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# DESIGN AND DEVELOPMENT OF ZIPRASIDONE LIPOSOMES FOR INTRANSAL ADMINISTRATION

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## ABSTRACT

*Ziprasidone is an atypical antipsychotic agent used for the treatment of schizophrenia. Sustained release of the ziprasidone liposome may reduce the frequency of administration. The blood brain barrier showed one of the difficult barriers for drug delivery. The barrier is limited for exchange of hydrophilic compounds, proteins between central nervous system and plasma. To overcome these limitations carrier system like liposomes, niosomes, microspheres, microparticles drug deliveries mainly used for brain targeting because these methods of preparation are easy, simple and easy to scale up. Intranasal drug delivery is one of the focused deliveries for brain targeting. Drugs which under goes first pass metabolism to avoid these and increases their bioavailability of drug nasal route is preferred. Liposomes are prepared by ethanol injection method and material used are cholesterol, lecithin. These liposomal preparations are characterized by FTIR and DSC analysis, pH, Drug content, vesicle size, PDI, zeta potential, % entrapment efficiency and percentage of drug release, release kinetics, ex-vivo nasal permeation studies. Optimized F4 ziprasidone liposomal formulation showed 67.6±1.97% Entrapment efficiency and 84.6±0.33% of drug release at 12$^{th}$ hour. Intranasal administration of ziprasidone liposomes significantly increased the bioavailability.*

## KEY WORDS

*Cholesterol, Ethanol, Intranasal delivery, Lecithin, Ziprasidone*

## 1. INTRODUCTION

Schizophrenia is a disorder of central nervous system followed by disordered thinking and impulsive psychological depression. In the case of schizophrenia, it requires psychological treatment along with the pharmacotherapy (Pratik Upadhyay et al., 2006). Though many antipsychotic drugs are available for treating schizophrenia, poor bioavailability and adverse drug reactions, where the major limitations.

The major limitation in the treatment of Central nervous system disorder is due to presence of protective barriers which restricts the entry of drugs in to the brain to produce significant pharmacological action. Though there are different techniques for delivering of drugs across blood brain barrier, such as change in therapeutic agent, altering barrier integrity, invasive techniques, there are various drawbacks to the above said methods (Reema Narayan et al., 2016). However, opening of the barrier, toxins and undesirable particles are enters into the central nervous system, thus resulting in damage of BBB (Kapil Kulkarni et al., 2013).

To overcome these limitations non-invasive techniques was developed. Which avoid drug transport across BBB. Intranasal route has gained popularity as a non-invasive route in treating the CNS disorder. Despite of significant popularity of oral route, there are limitations like drugs fail to achieve systemic absorption through GIT and first pass metabolism. Led to alternative investigations such

as intranasal and parenteral in order to achieve faster and higher drug absorption. Enhance results in improved bioavailability enhanced therapeutic effect and promote patient compliance. Significant enhancement in drug absorption through nasal route compare to oral delivery has been reported by many researches (Eskandan et al., 2011).

Many drugs fail to reach the brain following oral (or) parenteral administration due to the BBB, which restricts the transport of drugs from systemic circulation into central nervous system. Drugs effective against CNS disorders most pass these barriers to reach the brain. There are two approaches to delivery of drugs to brain may be invasive and non- invasive approaches (Reema Narayan et al., 2016). The invasive methods are not practical for use in human for several reasons like convenient, safety. The non-invasive techniques used for by passing the BBB, provides alternative to invasive method of drug administration. Advances in pharmaceutical technology has led to development of different drug delivery techniques, that allowed drug to be delivered through the skin, ocular, transmucosal membranes (Saranageeb EI-Helaly et al.,2017).

The important approaches bypass BBB by intranasal delivery, which provides practical, non-invasive, rapid and simple methods to deliver the therapeutic agents to CNS. Drug delivery to the brain via nasal route can be achieved as the olfactory region and trigeminal region are in directly connect with CNS and as well as outer environment (Prathik Upadhyay et al., 2006).

