

**SURFACE MODIFIED LIPOSOMES FOR TARGETED DRUG DELIVERY**

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Corresponding author e-mail:** anupama.ssp5@gmail.com*Received on: 27-02-2016; Revised on: 28-04-2016; Accepted on: 24-06-2016ABSTRACT**

The aim of the present study was to design and develop a suitable intranasal spray formulation of surface modified liposomes to improve the concentration of anti-psychotic drug into the brain tissue for therapy of schizophrenia. Liposomal spray solution was optimized; particle size, zeta potential and polydispersity index were measured using Malvern Zetasizer. Morphology of liposomal spray droplets was examined using scanning electron microscopy. Drug diffusion studies were performed and drug diffused was estimated using UV spectroscopic analysis. Stable liposomal spray was formulated. The optimized liposomal spray showed uniform vesicles in the size range 155-170 nm and zeta potential of optimized formulation was found to be 29.77. Surface coating was done using chitosan solution in glacial acetic acid and evaluated. Intranasal surface modified liposomal spray exhibited higher deposition in brain tissues compared to conventional solution after intranasal and intramuscular administration indicating potential for nose to brain targeting. Thus nasal drug delivery offers a viable alternative to oral and injectable routes of administration.

Keywords: Surface modified liposomes, intranasal, brain deposition, targeted delivery**INTRODUCTION**

Schizophrenia is a mental disorder often characterized by abnormal social behavior and failure to recognize what is real. In this study, formulation development of a drug that belongs to the new generation of neuroleptics, used in the treatment of schizophrenia was investigated. Some of these drugs could lead to increased weight gain and preliminary evidence indicates after oral administration, a dose dependent relationship between drug and metabolic issues such as diabetes. So there is a need for alternative routes of administration to increase bioavailability and reduce drug dose and in turn toxic effects.

Intranasal route has been successfully exploited for systemic delivery of drugs and more specifically, for preferential targeting to the Central Nervous System. Intranasal route has received immense attention,

because it offers advantages including rapid absorption, avoidance of hepatic first-pass metabolism and preferential drug delivery to the brain via the olfactory region. Intranasal route allows transport of drugs to the brain circumventing the Blood Brain Barrier thus providing a unique feature and better option to target drugs.

Liposomes have emerged as a potential tool for the delivery of multiple therapeutic and diagnostic agents in a single dosage form, given their biocompatibility, biodegradability and targeting ability. Liposomal formulations have been used to both enhance absorption and to regulate release of incorporated drugs, thus localizing the effect of the drugs. The flexibility of their behavior can be exploited for the drug delivery through any route of administration.

Mucoadhesive properties in dosage forms have received substantial attention in novel drug delivery

systems as they improve the bioavailability of drugs by prolonging their residence time and controlling the drug release characteristics. Mucoadhesive nanoparticulate systems such as polymercoated liposomes are found to be useful carriers for improved delivery because of their prolonged retention on the mucosal surface and excellent penetration into the mucus layer. The mucoadhesive liposomes can be prepared by coating drug carrier surface by various mucoadhesive polymers such as chitosan, carbopol and eudragit.

This research project was taken up with an objective to develop and evaluate a sustained release intranasal formulation of a thienobenzodiazepine as a mucoadhesive liposomal spray. The aim was to attain direct nose to brain targeting of the drug for therapy of schizophrenia.

MATERIALS AND METHODS

Materials: Phosphatidylcholine Leciva S70 was received as a gift sample from VAV Lifesciences; cholesterol was purchased from Qualigens Ltd; chitosan was purchased from Otto Ltd and solvents chloroform, methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from S.D. Fine Chemicals.

Formulation Development:

Preparation of liposomes: Liposomes were prepared by a method principally involving thin film hydration technique. Briefly, lecithin + cholesterol + Drug in a 3.5:0.5:1 ratio were weighed accurately and dissolved in chloroform in a round bottom flask together with glass beads. This round bottom flask (RBF) was connected to the rotary vacuum evaporator, and vacuum was applied. The RBF was rotated at 120 rpm and the bath temperature was maintained at 37°C to completely evaporate chloroform until the dried solid thin lipid film was deposited on the wall of flask. It was continued for 15 min after the dry residue first appeared.

After releasing the vacuum 5ml of phosphate buffer saline and 5g of glass beads were added to the round bottom flask. The flask was attached to the evaporator again. It was secured in position with a clip, and rotated at room temperature and pressure at the same speed as before ~120 rpm. The flask was rotated for 60 min, until the entire lipid had been removed from the walls of the flask, and a homogeneous off-white suspension was formed.

