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ORIGINAL RESEARCH

Self-Assembling Lecithin-Based Mixed Polymeric Micelles for Nose to Brain Delivery of Clozapine: In-vivo Assessment of Drug Efficacy via Radiobiological Evaluation

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Purpose: The research objective is to design intranasal brain targeted CLZ loaded lecithin based polymeric micelles (CLZ- LbPM) aiming to improve central systemic CLZ bioavailability.

Methods: In our study, intranasal CLZ loaded lecithin based polymeric micelles (CLZ- LbPM) were formulated using soya phosphatidyl choline (SPC) and sodium deoxycholate (SDC) with different CLZ:SPC:SDC ratios via thin film hydration technique aiming to enhance drug solubility, bioavailability and nose to brain targeting efficiency. Optimization of the prepared CLZ-LbPM using Design-Expert® software was achieved showing that M6 which composed of (CLZ:SPC: SDC) in respective ratios of 1:3:10 was selected as the optimized formula. The optimized formula was subjected to further evaluation tests as, Differential Scanning Calorimetry (DSC), TEM, in vitro release profile, ex vivo intranasal permeation and in vivo biodistribution.

Results: The optimized formula with the highest desirability exhibiting (0.845), small particle size (12.23±4.76 nm), Zeta potential of (−38 mV), percent entrapment efficiency of > 90% and percent drug loading of 6.47%. Ex vivo permeation test showed flux value of 27 µg/cm².h and the enhancement ratio was about 3 when compared to the drug suspension, without any histological alteration. The radioiodinated clozapine ([^{131}I] iodo-CLZ) and radioiodinated optimized formula ([^{131}I] iodo-CLZ-LbPM) were formulated in an excellent radioiodination yield more than 95%. In vivo biodistribution studies of [^{131}I] iodo-CLZ-LbPM showed higher brain uptake (7.8%± 0.1%ID/g) for intranasal administration with rapid onset of action (at 0.25 h) than the intravenous formula. Its pharmacokinetic behavior showed relative bioavailability, direct transport percentage from nose to brain and drug targeting efficiency of 170.59%, 83.42% and 117% respectively.

Conclusion: The intranasal self-assembling lecithin based mixed polymeric micelles could be an encouraging way for CLZ brain targeting.

Keywords: clozapine, soy lecithin, self-assembling polymeric micelles, intranasal route, brain targeting, radioiodinated clozapine, ^{131}I

Introduction

Clozapine (CLZ) is an antipsychotic drug, atypical class, which is FDA approved for the treatment of schizophrenia, a chronic mental disorder which is described by thinking, emotions and behavior distortions.[1] Based on recent guidelines recommendation, CLZ is the only drug which can manage treatment-resistant schizophrenia (TRS).[2,3] TRS, is the failure to respond to at least two sufficient trials of antipsychotic drugs, which has a probability of 30% to affect the schizophrenic patients.[4] CLZ treatment has many outcomes as the majority of patients had reported better compliance, mood, satisfaction, better quality of life. In addition to several enhanced consequences including a reduced rate of hospitalization and mortality by lowering the suicidal behavior accompanied with TRS.[5]

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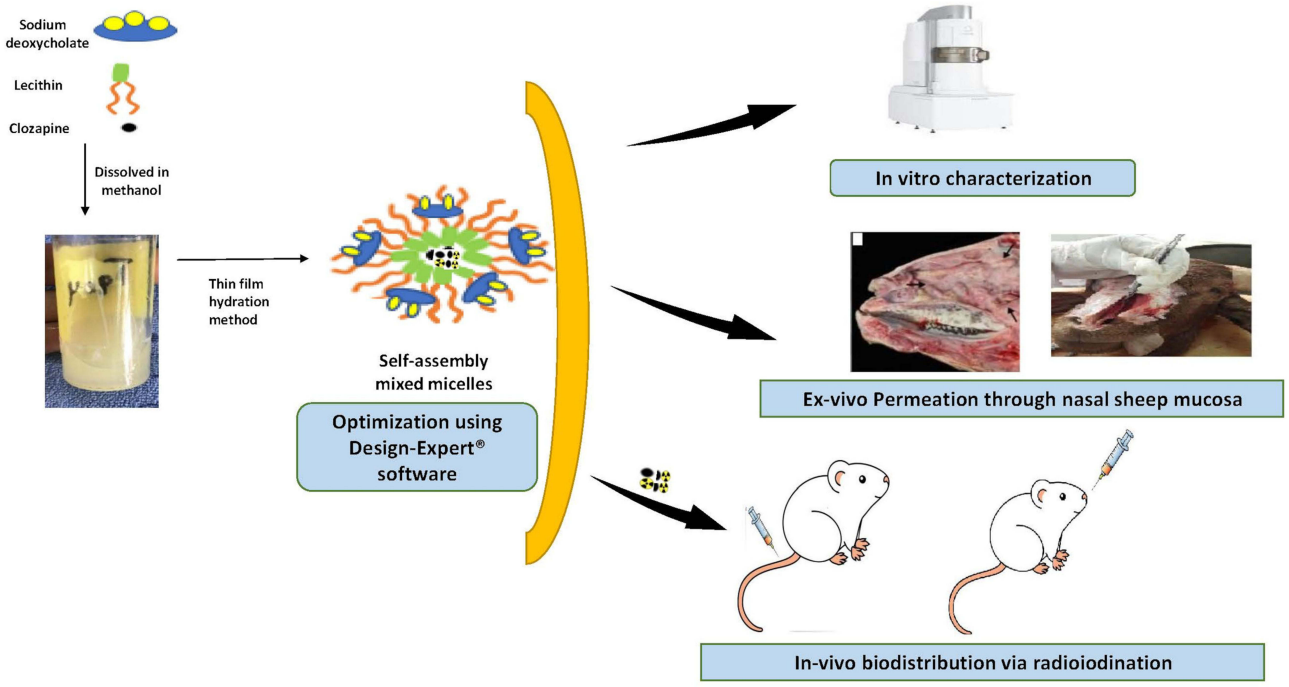
Graphical Abstract



Some receptors such as dopamine and serotonin have a vital role regarding the neurotransmission and any variation in their functions will result in many psychiatric diseases such as schizophrenia.[6] CLZ belongs to dibenzodiazepines group, which is considered as a multireceptorial antipsychotic drug due to its affinity to many receptors as dopamine "D_1, D_2 and D_4", serotonin "5-HT_{1A} and 5-HT_{2A}", norepinephrine, acetylcholine, and acetylcholine histamine receptors. The major action of CLZ was achieved through its binding effect to dopamine and serotonin receptors. D_1 receptors help regulate the neurons development, motor activity, leaning, and memory. The functions of D_2 and D_4 are impulse control, attention, and cognition.[7] CLZ has more affinity to D_1 and D_4 than D_2 in addition to fast dissociation from it which helps in lowering the incidence of extrapyramidal symptoms.[8,9] EPS, is also termed as drug-induced movement disorders, which describe the side effects results from treatment by dopamine receptor blocking agents, usually antipsychotic drugs, which are often used to control psychosis, especially schizophrenia. The side effects include involuntary movements such as bradykinesia, tardive dyskinesia, or akathisia.[10] Concerning serotonin receptors, CLZ increases the dopamine release through binding to 5-HT_{1A} receptor.[6]

Clozapine is a yellow powder with a crystalline state, low solubility in water but highly permeable so according to Biopharmaceutical Classification System (BCS) it belongs to class II. It is a lipophilic drug having massive first pass metabolism following its oral administration which results in poor bioavailability ranged from 27 to 50%.[11]

Recently many nasal CLZ nano preparations have been developed; nanosized binary mixed micelles intranasal gel was developed using poloxamer/polysorbate which improved both drug permeation rate through nasal sheep mucosa and system stability.[12] Patel et al formulated CLZ nanosuspension for nose to brain delivery which demonstrated higher CLZ concentration and faster absorption in the brain compared with CLZ suspension.[13] Another approach for intranasal CLZ appeared in the formulation of polymeric nanomixed micelles using Tetronic® 904/Synperonic® PE/F127, the optimized formula was radiolabeled by ^{99m}Tc to ensure the direct delivery of CLZ from nose to brain and high bioavailability of the drug in the central nervous system (CNS).[14]

In recent years, a considerable attention has been directed towards the use of intranasal (I.N.) route for CNS drug delivery. Intranasal drug delivery provides numerous advantages over oral or intravenous (I.V.) administration, such as

patient convenience, large surface area for absorption, low enzymatic availability, rapid onset of action, increase in drug bioavailability through skipping the hepatic first pass metabolism, lower in systemic side effects as well as direct nose to brain drug delivery.[15,16]

The anatomy of the nasal cavity contributes to the absorption and penetration of the drugs. The nasal cavity consists of four regions: Olfactory Region, Respiratory Region, Atrium and Vestibule. The permeability of drugs through Atrium and Vestibule is moderate and very poor, due to lower vascularization compared to olfactory and respiratory regions respectively. Olfactory Region has high potential for nose to brain drug permeation due to its high vascularization nature offering a direct route of connection between nose and brain compartment.[17]

