doi.org/10.36721/PJPS.2022.35.5.REG.1363-1369.1

Brain targeted intra nasal acyclovir lipid nanoparticles; *in-vitro* characterization and *in-vivo* biodistribution studies

Kousalya Selvaraj, Gowthamarajan Kuppusamy*,
Vyshnavi Tallapaneni and Veera Venkata Satyanarayana Reddy Karri
Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Nilgiris, Tamilnadu, India

Abstract: Acyclovir (ACY) is an antiviral class of drugs used to treat herpes simplex virus infections such as herpes simplex encephalitis (HSE). ACY is widely distributed; Systemic exposure of ACY leads to serious adverse effects. Because of its high pH, intravenous ACY may cause phlebitis and local inflammation if extravasation occurs. This study aims to enhance acyclovir delivery to the brain via the intranasal route by formulating ACY nano lipid carriers (ACY-NLCs) to circumvent the side-effects, as mentioned earlier. ACY-NLCs were prepared by emulsification, followed by ultrasonication. A Box-Behnken statistical design with three factors, three levels and 17 runs was selected for the optimization study using Design- Expert Software. Nanoparticles were characterized for particle size, entrapment efficiency and *in-vitro* drug release. ACY- NLC showed biphasic release pattern i.e. an initial faster release followed by sustained release. Biodistribution study by imaging, Nanoparticles were slowly cleared and biodistributed to the other organs was observed in 2nd and 3rd hr post-administration. From the toxicity studies, NLC formulation is safe and non-toxic for the nasal administration. Rhodamine loaeded NLCs were quickly adsorbed by the olfactory tract and distributed mainly to the lungs through respiratory tract and were also detected in the trachea and olfactory bulb. Biodistribution study of dye loaded NLCs reach brain compared to the Rhodamine-solution.

Keywords: Acyclovir, blood-brain barrier, blood-cerebrospinal fluid barrier, central nervous system, intranasal, nano lipid carriers.

INTRODUCTION

Intranasal administration of active medicines in low dosages is a non-invasive method with no or low oral bioavailability. Intranasal administration of a wide range of Pharmaceutical compounds for localized region, systemic and central nervous system (CNS) actions is possible (Dhuria *et al*. 2009). To treat CNS illnesses while avoiding systemic exposure, drugs acquire direct access from such nasal mucosa towards brain and spinal cord via passageways mostly along the olfactory and trigeminal nerves (Pires *et al*., 2009 and Mittal 2014). Due to barriers in the brain that prohibit medicines from accessing the flow of blood to the brain or strongly isolates the brain from the bloodstream, systemic transport of therapeutics to the CNS is insufficient for almost 100% of big molecules and 49.8% of small molecule medications (Appasaheb *et al*., 2013). The hurdles are the blood-brain barrier (BBB) and the blood-cerebrospinal-fluid barrier (BCSFB) (Dhuria *et al*., 2010). As a result, invasive strategies such as intraparenchymal, intraventricular and intrathecal delivery (BBB disruption) have been used, as well as non-invasive strategies such as chemical changes, prodrug approaches, or even drug complexation with antibodies or ligands, to improve drug CNS specificity (Davis 1997). Several studies have explored nose-to-brain drug delivery to take use of the merits of this approach, such as trying to bypass the BBB, elimination of metabolism in liver, practicality and simplicity of administration or application, and non-invasive nature (Illim 2003),

The presence of the BBB complicates the design of effective therapeutics for CNS illnesses, causing most medications to be inconvenient to access the brain. As a result, finding appropriate brain targeting technologies has become a major issue for CNS medication development (Vyas 2005, Mukta *et al*., 2020). In this context, nanotechnology has increased in popularity in recent years since it provides promising solutions to this problem. Therapeutic chemicals can be formulated in biocompatible nanocarriers using a variety of ways, allowing them to be delivered into the brain. Furthermore, particular brain targeting moieties can be added to these biocompatible nanocomposites to increase CNS selectivity. Different delivery mechanisms, such as polymeric and lipidic nanoparticles, liposomes, are often employed nanocarriers (Illum 2003).