The suspension was allowed to stand for a further 2 hours at room temperature in order to complete the swelling process. The formed liposomal dispersion was stored at 4°C overnight for further

characterization.

Surface modification of prepared Liposomes:

Coating of liposomes was carried out by mixing liposomes with chitosan solution. 0.05% Glacial Acetic Acid (GAA) was used to dissolve chitosan. A 10% w/v solution of chitosan was prepared for coating the liposomes. Liposomal dispersion was subjected to sonication for 3 minutes for further size reduction. An equal volume of liposomal dispersion and chitosan solution were mixed using magnetic stirrer. Drop wise solution of chitosan in GAA was added to liposomal dispersion and stirring was continued for another 5 minutes.

Particle size and Zeta potential:

Particle size and zeta potential of surface modified liposomes were measured by particle size analyser and zetasizer (Malvern instrument) to observe increase in the particle size and changes by virtue of chitosan coating. The samples were diluted with water and placed in electrophoretic cell and measured in the automatic mode.

Preparation of conventional drug solution: The drug solution meant for comparative evaluation with liposomal spray solution was prepared by dissolving the drug in propylene glycol: ethanol mixture and stirred continuously until clear solution was obtained.

Preparation of blank nasal spray solution: 70 % of purified water was heated at 50° C and a known amount of sodium phosphate monobasic, sodium phosphate dibasic, sodium EDTA and benzyl alcohol were added to water. With continued mixing the water soluble polymers, i.e. polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) were dissolved. Then while mixing the benzalkonium chloride 17% solution was added and mixed for at least 5 minutes and cooled to 30° C. The final volume was adjusted with water, mixed and filtered until clear solution was obtained.

Preparation of liposomal spray solution: 3ml of liposomal dispersion was incorporated in 7ml of blank nasal spray solution with continuous stirring under a magnetic stirrer upto the concentration of drug of 10mg/ml in final spray solution.

Evaluation of Developed Formulations:

In-vitro diffusion study:

In-vitro diffusion study of different formulations was performed through nylon membrane (0.22u) using Franz diffusion cell. Phosphate Buffer Saline (PBS, pH 6.4) containing PEG 400 in the ratio of 7:3 was used as the receptor medium in Franz diffusion cell.

Nylon membrane (0.22 μ) was sandwiched between the receptor compartment and donor compartment. The receptor fluid was maintained at $37 \pm 1^\circ\text{C}$ by circulating water bath. Samples (1ml) were withdrawn at time intervals, 5, 10, 15, 30, 45, 60, 120, 180, 240, 360, 480, 720 min and replaced with an equivalent amount of the medium at definite intervals. The withdrawn samples were filtered and analyzed using UV-visible spectroscopy.

Ex-vivo diffusion study:

The freshly excised goat nasal mucosa, except the septum part, was collected from the slaughter house in PBS, pH 6.4. The membrane was kept in PBS (pH 6.4) for 15 min to equilibrate identified and separated from the nasal membrane. The excised superior nasal membrane was then mounted on Franz diffusion cell. The tissue was stabilized using phosphate buffer (pH 6.4) in both the compartments and allowed to stir for 15 min on a magnetic stirrer. After 15 min, solution from both the compartments was removed and fresh phosphate buffer (pH. 6.4) was filled in the acceptor compartment. The temperature of the receiver chamber containing 22 mL of diffusion media (phosphate buffer, pH 5.0) was controlled at $37^\circ\text{C} + 1$ under continuous stirring with Teflon-coated magnetic bar at a constant rate, in a way that the nasal membrane surface just flushed the diffusion fluid. A volume of 1.0 ml of each conventional drug spray, and liposome based spray was placed in the donor compartment of Franz diffusion cell. Samples from the receptor compartment were withdrawn at predetermined time intervals and analyzed. Each sample removed was replaced by an equal volume of diffusion media. The formulations were studied in triplicate for diffusion studies and the mean cumulative values for % drug diffused versus time were plotted against time. The slopes of the graphs were used to calculate the diffusion coefficients.