Nasal delivery is a convenient and effective system that provides absorption of drug into the systemic circulation which results in rapid onset of pharmacological action. To overcome the limitations of conventional dosage forms through intranasal drug delivery, colloid carriers (or) lipid-based systems are used (Reema Narayan et al.,2016).

Carrier system like liposomes, niosomes and nanoparticles are mainly used for the treatment of brain diseases because these methods of preparations are simple and easy to scale up. Liposomes are interesting candidates for brain targeting due to rapid uptake of brain, bio acceptability, bio degradability compared to the polymeric nano particles (Arumugam et al., 2008). Liposomes plays a key role in overcoming the limitation of nasal delivery like mucociliary clearance, enzymatic degradation and low bioavailability of drugs (Parthik Upadhyay et al., 2016).

## 2. MATERIALS AND METHODS

Ziprasidone was obtained as a gift sample from Hetero laboratories (India), Lecithin (Himedia Laboratories, India), Cholesterol extra pure (Burgoyne Burbidges, Mumbai), Ethanol, sodium chloride, potassium chloride, calcium chloride (Himedia Laboratories, India).

**Preparation of ziprasidone liposomes by ethanol injection method:**

Ethanol injection method suitable for encapsulation of hydrophilic drugs as small vesicles, and higher entrapment efficacy can achieve by this method. Accurately weighed amount of soya lecithin and cholesterol were taken in 10ml beaker and dissolved in sufficient amount of ethanol with slight heating (50-60$^{o}$C). Simultaneously ziprasidone was dissolving in pH 6.7 simulated nasal fluid by remi magnetic stirrer which was maintained at 1000-1200 rpm at room temperature using Teflon coated bead (Miquel pons et al., 1993). Monophasic ethanol lipid mixture was injected to drug solution kept under stirring at the rate of 0.25 ml/min using 14-gauge size needle. Aqueous phase terms milky indicates vesicle formation in order to remove the ethanol the system was kept under stirring for 2 hours. The vesicular dispersion was made up to 10 ml with SNF (pH 6.7) and dispersion was filter with grade filter to obtain stable vesicle (J.M.H. Kremer et al., 1977). The vesicles were transferred to 10 cc vials by nitrogen sparging and sealing. The prepared liposomes were stored at 4˚C until analysis (Kartik Arumugam et al., 2008).

**Table 1. Formulation of ziprasidone loaded liposomes**

| Formulation | Ziprasidone (mg) | Cholesterol (mg) | Lecithin (mg) | Ethanol (ml) | SNF buffer(ml) |
|---|---|---|---|---|---|
| F1 | 200 | 20 | 160 | 2 | 8 |
| F2 | 200 | 40 | 120 | 2 | 8 |
| F3 | 200 | 40 | 160 | 2 | 8 |
| F4 | 200 | 40 | 200 | 3 | 7 |
| F5 | 200 | 50 | 150 | 2 | 8 |
| F6 | 200 | 50 | 200 | 2 | 8 |

**3. Characterization of liposomal dispersion:**

The prepared liposomes were characterized for drug content, vesicle size, zeta potential, % entrapment efficacy, in-vitro drug release studies. All the determinations were done in triplicate average values are represented with standard deviations.

**FTIR and DSC studies**

Compatibility of drug and excipients in formulation were determined by using FTIR spectroscopy. Appropriate quantities ziprasidone, lecithin, cholesterol, physical mixture, formulation was studied to elucidate the functional group positions between the drug and other excipients. The IR spectrum of drug and excipient were recorded in the range of 4000 to 40 $cm^{-1}$ using KBR disk method (Reema Narayan et al., 2016).

Phase transition and thermotropic characteristics of pure drug and phosphatidyl choline was measured by DSC analysis. 5±2 mg average weight of samples was heated on sealed aluminum pan at temperature 20˚C-300˚C under nitrogen gas flow 30 ml/min at heating rate of 10˚C/min (Reema Narayan et al., 2016).

**Morphological studies**

Lamellarity: The formation of unilamellar vesicles were confirmed by examine liposomal suspension under inverted microscope 40x Boeco nib-100, Germany (Pratik Upadhyay et al.,2017).

