permeation profiles revealed that the drug permeation followed a biphasic pattern with rapid phase for first 2 hour followed by slow phase from 2 to 24hr. The drug permeation was rapid for optimized AT-SLN gel and more when compared to the AT-Plain gel and the drug release was prolonged up to 24hr (Figure 7).

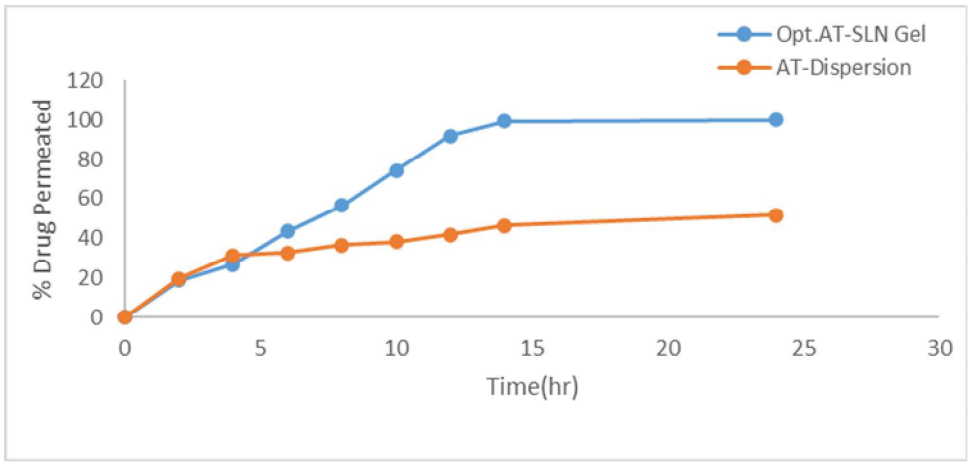
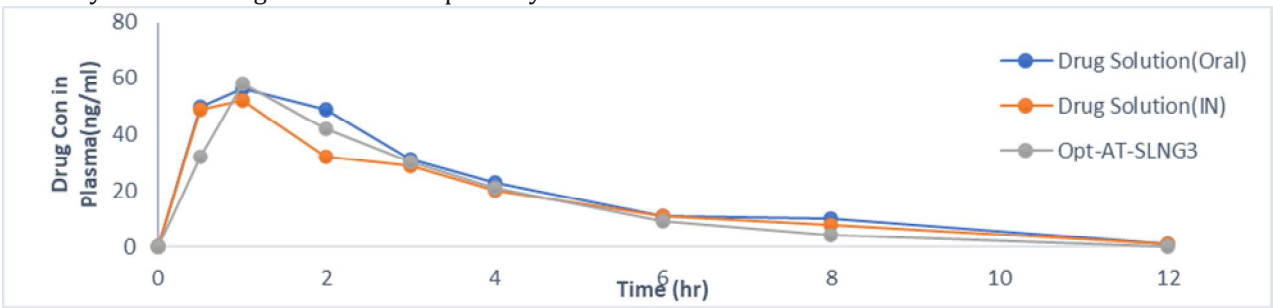


Figure 7: *Ex-vivo* Nasal Permeation studies for SLN dispersion and Opt.SLN formulation

***In-vivo* Drug Permeation study**

The plasma C_{max} of optimized AT-SLNG3 gel was found to be significantly high ($p<0.001$) compared to the oral and nasal drug solutions with in 15 min. However, the C_{max} of optimized AT SLN gel in brain was found to be 3.0 folds more than the plasma C_{max}, this could be due to the rapid absorption of drug by olfactory nerves through nose to blood pathway.