Bio-adhesion Test:

This test is used to ensure that the spray solution inside the nasal mucosa remains adhered to the mucous membrane. Goat nasal mucosa was used for this study. Isolated goat nasal tissue obtained immediately after killing of goat at slaughter house was cleaned and then separated from the supporting muscular and connective tissues taking care to maintain integrity of mucosa. Goat nasal tissue was kept in Tyrode solution under refrigerated condition. The mucosa was then mounted on a glass slide in such a way that mucosal layer was on outer side. Four actuations of developed formulation mixed with methylene blue dye were sprayed on to the exposed mucosal side. Then the glass slide was kept on a glass beaker containing PBS pH 6.4 and ethanol (1:1) in

such a way that the mucosal side was in continuous contact with diffusion medium. This arrangement was subject to continuous stirring on a magnetic stirrer for 6 hours.

Histopathological studies:

Irritation potential of developed formulations was studied using Male Sprague Dawley rats between the age of 3-4 months weighing 200-250g. The animals were housed in propylene cages with free access to standard laboratory diet and water and were acclimatized for 7 days before experimentation. Rats were administered with the liposomal spray intranasally while keeping them in supine position. After 4 hrs the test and the control rat were sacrificed. The nasal mucosa of rats were excised and placed in formalin for 24 hrs for fixation. The mucosae were then studied for histopathological inflammation or damage at the site of application of formulation.

Brain Deposition study: Male Sprague–Dawley rats weighing 200–250g were anesthetized with an intraperitoneal injection of urethane (40 mg/kg) and were kept on a heating pad to maintain the body temperature. The anaesthetized rats were placed in a supine position. The thoracic region was prepared by wetting it with 70% alcohol. A skin incision made in the thoracic region was extended from the neck to the base of the anterior end of the abdomen over the occipital bone. The first layer of muscle was cut, and the atlanto-occipital membrane was exposed. The trachea was cannulated with a polyethylene tube (PE 200) to allow free breathing. All of the incisions were covered with wet gauze. For the intranasal administration, about 30 min after operation, 100 μl of the nasal formulation was administered via a PE 10 tube attached to a microlitre syringe inserted 1 cm into each nostril of rat at a dose of 4 mg/kg. For the intramuscular administration, the drug solution was delivered (dose equivalent to 4 mg/kg) through the cranial thigh muscle, volumes were between 0.46 and 0.54 ml. At 15min, 30min, 60min, 120min, 180min and 240min after the dose, the rats were euthanised and brain tissue was withdrawn by cisternal puncture. Each brain tissue was quickly rinsed with saline and blotted up with filter paper to get rid of blood-taint and macroscopic blood vessels as much as possible. The brain tissue samples were then weighed and homogenized with Tissue Homogenizer (Remi Motors) at about 100 rpm for 10 min. Resulting mixture was centrifuged at 15,000 rpm (Eppendorf 5810 R, Rotor F-45-30-11) for 20 min at a temperature of 40°C . The supernatant was filtered through 0.45 μm syringe filter and stored at -800°C until the time of analysis.

Samples were injected and the chromatographic

separations were achieved on analytical column (Kromasil C-8 (25 x 0.46 mm, 5 µm). The mobile phase was prepared using methanol, acetonitrile and millipore water in the ratio of 40:20:40 v/v at pH3 and were filtered through a 0.45 µ filter. Flow rate of mobile phase was kept at 1.5 ml/min. Drug was detected at a wavelength of 260 nm. The concentration of drug in the samples was analysed according to their area under curve and using respective straight line equation.

RESULTS AND DISCUSSION

Formulation development:

Liposomes were prepared by thin film Hydration Technique and surface modification was done under a magnetic stirrer.

Particle size and Zeta potential:

Particle size of drug loaded liposomes was in the size range 155-170 nm. The zeta potential of optimized formulation was found to be -29.77. Whereas the particle size of surface coated liposomes with chitosan was found to be in the range 174.4- 192.9 nm and zeta potential was found to be -21.66.

In-vitro drug diffusion study:

The cumulative amount of drug diffused from Drug liposomal spray across the Nylon membrane 0.25µ in 10 hrs was found to be 54.74 % while amount of drug diffused from Drug conventional solution was found to be 96.19 % at the end of 10 hours. The results demonstrated that the permeation rate and permeation coefficient of the drug from the optimized Drug liposomal spray formulation through dialysis membrane 0.25 was lower in comparison to conventional solution formulation. Thus, the Drug liposomal spray formulation exhibited sustained release of drug.

Ex vivo drug diffusion study:

Results obtained after *ex vivo* drug diffusion study using goat nasal mucosa, showed sustained release of drug from intranasal spray, 56 % of drug was diffused whereas in case of conventional gel, almost 92 % of drug was diffused within 10hrs.

