



Received on 22 February 2020; received in revised form, 25 November 2020; accepted, 30 November 2020; published 01 February 2021

## BRAIN TARGETING OF WATER SOLUBLE DRUG THROUGH NASAL ROUTE USING FATTY ACID VESICLES

K. Gayathri Devi and P. K. Lakshmi \*

Department of Pharmaceutics, G. Pulla Reddy College of Pharmacy, Mehdiapatnam, Hyderabad - 500028, Telangana, India.

### Keywords:

Fatty acid vesicles, Blood brain barrier, Full factorial design, Brain targeting, *Ex-vivo* studies

### Correspondence to Author:

**Dr. P. K. Lakshmi**

Professor,  
Department of Pharmaceutics, G.  
Pulla Reddy College of Pharmacy,  
Mehdiapatnam, Hyderabad - 500028,  
Telangana, India.

**E-mail:** drlakshmisuresh@gmail.com

**ABSTRACT:** Fatty acid vesicles are novel vesicles incorporating fatty acids which enhance the brain targeting efficiency when compared to conventional liposomes. This study's objective was to prepare, characterize, and evaluate fatty acid vesicles for brain targeting of a water-soluble drug, atomoxetine HCl through nasal route. The atomoxetine HCl loaded fatty acid vesicles were prepared by using the reverse phase evaporation method using Phospholipon 90 H, oleic acid, and ethanol. Prepared formulations were evaluated for parameters like particle size (152.9 nm), drug entrapment (90.4%), zeta potential (-9.41 mv), and *in-vitro* release was found to be 98% up to 4 h. SEM results showed spherical shaped vesicles. The ability of the prepared atomoxetine HCl loaded fatty acid vesicles to deliver atomoxetine HCl through the nasal route was studied by *ex-vivo* studies using goat nasal mucosa, which showed sustained release of 51% of the drug up to 4 h.

**INTRODUCTION:** Attention-deficit / hyperactivity disorder (ADHD) is a neurobehavioural condition resulting in impairment of concentration, impulsivity and cognitive processing. The symptomology of ADHD was initially believed to be caused by brain damage from illness or traumatic injury. But functional and structural studies of the ADHD brain compared with the typical brain showed an activity deficit in the prefrontal cortex. The prefrontal cortex in the brain is known for its involvement in working, attention, organization, constraint, and impulsivity, all of which can be impaired in the individual with ADHD.

ADHD always begins in childhood, and symptoms may continue beyond adolescence. The symptoms of ADHD include anxiety, depression, emotional trauma, bipolar disorder, and many other psychological disorders<sup>1</sup>. Delivery of drugs to the central nervous system (CNS) remains a challenge in the development of therapeutic agents for central targets due to the impenetrable nature of the drugs through the blood-brain barrier (BBB).

The BBB blocks the substrate penetration based on several characteristics, including lipophilicity, molecular size, and specificity for a variety of ATP-dependent transport systems. Injection of dyes in the ventricles of rabbits and monkeys showed that the cerebrospinal fluid (CSF) is drained via the olfactory neurons, originating from the olfactory bulb and connect the brain with the nasal cavity by penetrating the cribriform plate, which brings the neurons into the nasal mucosa. This coined the idea that this transport route could also exist in the

<b>QUICK RESPONSE CODE</b> 	<b>DOI:</b> 10.13040/IJPSR.0975-8232.12(2).1177-83
This article can be accessed online on <a href="http://www.ijpsr.com">www.ijpsr.com</a>	
<b>DOI link:</b> <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.12(2).1177-83">http://dx.doi.org/10.13040/IJPSR.0975-8232.12(2).1177-83</a>	

opposite direction that would imply direct access from the nasal cavity to the brain and thus circumventing the BBB<sup>2</sup>. In recent years, vesicular systems became a formulation scientist's choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, and diagnostic techniques along with targeted drug delivery systems (especially brain). They enhance effective drug permeation into cells, prolonged existence of drugs in the systemic circulation, selective uptake by reducing toxicity, effective for treating intracellular infections, sustains drug release, and lessens the therapeutic cost<sup>3</sup>.