Micelles are considered an attractive way to deliver drugs especially hydrophobic one with poor water solubility and bioavailability.[18] Micelles' formation occurs following amphiphilic molecules self-assembly, whose structure is characterized by the presence of hydrophobic center (core) and hydrophilic region. The hydrophilic part of micelles makes them water soluble which facilitates the delivery of hydrophobic drugs after encapsulating them in the hydrophobic core. Mixed micelles are formed from a combination between different amphiphiles resulting in micellar aggregates with small particle size which overcome the solubility limitation by one polymer in the traditional single micelles but maintain the advantages of single micelles.[19]

In our research, mixed micelles were formulated of soy lecithin or soy phosphatidylcholine (SPC) and sodium deoxycholate (SDC). In aqueous medium, they show self-assembly characteristic and play a crucial role in drug transport.[20] SPC structure is a mix between phospholipid and phosphatidylcholine (PC) as a major component (up to 98% w/w). SPC is characterized by lipophilic-hydrophilic balance, assembly and solubilization properties.[21] Using alone, SPC forms unstable vesicles due to bulky hydrophobic lipid tails which hinder its solubility in aqueous medium.[22] Furthermore, SDC belongs to anionic bile salts group, being an amphiphile which differs in structure in having polar and non-polar faces not head and tails. Accordingly, SDC facial structure results in forming unusual micelles with low aggregation number when used to form single micelles.[22] The formation of self-assembly lecithin-based polymeric micelles (LbPM) offered the advantages of combined SPC and SDC giving rise to higher therapeutic activity supported by the presence of SPC lipid layer which encapsulates more of CLZ, in addition, marked increase in the formulation stability through SDC hydrophilic part. This combined effect is more pronounced than using single micelles of either SPC or SDC alone.[20,23]

Consequently, our study aims to formulate of intranasal CLZ-LbPM to overcome all drawbacks arising from administration of CLZ in traditional dosage forms. Furthermore, in vitro characterization, ex vivo permeation and in vivo radiolabeling biodistribution studies of radioiodinated formulations were conducted on the optimum polymeric micelle formula.

Experimental

Materials

Clozapine was a kind gift from COPAD Pharma (Cairo, Egypt). Bile salt sodium deoxycholate (SDC) and Soy lecithin (SPC) were purchased from Sigma Chemical Company. Acetonitrile HPLC grade and dialysis tubing cellulose membrane (molecular weight cut-off 12,000 g/mole) were procured from Sigma Aldrich (St. Louis, MO, USA). Methanol, sodium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate were acquired from El Nasr pharmaceutical company (Cairo, Egypt). Iodine-131 (^{131}I) was a gift from Radioisotope Production Facility (RPF), Egypt. The whole other reagents were of analytical grade.

Methods

Study Design

A 3^2 factorial design was applied to prepare CLZ- LbPM formulae (M1-M9) via Design-Expert® software Version 11 (Stat-Ease Inc., MN, USA). The predictor variables were (A) SPC at three levels and (B) SDC at three levels. The levels were expressed as (−1, 0, +1) (Table 1). The examined dependent variables were: particle size (PS), zeta potential (ZP), percent encapsulation efficiency (EE%) and percent drug loading (DL%), (Y1, Y2, Y3, and Y4, respectively).

Table 1 A 3^2 Full Factorial Design Used in the Formulation of CLZ-LbPM Formulae

	Level		
	Low (−1)	**Medium (0)**	**gHigh (1)**
Factors (independent variables)			
A: SPC (mg)	10	20	30
B: SDC (mg)	50	100	150
Responses (dependant variables)		Constraints	
Y1: PS (nm)		Minimize	
Y2: ZP (mV)		Maximize	
Y3: EE %		Maximize	
Y4: DL %		Maximize	

Preparation of CLZ- LbPM

The technique of thin film hydration was utilized for preparation of CLZ- LbPM. In a prearranged weight ratio; the CLZ, SPC and SDC were added to 10 mL methanol in 1 L round-bottom flask. The mixture was sonicated for one min and then allowed to be evaporated by rotary evaporation (Rotavapor, Heidolph VV 2000, Burladingen, Germany) by the mean of low pressure revolving at 120 rpm for 10 min at 60 ±0.5°C, whereby a thin dry film was formed on the inner wall of the rotating flask. About 10 mL of distilled water was added to hydrate the thin film self-assembly leading to micelle formation and the round flask was let to rotate at steady hydration temperature of 60°C ±0.5°C for 30 min under normal pressure.[23] A 0.22 µm filter was used to remove the non-capsulated CLZ aggregates from the micellar solution. Table 2 presents the different prepared CLZ- LbPM composition.

In-Vitro Characterization of CLZ- LbPM

Micellar Particle Size (PS), Polydispersity Index (PDI) and Zeta Potential (ZP) Determination

Assessment was carried out by diluting 1mL of each dispersion with distilled water (10X). Then the formulae PS, PDI and ZP were detected by photon correlation spectroscopy (PCS) via a Zetasizer Nano ZS-90 instrument (Malvern Instruments, Worcestershire, UK). All measurements were repeated in triplicates (n=3).[24]

Percent Encapsulation Efficiency (EE%) and Percent Drug Loading (DL%) Determination

One mL of the filtrate dispersion was diluted by methanol then CLZ concentration was determined spectrophotometrically

Table 2 Composition of the Prepared CLZ-LbPM Formulae

Formula Code	CLZ (mg)	SPC (mg)	SDC (mg)
M1	10	10	50
M2	10	20	50
M3	10	30	50
M4	10	10	100
M5	10	20	100
M6	10	30	100
M7	10	10	150
M8	10	20	150
M9	10	30	150

(Shimadzu, model UV-1601 PC, Kyoto, Japan) at λmax 292 nm.[25,26] The EE% and DL% were calculated according to equations 1 and 2, respectively:[27,28]

$$EE(\%) = \frac{\text{weight of the drug in micelles}}{\text{weight of the feeding drug}} \times 100 \quad (1)$$

$$DL(\%) = \frac{\text{Amount of drug encapsulated in micelles}}{\text{Total weight of micelles}} \times 100 \quad (2)$$

Statistical Analysis and Optimization of the Prepared CLZ- LbPM Formulae

The design analysis was performed using Design-Expert software. Each measurement result was done in triplicates and represented as mean ± standard deviation (SD).[29] The significance of the results was assessed by one-way ANOVA test via SPSS program (IBM SPSS statistics, virgin 22) ($p < 0.05$ indicated significance). The optimized formula was selected using Design-Expert software by choosing the formula obtaining the greatest desirability. The desirability was determined by the maximum EE%, DL% and ZP, in addition to minimum PS.

In-vitro Characterization of the Optimized CLZ- LbPM

Differential Scanning Calorimetry (DSC)

Determination of thermal properties of powdered CLZ, SDC, SPC and CLZ- LbPM was conducted through each sample (2 mg) heating in aluminum pan at a heating rate of 10 °C/min with 25 mL/min inert nitrogen flow at temperature range of 0 °C to 250 °C using Shimadzu differential scanning calorimeter (DSC-50, Kyoto, Japan).[30]

Transmission Electron Microscopy (TEM)

The optimized CLZ- LbPM formula was appropriately diluted ten times with distilled water followed by one drop of the optimized formula deposition on a negatively stained copper grid with 2% w/v phosphotungstic acid and allowed to be dried at temperature of 25±0.5 °C. Morphological examination was carried out by TEM (JEOL, Tokyo, Japan).[14,31]

In-vitro Release Study

The release of CLZ from the optimized formula was examined via dialysis bag technique. Two mL (containing 2mg CLZ) of the optimum CLZ- LbPM formula were transferred into a cellulose dialysis bag (molecular weight cut off 12,000–14,000 Da) in 50 mL phosphate buffer saline PBS (pH 7.4) as a release medium in amber glass bottles. The bottles were placed at 37±0.5 °C using a shaker with controlled thermostatic and a shaking rate of 100 strokes/minute. Samples were withdrawn at prearranged time intervals (0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h) and assayed spectrophotometrically at λmax of 292 nm compared with PBS (pH 7.4) as a blank. The pulled out samples were substituted by the addition of same volume of the freshly prepared release medium.[14]

pH Evaluation

The pH of the optimized formula was measured by pH meter (Mettler Toledo, Columbus, OH, USA). The prepared formula was placed in beaker of 10 mL then the pH was measured at room temperature.[32] All measurements were done in triplicates (n=3).

Effect of Storage

The optimized formula was maintained in a sealed glass vial and its stability was assessed for three months at (4°C –8°C).[33] The assessment included physical appearance, EE%, DL%, PS, % drug release (6 h), and % drug release (24 h). All evaluation tests were done in triplicates (n=3).