Nasal Ciliotoxicity studies and irritation potential of formulation:

Minimal degree desquamation of epithelium and mild degree hyperaemia was observed. The nasal membrane remained intact substantiating the safety of excipients used in the formulation. Thus,

formulation components can be considered to be biocompatible and do not induce serious histological changes in the nasal mucosa.

Bio-adhesion test:

After magnetic stirring of 6 hours, formulation mixed with methylene blue dye sprayed to the exposed mucosal side was visible on the mucosa, confirming the bio-adhesive properties.

Brain Deposition study:

To evaluate brain targeting efficiency, after nasal dosing, statistical differences between intramuscular conventional solution, intranasal conventional solution and intranasal liposomal spray were assessed by comparison between Concentration vs AUC plots after administration of formulation intranasally. The drug concentration reaching the brain after administration of intranasal liposomal spray was significantly more as compared to conventional intramuscular administration of drug. This confirmed the definite merits of intranasally administered liposomal spray formulation of drug. It has greater potential of prolonged action and enhanced bioavailability.

CONCLUSION

The delivery of drug molecules across the nasal mucosa opens a new hope for the systemic delivery of medicaments especially to the central nervous system. Liposomes of an antipsychotic drug were prepared that exhibited good stability and entrapment efficiency. Prepared liposomes were surface modified with chitosan and mucoadhesive surface modified liposomal spray delivery system was prepared and evaluated. *In vitro* characterization was established and the developed intranasal delivery system produced desired nasal residence. The formulation showed good bioavailability. Intranasal surface modified liposomal spray exhibited higher deposition in brain tissues compared to conventional solution after intranasal and intramuscular administration indicating potential for nose to brain targeting. Thus nasal drug delivery offers a viable alternative to oral and injectable routes of administration.

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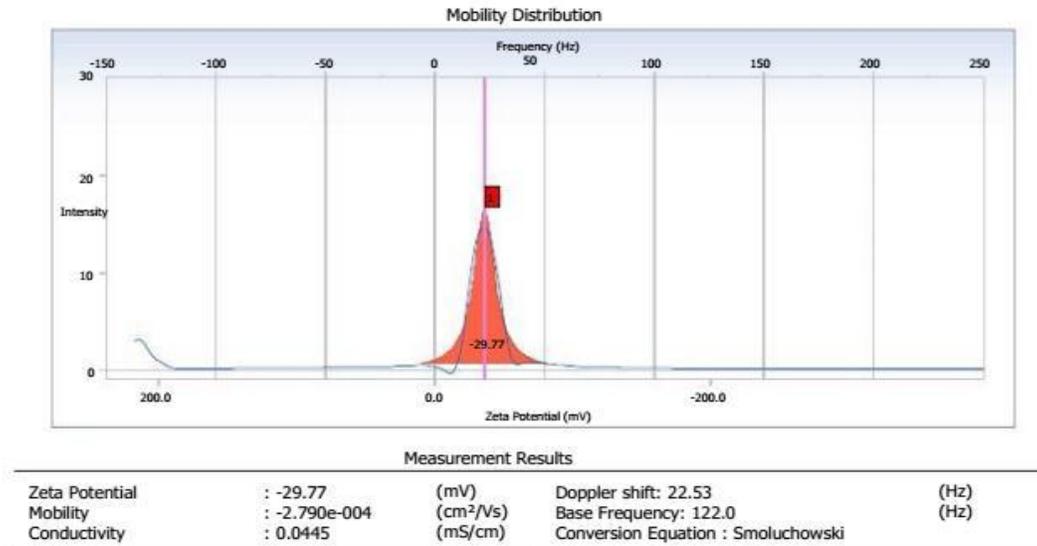


Figure 1: Graphical representation of zeta potential of optimized drug loaded liposomes