Delivery techniques for medicines based on lipids (lipid-based) are becoming more common. These may be regarded as potential carriers due to expressing personal ability to boost the solubility and bioavailability of medications that are weakly water-soluble or lipid soluble. Nano lipid carriers (NLCs), a new type of lipid nanoparticle, have overcome the limitations of classic lipid-based compositions and solid lipid nanoparticles

*Corresponding author: e-mail: gowthamsang@jssuni.edu.in

(SLNs) (Mehnert 2012). Because of their compatible biological properties, drug attacking (targeting), customizable delivery (deployment) and efficiency of massive fabrication, SLNs have previously piqued interest as a drug delivery technique. However, concerns such as medication leakage during preservation (storage) and inadequate drug loading may emerge depending on the medicine (Ghasemiyeh 2018, Radtke 2001). NLCs are then made by mixing solid and liquid lipids, resulting in new nanostructures with improved therapeutic loading capabilities, drug release profile changes and long-term stability. This type of transporting system has the ability to include massive quantities of medicines due to the production of a weak organized lipid matrix comprising various flaws (Illum 2003).

Lipid-based medication delivery methods are becoming increasingly prevalent. Despite their propensity to maximize solubility and bioavailability for weakly water-soluble or lipid soluble (lipophilic) medicines, they are considered to be potential carriers. The introduction of nano lipid carriers (NLCs), a new generation of lipid nanoparticles, has addressed the constraints of traditional lipid-based formulations and solid lipid nanoparticles (SLNs) (Mehnert 2012). SLNs have previously gained a piqued interest as a mediation delivery method due to their biocompatibility, drug targeting, customizable release, and ease of large-scale manufacture. However, depending on the medicine, issues such as drug leakage at the time of preservation and inadequate drug load may arise (Ghasemiyeh 2018, Radtke 2001). Later, NLCs are created by combining solid and liquid lipids, resulting in novel nanostructures with increased therapeutic loading capabilities, drug release profile change, and long-term stability. Because of the creation of a less organized lipid matrix with various defects, this sort of carrier/delivery giving system has the potential to integrate massive amount of medicine (Illum 2003).

Acyclovir (ACY) is an antiviral medication that is used to treat illnesses bring out by the herpes simplex virus, like herpes simplex encephalitis (HSE). If a patient is diagnosed with HSE, treatment begins with 30mg/kg/day intravenous (IV) acyclovir injections for 14-21 days. Higher intravenous ACY dosages (45-60 mg/kg per day) are recommended by some experts. ACY is broadly distributed, with large quantities detected with in kidneys, lungs, liver, heart, and skin vesicles; CSF concentrations are typically half that of plasma (Wagstaff *et al*., 1994). When ACY is ingested, it has substantial side effects. If IV ACY extravasates due to its high pH, it might cause phlebitis and local irritation (Peterslund *et al*., 1981). The most common adverse events include gastrointestinal (GI) disorders, headaches and rash.

Direct transport of ACY to the brain, on the other hand, has a more pronounced toxic effect than the systemic route. Because of the drug's restricted absorption through blood-brain barriers, the amount of ACY that reaches brain tissue during therapy is quite low (Lycke *et al*., 1989). A nanoparticle can help you break through the barrier (Lockman *et al*., 2002). The introduction of therapeutic compounds to the brain via intranasal route allows for a non-invasive bypass of the blood-brain barrier. Nano-sized drug carriers have been discovered to improve medication delivery to the CNS (Varsha *et al*., 2017), when contrasted to comparable drug solution formulations.

MATERIALS AND METHODS

ACY from BMR chemicals, Odisha, India. Gattefosse provided tripalmitin as a gift sample (Colorcon, India). Phosphate buffer saline solution, Tween 80, transcutol HP and chitosan were acquired from Sigma (India). All of the other compounds were analytical grade and were utilised without further purification.

Methods

Preparation of ACY-NLCs

Emulsification and ultrasonication were used to make acyclovir-loaded NLCs (ACY-NLCs) (Jessie *et al*., 2016). ACY was dissolved by mixing liquid lipid (Campul MCM) with melting solid lipid (palmitic acid). A magnetic stirrer was used to scatter the lipid phase in a heated surfactant aqueous phase heated to the same temperature for 20minutes at 1000rpm. A probe sonicator with a500 W amplitude and a 20 kHz frequency was used to ultrasonicate the acquired main emulsion for 15minutes. Tween-80 and pluronic F-68 were used as surfactants and co-surfactants in water. The dispersion was then cooled to become NLC dispersion.

