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Formulation and Evaluation of Thermosensitive In situ Nasal Gel of Mirtazapine Loaded Aquasomes

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Abstract: The objective of the study was to formulate and evaluate a stable thermoreversible mucoadhesive nasal gel of Mirtazapine (MRT)-loaded aquasomes for depression treatment. One of the major drawbacks of commercial MRT is extensive removal by first pass effect, which we try to avoid by nasal route. Aquasomes formulae were prepared by the co-precipitation sonication method and assessed for their particle size and loading efficiency. The formula with the minimum particle size and the highest loading efficiency was chosen for further measuring their zeta potential and investigating its internal structure using the transmission electron microscope (TEM). MRT-loaded aquasomes were incorporated into in situ nasal gel by the cold process by utilizing Poloxamer 407 and various mucoadhesive polymers and tested for clarity, pH, mucoadhesive strength, viscosity, MRT content, and in vitro MRT release. The formulations that showed the highest drug release were selected for stability studies at temperatures of 4±2°C and 25±2°C/60±5%RH. The chosen formula showed the smallest particle size of 219.3±0.9 nm and the highest loading efficiency of 82.6±2.8%. TEM images revealed an almost nano size spherical shape with acceptable zeta potential (-15.9 m V). Nasal gel characterizations were confirmed to be within permissible limits, such as drug content and gelling temperature, which were found to be 96.56 to 99.55% and 29.5–32.1°C, respectively. It was found that the tested formulae were physically stable during the period of storage. These results suggested that MRT-loaded aquasomes in situ gel was found to be a promising intranasal formulation for the management of depression.

Keywords: Mirtazapine; Aquasomes; In situ nasal gel; Depression; Poloxamer 407; Mucoadhesive polymers.

1. INTRODUCTION

Oral administration is the most preferred and convenient route, so tablets and capsules are the most popular dosage forms. Nevertheless, some drugs' limited oral bioavailability encouraged researchers to look for more efficient ways to deliver them to systemic circulation[1]. Because of the enhanced vascular supply in the nose cavity, intranasal drug administration is characterized by higher bioavailability, particularly for drugs that readily cross the mucosal membranes. Through this route, the drug is not degraded by hepatic first-pass metabolism or gastrointestinal enzymes. In terms of absorption rate and plasma concentration, intranasal drug delivery is superior to subcutaneous or intramuscular methods and comparable to intravenous delivery [2].

Intranasal drug administration is regarded as one of the targeted delivery routes to the brain, as the nose and brain are connected to each other through the olfactory/trigeminal channel through peripheral circulation. This reduces drug distribution to nontargeted areas and delivers the drugs directly to the brain [3].

However, it was shown that nasal liquids quickly evacuate the nasal cavity and do not let the drug remain at the absorption site for an extended period of time. Furthermore, because of their high viscosity, traditional gels rather than liquids may make it difficult to provide a precise amount of the drug. In situ, mucoadhesive gels might be developed to address these problems [4].

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In situ gels are forms of drug delivery methods that, before administration in the body, are in solution form; but, after administration, they go through a phase transition to become a hard gel. The consistency of the in situ gel system is characterized by delayed release, enhanced predictable results, an exceptional stability profile, biocompatibility, and dosage precision. A mucoadhesive polymer that forms a gel simulates the mucosal delivery of multiple substances, providing prolonged release in the nasal cavity. The mucoadhesive gel may have a higher bioavailability than an oral dose form due to targeted distribution [5].

Mirtazapine (MRT), an antidepressant, is often used to treat mild to severe depression. There is just one other tetracyclic antidepressant that the Food and Drug Administration (FDA) has authorized for this usage [6]. Mirtazapine only has a 50% absolute bioavailability because of its high first-pass metabolism. This is because the liver metabolizes a significant portion of the drug before it reaches circulation. To solve this constraint and boost the bioavailability of mirtazapine, many administration techniques have been researched, such as transdermal or intranasal delivery [3].