Ex-vivo Characterization of the Optimized CLZ- LbPM Formula

Sheep Nasal Mucosa Isolation

The Research Ethics Committee accepted the animal study protocol, (code PI 2983), Faculty of pharmacy, Cairo University, Egypt (REC-FOPCU). The head was freshly obtained from 55 kg of one 1-year-old sheep which was gained

from a local slaughterhouse (Cairo, Egypt). Removal of the mucosa is preferred to be within 10 min after sacrifice to make the tissue viable during the experiment. The longitudinal cut was applied across the lateral part of the nasal wall, to get freshly excised nasal sheep mucosa. Then with caution, the nasal membrane was detached, cleansed and put in ice-cold Ringer's solution.[14]

Histopathological Studies

Histopathological examination was executed to study the effect of the optimized CLZ- LbPM formula on the integrity of the nasal sheep mucosa and the probability of the cytotoxic effect. First, the anterior and posterior mucosal sections of nasal cavity were detached. Each section split into segments and randomly divided into 2 groups. The mucosa of the negative group treated with two mL of PBS pH 7.4, while the positive group treated with two mL of the optimized CLZ- LbPM formula.[34] After 2 h, each treatment was washed away from the tissues with distilled water and preserved for 24 h in saline solution: formalin (90:10) v/v.[14,35]

Specimens were washed by tap water and serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Xylene was used to clear the specimens then specimens were inserted in paraffin in hot air oven at 56 °C for 24 h. Paraffin bees wax tissue units were prepared at 4 microns' thickness using sledge microtome. On glass slides the tissue sections were collected then deparaffinized. Assessment was done using light electric microscope (Leica, Cambridge, UK) after staining the tissue sections by hematoxylin and eosin.[14]

Ex-vivo Permeation Investigation

The ex vivo permeation studies were investigated for the optimized CLZ- LbPM formula and pure CLZ suspension using Franz diffusion cell. The Franz diffusion cell divided into receptor and donor and compartments where the nasal sheep mucosa (permeation area of 1 cm^2) was fixed into the donor one. The optimized CLZ- LbPM formula (Each one mL formula containing 1 mg CLZ) was charged into the donor compartment while the receptor compartment composed of methanolic phosphate buffer saline pH 7.4 (40:60% v/v) and the whole system were kept at 37 ± 0.5 °C.[14,36,37] At listed time points (1, 2, 3, 4, 6, 8, 10, 12 and 24 h), one mL sample was withdrawn from the receptor compartment and replaced with the same volume of fresh medium. The cumulative amount of the drug permeated was determined using sensitive HPLC method.[14]

The HPLC system involved Agilent Model Number: G1311A (Quaternary Pump) connected to Agilent Model Number: G1311A (UV Detector). The study was carried out utilizing C18 column, having the following properties; 4 mm internal diameter, 250 mm length with 5 µm particle size. The mobile phase consisted of acetonitrile: 10 mM potassium dihydrogen orthophosphate (using *o*-phosphoric acid for pH adjustment to 3.0): in ratio 35:65, v/v at a flow rate of 1 m/min with detection peak of 292 nm.

Determination of ex vivo permeation profile was done by plotting CLZ cumulative amount (µg/ cm^2) that permeated via nasal sheep mucosa versus time (h). Flux value after 24 h, J (µg/cm^2.h) was calculated as the CLZ quantity permeated through nasal sheep membrane having an area of 1 cm^2 per unit time. The slope of the linear section of the curve was used for flux value (J) calculation according to the following equation:[38]

$$J = \frac{\text{Amount permeated}}{\text{A.T.}} \tag{3}$$

where T is the permeation time and A is the surface area of the used membrane.

Enhancement ratio was assessed by dividing the flux of the optimized formula by the flux of CLZ suspension as follows:[39]

$$ER = \frac{\text{J of the formulation}}{\text{J of CLZ suspension}} \tag{4}$$

In-vivo Characterization of the Optimized CLZ- LbPM Formula

There are different methods of radiological technique to evaluate the in-vivo biodistribution as the drug radiolabeling,[40] radiolabeling of the optimized formula[14] or using radiolabeling indicator.[41] Merging between the radioisotopes (imaging

or therapy) and nanoformulations to formulate nano radiopharmaceutical become an urgent necessity to be used as a theragnostic agent.[42]

Preparation of Radioiodinated Clozapine ([^{131}I] Iodo-CLZ)

CLZ radioiodination was performed using the oxidizing agent chloramine-T (CAT)[43] with 10 µL of Na^{131}I (30 MBq). The parameters with an impact on the radioiodination yield were investigated, such as CLZ concentration (0.2–1.6 mg/mL), CAT concentration (50–250 µg/mL), pH range (3–10) and the reaction duration (5–60 min). 0.1 N sodium hydroxide or 0.1 N hydrochloric acid solutions were used to adjust the pH of the reaction in the range of 3–10 and the reaction volume was completed to 1 mL. Each parameter examination was repeated three times, and the data differences were estimated using one-way ANOVA test. The significance (*P*) of the results was stated at <0.05, and all data were reported as mean ± standard deviation (SD).

The determination of ([^{131}I] iodo-CLZ) radioiodination yield was assessed using ascending paper chromatography with Whatman paper no. 1 (Whatman International Ltd, Maidstone, Kent, UK). The reaction mixture (1–2 drop) was placed 2 cm above the lower edge of a paper strip (1-cm width and 13-cm length) and allowed to evaporate spontaneously. The strip was developed in an ascending manner in a closed jar, and a fresh mixture of chloroform: methanol 3:1 (v/v) was used as a mobile phase, where the free radioiodide (I^-) at R_f = 0.1–0.2, while radioiodinated clozapine moved with the solvent front (R_f = 0.9). The paper strip was detached after development then dried, divided into 1 cm pieces, and counted in a NaI (Tl) γ-scintillation counter. The ratio of radioactivity of [^{131}I] iodo-CLZ to total activity multiplied by 100 was used to calculate the percentage of radioiodination yield.

Preparation of [^{131}I] Iodo- CLZ- LbPM Formula

[^{131}I] iodo-CLZ-LbPM was formulated using thin film hydration method previously discussed but with replacing CLZ by ([^{131}I] iodo-CLZ). The radioiodination yield of [^{131}I] iodo-CLZ-LbPM was evaluated by paper chromatography to confirm the in vitro stability of the optimum formula.

Biodistribution Study in Mice

The protocol of animal studies was approved by both animal ethics committee of Faculty of Pharmacy, Cairo University, Egypt (REC-FOPCU) (PI 2983), and Egyptian Atomic Energy Authority (EAEA) Committee (PI 216/2022). The biodistribution study was in accordance with the guidelines set out by the EAEA animal ethics committee. The optimized formula biodistribution and pharmacokinetics parameters were determined by evaluation of the biological results in normal male Swiss albino mice after I.N. and I.V. administration of ([^{131}] iodo-CLZ) drug solution and ([^{131}I] iodo-CLZ-LbPM).

The experimental animals (normal mice weighing 25–30 g) were divided into three groups: Group I for I.N. administration of radioiodinated clozapine drug solution (([^{131}I] iodo-CLZ-DS), Group II for I.V. injection of ([^{131}I] iodo-CLZ-LbPM) via mice tail vein and Group III for I.N. administration of [^{131}I] iodo-CLZ-LbPM. Each group consisted of eighteen mice, three mice for each time point (5 min, 0.25, 0.5, 1, 2, and 4 h). All the mice groups were assimilated under maintained nutritional and environmental conditions throughout the study time.

A volume of different radioiodinated formulations (10 MBq) having ~ 64 µg CLZ (equivalent to 2.1–2.6 mg/Kg body weight) was administrated in each group. I.N. administration was administered into the nostril openings of mice using a Hamilton syringe with a 0.1 mm inner diameter polyethylene tube at the site of delivery, Mice were placed in an inclined position during administration to allow them to inhale the solution.[44]

Mice were anesthetized, weighed and dissected at pre-indicated time points. The blood was taken by cardiac puncture while the other organs including the brain were separated and cleaned from blood and any attached tissue using normal saline. The blood and organs were weighed and counted by a NaI (Tl) γ-ray scintillation counter.[45] Samples of muscles, bone and blood were weighed as their complete separation is impossible so it can assume to be 40, 10 and 7% of the total mice mass, respectively to detect their total weight.[46] The percent of administrated or injected dose per gram (% ID/g) of organ or blood per each time point were calculated by the following equation:[47]

$$\%\ \mathrm{ID/g} = \frac{\text{Activity of tissue or fluid or organ} \times 100}{\text{Total injected activity} \times \text{Weight of tissue or fluid or organ}} \quad (5)$$

Phoenix® WinNonlin® 6.4 (Certara, L.P, St. Louis, MO) was used to assess the pharmacokinetics behavior of the three radioiodinated formulations. The following parameters were determined; the maximum [^{131}I] iodo-CLZ uptake % of administrated dose per gram (%ID/g) for blood or brain equivalent to C_{max} and time to reach C_{max} is T_{max}. The area under the concentration-time curves from zero to 4 h (AUC_{0-4} h%ID/g) and area under the curve from zero to infinity ($AUC_{0-\infty}$ h%ID/g).