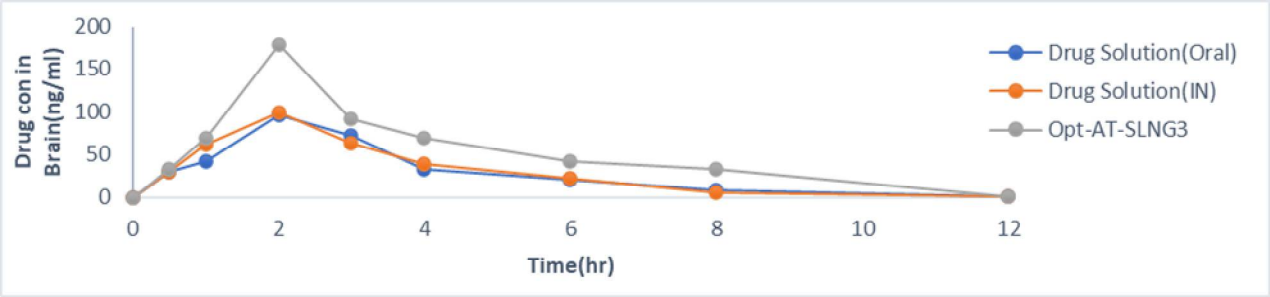


Figure 8: a) Plasma Drug Concentration graph of Drug Solution (oral), Drug Solution(in), Opt-AT-SLNG3(i.n) b) Brain Drug Concentration graph of drug solution(oral), Drug Solution(in), Opt-AT-SLNG3(i.n)

Pharmacokinetic & Neuro-pharmacokinetic Study

Pharmacokinetics Atomoxetine loaded SLN formulations in plasma and brain samples were studied in the rat model. Atomoxetine equivalent to Atomoxetine 10mg/kg was administered to rats by oral and nasal route. The mean plasma concentration-time profiles and brain concentration vs. time profile were shown in **Figure 8a and Figure 8b**, respectively. Pharmacokinetic parameters of plasma and brain were shown in Table 3 C max of Plasma. There was no significant difference in C max, plasma of treatments optimised-AT-SLNG3, Atomoxetine Solution oral and Atomoxetine nasal C max, brain C max, brain of optimised-AT-SLNG3 were significantly high at P< 0.01. As expected, the drug concentration was significantly high in the brain in case of nasal formulations as it bypasses the blood-brain barrier. The olfactory and trigeminal neural pathway plays a vital role in drug absorption into the brain[15-16]. So, the placing of formulation on the olfactory lobe and formulation is a key factor, influencing brain concentration. For this purpose, specially, designed rat nasal catheter was used; the catheter tube was inserted in such a manner that the maximum amount of formulation was placed on the olfactory region. The AUC of optimized-AT-SLNG3 in plasma and brain was significantly high compared to the oral and nasal drug solution. Moreover, the AUC values in brain were significantly high when compared to the plasma[17-18]. This could be due to the higher C_{max} values, obtained in brain as compared to the plasma. These values were also significantly high when compared to the nasal drug solution. The optimised AT-SLNG3 gel and drug solution were also estimated for DTE and % DTP as described in table optimised AT SLNG3 gel showed significantly high DTE at P<0.001, at P<0.01 and at p<0.05respectively than oral drug solution. The % DTP of optimized ATSLNG3 gel was significantly high compared with Atomoxetine nasal solution at P<0.0001.

TABLE 3: PHARMACOKINETIC PARAMETERS OF ATOMOXETINE AFTER ADMINISTRATION OF AT-SLNG3 (I.N), AT SOL (I.N) AND AT SOL (ORAL) ADMINISTRATION TO RATS IN BRAIN AND PLASMA.