Aquasome is an effective carrier system for bioactive molecules and drugs to target the sites. Aquasomes nanoparticles consist of three layers: a core, carbohydrate coat, and the absorbed drug. Because it displays a high degree of surface exposure while still maintaining conformational integrity. The molecule's solid core provided structural stability, while the carbohydrate covering protected it from dehydration. Because aquasomes have characteristics that are similar to those of water, they can retain and protect pharmaceutical drugs [7]. Aquasomes provides a water like environment due to the presence of carbohydrate coating and preserves the conformational stability of the biochemically active molecule. Polysaccharide film stabilizes the ceramic core through ionic, non-covalent, and entropic forces [8].

To the best of our knowledge, no papers in the literature have addressed the benefit of aquasomes to improve MRT nasal gel distribution among earlier work concentrating on this topic. Thus, the creation of MRT-loaded aquasomes nasal gel was intended to increase therapeutic efficacy and decrease the adverse effects of oral MRT administration as a potential depression therapy. The goal of the current work was to prepare MRT-loaded aquasomes as a vesicular carrier system and incorporate them into in situ nasal gel. It will prolong nasal residence time by addressing the issue of rapid nasal clearance of the administered drug in the conventional nasal formulation. Furthermore, improves the bioavailability of MRT by bypassing extensive first-pass metabolism. Nasal gel formulations were assessed for their mucoadhesive strength, viscosity, pH, stability, and release tests.

2. METHODS

2.1. Materials

Mirtazapine was provided as a present from Al-Debeiky © Pharma (DBK) located in Cairo, Egypt. Calcium chloride, Disodium Hydrogen Phosphate, and lactose were purchased from El-Nasr Pharmaceutical Chemicals Co© (ADWIC) in Cairo, Egypt. Additionally, Poloxamer 407, Chitosan, and Carrageenan were sent as a trial gift from EIPICO Pharma©, also located in Cairo, Egypt. Dihydrogen potassium orthophosphate was obtained from EL-Gomhouria Pharmaceutical Co© in Cairo, Egypt. Lastly, Benzalkonium chloride, Sodium chloride, and ethanol were provided as gifts from PHARCO Pharma Co©.

2.2. Preparation of MRT-loaded aquasomes

The preparation of MRT-loaded aquasomes involved three steps. Firstly, an inorganic ceramic core was created using calcium phosphate. This was achieved by reacting 0.75 M of disodium hydrogen phosphate (Na2HPO4) and 0.25 M of calcium chloride (CaCl2) as reported earlier by Kamaljeet Kaur 2015 [9] with sonication using an ultrasonicator (Sonix IV, bath sonicator, and Model SS101H, USA) for varying times of 30, 60, and 90 minutes [9]. After sonication, we centrifuged the suspension at 1500 rpm for one hour using a centrifuge (Centurion Scientific, Model K240, UK). We carefully washed the precipitate three times with double-distilled water and then drained off the supernatant. Next, we dissolved the precipitate in 50 ml of distilled water before filtering it through a membrane with a pore size of 0.2 µm. Finally, we dried the core for 48 hours at 70 °C [10].

Secondly, a carbohydrate coating was applied to the core (100 mg) using a simple mixing process. Lactose solution in different concentrations (100, 200, and 300 mg) in water was used as a carbohydrate coating, and the aqueous dispersion of the coated core was sonicated to enhance the coating process. Lastly, MRT was loaded onto the coated core assembly by incubating MRT solution (2.5% w/v in acetone) in the coated core solution for 24 hours at 4°C, as shown in Table (1) [11]. We then performed centrifugation on the dispersion and decanted the supernatant liquid to obtain MRT-loaded aquasomes. Finally, we air-dried the MRT-loaded aquasomes for 24 hours to remove any

remaining moisture and obtained it in powder form [12]. Formulas were formulated using the Vengala, et al. 2013 approach with some modifications [13].