Fatty acid vesicles nowadays are widely used for the brain targeting drug delivery systems, mainly for the drugs that is unable to cross the BBB due to their physical characteristics<sup>4</sup>. They are useful as carriers for the oral administration of poorly absorbable drugs, drugs that are unable to cross BBB, drugs having hindrance for passing through stratum corneum, and for the horizontal transfer of genes from plants.

This study's prime objective was to prepare, characterize, and evaluate the capability of fatty acid vesicles as a base component for nanocarrier development to deliver highly water-soluble drugs into the brain across BBB in a sustained manner. Further, its efficiency was evaluated by performing *ex-vivo* studies.

## MATERIALS AND METHODS:

**Materials:** Atomoxetine was obtained as a gift sample from Suven pharmaceuticals, Andhra Pradesh, Phospholipon 80 H and Phospholipon 90 H from lipoid GmbH, Oleic acid from Moly Chem, chloroform, and ethanol from S. D. Fine Chemicals Limited.

**Preparation of Fatty Acid Vesicles by Reverse Phase Evaporation Method:** Reverse phase evaporation method is used for the preparation of fatty acid vesicles. The organic phase (phospholipid, fatty acid, and organic solvent) and the aqueous phase (drug and buffer) in respected

ratios were mixed and probe sonicated to form o/w emulsion. Then the emulsion formed is evaporated by rotavap to get viscous gel formulation. The gel formulation is mixed with 20 ml buffer and bath sonicated to form fine dispersion. The obtained vesicles were ultrasonicated and sized subsequently by polycarbonate membrane<sup>5</sup>.

**Experimental Design:** Full factorial design method was used to optimize the fatty acid vesicular formulation. A factorial design is an effective method in indicating the relative significance of several variables and their interactions.

**TABLE 1: SELECTION OF INDEPENDENT VARIABLES FOR A (3<sup>2</sup>) FULL FACTORIAL DESIGN OF FATTY ACID VESICLES**

Factor	Level		
	-1	0	+1
Phospholipon 90H	100 mg	150 mg	200 mg
Oleic acid	100 mg	150 mg	200 mg

In this experimental design, the number of experiments required for studying the significance of variables and their interactions is dependent on the number of independent variables selected<sup>6</sup>.

**TABLE 2: FULL FACTORIAL DESIGN (3<sup>2</sup>) EXPERIMENTAL TRIALS**

Formulation Code	Phospholipon 90H (mg)	Oleic Acid (mg)
F1	150	150
F2	200	200
F3	200	150
F4	100	150
F5	150	100
F6	200	100
F7	100	100
F8	100	200
F9	150	200

A 3<sup>2</sup> full factorial design was utilized in the optimization study. In the design, two factors were evaluated each at three levels, and experimental trials were carried out at nine possible combinations. The design layout and coded value of the independent factor are shown in **Table 1**.

**TABLE 3: FULL FACTORIAL DESIGN (3<sup>2</sup>) EXPERIMENTAL RUNS FORMULAE**

Materials	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (mg)	25	25	25	25	25	25	25	25	25
Phospholipon 90H	150	200	200	100	150	200	100	100	150
Oleic acid (mg)	150	200	150	150	100	100	100	200	200
6.4 PBS (ml)	20	20	20	20	20	20	20	20	20
% Entrapment efficiency	56	84	32.4	40	48	76.4	81.2	90.4	62

The factors were selected based on screening study. Phospholipon 90 H and oleic acid were selected as independent variables, while entrapment efficiency was selected as the dependent variable. FFD (full factorial design) experimental run formulae are given in **Table 3**, and formulation of the factorial batches (F1 to F9) are shown in **Table 2**.

### **Characterization and Evaluation of Fatty Acid Vesicles:**

#### **Drug Excipient Compatibility Study by FTIR:**

The spectrum analysis of atomoxetine HCl and phospholipids which employed in the preparation of gels was studied by Fourier Transform Infra-Red (FTIR) Spectroscopy. FTIR spectra were recorded by preparing potassium bromide (KBr) disks using a Shimadzu Corporation (Kyoto, Japan) facility (model - 8400S).