Parameter (units)	ATSLNG3 i.n.		Drug solution i.n.		Drug solution oral	
	Brain	Plasma	Brain	Plasma	Brain	Plasma
C_{max} (ng/ml)	178.32±21.03	58.34±3.25	99.02±11.34	52.11±1.02	96.32±14.32	56.44±4.32
T_{max} (h)	4.03±0.1	4.52±0.13	2.66±0.10	1.89±0.23	3.65±0.04	3.27±0.01
$AUC_{0-24\,h}$ (ng.h/ml)	1490±132.4	610±25.34	900±98.31	531±18.31	781±52.14	480±22.14
K_e (h^{-1})	0.1719	0.1533	0.2605	0.3666	0.1898	0.2119
MRT (h)	8.87±0.24	8.4±0.21	8.09±0.20	7.5±0.17	7.64±0.07	6.7±0.11

DTE-Drug Targeting Efficiency; DTP-Direct nose to brain transport; F-Relative bioavailability; AUC-Area under Curve;

MRT-Mean Residence Time; IN-Intra nasal; Data expressed mean ± SD (n=6 for Plasma; n=3 for Brain); p<0.00001; p<0.0001, p<0.001; p<0.01, p<0.05 statistically significant to Drug solution oral /nasal.; #-indicates that very high significant value

The Percent brain targeting efficiency (DTE%)and nose-to-brain direct transport percentage (DTP%):

The optimized AT-SLNG3 gel and drug solution were also estimated for DTE and % DTP as described in table 5. optimized AT-SLNG3 gel i.e. showed significantly high DTE at P<0.001, at P<0.01 and at p<0.05 respectively than drug solution i.e. The % DTP of optimized AT-SLNG3 gel i.e. was significantly high compared with drug solution i.e. at P<0.0001.

Table 4: DTE (%) and DTP (%) of optimized AT-SLNG3 Gel i.n. and AT-sol. i.n.

Formulation and route of Administration	DTE (%)	DTP (%)
Opt. AT SLNG3 gel i.n.	2.44	51.91
AT sol. i.n.	1.48	28.64

CONCLUSION

In this study, a successful design of Solid lipid Nano particulate drug delivery system (SLNs) for encapsulating Atomoxetine for intranasal delivery to treat ADHD disorder was achieved using micro-emulsification method. The SLN's were assessed for their particle size, particle size distribution (PDI), zeta potential, and entrapment efficiency. The optimized SLN dispersion was used to create in-situ nasal gel which was then evaluated for in-vitro release, in-vivo studies, and stability studies. All findings were determined to fall within acceptable boundaries. A controlled and sustained release profile of AT-SLN's was observed with a 98.0% in-vitro drug release over 24 hours. Pharmacokinetic research was done on Wistar rats, where the DTE (%) and DTP (%) are better indicators of direct delivery from the nose to the brain without crossing the BBB, demonstrating the superiority of AT-SLN compared to AT sol. i.n. and oral. The effectiveness of AT-SLN's for treating ADHD through the nose to brain route can be inferred. Nonetheless, more clinical evidence is required to assess the balance between risks and benefits.