2.3. Characterization of MRT loaded aquasomes

2.3.1. Determination of mean particle size

The particle size of MRT-loaded aquasomes formulations was evaluated using a Malvern Mastersizer (X ver. 2.15, Malvern Instruments Ltd., Worcestershire, UK). For each formula, 10 mg of the aquasomes were suspended in 10 mL of water and then evaluated in triplicate at room temperature [9]. Prior to measurement, the samples were sonicated for five minutes to ensure proper dispersion [14].

2.3.2. Determination of MRT loading efficiency

We incubated the coated ceramic core with a predetermined quantity of MRT solution for 24 hours at 4°C to ascertain the amount of MRT adsorbed onto the aquasomes' surfaces. After that, we separated the supernatant from the suspension using a cooling centrifuge (Centrifuge, Centurion Scientific, Model K240, UK) for an hour at a low temperature. The quantity of MRT present was then determined by filtering the clear supernatant, and the filtrate was examined spectrophotometrically at 290 nm. This enabled us to determine how well the aquasomes loaded the drug [15, 16].

Calculation of MRT loading in the nanoparticles was done according to equation [7].

$$\% \text{ Drug loading} = \frac{(\text{Weight of total added drug} - \text{weight of the unentrapped drug})}{\text{Weight of aquasomes}} \text{X100}$$

2.4. Zeta potential and morphology of MRT-loaded aquasomes

Both tests were performed using the selected formula, F2. Zeta potential is an essential physicochemical character of nanoparticles and can impact the physical stability of MRT. Zeta potential was evaluated by Malvern Mastersizer (X ver. 2.15, Malvern Instruments Ltd., Worcestershire, UK)) [9]. Morphological studies of the selected MRT-loaded aquasomes were done using transmission electron microscopy (TEM) (JEOL GEM-1010). We placed MRT-loaded aquasomes on a copper grid at room temperature, stained them with phosphate-tungsten acid, and evaluated them using TEM at 80 KV [17, 18].

2.5. Fourier Transport Infrared Spectroscopy (FTIR)

FTIR analysis was done to evaluate the compatibility between pure MRT, MRT-loaded aquasomes and polymers used in the preparation of in situ nasal gel. The FTIR spectra of pure MRT, MRT-loaded aquasomes, Plx407, Chitosan, Carrageenan, and the final gel formula were measured in the wavenumber range of 650-4000 cm-1 using a potassium bromide sample disc process (BX (Perkin Elmer, Waltham, MA) infrared spectrophotometer [19].

2.6. Differential Scanning Calorimetry (DSC)

DSC analysis was performed for pure MRT, MRT-loaded aquasomes, Plx407, Chitosan, Carrageenan, and the final gel formula to study the thermal behavior and determine the compatibility using the Calorimeter (DSC60, Shimadzu, Japan). For each sample, 2 mg was consistently used, and they were put in covered aluminum pans before being heated under nitrogen flow at a rate of scanning of 10 °C/min across the temperature range of 20–400 °C [20].

2.7. Preparation of in situ nasal gel of MRT-loaded aquasomes

In situ nasal gel was prepared using a cold approach established by Schmolka [21]. Plx407 (18% w/v) was dissolved in cold water while being constantly stirred (RQ-122, Remi, India), and it was then left at 4°C overnight [6].

To achieve the concentrations given in Table (2), particular quantities of the mucoadhesive polymers (Chitosan and Carrageenan) were dispersed in 10 ml volume of distilled water at room temperature using a magnetic stirrer. The gels were chilled in a refrigerator to 4°C. Then, 5 ml 18% w/v Plx407 was gradually added while continuously stirring in an ice jacket with a thermostat. Dispersions were then refrigerated for a full night to provide clear solutions for further evaluation [22]. We used sodium chloride (0.9% w/v) to adjust the isotonicity [23]. To increase preservation, benzalkonium chloride (0.01% w/v) was used [24]. The chitosan was dispersed in 1 ml of 0.1N acetic acid before being prepared as directed [25].