The relative bioavailability of intranasal [^{131}I] iodo-CLZ-LbPM in comparison to [^{131}I] iodo-CLZ-DS was calculated using the following equation:[48]

$$\text{Relative bioavailability}\ \% = \frac{(AUC_{\text{LbPM}0-\infty})\text{IN}}{(AUC_{\text{solution}0-\infty})\text{IN}} \times 100 \quad (6)$$

Drug targeting efficiency (DTE) and drug targeting index (DTI) can be presented from the potency of brain targeting for [^{131}I] iodo-CLZ-LbPM formula after the intranasal administration,[49,50] and nose-to-brain direct transport percentage (DTP).[51] DTE stands for time average partitioning ratio of the drug between brain and blood and can be assessed according to the following equation:

$$\text{DTE}\ \% = \frac{AUC_{\text{brain I.N.}}}{AUC_{\text{blood I.N.}}} \times 100 \quad (7)$$

Determination of DTI can be done through the following equation:

$$\text{DTI} = \frac{(AUC_{\text{brain}}/AUC_{\text{blood}})\,\text{I.N}}{(AUC_{\text{brain}}/AUC_{\text{blood}})\,\text{I.V}} \quad (8)$$

Where AUC_{brain} is the area under the curve for CLZ concentration in brain from zero to 4 h while AUC_{blood} is the area under the curve for CLZ concentration in blood from zero to 4 h.

The percentage of the drug transported via trigeminal and olfactory pathway directly to the brain can be expressed as DTP (direct nose to brain transport), and the calculation through this equation:[52]

$$\text{DTP}\ \% = \frac{B_{IN} - B_X}{B_{IN}} \times 100 \quad (9)$$

Where $B_{I.N.}$ is the sum of brain $AUC_{(0-4)}$ after I.N. administration and B_x is a fraction of the brain $AUC_{(0-4)}$ supplied by the systemic circulation via the BBB following the I.N. administration and was determined according to Equation 10:

$$B_X = \frac{B_{IV}}{P_{IV}} \times P_{IN} \quad (10)$$

Where B_{IV} is the brain $AUC_{(0-4)}$ after I.V. administration, P_{IV} is the blood $AUC_{(0-4)}$ following I.V. administration and P_{IN} is the blood $AUC_{(0-4)}$ following I.N. administration.

Statistical examination for the three determinations was done by one-way ANOVA test via SPSS program (IBM SPSS statistics, virgin 22), and the significance limit was appointed as ($p<0.05$).

Results and Discussion

Analysis of 3^2 Factorial Design

A Factorial design was implemented to evaluate the effect of CLZ:SPC: SDC variable ratios in three levels (Table 1) on the characteristics of CLZ-LbPM formulae regarding their P.S, ZP, E.E%, and DL% respectively (Table 3). Each factor was tested separately and fitted to different order models. To determine the significance of each response, ANOVA was applied by setting the level of significance to be 5%, in another words if $p<0.05$, the model is considered significant.[53] Analysis of the adjusted and predicted R^2 for each response is shown in Table 4 together with the adequate precision for all responses which indicates the model adequacy and discrimination.

Table 3 Measured Parameters of the Prepared CLZ-LbPM Formulae

Formula Code	PS (nm)	PDI	ZP (mV)	EE%	DL%
M1	17.4 ±1.47	0.20 ±0.03	−26.50 ±2.21	75.00±1.68	14.43±0.91
M2	20.12±0.66	0.22±0.06	−27.40±2.50	86.92±0.21	11.90±0.30
M3	25.66±9.10	0.31±0.01	−30.50±1.53	95.57±0.13	08.11±1.45
M4	10.39±0.81	0.23±0.02	−33.45±3.21	78.42±1.11	08.40±1.06
M5	11.20±1.89	0.32±0.01	−34.15±4.20	85.00±2.03	06.55±1.23
M6	12.23±4.76	0.24±0.02	−38.60±2.10	93.00±0.05	06.47±0.15
M7	26.12±1.94	0.41±0.06	−34.30±1.40	71.15±0.35	05.38±0.34
M8	29.23±5.10	0.48±0.03	−35.75±3.21	86.26±1.30	04.79±1.40
M9	34.23±0.32	0.43±0.07	−41.70±1.53	91.50±0.15	04.81±1.82

Table 4 Output Data of the 3^2 Factorial Design Analysis CLZ-LbPM

Responses	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	Significant Factors & Probability (*p* value)
PS	0.9956	0.9912	0.9777	38.768	A (0.0052), B (<0.0001)
ZP	0.9938	0.9877	0.9689	35.683	A (0.0006), B (<0.0001)
EE %	0.9340	0.9120	0.8514	12.932	A (0.003)
DL %	0.8679	0.8239	0.7028	8.869	B (0.0023)

Note: A is SPC amount, B is SDC amount in the total polymer (w/w).

In-vitro Characterization of the Prepared CLZ- LbPM

Micellar Particle Size (PS), Polydispersity Index (PDI) and Zeta Potential (ZP) Determination

Particle size has a direct impact on the physical stability, drug release from micelles, cellular uptake and biodistribution, so PS is considered as a crucial parameter. ANOVA results in Table 4 displayed that the two factors A and B (SPC and SDC), respectively, had a significant effect on the PS (p=0.0052, <0.0001), respectively.

As displayed in Table 3 and Figure 1a that increasing the SPC amount from 10 to 30 mg resulted in increasing the PM size, this is due to the increase in phospholipid lipophilic content of SPC consisting of 15% Phosphatidyl choline (PC), 10% Phosphatidylinositol (PI), 11% Phosphatidylethanolamine (PE) and 4% Phosphatidic acid (PA),[54] leading to an increase in the hydrophobic core of micelles allowing more drug to be entrapped and thereby increase in PS.[23,55]

Also, factor B (amount of SDC) (Figure 1a) had significant impact on PS, as SDC being negatively charged due to the presence of cholate group in its structure, in addition, SPC composed of linoleic (C18), oleic (C18), palmitic (C16) and α-linolenic (C18) acids account for 55, 17, 16, and 7%, respectively, of the total fatty acids[54] constituting the head hydrophilic groups which by increasing SDC concentration, will increase the repulsion between the micelles resulted in an increase in PS of the formed polymeric mixed micelles.[33]

Polydispersity index was used for size distribution evaluation when being close to 0. The formulae PDI values ranged from (0.20 ±0.03 to 0.48±0.03) (Table 3) which gives a good indication regarding size similarity.[33]

ZP is a good indicator of stability of the prepared formulae, usually the system is considered stable when having ZP value nearby ±30 mV.[56] ZP values was from (−26.50±2.21 to −41.70±1.53) which explained that all formulations had been charged enough that prevent particles aggregation. Both factors A and B had a significant effect on ZP (p=0.0006, <0.0001) (Table 4) (Figure 1b and c). By increasing the SPC amount, the ZP increased because soy lecithin is an

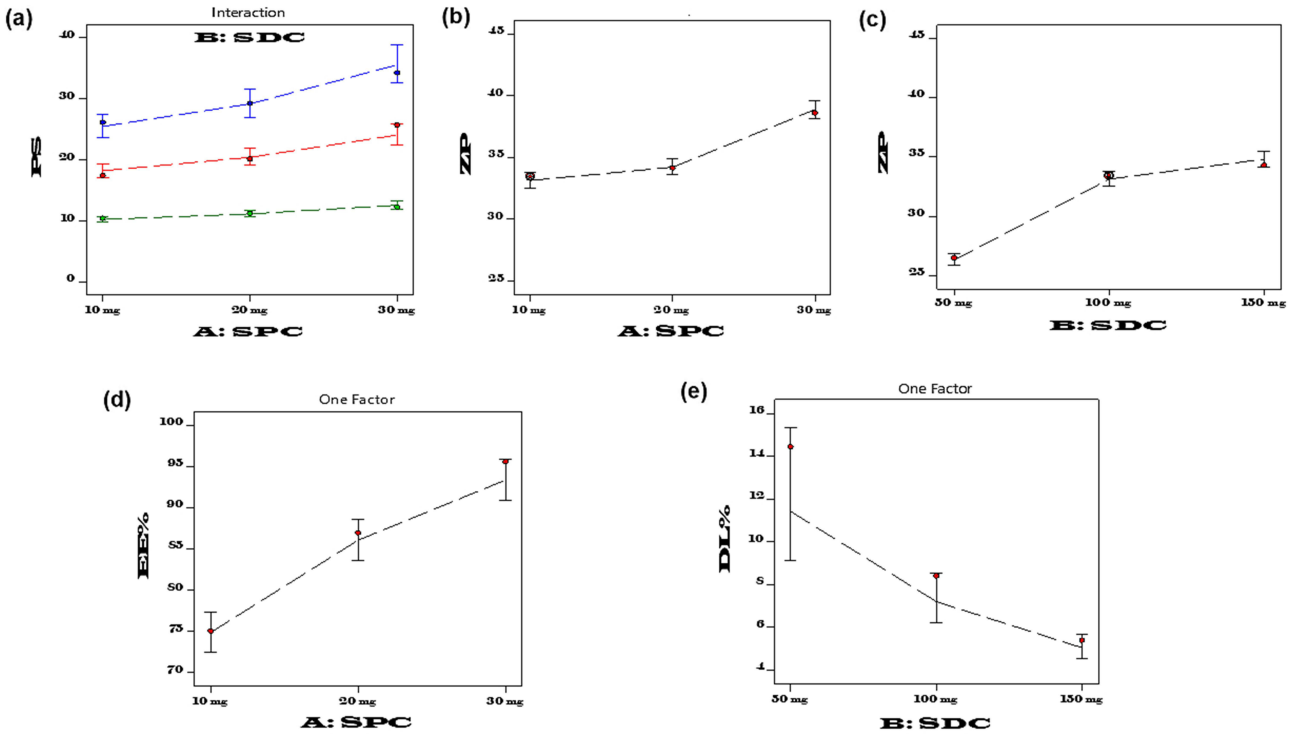


Figure 1 Response plots of the effect of variables (SPC and SDC amount) on the studied responses (**a**): PS, (**b** and **c**): ZP, (**d**): EE%, and (**e**): DL%.