Scanning electron microscope: SEM was performed to determine the morphology, size and shape of formulation. The surface morphology of the lipsomes was studied using scanning electron microscopy (Karthik Arumugam et al., 2008).

**Determination of drug content**

A weighed amount of the prepared liposomes equivalent of 10 mg ziprasidone was taken in a test tube and lysed with 9 ml methanol. The resultant solution was diluted with SNF (pH 6.7) and absorbance was measured at λ max of 319 nm using UV-Spectrophoto meter (Shimadzu, Uv-1800 Japan). The study was performed in triplicates.

**% Drug content =Observed concentration/ Expected concentration x 100**

**Determination of pH**

The pH values of ziprasidone liposomes were measured by using pH meter (cyber lab, mumbai, india).

**Determination size, zeta potential and poly dispersity index**

Vesicle size and poly dispersity index of ziprasidone loaded liposome determined by photon correlation spectroscopy (Malver instruments Ltd, UK). Analysis was carried at 25˚C and keeping the angle 90˚C. The mean vesicle size is expressed interms of diameter (nm). The size distribution of vesicles expressed inters of polydispersity index (PDI). Zetapotential measurement of carried out using the same instrument laser-Doppler electrophoresis technique. The zetapotential expressed interms of surface charge system (Malver instrument). All the measures carried out in triplicates at 25±0.1˚C (Reema naryan et al., 2016).

**Determination of % entrapment efficiency**

The free drug was separated from the liposomes by using ultracentrifugation technique. A measured value 5 ml of the liposomal dispersion was taken and centrifuged at 10,000 RPM (Hettich mikro 220r, germany) for 90 minutes at controlled temperature of 40˚C. Supernatant containing the free drug was withdrawn and absorbance was measured using Uv-spectrophoto meter at 319nm against SNF buffer. All the determinations made in triplicates. The amount of drug entrapped in liposomes were determines by following equation (Reema naryan et al., 2016).

$$\% \text{ Entapment efficiency} = (C_d\text{-}C)/C_d \times 100$$

Where $C_d$ is the concentration of total drug and C is the concentration of unentrapped drug (sara nageeb EI-helaly et al., 2017).

***In vitro* release study**

The in-vitro drug release of ziprasidone from the liposomal formulation was determined by using dialysis sac method (Himedia membrane, molecular weight cut off 10 KDa). For in-vitro drug release studies simulated nasal fluid (pH 6.7) was used. The pH of solution was mainatained between ranges of 6.2-6.8. The dialysis tube was washed and soaked in SNF for 12 hours before use. Two milli liters (2 ml) of ziprasidone liposomal dispersion was ultra centrifuged (to remove any free drug ) and the preparation was reconstituted to 2 ml using SNF. Then, one end of the tube was tied, and 1 ml of reconstituted ziprasidone loaded liposomes equivalent to 20 mg was placed in the dialysis bag and the other end was also fixed with the thread. This dialysis bag was dipped in beaker containing 25ml simulated nasal fluid maintained at 37˚c and stirred on a magnetic stirrer with 300 rpm. One ml of sample was withdrawn at each time interval of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 hours and same volume of SNF was added to the beaker to maintain sink condition. The samples were analyzed by using UV-spectrophotometer at λ max of 319 nm. The experiment was carried out in triplicates and the average percentage of drug release was calculated (Karthik Arumugam et al 2008).

**Ex- vivo nasal permeation studies**

Ex- vivo drug permeation studies were carried out by using freshly sheep nasal mucosal epithelium from slaughter house. Mucosal tissue has 0.2 mm thick, 10 mm diameter and 78.5 $mm^{2.}$ The tissue fixed in Franz diffusion cell between donor and receptor compartments (Pratik Upadhyay et al., 2016). The study was carried out using SNF (pH 6.7) as diffusion media 20 ml filled in donor compartment, and receptor compartment filled with 1 ml of ziprasidone liposomes, under mild agitation, on magnetic stirrer (300 rpm) and the temperature was maintained at 37±0.5˚C. The samples were withdrawn for 1 to 12 hours and same volume added to donor compartment to maintain sinks conditions. The samples absorbances were analyzed at 319 nm by using uv-spectrophotometer (Omidreza jafarieh et al 2014). The experiment was carried out in triplicates and the amount of drug release from tissue was calculated (Karthik Arumugam et al., 2008).