MRT-loaded aquasomes (containing 150 mg equivalent of MRT) were added to 10 ml of mucoadhesive Plx407-based in-situ gel formulations under magnetic stirring. The produced gels were placed in glass vials and chilled to 4 °C before being used for further evaluation [26].

2.8. Evaluation of MRT loaded aquasomes in situ nasal gel

2.8.1. Physical investigation

Clarity: Each nasal gel preparation was visually inspected against a white and black backdrop, and if it was determined to be clear, free of turbidity, and flowing freely, it was classified as turbid (+), clear (+ +), or very clear (+++) [24].

PH of nasal gel: The pH measurement was calibrated using a digital pH meter (PH. Meter, Model 420, ORION). To confirm the validity of the data, use pH buffers of 4 and 7 prior to beginning the experiment [27]. Gel (5 ml) was diluted with distilled water in a volumetric flask, and an electrode was submerged in it to determine the PH of the gel [6]. For nasal formulations, PH is a crucial consideration. Since the nose's mucous membranes can become damaged by an acidic or alkaline pH [28]. The nasal mucosa's physiological pH normally falls between 4.5 and 6.5. Nevertheless, the nasal mucosa can withstand pH levels between 3 and 10 [24]. Lower pH functions as a hypertonic solution, which shrinks epithelial cells and also reduces ciliary action. Alkaline pH inactivates the lysozyme released by nasal cells, rendering the nasal tissue prone to microbial infection [29].

2.8.2. Drug content

Ethanol was added to lyse the vesicles, 0.5 mL of MRT-loaded aquasomes nasal gel was diluted in 100 mL of phosphate buffer solution (PBS) pH 6.4 and the absorbance was evaluated against the blank at 290 nm [29]. Furthermore, % MRT content was calculated using the following equation:

% MRT content= (Concentration of MRT in sample solution /conc. of MRT taken) ×100

2.8.3. Gelation temperature

In order to create in-situ gelling formulations, it is essential to determine the gelation temperature. The formulation should have an amount of gelling polymer such that it was in a solution condition before application and changed to a gel once it reached the in-situ temperature. Typically, the thermos-reversible nasal gels should gel at a temperature between 32 and 34 °C [24]. Gelation occurs at room temperature at temperatures below 25°C, which causes difficulties with production, handling, and administration. A thermos-gelling formulation will therefore remain liquid at body temperature if it does not gel at a temperature below 34°C, leading to a greater rate of dosage form clearance [28].

To evaluate the gelation temperature, two milliliters of each formula were put into a test tube that had been parafilm-wrapped and set in a thermostatically controlled shaking water bath (Gallent Kamp, England). The temperature of the water bath was gradually increased in 0.5 °C increments from 20 to 40 °C [30].

2.8.4. Gelation time

The mucociliary clearance half-life (21 min) as well as the nasal cavity's physiological temperature (32–37 °C) both place restrictions on the nasal administration formulations' efficacy. When exposed to the system's gelation temperature, it is ideal to expect the system to gel instantly or quickly.

The gelation time was investigated using the tube inversion technique. A test tube contained two ml of nasal gel that was kept at 4°C. The test tube was then put in a water bath that had been set at 37°C (the gelation temperature) and periodically reversed. The gelation time was defined as the moment when the gel stopped flowing when the tube was turned upside down [31].

2.8.5. Mucoadhesive strength

We assessed the ex vivo mucoadhesive strength using goat nasal mucosa. The protocol for using nasal goat mucosa was reviewed and approved by the ethical committee, Faculty of Pharmacy, Al-Azhar University (Approval number: 352). The experiments performed followed the regulations of the Guide for the Care and Use of Laboratory Animals. In order to separate the mucosal membrane, The loose tissues and fat underneath the mucosal membrane must be removed. With PBS (pH 6.4) and distilled water, the membrane was washed three times. The experiment's design was based on the modified balancing technique [32].