ampholytic surfactant which contains amino and phosphate groups, phosphate groups negative charges led to the increase in ZP.[57] SDC has already a negative charge, so by increasing its ratio resulted on induction of negative charges on the mixed micelles, The presence of high negative charge will increase the repulsion between the leading to further decrease in their aggregation resulting in less particles growth and more system stability.[58]

Percent Encapsulation Efficiency (EE%) and Percent Drug Loading (DL%)

Encapsulation efficiency is the percentage of the drug successfully entrapped within the mixed micelles. As shown in Table 3, the EE% ranged from (71.15±0.35 to 95.57±0.13%). Results of ANOVA factorial revealed that SPC had a positive significant effect on EE% (p=0.0003) (Table 4) (Figure 1d). This might be due to the increase in the hydrophobic core volume of micelles which encapsulate and solubilize higher amount of CLZ.[23] Another reason is the presence of the lipophilic benzodiazepine aromatic ring in CLZ which facilitates the entrapment of CLZ within the lipophilic core of micelles.[59]

Regarding the increase in bile salt (SDC) concentration, no significant effect on EE%. This might be due to the increase in lipid bilayer fluidization resulting in more drug loss.[33]

Drug loading is defined as the amount of solubilized CLZ in the mixed micelles from the initial amount of CLZ and polymers. Table 4 and Figure 1e showed that SDC amount had a negative significant effect on %DL (p=0.0023). The increase in bile salts concentration led to micelles lipid bilayer fluidization and entrapped drug loss.[33]

Optimization of the Prepared CLZ- LbPM

Numerical optimization was performed using Design-Expert software for desirability determination, taking into account the significant factors only. The choice criteria of the optimized formula with maximum desirability were based on maximum ZP (Y2), EE% (Y3) and DL% (Y4) in addition to minimum PS (Y1) (Table 1). The optimized formula M6; CLZ:SPC: SDC with the ratio of 1:3:10 mg was chosen as the optimized formula gaining the highest desirability of 0.845 and was subjected for further investigations as will discussed below.

In-vitro Characterization of the Optimized CLZ- LbPM Formula

Differential Scanning Calorimetry (DSC)

Figure 2 shows the pure CLZ, SDC, SPC and the optimized formula (M6) DSC patterns. The DSC pattern of pure CLZ showed an endothermic peak at 184.4 °C which give confirmation of the crystalline state and melting point presentation.[14] Concerning the DSC thermograms of SDC and SPC, an exothermic peak at 214 °C[60] and 165°C[33] corresponding to their melting points respectively. The disappearance of the featured endothermic peak of CLZ indicated that the drug was completely entrapped within the formed lecithin based polymeric micelles formula.[14]

Transmission Electron Microscopy (TEM)

TEM gives a confirmation of PS obtained from Malvern Zetasizer (Figure 3a).[61] The M6 morphology is demonstrated in Figure 3b, which indicated that the morphology of the optimized CLZ- LbPM showed spherical, self-assembled and well distributed shape.

In-vitro Release Study

The release profile of CLZ from M6 is shown in Figure 4 compared with CLZ suspension having the same drug load (1 mg/mL). It is clear that the release of CLZ from M6 and CLZ suspension showed cumulative amount of CLZ released up to 93.235 and 20.36% at the end of 24 h, respectively. This might be attributed to many reasons; First, the solubilizing effect of CLZ within the hydrophobic core of the formed micelles resulted in higher released amount of CLZ and sustain its release from M6.[52] Second, SPC is a penetration enhancer which improves CLZ release from M6.[62]

Figure 4 shows that CLZ release from LbPM demonstrates a biphasic release model with initial fast release within the first 8 h followed by sustained release up to 24 h. The initial burst might be the result of rapid CLZ diffusion from PM surface while, the sustained release phase might be attributed to the hydrophobic interaction between the drug and the hydrophobic PM core.[52]

pH Evaluation

The pH factor affects the mucociliary action as any deviation in the pH may cause irritation to the nasal mucosa. The pH of the optimized formula was 5.3±0.24 which is close to the nasal secretions pH (4.5–6.5)[63] ensuring that the formula not irritant to the nasal mucosa.

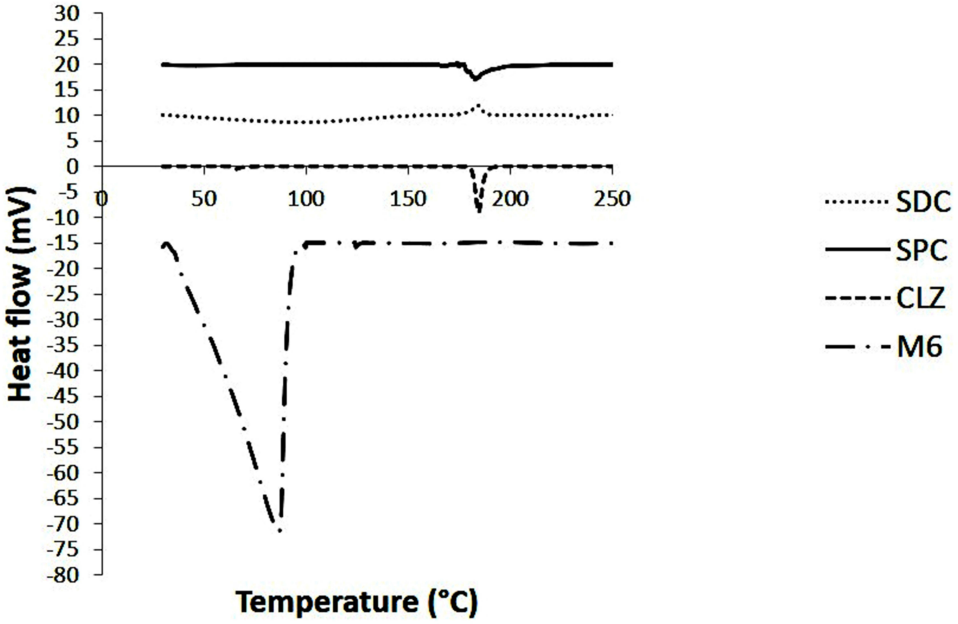


Figure 2 DSC thermograms of CLZ, Sodium deoxycholate (SDC), Soy phosphatidyl choline (SPC) and optimum CLZ-loaded LbPM (M6).

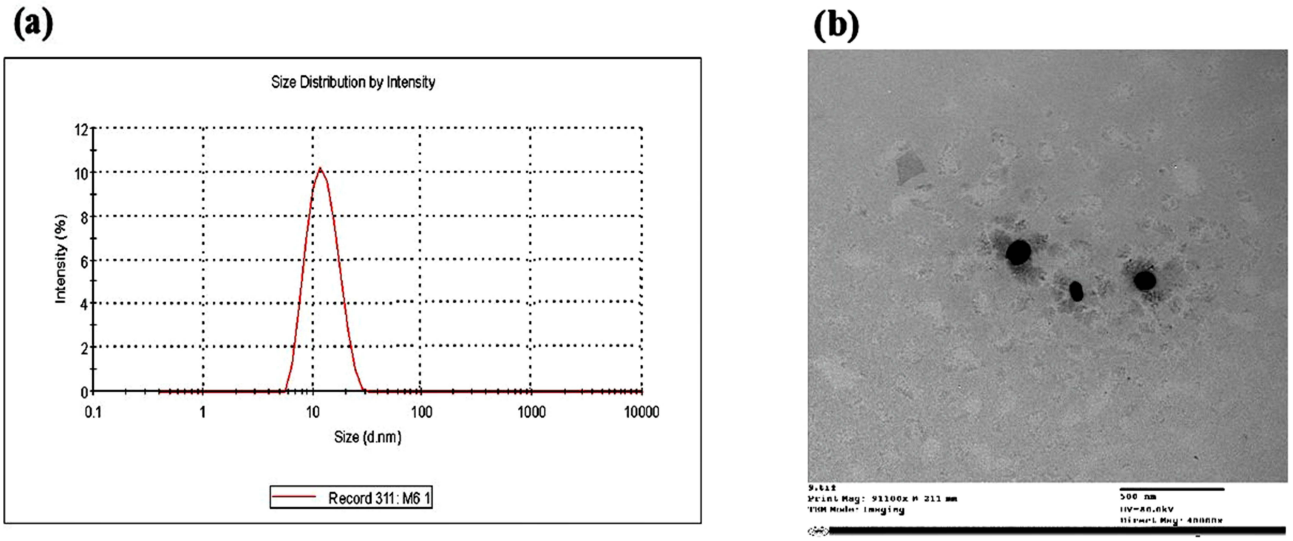


Figure 3 (**a**) Particle size distribution of the optimum CLZ-loaded LbPM formula (M6) and (**b**) transmission electron micrograph of the optimum CLZ-loaded LbPM formula (M6).