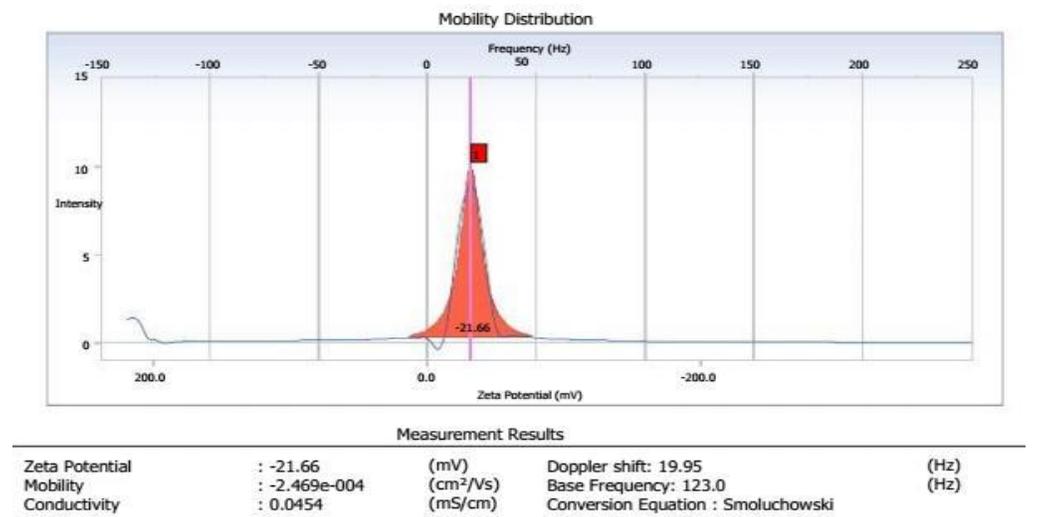


Figure 2: Graphical representation of zeta potential of surface modified liposomes



Figure 3: Formulation on goat nasal mucosa Figure 4: Mucosal surface in contact with medium

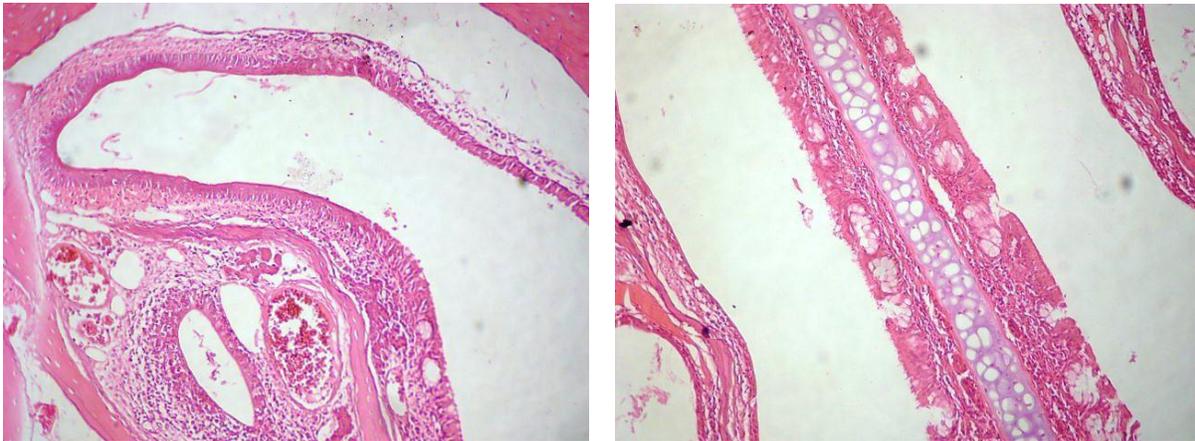


Figure 5: Photographs of goat nasal mucosa demonstrating histological characteristics when treated with 1):control (Phosphate buffer saline pH 6.4) 2):Drug liposomal spray