By putting one beaker on the left pan and weight (5 g) on the right pan, the balance was brought into equilibrium on both sides. To allow the nasal mucosa's flat surface to face the upper side of the glass, the nasal mucosa was cut into 1 cm^2 and adhered to the glass support with cyanoacrylate. By lowering the glass support, PBS (pH 6.4) was added to the beaker on the right side of the balance to moisten the bonded sheep nasal mucosa. The aforementioned setup was positioned beneath the pan's right side. On the bottom of the right pan, a 1 g thin sheet of the produced gel was applied. The right pan was lowered and spread with gel by removing the beaker from the left pan. To achieve appropriate contact between the gel and the nasal mucosa, the pan was left unattended for two minutes. The nasal mucosa was then gradually detached from the gel film by adding water with a burette to the left pan. The weight necessary to separate the mucosa was estimated to determine the mucoadhesive force. Dynes per square centimeter (dyne/cm^2) were used to measure the force.

Mucoadhesive strength (Dyne/cm^2) = mg/A

where A is the area of the mucosa exposed (cm^2), g is the acceleration caused by gravity (980 cm/s^2), and m is the weight necessary for separation in grams [31].

2.8.6. Viscosity and rheological study

The viscosity of the gels was measured using an Anton Paar MCR502, SN81750818, measuring cell P-PTD200/TG, SN81720491, and measuring system CP50-1/TG, SN31451. The viscosity measurement was carried out at various angular velocities of 20, 40, 60, 80, and 100 rpm. The prepared formulae's rheograms shear rate versus viscosity at 35 °C were plotted [33].

2.8.7. In vitro study of MRT loaded aquasomes In Situ Nasal gel formulations

The USP II method (USP dissolution test apparatus, USP Standard (scientific), DA-6D, Bomby 400-069; India) was used to monitor the in vitro release of MRT from in situ nasal gel preparations. A cellulose membrane tube dialysis bag (VISKING® dialysis tubing, cutoff: 12000–14000, SERVA GmbH; D–69115 Heidelberg) was attached to the paddle and submerged in 500 ml of PBS of pH 6.4 as a dissolution medium at 35–0.5 °C while maintaining a rotational speed of 50 rpm. The gel preparation contained 15 mg of MRT per milliliter [34]. At intervals of 1, 2, 3, 4, 5, 6, 7, and 8 hours, aliquots of 5 ml from the release medium were removed and replaced with an equivalent volume of fresh PBS with a pH of 6.4. The amount of drug released was calculated using a UV spectrophotometer set to its maximum wavelength of 290 nm [6].

2.8.8. Kinetic analysis of the in vitro release data

The *in vitro* release data were fitted using a number of kinetic models and formulas. The best-fitting model had the highest correlation coefficient (R^2) value [35].

- Zero-order reaction: C = K0t,

where t is the time and K0 is the rate constant expressed in concentration/time units.

- First-order reaction: $\log C = log\ C0 - Kt/2.303$

Where k is the rate constant and C0 is the MRT's starting concentration.

- The Higuchi reaction: $Q = Kt\ 1/2$

where K is a constant that reflects the system's design variables [35].

2.9. Stability studies

Stability testing was performed on *in situ* gel preparations in accordance with the International Conference on Harmonization's recommendations [36, 37]. Adequate quantities of each formulation were stored in tightly sealed glass containers and maintained in a chiller at 4°C and in an oven (Clifton cerastir, Model C/stir, UK.) at 25°C/60°±5% RH. At the start of the trial and at intervals of 7, 14, 30, 45, 60, 75, and 90 days, sufficient amounts of these formulations at each temperature were taken and tested for drug content [38]**.** Measuring the pH and gelation temperature of freshly manufactured gel both before and after storage. The MRT concentrations in the gel formulation were assessed at various time points. Zero, first, and second orders were used to determine the correlation coefficient.

3. RESULTS

3.1. Characterization of MRT loaded aquasomes

The resulting particle size of the aquasomes produced varied from 219.3 nm for formulation F2 to 434.3nm for formulation F9, while efficient MRT loading rates ranged from 61.2±1.5% to 82.6±2.8%, with Formula F2 having the highest value and Formula F9 having the lowest, as shown in Table (1).