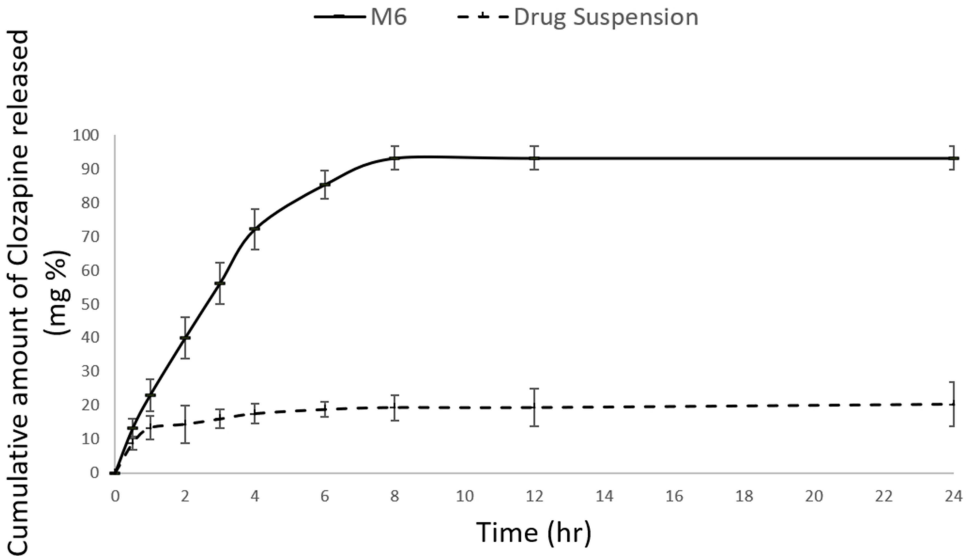


Figure 4 In-vitro cumulative release profiles of optimized CLZ-LbPM formula (M6) and CLZ suspension.

Physical Stability

The optimized formula (M6) had been stored for three months at (4–8°C). After storage period, no significant change (*p*>0.05) in its appearance, PS, ZP, EE%, DL%, % drug release (6 h), and % drug release (24 h) when compared with the freshly prepared M6 (Table 5). One-way ANOVA test ensures that no crucial change between the fresh and stored optimized formula (M6) after three months of storage.

Ex-vivo Characterization of the Optimized CLZ- LbPM Formula

Histopathological Studies

Examination of any alteration in nasal sheep mucosa was thru comparing both negative and positive groups following treatment by PBS PH 7.4 and M6 respectively. Figure 5a and b demonstrates that no significant change regarding the histopathological structure of the anterior parts of the positive group compared to the negative one. Concerning the

Table 5 Effect of Storage on the Physical Properties of the Optimized CLZ-LbPM Formula (M6)

Parameters	Fresh	After 3 Months of Storage at (4°C –8°C).
PS (nm)	12.23±4.76	15.12±1.31
ZP	−38±2.10	−40±1.31
EE %	93±0.05	91±0.15
DL %	6.47±0.15	6.12±2.10
% Drug release (6 h)	85.32±4.26	82.56±1.15
% Drug release (24 h)	93.20±3.53	91.53±2.35

posterior parts, no signs of damage, inflammation, epithelial mucosa alteration nor irritation were found in both group segments. (Figure 5c and d).

These results were complied with Sayed et al who examined the local toxicity effect of CLZ from the optimized CLZ polymeric nanomixed micelles formula on nasal sheep mucosa using PBS (pH 7.4) as a negative control confirming that the selected formula can be applied safely with no changes in the nasal sheep mucosa histopathological structure.[14]

Permeation Through Nasal Sheep Mucosa

Ex-vivo permeation experiment was done through nasal sheep mucosa due to its similarity with human nasal mucosa regarding both morphological and histological structures.[64] CLZ suspension was used as a control to confirm the privilege of M6 over drug suspension in ex-vivo permeation. Figure 6 shows that the permeation of CLZ from M6 was significantly higher compared to drug suspension containing same amount of CLZ (1mg/mL) after 24 h.

Regarding the steady state flux of M6, it had higher significance level ($p<0.05$) than CLZ suspension flux (26.7 µg/cm².h and 9.4 µg/cm².h), respectively. The enhancement ratio was about 3, suggesting that the nasal uptake from M6 was achieved in higher extent compared to drug suspension confirming the superiority of polymeric mixed micelles formula in the improvement of ex-vivo permeation. The nanosized formula with high solubilizing power allowing large surface area for drug permeation, thereby facilitating the direct passage of CLZ from the nasal membrane to the brain. Also

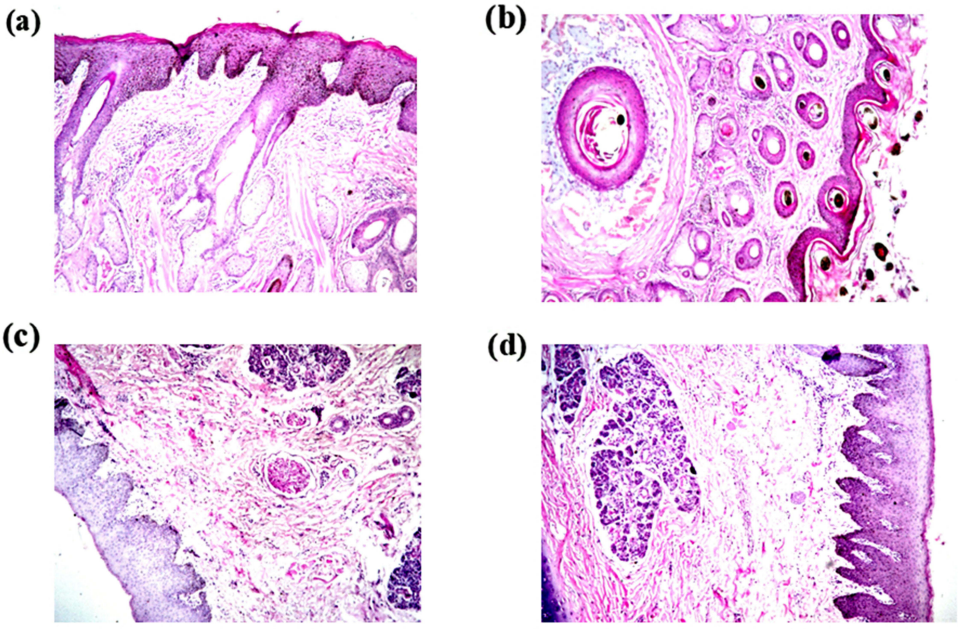


Figure 5 Photomicrographs showing histopathological sections of the anterior parts of sheep nasal mucosa treated with PBS PH 7.4 (negative control, **a**) and M6 (optimized CLZ-loaded LbPM, **b**), And the posterior parts of sheep nasal mucosa treated with PBS PH 7.4 (negative control, **c**) and M6 (CLZ-loaded LbPM, **d**).

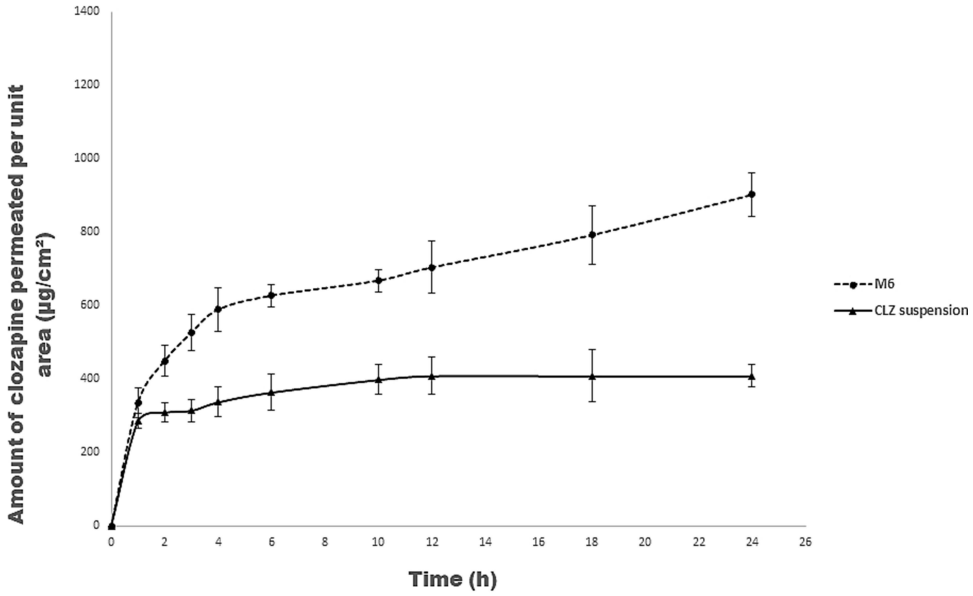


Figure 6 Ex-vivo permeation profiles of the optimized CLZ-loaded LbPM formula (M6) and CLZ suspension through nasal sheep mucosa.

permeability results were in agreement with Saleh et al outcomes, who formulated Zolmitriptan as intranasal surfactant-based elastic vesicular drug carrier systems showing higher permeability rate, percent of drug permeated and steady state flux compared to Zolmitriptan control formulation.[65]

In-Vivo Characterization of the Optimized CLZ- LbPM Formula

Preparation of ([^{131}I] Iodo-CLZ)

The [^{131}I] iodo-CLZ was formulated via direct electrophilic substitution reaction with iodine-131 under oxidative conditions in the presence of CAT.[66] All reaction parameters were tuned to improve radioiodination yield, and the results are graphically depicted in Figure 7A–D. The highest radioiodination yield (96.4 ± 0.29%) was achieved at CLZ concentration (1mg/mL), 150 µg/mL of CAT, pH 7 and 15 min as reaction time.