Optimization of ACY- NLCs

Design-Expert Software chose a Box–Behnken statistical design involving three independent variables at three extents, and 17 experimental runs for the optimization study (Design-Expert 8.0.4). This model can be used to investigate polynomial equations and examine quadratic response surfaces. The independent and dependent variables are listed in table 1. This experimental design yielded the following polynomial equation.

b0 represents an intercept; The regression coefficients b1 to b33 are calculated out from recorded experiment data of “Responses,” and the encoded values of independent variables are A, B and C. The terms AB, AC, or BC and A2, B2, or C2, correspondingly, denote the interaction and quadratic terms. The variables studied are listed in table 1 together with their minimum (-1), intermediate (0) and high (+1) levels. The experimental range on every factor was determined using the preliminary trial data.

Characterization of NLC

Particle size and Zeta potential (ZP)

Photon correlation spectroscopy was used to calculate the mean particle size, distribution of size, and zeta potential (PCS; Zeta sizer, Malvern Instruments, Malvern, UK). The average particle/globule dimensions were determined using a technique called photon correlation spectroscopy, which analyses that Brownian flow pattern causes irregularities in dynamic light scattering. In 10 mm diameter cells, the average diameter was measured once at 90° angle at 25°C. Because it displays the electric charge on the surface of the particle, the ZP is useful for measuring the physical stability of almost any colloidal system. An electrophoretic light scattering approach was used to determine it. After dilution of all samples with the original dispersion media, overall size and ZP assessments were performed in 25°C in making use of disposable polystyrene cells and disposable plain folded capillary zeta cells, correspondingly. Each value was measured three times.

Drug entrapment efficiency and drug loading

The amount of unentrapped medication in the supernatant obtained after the filtration/centrifugation procedure was used to calculate the entrapment efficiency of ACY-NLCs. The NLC dispersion was centrifuged at 10000 rpm over a duration of 30 minutes, rinsed twice with water, then, the centrifuged floating fluid was gathered (Remi, Mumbai, India). The centrifuged floating material was adjusted in an appropriate ratio of acetonitrile to water (50:50). The amount of unentrapped drug, as well as the percentage of encapsulation capacity and drug filling of NLCs, were assessed by making use of HPLC.

$$\text{Encapsulation efficiency}\,(\%) = \frac{\text{Total amount of drug - the amount of free drug}}{\text{Total drug added}} \times 100$$

In-vitro drug release

The dialysis bag technique (Marina *et al*., 2016, S Brito *et al*., 2019) was used to study the drug release of NLC *in vitro*. For dialysis membrane activation, the dialysis membrane was immersed in a buffer overnight. The tests were conducted out in a sink environment. ACY-NLC, ACY drug solution (0.17mg/mL) and ACY drug solution (0.17mg/mL) were individually placed into a dialysis bag and sealed. The bags were placed in a beaker containing 100mL of the release media's simulated nasal fluid pH 6.4. The temperature was kept constant at 37°C and the mixture was agitated at 100rpm. Until 48 hours after the start of the experiment, 2mL of the release media was removed at predefined intervals. To keep the sink conditions the same, the same volume of fresh media was replaced every 72 hours. HPLC was used to examine the aliquots (n=3).

In-vitro release tests of optimized freeze-dried nanoparticles in SNF pH 6.4 were conducted for 48hours. The %cumulative drug release was calculated using samples taken over different intervals of time. The percentage of cumulative drug release was displayed against time in this graph. To investigate the likely mechanism of drug release by NLCs, drug release data was submitted to various models. At the conclusion of 48 hours, the percentage cumulative drug release was found to be in the range of 76-89 percent in all cases.

In vivo Biodistribution study

Animals will be grouped into two groups, with six animals in each group.

i. Grouping of animals

Group I: Rhodamine in saline (IN)

Group II: Rhodamine loaded NLC Formulation (IN)

Treatment

Male mice weighing 20-30 g were procured from the JSS College of Pharmacy's central animal facility in Udhagamandalam, Tamilnadu, India. The tests were conducted out with the agreement of the JSS College of Pharmacy's institutional animal ethics committee (IAEC) in Udhagamandalam, India. (Proposal no. JSSCP/IAEC/ OT/Ph.Ceutics/01/2017,18,). The animals were given a single dosage of formulation and rhodamine in saline, with the vehicle supplied via nose using a micropipette to each group. Fluorescence imaging monitoring the rat views at 1, 2 and 3hours after injection was used to quantify *in vivo* brain accumulation and whole-body biodistribution. The software was used to examine images and measurements of fluorescence signals. The intranasal method was used to conduct a biodistribution investigation and it was discovered that NLC formulations were absorbed into the brain, but rhodamine solutions were absorbed into the systemic circulation.