Table1 1. Formulation and evaluation of MRT-loaded aquasome.

Formula	Core concentration (mg)	Coat concentration (mg)	Sonication time (minute)	MRT Concentration %(w/v)	Particle size (nm)	Drug loading efficiency (%)
F1	100	100	30	2.5	281.4±1.6	78.3±2.3
F2	100	100	60	2.5	219.3±0.9	82.6±2.8
F3	100	100	90	2.5	367.5±0.4	74.9±1.9
F4	100	200	30	2.5	310.2±1.3	79.5±1.67
F5	100	200	60	2.5	254.3±1.1	80.1±1.89
F6	100	200	90	2.5	402.0±0.66	67.4±2.23
F7	100	300	30	2.5	369.6±1.6	70.5±3.20
F8	100	300	60	2.5	336.3±0.89	78.4±1.0
F9	100	300	90	2.5	434.3±0.49	61.2±1.5

Core: CaHPO4; coat: lactose

3.2. Characterization of selected MRT loaded aquasomes

Zeta potential was determined to be -15.9 mV, and transmission electron microscopy showed a clear, spherical shape and nanosized particles as shown in Figure 1.

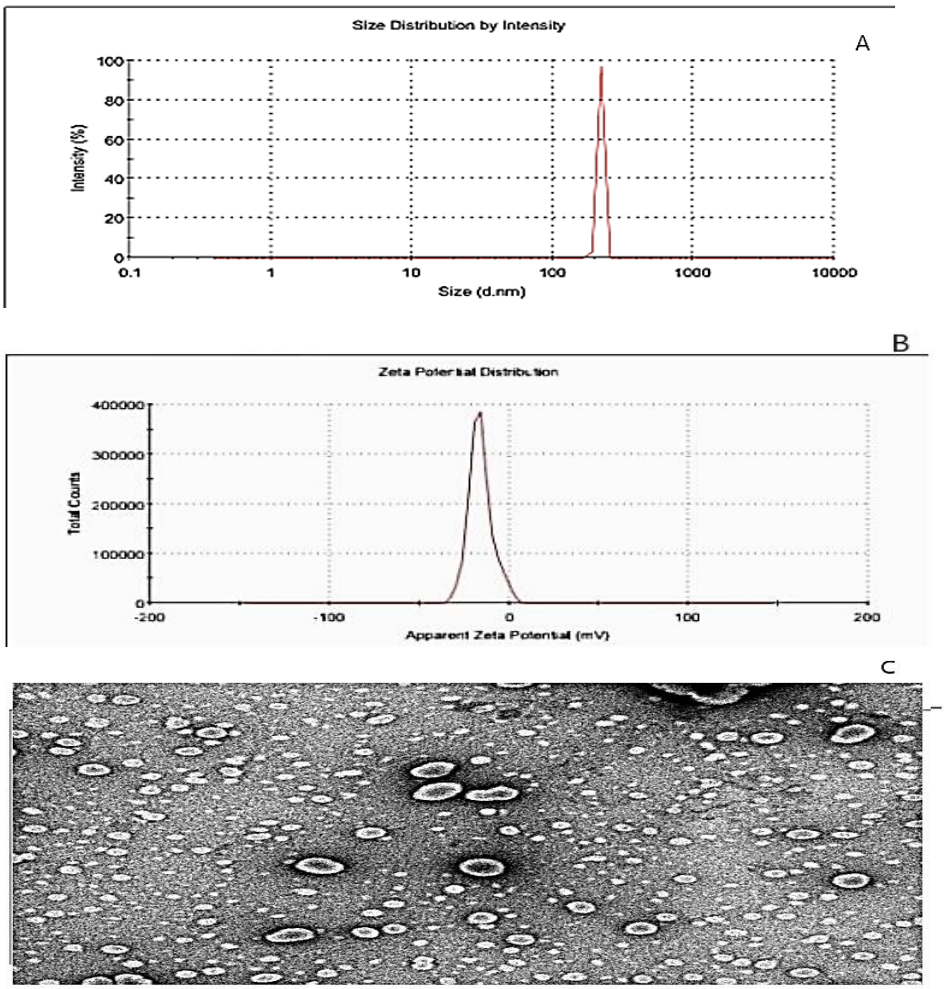

Figure 1. Characterization of selected formula (F2) as particle size (A), zeta potential (B), and Morphological study by TEM(C).