Potassium bromide (KBr) disks were prepared by mixing a few mg of a sample with potassium bromide and by compacting that in a hydrostatic press under vacuum at 6-8 tons pressure. Then the resultant disc was mounted in a suitable holder in IR spectrophotometer, and the IR spectrum was recorded from  $4000\text{ cm}^{-1}$  to  $200\text{ cm}^{-1}$ . The resultant spectrum was compared for any spectral changes. The spectra were observed for the presence of characteristic peaks for the respective functional group in the compound.

**Entrapment Efficiency:** Fatty acid vesicular formulation of about 1.5 ml was transferred into Eppendorf tubes. Then it was centrifuged for 30 minutes at  $4\text{ }^{\circ}\text{C}$  at 8,000 rpm for two cycles, followed by 15 minutes at 6,000 rpm for one cycle to separate free drug and atomoxetine HCl loaded fatty acid vesicles. The clear fraction was used to determine the un-entrapped drug with the following formula <sup>7</sup>.

Entrapment efficiency % = Amount of entrapped drug / Amount of total drug added  $\times$  100

**Vesicle Shape:** Fatty acid vesicles can be easily visualized by using scanning electron microscopy (SEM). A scanning electron microscope or SEM is a powerful microscope that uses electrons to form an image and allows for imaging of conductive samples at magnifications that cannot be achieved using traditional microscopes <sup>8</sup>. As the SEM doesn't use light to create images, the resulting

pictures it forms are colorless (black & white). SEM was performed for the optimized formulation for determining the surface morphology, size, and shape of the formulation.

**Vesicle size and Zeta Potential:** The particle size of the fatty acid vesicles can be determined by dynamic light scattering (DLS). Particle size can be determined by measuring the random changes in the intensity of light that is scattered from a suspension or solution. Small particles in suspension undergo random thermal motion, which is known as Brownian motion. The Zeta potential of the formulation was measured by Zetasizer. The electrophoretic light scattering method was utilized for zeta potential measurement. A dip cell (zen1002, Malvern Instruments) with a pair of parallel Pd electrodes was used to provide an electrical trigger on charged particles. The signals were collected at  $12.8^{\circ}$ , and the data were analyzed using Zetasizer software.

#### **In-vitro Diffusion Studies (Dialysis Membrane):**

Diffusion studies were performed using two sides open-ended boiling tube. The boiling tube acts as a donor compartment, and the receptor compartment was a beaker of volume 250 ml. The dialysis membrane used for diffusion studies was placed between donor and receptor compartment <sup>9</sup>. The fatty acid vesicular suspension was added to the membrane and clamped together. The receptor compartment was filled with pH 6.4 phosphate buffer saline and maintained by continuous stirring at 50 rpm with a magnetic bead and maintained at  $37\text{ }^{\circ}\text{C}$ . At pre-determined time intervals, 1ml samples were withdrawn and replaced with an equal volume of buffer. The samples were analyzed after appropriate dilution at  $\lambda_{\text{max}}$  of 273 using a spectrophotometer.

#### **Ex-vivo Permeation Studies (Nasal Mucosa):**

*Ex-vivo* drug permeation study was carried out for the optimized formulations. Fresh nasal tissue was removed from the nasal cavity of the goat obtained from a local slaughterhouse. The mucosal tissue was stored in saline water in frozen conditions. It was placed in between the donor and the receptor compartment, and above same procedure was followed. *Ex-vivo* permeation rate studies such as % drug release, steady-state flux (SSTF) for atomoxetine HCl were calculated <sup>10</sup>.

**Release Kinetics:** Various models like linear and non-linear kinetic models that are dependent on the curve fitting procedure were tested for explaining the kinetics of drug release. To analyze the dosage form's drug release rate kinetics, the obtained data was fitted into zero-order, first-order, Higuchi, and Korsmeyer peppas release model, to study the drug release from the dosage form. The dissolution profiles of the optimized formulations were subjected to zero-order, first-order, Higuchi, and Korsmeyer peppas models to ascertain the kinetic modeling of drug release.