RESULTS

For the formulation optimization, the lipid with the highest acyclovir solubility was chosen. Acyclovir's solubility in solid and liquid lipids was investigated. Campul MCM (liquid lipid) and palmitic acid (solid lipid) were chosen.

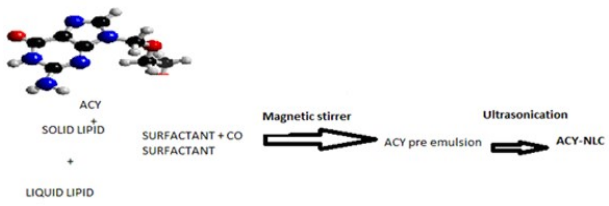

Fig. 1: Preparation of ACY-NLC

Experimental Design

Effect of variables on particle size

The interaction between the A drug: lipid ratio and the B drug: lipid ratio was shown by plot analysis. The effect of

increasing particle size on the solid lipid: liquid lipid ratio was found. Based on the response, the following The Statistical analysis of the data produced polynomial equations:

Particle size =88.60 + 21.13A + 63.13B-2.00C - 28.50AB +37.75AC+11.75BC + 19.20A^2 + 71.20B^2 + 26.95 C^2 (2)

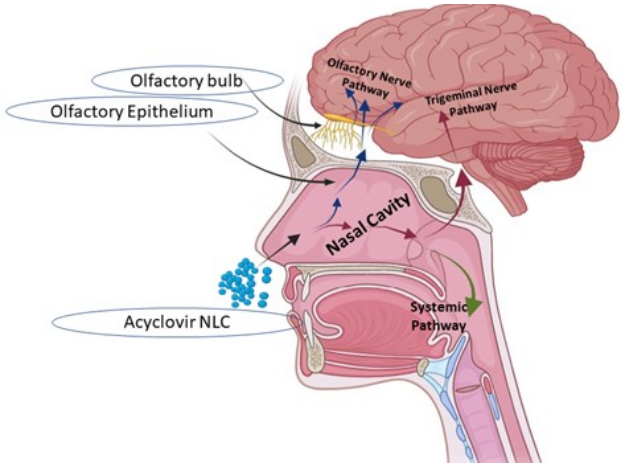

Fig. 2: ACY-NLCs reaching brain

The positive value just before the regression equation factor indicates that the output grows as the factor keeps increasing and vice versa. The value R^2 of equation parameter two was obtained to be 0.9664, suggesting an excellent fit model.

Effect of variables on entrapment efficiency

The plot showed the interaction between three factors on entrapment efficiency, high ratio of solid lipid: liquid lipid rapidly increases the entrapment efficiency. Increasing the amount of lipid acts as solubilizing agents for lipophilic drugs and provides more space to accommodate excessive drugs. The polynomial equation was constructed by statistical interpretation of the findings depending on the feedback.

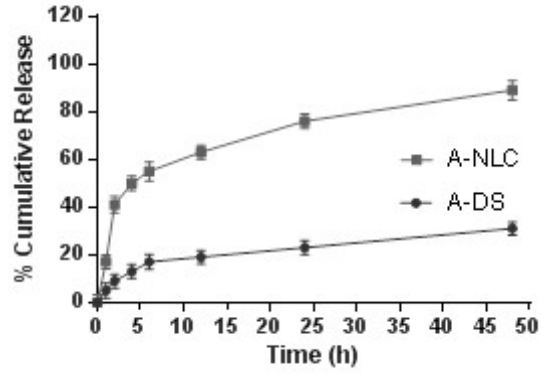

Fig. 3: *In vitro* drug release of Acyclovir

Entrapment Efficiency = 95.60 -1.37A-7.37B - 0.25C +1.25 AB-3.50 AC -1.00 BC-1.68 A^2 - 6.18 B^2-1.42 C^2. The positive sign showed that an AB factor has more impact on the entrapment efficiency. The regression coefficient value from the results was R^2 0.9737; it represents the model fit.