3.3. Compatibility study

3.3.1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a widely used spectroscopic technique for characterizing solid-state materials. Figure 2 displays the IR spectra of pure MRT, indicating N-H stretching at 3324 cm^{-1}. The addition of a methyl group to an N2 atom creates a band at 2937 cm^{-1}, while phenyl group C-C stretching bands were observed at 1587 cm^{-1} and 1446 cm^{-1}. The major aromatic amines with N directly on the ring produced bands at 1334^{-1}-1250 cm^{-1} [39]. MRT-loaded aquasomes generated incredibly precise signals for functional groups including phosphate (PO4), hydroxide (OH), and carbonate ions. The presence of PO4 in the core was indicated by the significant peak at 1073 cm^{-1}. The existence of the OH groups was indicated by the appearance of a significant peak in the 3200–3400 cm^{-1} range. At 1699 cm^{-1}, a distinctive peak suggesting the C=O group is found [40]. The FTIR spectrum of the final nasal gel of MRT-loaded aquasomes in Figure (2) revealed that all of the distinctive bands of the MRT-loaded aquasomes were present in the same locations with no appreciable spectral shift. The results showed that there was no physical or chemical interaction between MRT-loaded aquasomes and excipients.

3.3.2. Differential scanning colorimetry (DSC)

The DSC spectrum of the pure MRT has a clear, sharp endothermic peak at 115, illuminating the MRT's crystalline nature in its purest form [19]. The thermal behavior of MRT-loaded aquasomes exhibited a very distinct endothermic peak at 50 ºC. This peak reflects the melting transition of MRT-loaded aquasomes. DSC thermogram of poloxamer 407 showed a very sharp endothermic

peak at 58 ºC [41]. The DSC of chitosan shows an endothermic peak at 78 ºC and at 305 ºC [42] .The DSC thermogram of carrageenan exhibits abroad endothermic peak at 90.8 ºC, this peak arises because of small amount of absorbed moisture [43]. The DSC thermogram of MRT loaded aquasomes' final gel formula showed the characteristic peak seen in MRT loaded aquasomes at 50 ºC. Results indicated the absence of physical and chemical interaction between MRT-loaded aquasomes and excipients.