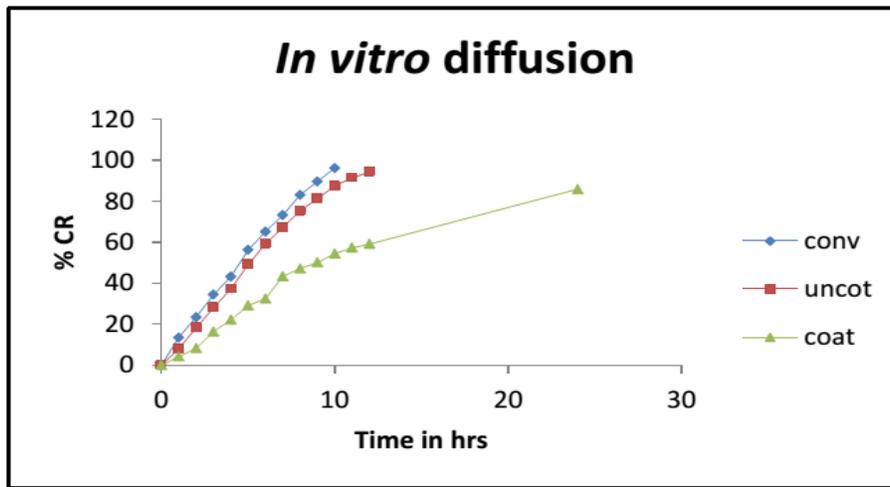


Figure 6: *in vitro* drug diffusion

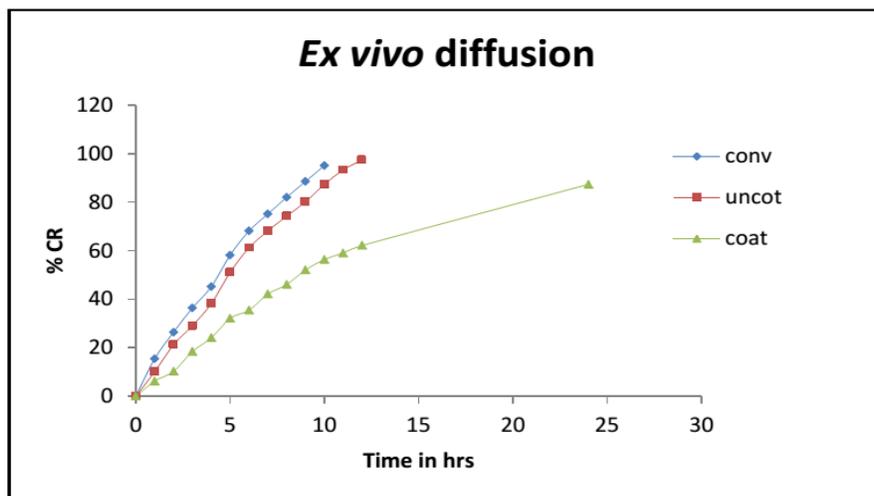


Figure 7: *ex vivo* drug diffusion

Table 1: Comparative concentration of drug in brain in ng/mg at various time points after intranasal administration of liposomal spray, intranasal administration of conventional solution and intramuscular administration of conventional solution.

Time points	Drug concentration in brain tissue in ng/mg		
	Intranasal liposomal spray	Intranasal conventional solution	Intramuscular conventional solution
	0	0	0
15	1040	760	730
30	1340	830	770
60	1310	710	660
120	960	670	620
180	810	610	580
240	730	570	540

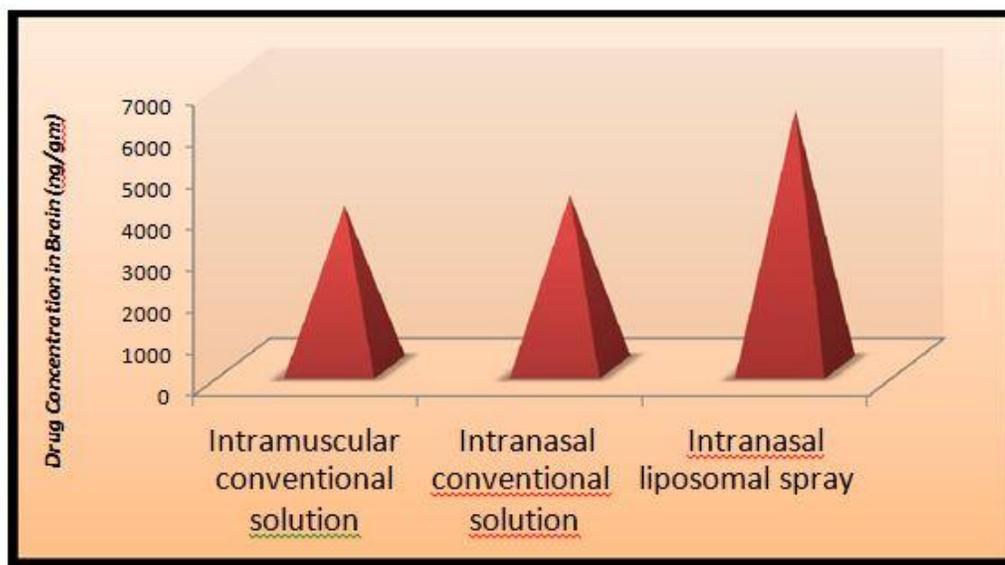


Figure 8: Comparative plot of drug reaching brain after all three modes of administration

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