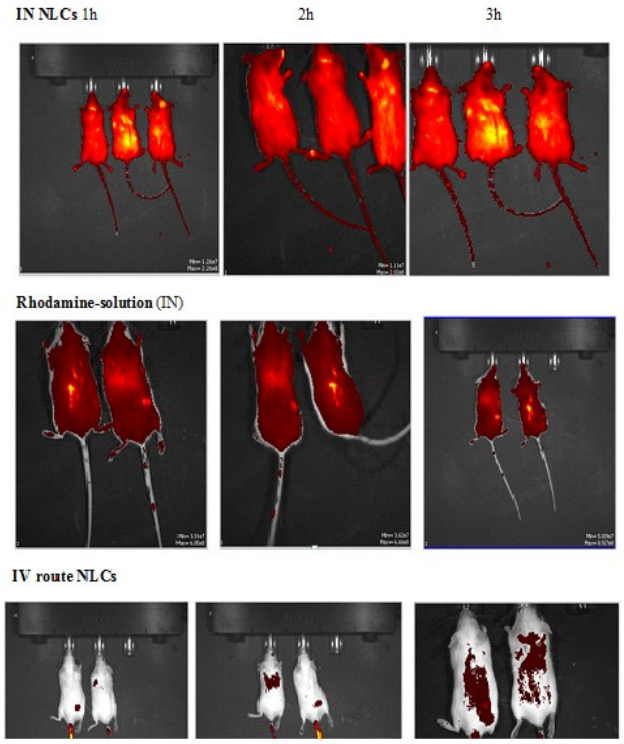

Fig. 4: Biodistribution of NLCs through nasal and intravenous route

Optimization

To obtain the optimum formulation, the desirability function was explored using Design-Expert software 8.0.4 version. The best formulation was determined by a set of criteria that included the smallest particle size and the highest entrapment efficiency.

To demonstrate the correctness of the optimization approach, optimal batches of nanoparticles with the predicted levels of formulation factors were generated. There was no difference between expected and actual values, indicating that the experiment's design was sound. To make NLCs, the following optimised and validated concentrations were used: drug: lipid ratio (60mg), solid lipid: liquid ratio (50mg), and surfactant concentration of 0.63 percent v/v. The size of particle and zeta potential of improved batch NLCs were evaluated.

Particle size

Particle size and EE are 46.25 nm and 85.5± 2.5 %.

In vitro drug release

ACY- NLC exhibited a biphasic delivery technique, through a quicker early discharge and by a slower

persistent discharge. 18.00 2.51% of the medication was released from A-DS in the first 8hours, followed by release up to 48hours (30 percent). The percentage of cumulative drug release was displayed against time in this graph. To investigate the likely mechanism of NLC drug release, data on drug release was submitted to various models. At the end of 48 hours, the percentage cumulative drug release was found to be in the range of ACY-NLC 76-89 percent in all cases (fig. 3)

In vivo Biodistribution study

Brain targeting Efficacy of NLCs via an intranasal route

The BBB obstructs delivery of drugs to the brain for therapy of brain disorders, restricting drug access to the CNS. After intranasal injection, rhodamine fluorescence was identified in brain mice as demonstrated by *in vivo* biodistribution monitoring done at 1h, 2h and 3h, as validated by the whole animal imaging system. The fluorescent signal in group I treated mice was localised with the brain after the photos were overexposed, confirming an *in vivo* brain deposition of NLCs (fig. 4.). After intranasal delivery, the fluorescence signal was monitored for 3 hours, indicating that our formulation (NLCs) would reach the brain without being cleaned by nasal mucosal cilia.

In two of the three animals treated with NLCs, the NLCs reached the brain via the nasal route. The other animal image depicts NLCs in the systemic circulation as a result of error in animal handling while administering the dose via nasal and mucociliary clearance. Nanoparticles removed and biodistributed to different organs over time, as seen in the 2nd and 3rd hours after treatment.