## 4. RESULT AND DISCUSSION

**FT-IR and DSC studies**

FT-IR study mainly used for investigates the possible interactions between ziprasidone and other excipients. The interpenetration may be done based on absorption bonds of the ziprasidone and excipients. The FTIR spectrum of drug excipient and optimized formulations was shown in Figure No (1, 2). There was no evident of new peaks. Hence there was expect that of drug and excipient. No interaction occurs between drug and polymer.

Phase transition observed for ziprasidone, cholesterol, lecithin and liposomal drug loaded formulation. Ziprasidone exothermic peak observed at 278.57˚c and physical mixture cholesterol and lecithin was observed at 122.77° C. Ziprasidone loaded liposomal formulation peak observed at 245.28˚c. The phase transition temperature proved stability of liposomes, drug entrapment in bilayers (or) aqueous compartments. The DSC results shows increase the entrapment efficiency of liposomal formulation (Olga Popov ska et al.,2013).

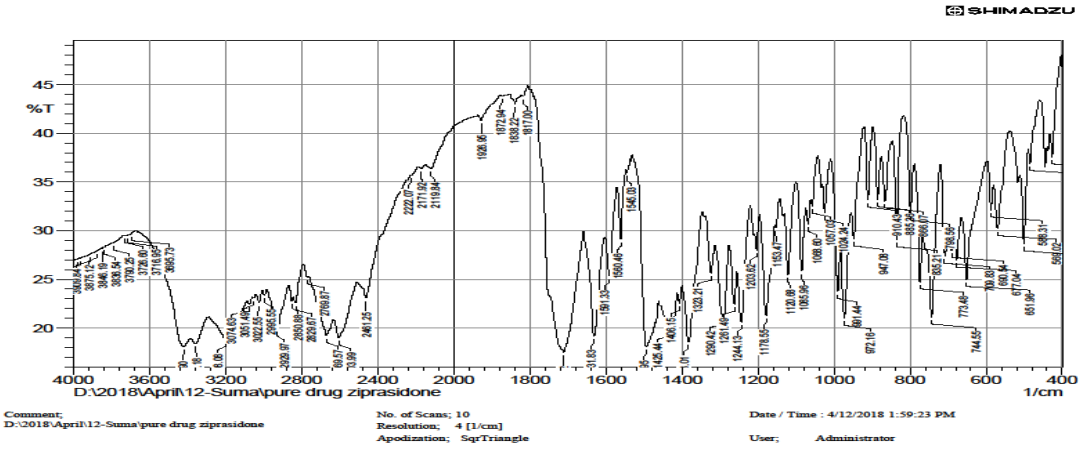