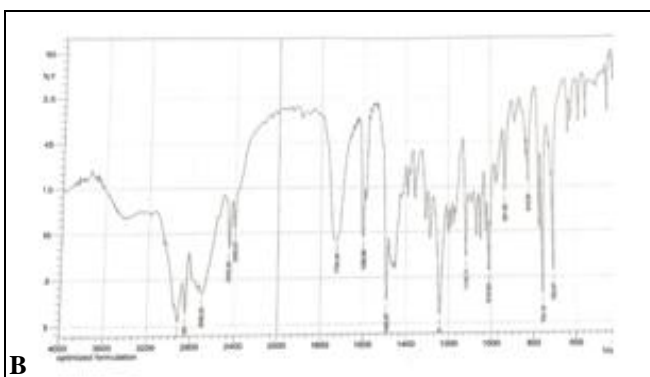
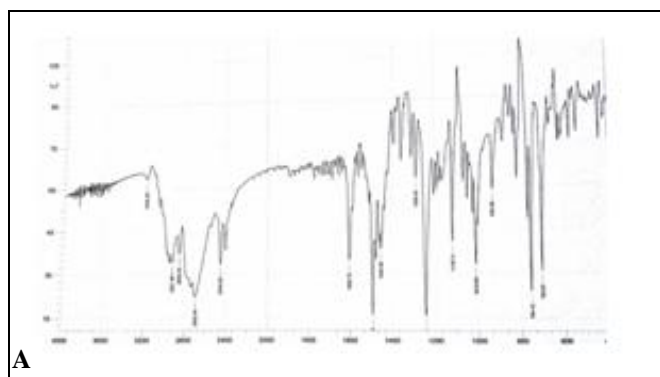


FIG. 1: FTIR SPECTRA OF PURE DRUG (A) AND OPTIMIZED FORMULATION (B)

**Entrapment Efficiency:** Phospholipids used for fatty acid vesicle preparation influenced the entrapment efficiency of the colloidal carrier<sup>12</sup>. Namely, Phospholipon 90 H was found to have better entrapment efficiency than Phospholipon 80 H, and the Phospholipon 90 H and oleic acid combinations were found to have high entrapment efficiency than Phospholipon 80 H and oleic acid combinations.

**Analysis of Factorial Designs:** It is inferred that there is an interrelationship between the predictor and its response as the plotted lines are not parallel to the x-axis. The response value is not the same for all predictors<sup>13</sup>. The magnitude of the inter-relationship effect is high as the slope of the line is steeper. Thus, we can conclude that oleic acid's inter-relationship effect is very high compared to Phospholipon 90H from Fig. 2.



FIG. 2: MAIN EFFECT PLOTS

**Surface Morphology:** Surface morphology and the three-dimensional nature of the fatty acid vesicles studied through SEM, confirmed the preparation as smooth-surfaced having vesicular characteristics.

The shape of fatty acid vesicles was found to be a spherical structure having nanosize with a smooth surface and a homogeneous distribution pattern. The SEM images of fatty acid vesicles revealed



that the vesicles were in a nanosize, spherical shape with smooth surface and homogenous distribution.

**Vesicle Size Analysis and Zeta Potential:** The vesicles obtained had a diameter of  $152.9 \pm 90.03$  d. nm and the polydispersity index 0.381, indicating that the optimized formulation is polydisperse in nature.

The particle size analysis revealed that the fatty acid vesicles were nano-size, which is an ideal characteristic for the brain's targeted delivery of the drug<sup>14</sup>.

It was found that with a decrease in the particle size, the penetration ability of the encapsulated drug increases. Therefore, the size of the vesicles is an important character that ensures a positive outcome of the preparation. Zeta potential was found to be -9.41 mV indicating that the formulation stable. The negative charge on the particle may be due to ethanol's presence in the sample, which prevents vesicle aggregation due to electrostatic repulsion, ensuring the stability of fatty acid vesicular dispersion and avoidance of aggregation of the vesicles<sup>15</sup>.