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REFERENCES

1. Vazquez J.C, de la Torre O.M, Palome J.L. Redolar-Ripoll D (2022 Feb 10). Effects of Caffeine Consumption on Attention Deficit Hyperactivity Disorder (ADHD) Treatment: A Systematic Review of Animal Studies. Nutrients;14(4):739. 2022, 14: 739.
2. Cuffe S.P., Moore C.G, McKeown R.E. (2005). Prevalence and Correlates of ADHD Symptoms in the National Health Interview Survey. J. Atten. Disord, 9(2): 392–401.
3. Arnsten A.F., Pliszka S.R. (2011). Catecholamine influences on prefrontal cortical function: Relevance to treatment of attention deficit/hyperactivity disorder and related disorders. Pharmacol. Biochem. Behav, 99(2): 211–216.
4. Bowton E., Saunders C., Erreger K., Sakrikar D., Matthies H.J., Sen N, Jessen T., Colbran, R.J. Caron, M.G., Javitch, J.A. (2010). Dysregulation of Dopamine Transporters via Dopamine D2 Autoreceptors Triggers Anomalous Dopamine Efflux Associated with Attention-Deficit Hyperactivity Disorder. J. Neurosci, 30: 6048–6057.
5. Brown J.T, Bishop J.R, Sangkuhl K, Nurmi E.L, Mueller D.J, Dinh J.C, Gaedigk A. Klein T.E, Caudle K.E, McCracken J.T. (2019). Clinical Pharmacogenetics Implementation Consortium Guideline for Cytochrome P450 (CYP) 2D6 Genotype and Atomoxetine Therapy. Clin. Pharmacol. Ther. 106: 94–102.
6. Crespo C. Liberia, T. Nacher, J. Varea, E.; Blasco-Ibanez J.M. Cranial Pair I (2019): The Olfactory Nerve. Anat. Rec., 302: 405–427.
7. Bonferoni M.C, Rossi, S, Sandri, G, Ferrari F, Gavini E, Rassu G, Giunchedi P. (2019). Nanoemulsions for "nose-to-brain" drug delivery. Pharmaceutics, 11: 84.
8. Sun Y, Li L, Xie H, Wang Y, Gao S, Zhang L, Bo F, Yang S, Feng A (2020). Primary Studies on Construction and Evaluation of Ion-Sensitive in situ Gel Loaded with Paeonol-Solid Lipid Nanoparticles for Intranasal Drug Delivery. Int. J. Nanomed.15: 3137–3160.
9. Gastaldi L, Battaglia L, Peira E, Chirio D, Muntoni E, Solazzi I, allarate M, Dosio F (2014). Solid lipid nanoparticles as vehicles of drugs to the brain: Current state of the art. Eur. J. Pharm. Biopharm. 87: 433–444.
10. Ozturk A.A, Aygul A, Senel B (2019). Influence of glyceryl behenate, tripalmitin and stearic acid on the properties of clarithromycin incorporated solid lipid nanoparticles (SLNs): Formulation, characterization, antibacterial activity and cytotoxicity. J. Drug Deliv. Sci. Technol. 54: 101240.
11. Mohanty D, Zafar A, Jafar M, Upadhyay AK, Haque MA, Gupta JK, Bakshi V, Ghoneim MM, Alshehri S, Jahangir MA, Ansari MJ. Development, in-vitro characterization and preclinical evaluation of esomeprazole-encapsulated proniosomal formulation for the enhancement of anti-ulcer activity. Molecules. 2022 Apr 25;27(9):2748.
12. Li Y, Jin M, Shao S, Huang W, Yang F, Chen W (2014). Small-sized polymeric micelles incorporating docetaxel suppress distant metastases in the clinically-relevant 4T1 mouse breast cancer model. BMC Cancer, 14:329.
13. Patra S, Dey J, Chakraborty A. (2023). Physicochemical Characterization, Stability, and In Vitro Evaluation of Curcumin-Loaded Solid Lipid Nanoparticles Prepared Using Biocompatible Synthetic Lipids. ACS Applied Bio Materials. Jul 5;6(7):2785-94.
14. Jeong SH, Jang JH, Lee YB. (2023). Drug delivery to the brain via the nasal route of administration: exploration of key targets and major consideration factors. Journal of pharmaceutical investigation. Jan;53(1):119-52.

15. Agrawal SS, Soni G, Pethe A, (2022). Yadav KS. Doxorubicin Hydrochloride-loaded Nanoparticles for Oral Delivery: Optimization using Design of Experiments. International Journal of Drug Delivery Technology.;12(4):1521-1526.
16. Kondapuram P, Sahoo SK. Development and Characterization of Eudragit Rl100 Nanoparticle Loaded Duloxetine Hydrochloride Gel for Transdermal Drug Delivery. International Journal of Drug Delivery Technology.
17. Bhargavi C, Raghuveer P. (2024). Development of Piribedil Solid Lipid Nanoparticles Incorporated in Nasal In-situ Gel by DoE. International Journal of Pharmaceutical Quality Assurance.;15(1):199-209
18. Gayathri H, S. Sangeetha, N. Damodharan. (2022). Formulation and Ex-vivo Permeation Study of Tamoxifen Citrate Loaded Transfersomal Gel. International Journal of Pharmaceutical Quality Assurance.;13(4):354-357