**Figure 1: FT-IR analysis of ziprasidone**

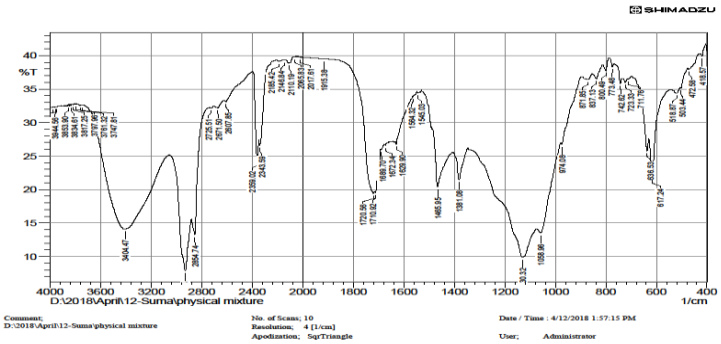


**Figure 2: FT-IR analysis of cholesterol and lecithin**

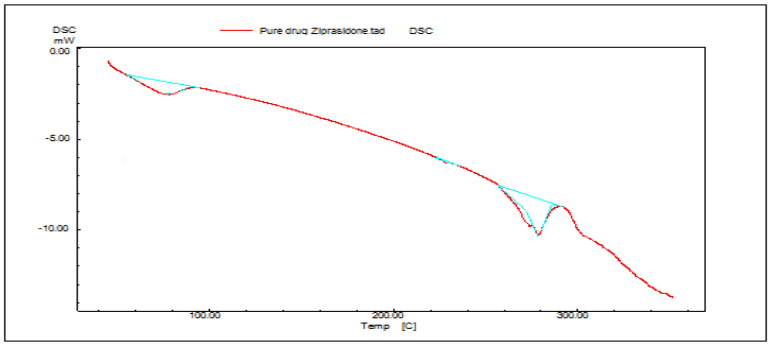


**Figure 3: Thermogram of ziprasidone loaded liposomal formulation**

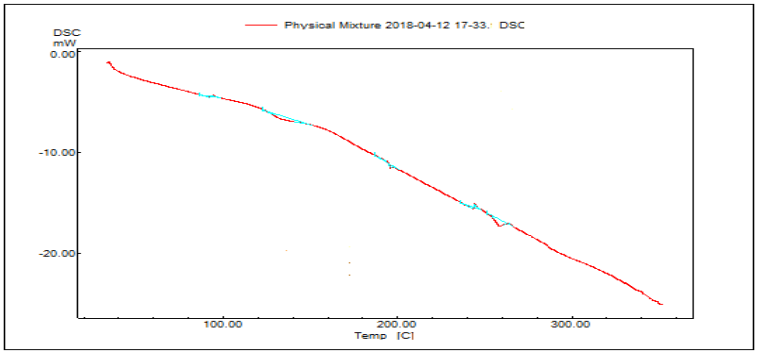


**Figure 4: Thermogram of physical mixture (cholesterol and lecithin)**

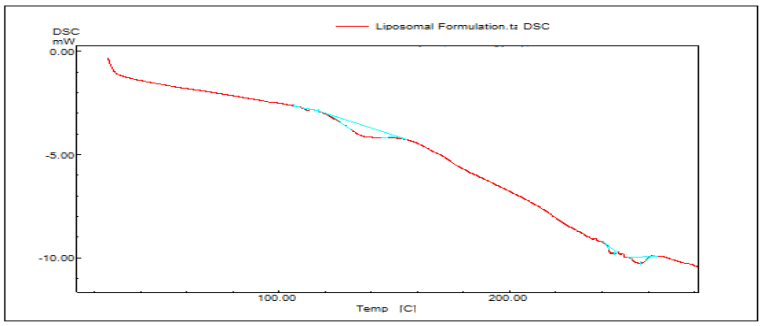


**Figure 5: Thermogram of ziprasidone drug**

**Morphological studies**

The morphological preparation of prepared liposomes investigated using optical microscope Inverted microscope and scanning electron microscope. All the prepared liposomes were spherical in shape and there was no evidence of aggregation showed that the formulated liposomes posses unilamellarity with large internal aqueous phase. The surface morphological studies using SEM (Scanning electron microscope) indicated that the vesicles exhibited smooth surface without any amorphous arrangement on the vesicle size (Kartik Arumugam et al., 2008).