DISCUSSION

Effect of variables on particle size

The blue zone was more common when the lipid ratio was low. The blue zone shrank as the gradient inclination of the drug lipid ratio increased, indicating that the size increased dramatically. It was discovered that raising the drug-to-lipid ratio increased particle size. With a high B ratio, the particle size increased when considering the solid lipid: liquid ratio. A good particle size range was provided by a medium level of A and B ratio. The solid lipid: liquid lipid ratio had a greater influence on particle size. The interaction between the surfactant, solid lipid: liquid and drug lipid ratios was shown in contour plots B and C. With an increase in the ratio of solid lipids, particle size increases. The particle size was visible in the medium surfactant range. Surfactant molecules prevent aggregation by forming a thick layer around the droplet, which helps to stabilize the formulation. There is a lack of ability to stabilize the dispersion system at low surfactant concentrations, resulting in a considerable size increase.

Effect of variables on Entrapment efficiency

At a low ratio of AB factors, the entrapment efficiency showed less entrapment efficiency. The plot showed that an adequate level of AB factors in the formulation gave favorable entrapment efficiency. Low ratio of Drug: lipid showed a decrease in entrapment efficiency.

Particle size

The particle size and entrapment efficiency results represented excellent concordance with the anticipated values.

***In vitro* drug release**

The presence of such a specific quantity of drug deposited onto the outer edge of NLC or allowed to settle mostly from simple lipid matrix in both NLC formulations implies the presence of a precise quantity of drug adhered to the outer surface of NLC or allowed to settle from the simple lipid matrix. The inner lipid matrix's inhomogeneity can also be explained by oil inhomogeneity.

In vivo biodistribution study

The olfactory tract immediately absorbed rhodamine-loaded NLCs, which were then transferred mostly to the lungs via the respiratory tract. They were also found in the trachea and olfactory bulb. It could be because rhodamine-NLCs cross the BBB and enter the brain via

Table 1: Variable and their constraints in the Box-Behnken Design

Factor	Levels Low Medium High (-1) (0) (+1)
Independent variables (mg)	
A = Drug: lipid	60 85 110
B =Solid-lipid: liquid-lipid ratio	50 75 100
C = Surfactant (%)	0.25 0.63 1
Dependent variables	
Y1 = Particle size (nm)	Minimize
Y2 = Entrapment efficiency (%)	Maximize

$Yi = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$

the intranasal route. The rhodamine-solution did not reach the brain in group II, but it did enter the systemic circulation.

They were also found in the trachea and olfactory bulb. It could be because rhodamine-NLCs cross the BBB and enter the brain via the intranasal route. The rhodamine-solution did not reach the brain in group II, but it did enter the systemic circulation. The IV treatment did not reach the brain in the mice. We found that prepared NLCs have higher penetration, and that treatments reach the brain. We found that prepared NLCs have higher penetration, and that treatments reach the brain directly via the nasal route

Conclusions summarizing the achievements and indication of scope for future work

The excipients' selection for the formulation of NLC was done based on the partition coefficient in various solid lipids and solubility studies in oils, surfactants and co-surfactants. These preliminary investigations helped select the excipients with the highest solubility for the selected drugs, an important criterion to maintain the drug in a solubilized state. Further, the compatibility of drugs and lipids was established by using FT-IR and DSC studies. Stability studies of the drugs were performed in SNF to ascertain that drugs are stable at nasal pH. Preliminary studies were carried out to optimize the formulation/ process variables affecting the particle size and PDI of NLCs. Based on the results obtained, further optimization of drug-loaded NLCs was carried out using Box-Behnken design using emulsification followed by sonication (ACY -NLCs) and microemulsion technique (DZP=NLCs).

The various physicochemical parameters characterized the developed NLCs. The results indicate that the particle size of ACY-NLC (46.25 ±14.58 nm) and DZP-NLC (117±3.6nm). From the toxicity studies, NLC formulation is safe and non-toxic for the nasal administration. Biodistribution study of dye Rhodamine 6G loaded NLCs reach brain compared to the Rhodamine-solution. The higher concentration was observed in the brain through the intranasal route.

Comparison - doi/abs/10.1080/10837450.2019.1667386?journalCode=iphd20

Comparing the paper ACY-SLN for brain targeting. Formulation comparison ACY-nanoparticles were coated with chitosan but our research ACY-NLC uncoated and route of administration is IV BOLUS. Our aim is to define whether drug or NLC reaches brain in non-invasive route. We selected nose to brain targeting. Nanoparticle were administered through nose and NLC reaches brain was observed by fluorescence imaging technique.

ACKNOWLEDGMENT

The authors would like to thank the Department of Science and Technology-Science and Engineering Research Board (DST-SERB) for their support for this work.