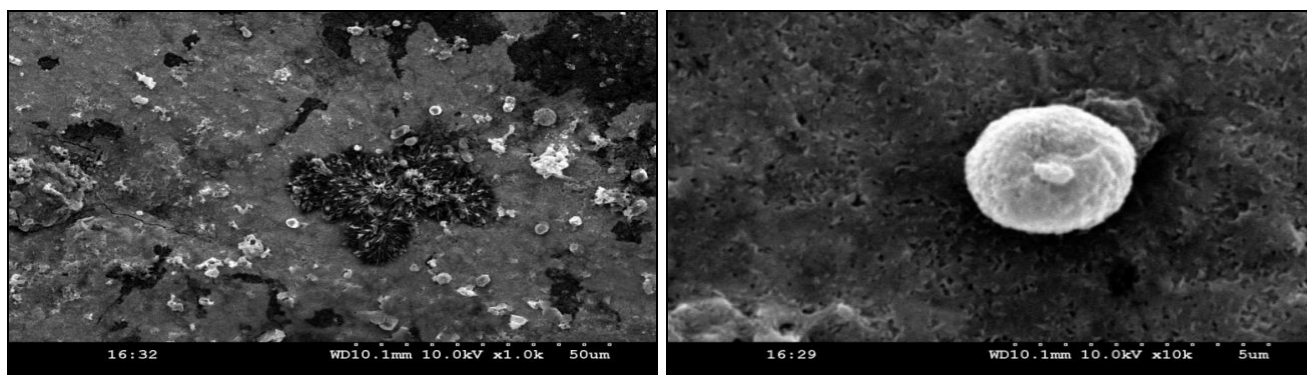


FIG. 3: SEM ANALYSIS OF OPTIMIZED FORMULATION (F8)

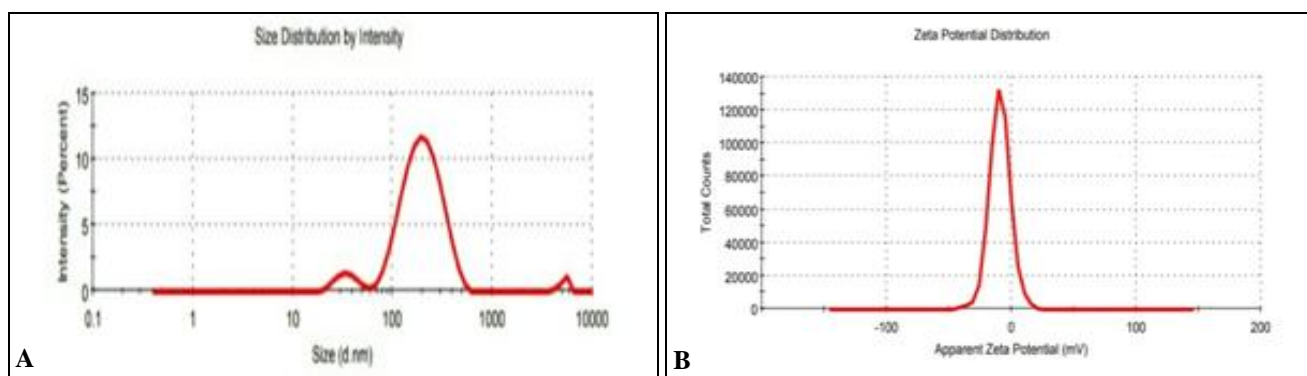


FIG. 4: PARTICLE SIZE ANALYSIS (A) AND ZETA POTENTIAL (B) OF OPTIMIZED FORMULATION (F8)

**In-vitro Diffusion Studies:** The *in-vitro* drug release data was given in **Table 4.18**, and the drug release profiles showed in Fig. 5. Formulations F2, F7, and F8 (200 mg 90 H + 200 mg oleic acid, 100 mg 90 H + 100 mg oleic acid, and 100 mg 90 H + 200 mg oleic acid, respectively) exhibited 88.5, 84.2, and 98.9% of drug release in 4 h respectively of all the 3 formulations; the release rate was high for F8 formulation. So, it is further selected for *ex-vivo* diffusion studies.

**Ex-vivo Diffusion Studies:** Results of permeation studies are shown in **Fig. 6**. The cumulative amount of drug permeated in 4 h from the pure drug was  $3278.061 \pm 0.03$  while from vesicular dispersion

was  $1278.432 \pm 0.21$ . Drug permeated from vesicular dispersion was low because maybe fatty acid vesicles increased the accumulation of the drug in the mucosal layer and thus decreased the permeation of the drug through the mucosa. Drug permeated from the pure drug was very high because it is in free form for penetration.