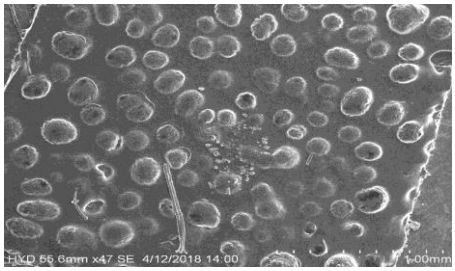

**Figure 6: Scanning electron microscopy of ziprasidone loaded liposomes shows spherical shape vesicles.**

**Table 2: Characteristics of ziprasidone loaded liposomes**

| Formulation | pH | Drug content (%) | Vesicle size (nm) | PDI | Zeta potential (mv) | % entrapment efficacy | % of drug release at 12$^{th}$ hour |
|---|---|---|---|---|---|---|---|
| F1 | 6.5±0.046 | 83.3±1.24 | 93.2±5.91 | 0.145±0.02 | -42.6±0.47 | 60.9±0.82 | 76.6±0.44 |
| F2 | 6.8±0.082 | 88.9±1.38 | 76.3±3.62 | 0.162±0.04 | -32.9 ±0.36 | 49.6±1.69 | 79.3±0.37 |
| F3 | 6.1±0.061 | 92.6±1.46 | 92.8±6.92 | 0.351±0.27 | -41.2±0.816 | 55.2±2.26 | 80.6±0.28 |
| F4 | 6.7±0.089 | 99.7±1.92 | 97.1±7.35 | 0.309±0.37 | -46.99±0.48 | 67.6±1.97 | 84.6±0.33 |
| F5 | 6.4±0.056 | 95.6±1.27 | 74.6±5.33 | 0.223±0.47 | -38.6±0.462 | 52.6±2.05 | 76.3±0.36 |
| F6 | 6.6±0.088 | 93.9±1.07 | 106.9±6.91 | 0.301±0.57 | -35.3 ±0.47 | 49.8±0.41 | 78.3±0.13 |

**Determination of pH**

The pH values of ziprasidone liposomes measured by a pH meter (Cyber lab, mumbai, india). The pH values of ziprasidone formulation ranged from 6.1 to 6.8. There was no siginicant change in pH values as a function of time for all formulation and non-irritant to nasal mucosa as the pH 6.8 of nasal cavity.

**Drug content**

All the prepared ziprasidone liposomes were performed for drug content uniformity. The drug content ranged from 83.3 to 96.7 %. From the results it could be concluded that ziprasidone drug was not lost during the formulation**.**

**Vesicle size, polydispersity index and zeta potential**

The physical stability and permeation are governed by mean vesicle size and polydispersity index of liposomes. The mean vesicle size of ziprasidone loaded liposomes ranged obtain 74.56 ± 6.9 nm to 106.7 ± 0.02 nm. The PDI was ranged from 0145 ± 0.02 suggesting that the prepared formulations were homogeneous formulations. The results of average vesicle size and PDI for the formulation are shown Table No (2).

From the results, it was observed that $F_1$ formulation containing 1:8 ratio of cholesterol and lecithin obtained 93.2±5.91 nm vesicle size. In $F_2$ formulation the ratio concentration was increased, the vesicle size decreased with 76.3±3.6 nm. Whereas as in F3 and F4 formulation, the ratio was increased to 1:4 and 1:5, the vesicle size increased 92.8±6.9 nm and 97.1±7.3 nm respectively. In $F_5$ formulation the ratio was 1:3 but the quantity of cholesterol and lecithin was increased, which lead to decreased vesicle size 74.6±5.3 nm. In $F_6$ when the ratio was increased to vesicle size increased 106.3±6.9 nm.From the results it could be concluded that the ratio as well as concentration of cholesterol and lecithin influenced the vesicle size (Kartik arumugan et al.,2018).

Zeta potential may influence adhesiveness on to the surface charge of membranes. In this study the prepared formulations showed zeta potential in range of -35.6 to -46.99 mv. The values of zeta potential showed that vesicles have sufficient charge to inhibits aggregation of vesicles due to electric repulsion which indicates the prepared vesicles are stable however no significant different, was observed in zeta potential based upon varying the ratio of ziprasidone (Reema Narayan et al.,2016)

**Determination of % entrapment efficiency**

Lipids and cholesterol concentrations are mainly effective on the % of entrapment efficiency. ziprasidone liposomal formulation can be ranges from 49.6% to 67.6%. The % drug release entrapment in liposomal formulation shown in Figure No (4.13). cholesterol, lecithin concentration is mainly influencing the % of drug entrapment. It was found that F4 formulation contains on increasing ratio of cholesterol and lecithin provides greater the % of drug entrapment compared to other formulations. It may be due to the inner core of the vesicle is large enough to coordinate with

ziprasidone liposomes. From the results it was observed that % entrapment efficacy with the increasing amount of molar ratio of cholesterol and lecithin (Reema Narayana et al.,2014).