REFERENCES

Appasaheb PS, Manohar SD, Bhanudas SR and Anjaneri N (2013). A review on intranasal drug delivery system. *J. Adv. Pharm. Edu. & Res,* **3**(4): 333-345.

Brito SR, Kothapalli BC and Kesavan BR (2019). Formulation, *in-vitro* and *in-vivo* pharmacokinetic evaluation of simvastatin nanostructured lipid carrier loaded transdermal drug delivery system. *Future J. Pharm. Sci.*. **5**(9): 2-14

Davis S (1997). Biomedical applications of nanotechnology-implications for drug targeting and gene therapy. *Trends Biotechnol,* **15**(16): 217-224.

Dhuria SV, Hanson LR and Frey WH (2009). Novel vasoconstrictor formulation to enhance intranasal targeting of neuropeptide therapeutics to the central nervous system. *J. Pharmacol. Exp. Ther.,* **328**(1): 312-320.

Dhuria SV, Hanson LR and Frey II WH (2010). Intranasal delivery to the central nervous system: Mechanisms and experimental considerations. *J. Pharm. Sci.,* **99**(4): 1654-1673.

Ghasemiyeh P and Mohammadi-Samani S (2018). Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: Applications, advantages and disadvantages. *Res. Pharm. Sci.,* **13**(4S): 288.

Hu QF, Jiang SP, Du YZ, Yuan H, Ye YQ and Zeng S (2005). Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. *Colloids Surf B Biointerfaces,* **45**(3-4): 167-173.

Illum L (2003). Nasal drug delivery-possibilities, problems and solutions. *J Control Release,* **87**(1-3): 187-198.

Jessie SP, Rachmat M and Nasya I (2016). Development of Nanostructured Lipid Carrier Formulation Containing Of Retinyl Palmitate. *Int. J. Pharm. Pharm. Sci.,* **8**(2): 256-260.

Lockman P, Mumper R, Khan M and Allen D (2002). Nanoparticle technology for drug delivery across the blood-brain barrier. *Drug Dev. Ind. Pharm.,* **28**(1): 1-13.

Lycke J, Andersen O, Svennerholm B, Appelgren L and Dahlof C (1989). Acyclovir concentrations in serum and cerebrospinal fluid at steady state. *J. Antimicrob. Chemother.,* **24**(6): 947-954.

Marina P, Ricardo R, Alexandre V, Fernanda A and Salette R (2016). Design of a nanostructured lipid carrier intended to improve the treatment of tuberculosis. *Drug Des. Devel. Ther.,* **10**: 2467-2475.

Mehnert W and Mader K (2001). Solid lipid nanoparticles: Production, characterization and applications. *Adv. Drug Deliv. Rev.,* **47**(2-3): 165-69.

Mittal D, Ali A, Md S, Baboota S, Sahni JK and Ali J (2014). Insights into direct nose to brain delivery: Current status and future perspective. *Drug Deliv.,* **21**(2): 75-86.

Mukta A, Swarnlata S, Shailendra S, Sunil KD, Anu P, Ravish JP, Ajazuddin, V Ravichandiran, Upadhyayula SM and Amit A (2020). Recent strategies and advances in the fabrication of nano lipid carriers and their application towards brain targeting. *J. Control Release,* **321**: 372-415.

Peterslund N, Ipsen J, Schonheyder H, Seyer-Hansen K, Esmann V, Juhl H (1981). Acyclovir in herpes zoster. *Lancet,* **2**(8251): 827-830.

Pires A, Fortuna A, Alves G and Falcao A (2009). Intranasal drug delivery: How, why and what for? *J. Pharm. Pharma. Sci,* **12**(3): 288-311.

Radtke M and Muller RH (2001). Nanostructured lipid drug carriers. *New Drugs.* **2**: 48-52.

Varsha P, Arpana P and Prathusha P (2017). Efavirenz loaded nanostructured lipid carrier engineered for brain targeting through intranasal route: *In-vivo* pharmacokinetic and toxicity study. *Biomed. Pharmacother.,* **94**: 150-164.

Vyas TK, Shahiwala A, Marathe S and Misra A (2005). Intranasal drug delivery for brain targeting. *Curr. Drug Deliv.,* **2**(2): 165-175.

Wagstaff AJ, Faulds D and Goa KL (1994). Aciclovir. *Drugs*, **47**: 153-205.