Preparation of [^{131}I] Iodo-CLZ- LbPM Formula

The [^{131}I] iodo-CLZ-LbPM was formulated in an excellent radioiodination yield more than 95% with in-vitro stability up to 8 h that is suitable for biodistribution study.

Biodistribution Study in Mice

The radioiodinated formula was administrated to all mice groups (I, II and III) as follows: I.N. [^{131}I] iodo-CLZ-DS, I.V. [^{131}I] iodo-CLZ-LbPM and I.N. [^{131}I] iodo-CLZ-LbPM, respectively. The [^{131}I] iodo-CLZ concentration uptake in brain and blood in addition to ratio of brain/blood were determined at predesigned time intervals up to 4 h that is equivalent to the CLZ concentration in organ or fluid (Table 6). Figure 8a clarified the maximum amount of [^{131}I] iodo-CLZ reaching the brain from I.N. [^{131}I] iodo-CLZ-LbPM mice group (7.8%± 0.1% ID/g at 0.25 h) with significant faster brain uptake compared to (I.N. [^{131}I] iodo-CLZ-DS and I.V. [^{131}I] iodo-CLZ-LbPM) (3.8% ± 0.18% ID/g at 0.5h and 2.3% ± 0.1% ID/g at 2h), respectively, which proves the intranasal brain targeting potency of the optimized formula (M6).

Figure 8b showed the blood results obtained from the three groups, accordingly the highest [^{131}I] iodo-CLZ concentration in blood was attained from the I.V. [^{131}I] iodo-CLZ-LbPM (14.9%±1.6% ID/g at 5 min) because the drug was directly delivered to the blood circulation, followed by I.N. [^{131}I] iodo-CLZ-DS (8.1%± 0.7% ID/g at 1 h) and finally I.N. [^{131}I] iodo-CLZ-LbPM (2.5%± 0.3% ID/g at 0.5 h), respectively. Concerning brain/blood ratio of the three radioiodinated formulations, I.N. [^{131}I] iodo-CLZ-LbPM has the maximum brain/blood ratio (6.00± 0.53 at 0.25 h)

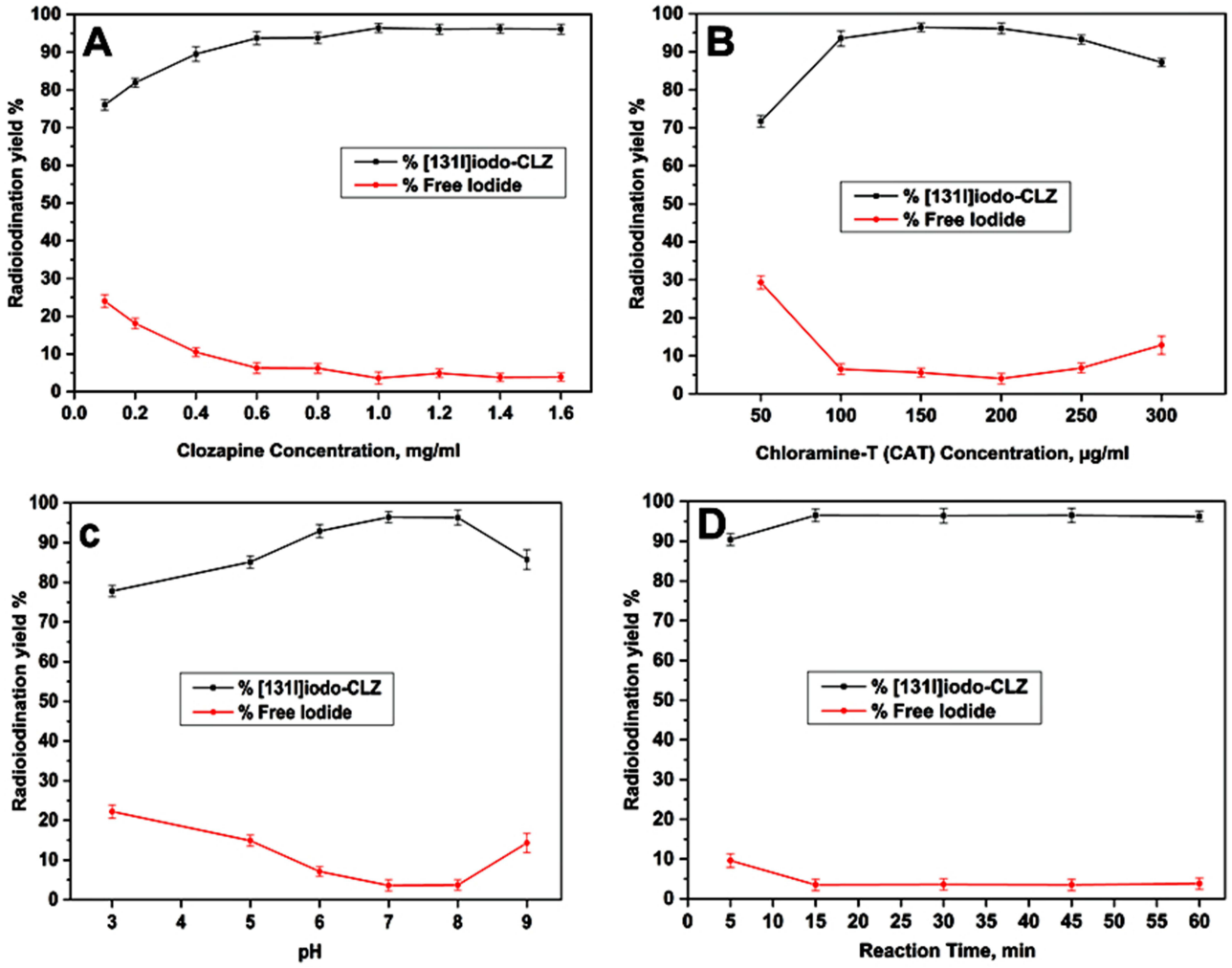


Figure 7 Variation of radioiodination yield of radioiodinated clozapine ([^{131}I] iodo-CLZ) at different reaction conditions: (**A**) Clozapine Conc., (**B**) Chloramine-T (CAT) Conc., (**C**) pH and (**D**) Reaction time.

compared to I.N. [^{131}I] iodo-CLZ-DS and I.V. [^{131}I] iodo-CLZ-LbPM (0.49± 0.04 at 0.5 h and 0.05± 0.01 at 0.25 h), respectively.

Therefore, we can conclude that I.N. [^{131}I] iodo-CLZ-LbPM can target the CLZ brain delivery efficiently on account of higher brain uptake %, brain/blood ratio and lower blood uptake % in comparison with I.N. [^{131}I] iodo-CLZ-DS and I.V.

Table 6 Brain/Blood Distribution of CLZ Administration as Intranasal [^{131}I] Iodo-CLZ-LbPM, Intranasal [^{131}I] Iodo-CLZ-DS, and Intravenous [^{131}I] Iodo-CLZ-LbPM in Male Swiss Albino Mice (Mean ±SD, n=3)

Formulation/Route of Administration	Organ or Tissue	Time					
		5 Min	0.25 h	0.5 h	1 h	2 h	4 h
I.N. Drug Solution (CLZ-DS)	Brain	0.6 ± 0.01	1.8 ± 0.10	3.8 ±0.18	1.4 ±0.1	0.7±0.01	0.4±0.00
	Blood	4.3 ± 0.5	6.2 ± 0.47	7.7 ±1.04	8.1 ±0.7	5.1±0.41	3.3 ± 0.2
	Brain/Blood	0.14±0.01	0.29 ±0.02	0.49±0.04	0.17±0.01	0.13±0.01	0.12±0.01
I.V. Drug Formula (CLZ- LbPM)	Brain	0.4 ±0.03	0.65 ±0.05	0.86 ± 0.1	1.7 ± 0.1	2.3 ± 0.1	1.09±0.08
	Blood	14.9 ± 1.6	12.2 ± 1.3	10.1 ± 1.1	9.9 ± 0.98	8.3 ± 0.7	5.2 ± 0.6
	Brain/Blood	0.03±0.00	0.05 ±0.01	0.09±0.02	0.17±0.09	0.28±0.02	0.21±0.02
I.N. Drug Formula (CLZ- LbPM)	Brain	3. 9 ± 0.1	7.8 ± 0.1	4.4 ± 0.5	2.3 ± 0.2	1 ± 0.2	0.7 ± 0.04
	Blood	0.9 ± 0.09	1.3 ± 0.1	2.5 ± 0.3	1.8 ± 0.2	1.6 ± 0.2	1.4 ± 0.2
	Brain/Blood	4.3 ± 0.4	6.0 ± 0.53	1.8 ± 0.13	1.3 ± 0.11	0.6 ± 0.08	0.5 ± 0.06