***In vitro* drug release of liposomal formulations**

**Table No 3*: In vitro* drug release of ziprasidone formulations**

| Time (hrs) | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| 0 | 0 | **0** | 0 | 0 | 0 | 0 |
| 0.5 | 2.6±0.08 | 9.7±0.04 | 1.91±0.014 | 7.36±0.04 | 4.3±0.22 | 11.4±0.12 |
| 1 | 10.7±0.37 | 13.6±0.5 | 7.6±0.26 | 10.93±0.09 | 5.8±0.21 | 18.6±0.47 |
| 2 | 25.3±0.85 | 34.3±0.47 | 19.19±0.14 | 21.1±0.62 | 14.1±0.12 | 27.6±0.44 |
| 4 | 42.3±1.65 | 51.5±0.41 | 42.7±0.37 | 35±0.09 | 39.2±0.202 | 45.4±0.37 |
| 6 | 58.8±1.68 | 67.9±0.14 | 61.1±0.67 | 45.3±0.41 | 44.5±0.412 | 63.8±0.28 |
| 8 | 67.3±2.40 | 72.7±0.51 | 65.3±0.42 | 68.4±0.41 | 67.2±1.35 | 69.1±0.33 |
| 10 | 75.7±0.55 | 76.9±0.42 | 71.6±0.41 | 80.4±0.24 | 74.3±0.49 | 73.9±0.36 |
| 12 | 76.6±0.62 | 79.3±0.14 | 80.6±0.24 | 84.6±0.41 | 76.3±0.14 | 78.3±0.13 |

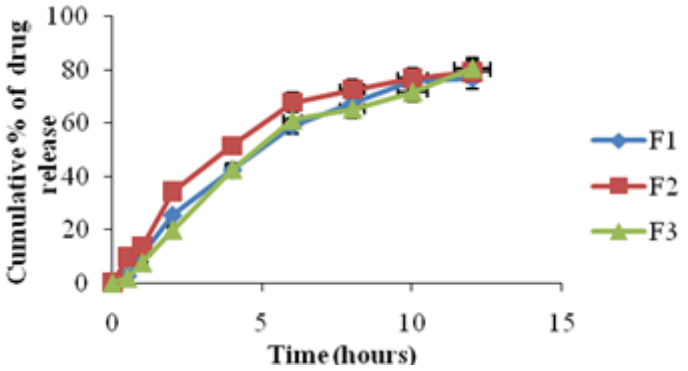


**Figure 7: Represent the cumulative % drug release of ziprasidone liposomes from F1 to F3 liposomal formlulation**

*In vitro* drug release studies of the ziprasidone loaded liposomes were done by dialysis sac method as described in chapter 3. The in-vitro drug release profile of liposomal formulations was performed, and the results shown in Table No (3). It was observed that from F1 to F6, The in vitro % drug release in the range of 11.9±0.12 % to 84.6±0.4%. Among all the six batches, F4 formulation showed the maximum cumulative % drug release. i.e. could be concluded that liposomal formulation showed sustained drug release. The lipid and cholesterol ratio have a direct impact on the drug release behavior. Depending upon the properties of cholesterol and lecithin used the release profile of liposomal extended to 12hrs. It can be concluded that liposomal formulation with 20% cholesterol Containing is beneficial for efficient encapsulation but achieve sustained release behavior, 40% cholesterol showed better results (Omidreza jafarieh et al., 2014).

***Ex-vivo* permeation study**

The *ex-vivo* study done for selected $F_4$ formulation. In this study isolated sheep nasal mucosa used. The drug diffusion was maximum of 69.1±1.72 % for formulation $F_4$ containing 1:4 ratio of cholesterol and lipid. The % of drug permeated for ziprasidone solution and ziprasidone loaded liposomes is presented in results shown in Table No (4.5). The % of drug permeated from ziprasidone solution was 97.6±1.24 % with in 8$^{th}$ hour, where as ziprasidone loaded liposomes showed 69.1±0.47 % of drug permeated through nasal mucosa at a time period of 12 hours. From the results it can be concluded that the release pattern was sustained release. It may be drug embedded within the lipid causing strong adhesion of formulation to be mucosal surface (Sara nageeb EI- helaly et al., 2017).