The drug release of nasal mucosa when compared to dialysis membrane was less; the reason was attributed to the high thickness of nasal mucosa. Slow drug permeation from vesicular dispersion indicates the release of drugs in a sustained manner for a prolonged period of time from fatty acid vesicles.

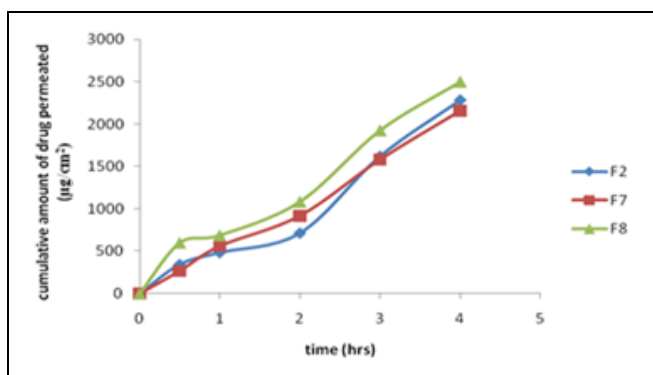


FIG. 5: *IN-VITRO* DIFFUSION STUDIES FOR OPTIMIZED FORMULATIONS

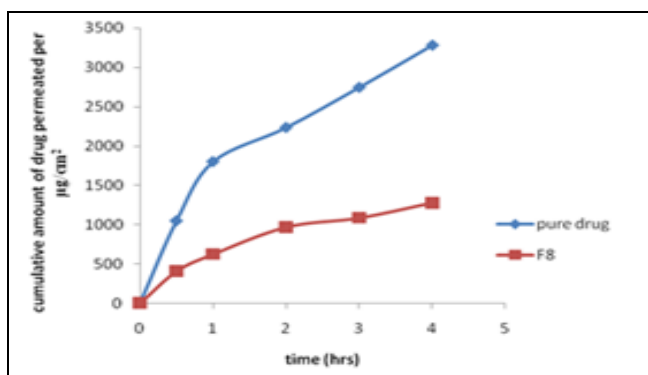


FIG. 6: *EX-VIVO* DIFFUSION STUDIES FOR PURE DRUG AND OPTIMIZED FORMULATION

**Release Kinetics:** Pure drug followed first-order kinetics while optimized formulation was found to follow first-order kinetics with Higuchi model-dependent kinetics. These release kinetics show the sustained drug release from the fatty acid vesicles. The first-order kinetics behavior of the fatty acid vesicles with the Higuchi mechanism might be due to the complex nature of the formulation, which is a sandwich model having a reservoir system incorporated in the matrix system.

**Stability Studies:** The stability studies were conducted for one month at room temperature and in the refrigerating temperature for 4 weeks<sup>16</sup>. A considerable decrease in the drug content and entrapment efficiency was observed when the formulation was stored at room temperature. Hence, it can be concluded that the formulation was stable at refrigerated conditions.

**CONCLUSION:** Nano-sized fatty acid vesicles were developed successfully using oleic acid to deliver the water-soluble drug atomoxetine HCl to the brain. The optimized formulation showed a sustained drug release profile. Thus, fatty acid vesicles may be utilized as a novel and effective carrier material to deliver atomoxetine HCl effectively into the brain. Fatty acid vesicles could be an emerging therapeutic strategy to deliver drugs to the brain and other organs.

Further, for many other CNS drugs that cannot cross BBB sufficiently, the developed formulation of fatty acid vesicles may be a suitable option to carry those drugs into the brain for better therapeutic management of CNS disorders.

**ACKNOWLEDGEMENT:** The authors acknowledge the Teaching, Non-teaching and Technical

staff of G. Pulla Reddy College of Pharmacy, Hyderabad, Telangana.