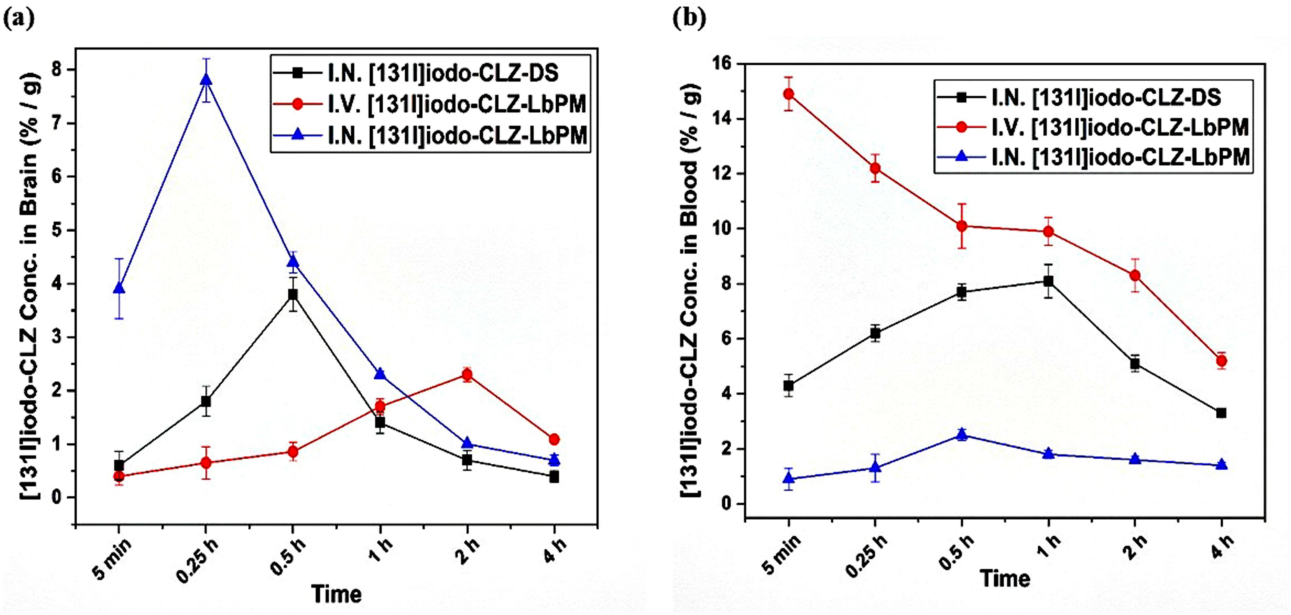


Figure 8 (**a**) Brain [^{131}I] iodo-CLZ and (**b**) Blood [^{131}I] iodo-CLZ following administration as intranasal [^{131}I] iodo-CLZ-DS, intranasal [^{131}I] iodo-CLZ-LbPM, and intravenous [^{131}I] iodo-CLZ-LbPM in male Swiss albino mice (mean ± SD, n=3).

[^{131}I] iodo-CLZ-LbPM. This might be attributed to the ability of I.N. [^{131}I] iodo-CLZ-LbPM to transport CLZ through the olfactory region directly to the brain avoidance of the first pass metabolism.[67]

Pharmacokinetics parameters estimation were determined by calculating Cmax, Tmax, and AUC (0–240 min) for blood and brain. By analyzing the brain data mentioned in Table 7, the I.N. [^{131}I] iodo-CLZ-LbPM showed higher significant values of Cmax (7.85±0.05%ID/g) when compared with I.N. [^{131}I] iodo-CLZ-DS and I.V. [^{131}I] iodo-CLZ-LbPM (3.89±0.09 and 2.35±0.05%ID/g) (p= 0.003 and <0.0001), respectively. Also, Tmax for the three formulations were; (15, 30 and 120 min) (p= <0.0001) for I.N. [^{131}I] iodo-CLZ-LbPM, I.N. [^{131}I] iodo-CLZ-DS and I.V. [^{131}I] iodo-CLZ-LbPM, respectively. The AUC (0–240 min) of I.N. [^{131}I] iodo-CLZ-LbPM was relatively higher than I.N. [^{131}I] iodo-CLZ-DS (482.57±21.32 and 267.88±5.38 (min. %ID/g)) (p= <0.0001) and greater than I.V. [^{131}I] iodo-CLZ-LbPM AUC (0–240 min) value (390.06±10.71 min. %ID/g) (p= 0.003).

Accordingly, I.N. [^{131}I] iodo-CLZ-LbPM can ensure the direct and effective delivery of the radiolabeled optimized loaded formula to the brain better than other route of administration (I.V.) of [^{131}I] iodo-CLZ-LbPM or IN delivery of the drug solution.[15,67] For further prove, the relative bioavailability for brain and blood were found to be 170.59% and 75.26%, respectively.

The evaluation of DTE, DTI and DTP were performed for both I.N. [^{131}I] iodo-CLZ-LbPM and I.N. [^{131}I] iodo-CLZ-DS (Table 8). DTE%, defined as average of partitioning time of drug between brain and blood,[52] was found to be 117% for I.N. [^{131}I] iodo-CLZ-LbPM which is significantly (p<0.05) more than the value of I.N. [^{131}I] iodo-CLZ-DS (19.68%).

Table 7 The Mean Pharmacokinetic Parameters of CLZ Administration as Intranasal [^{131}I] Iodo CLZ-LbPM, Intranasal [^{131}I] Iodo-CLZ -DS, and Intravenous [^{131}I] Iodo-CLZ-LbPM in Male Swiss Albino Mice (Mean ±SD, n=3)

Formulation/Route of Administration	Organ or Tissue	Pharmacokinetic Parameters			
		Cmax (%ID/g)	Tmax (Min)	AUC 0–240 (Min. %ID/g)	AUC 0–∞ (Min. %ID/g)
I.N. Drug Solution (CLZ-DS)	Brain	3.89±0.09	30	267.88±5.38	326.71±3.89
	Blood	8.45±0.35	60	1361.21±56.71	2112.22±57.25
I.V. Drug Formula (CLZ- LbPM)	Brain	2.35±0.05	120	390.06±10.71	772.67±30.62
	Blood	15.7±0.80	5	2009.67±13.67	3551.55±120.17
I.N. Drug Formula (CLZ- LbPM)	Brain	7.85±0.05	15	482.57±21.32	557.33±21.43
	Blood	2.65±0.15	30	412.08±23.83	1589.78±176.36

Table 8 The DTE%, DTI%, and DTP% of Intranasal [^{131}I] Iodo-CLZ-LbPM, Intranasal [^{131}I] Iodo-CLZ-DS Relative to the Intravenous [^{131}I] Iodo-CLZ-LbPM in Male Swiss Albino Mice (Mean ±SD, n=3)

Formulation/Route of Administration	DTE%	DTI	DTP%
I.N. Drug Solution (CLZ-DS)	19.68	1.00	1.37
I.N. Drug Formula (CLZ- LbPM)	117	6.03	83.42

Abbreviations: DTE, drug targeting efficiency; DTI, drug targeting index; DTP, nose-to-brain direct transport percentage.

Drug targeting index (DTI) showed the difference in targeting after I.N. and I.V. administration of [^{131}I] iodo-CLZ-LbPM, the highest values indicating the effective brain targeting.[68] Its values were found to be (6.03 and 1) for I.N. [^{131}I] iodo-CLZ-LbPM and I.N. [^{131}I] iodo-CLZ-DS, respectively.

The [^{131}I] iodo-CLZ percent that transport directly to the brain can be evaluated by calculation of DTP%,[69] was found to be equal to 83.42% and 1.37% for I.N. [^{131}I] iodo-CLZ-LbPM and I.N. [^{131}I] iodo-CLZ-DS, respectively. The significant higher value ($p<0.05$) explained the higher nasal permeation rate of M6 when compared with its drug solution, resulted in efficient brain targeting.

Conclusion

Clozapine can be effectively loaded within LbPM intranasal formula showing high encapsulation efficiency percent of (93.00±0.05%) with small P.S. (12.23±4.76 nm) and better stability with in-vitro CLZ sustained release and ex-vivo permeation profiles up to 24 h. the presence of nanosized range of the prepared polymeric mixed micelles facilitates perfect drug transport through nasal cavity which in consequence resulted in biodistribution and pharmacokinetics improvement of the radioiodinated clozapine ([^{131}I] iodo-CLZ). Intranasal administration of [^{131}I] iodo-CLZ-LbPM showing superior results of brain uptake (3.8%± 0.18%ID/g) with brain/blood ratio (0.49±0.04) at 0.5 h over the intravenous route (2.3%± 0.1%ID/g) and (0.28±0.02) at 2 h, respectively. Perspicuously, lecithin based polymeric mixed micelles could be a promising and preferable way for CLZ from nose to brain targeting.

Disclosure

The authors declare that they have no conflict of interest.

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