**Table No 4: *Ex-vivo* nasal permeation study**

| Time (hrs) | Ziprasidone drug solution | F4 (Ziprasidone liposomal formulation) |
|---|---|---|
| 0 | 0 | 0 |
| 0.5 | 29.3±1.69 | 8.6±0.23 |
| 1 | 36.3±2.31 | 12.2±0.81 |
| 2 | 48.2±2.67 | 23.6±0.94 |
| 4 | 67.6±3.47 | 37.2±1.41 |
| 6 | 83.9±0.49 | 45.6±2.82 |
| 8 | 97.6±1.24 | 61.5±2.45 |
| 10 | - | 64.6±4.18 |
| 12 | - | 69.1±0.47 |

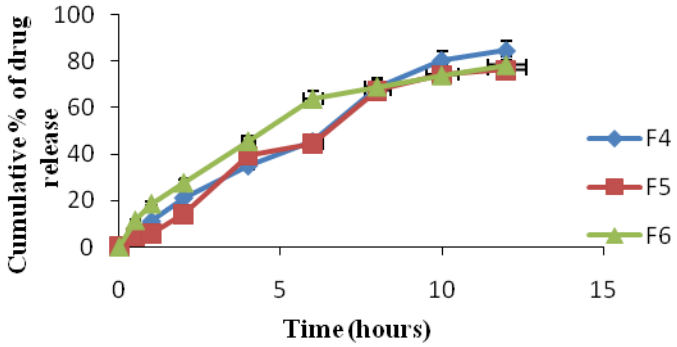


**Figure 8: Represent the cumulative % drug release ziprasidone liposomes from F4 to F6 liposomal formulation**

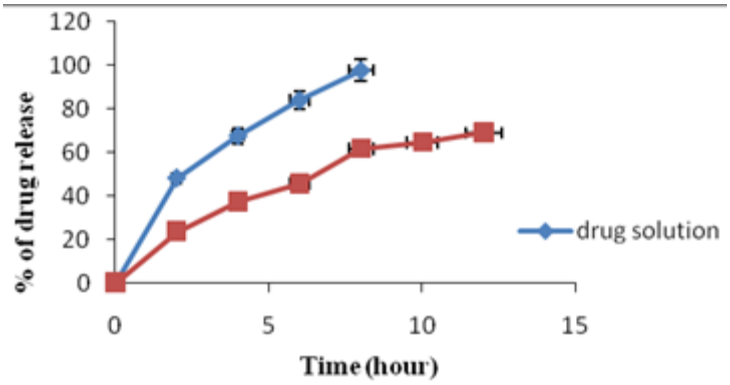


**Figure 9: *Ex-vivo* drug release of optimized ziprasidone liposomal formulation (F4)**

## CONCLUSION

Ziprasidone loaded liposomes were prepared successfully using ethanol injection method. The various formulation variables like lipid and cholesterol were formed to effect liposomal characteristics like vesicle size, entrapment efficiency, *in-vitro* drug release profile. The prepared liposomes were characterized for shape and structure using inverted microscope. The morphological studies revealed liposomes exhibited spherical and smooth surface area with unilamellarity. The entrapment efficiency was found to be influenced by proportion of lipid and cholesterol used. The in-vitro drug release study developed the formulation extended for drug release over 12 hours, which could be useful for controlled drug delivery. The *in-vitro* drug release fitted into first order kinetics. FTIR and DSC studies revealed that there were no interactions between drug and excipients. i.e it can be concluded the ziprasidone loaded liposomes are suitable for treatment of schizophrenia. Optimized (F4) ziprasidone liposomes can be further studied for pharmacokinetics and pharmacodynamics studies in suitable animal models. Bio distribution to brain can be carried out in suitable animal models.

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