**CONFLICTS OF INTEREST:** The authors declare that there was no conflicting interest.

#### REFERENCES:

- Keilow M, Holm A and Fallesen P: Medical treatment of attention deficit/hyperactivity disorder (adhd) and children's academic performance. *PLoS ONE* 2018; 13(11): 1-17.
- Dong X: Current strategies for brain drug delivery. *Theranostics* 2018; 8(6): 1481-93.
- Ashara KC, Paun SJ, Soniwala MM, Chowda JR, Nathawani SV, Nitin Mori M and Mendapara VP: Vesicular drug delivery system: a novel approach. *Mintage Journal of Pharmaceutical and Medical Sciences* 2014; 3(3): 1-14.
- Shaw TK, Mandal D, Dey G, Pal MM, Paul P, Chakraborty C, Ali AK, Mukherjee B, Bandyopadhyay AK and Mandal M: Successful delivery of docetaxel to rat brain using experimentally developed nanoliposome: a treatment strategy for brain tumor. *Drug Delivery* 2016; 24 (1): 346-57.
- Zarei M, Norozuian D, Honarvar B, Mohammadi M, Shamabadi EH and Akbarzadeh A: Paclitaxel loaded noisome nanoparticle formulation prepared via Reverse phase evaporation method: Anss *in-vitro* evaluation. *Pakistan J of Biological Sciences* 2013; 16(6): 295-98.
- Lakshmi PK and Vidya K: Cytotoxic effect of transdermal invasomalnanastrozole gel on MCF-7 breast cancer cell line. *Journal of Applied Pharmaceutical Science* 2019; 9(3): 50-58.
- Lakshmi PK, Mounica V, Manoj Kumar Y and Prasanthi D: Preparation and Evaluation of Curcumin Invasomes. *International Journal of Drug Delivery* 2014; 6: 113-20.
- Lakshmi PK, Kalpana B and Prasanthi D: Invasomes-novel vesicular carriers for enhanced skin permeation. *Syst Rev Pharm* 2013; 4: 26-30.
- Zsiko S, Csanyi E, Kovacs A, Szucs MB, Gacsi A and Berco S: Methods to evaluate skin permeation *in-vitro*. *Sci Pharm* 2019; 87(19): 2-21.
- Deepika D, Dewangan KH, Maurya L and Singh S: Intranasal drug delivery of frovatriptan succinate – loaded polymeric nanoparticles for brain targeting. *J Pharm Sci* 2019; 108(2): 851-59.
- Pramod K, Suneesh CV, Shanavas S, Ansari SH and Ali J: Unveiling the compatibility of eugenol with formulation

- excipients by systematic drug excipient compatibility studies. *J Anal Sci Technol* 2015; 6(34). <https://doi.org/10.1186/s40543-015-0073-2>.
12. Tatode AA, Arun AT, Umekar MJ and Telang DR: Investigation of effect of phospholipids on physical and functional characterization of paclitaxel liposomes. *Int J Pharm Pharm Sci* 2017; 9(12): 141-46.
  13. Dutta L, Mukherjee B, Chakraborty T, Das MK, Mondal L, Bhattacharya S, Raghuvir H, Gaonkar and Debnath CM: Lipid-based nanocarrier efficiently delivers highly water soluble drug across the blood–brain barrier into brain. *Drug Delivery* 2018; 25 (1): 504-16.
  14. Prajapati A, Shah D, Jani J and Singh S: Fabrication of Sustained release matrix tablet of lornoxicam: influence of hydrophilic and hydrophobic polymers on the release rate and *in-vitro* evaluation by 3<sup>2</sup> full factorial design. *Inventi Rapid NDDS* 2012; 4: 1-14.
  15. Wang Y, Jiang S, Wang H and Bie H: A mucoadhesive, thermoreversible in situ nasal gel of geniposide for neurodegenerative diseases. *PLoS ON* 2017; 12(12): 1-17.
  16. Kaur N and Garg R: Optimization and evaluation of oleic acid based unsaturated fatty acid liposomes gel. *J Bioequiv Availab* 2017; 9(3): 424-29.

**How to cite this article:**

Devi KG and Lakshmi PK: Brain targeting of water soluble drug through nasal route using fatty acid vesicles. *Int J Pharm Sci & Res* 2021; 12(2): 1177-83. doi: 10.13040/IJPSR.0975-8232.12(2).1177-83.

All © 2013 are reserved by the International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)