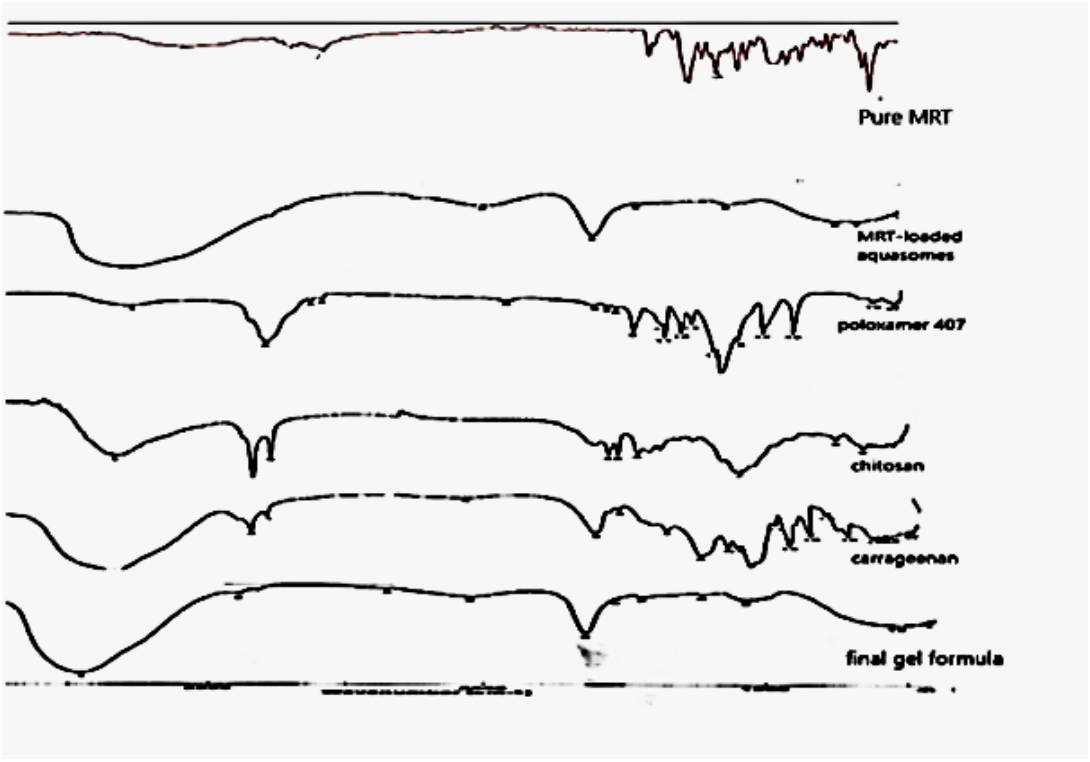

Figure 2. IR spectrum of MRT loaded aquasomes, PLX 407, chitosan, carrageenan, and final gel formula.

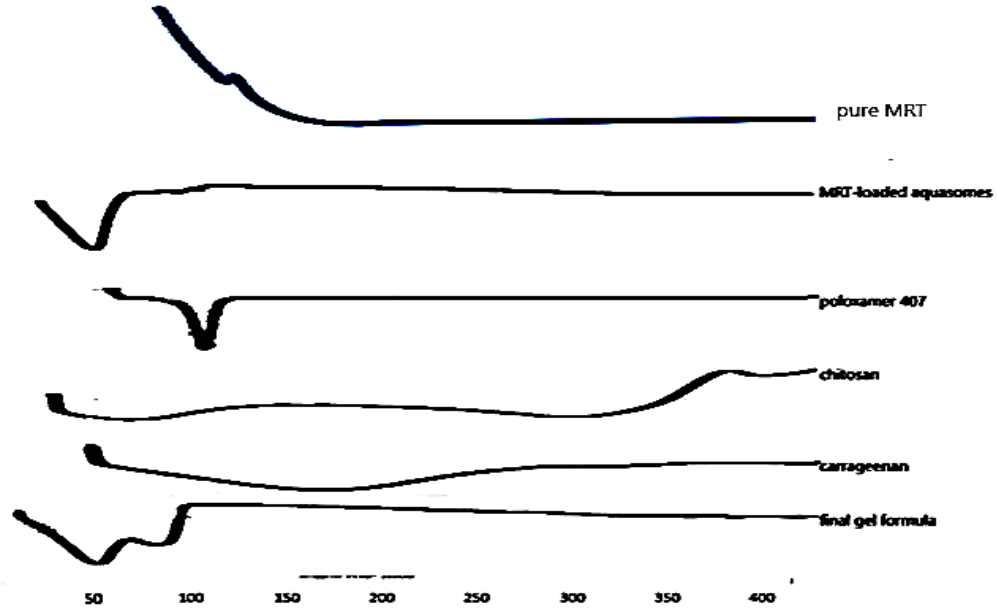

Figure 3. DSC thermogram of MRT loaded aquasomes, PLX 407, chitosan, carrageenan, and final gel formula.

3.4. Preparation of MRT loading aquasomes IN Situ Nasal gel

The concentration of Plx407 (18% w/v) was added to create the in situ nasal gel using mucoadhesive polymers (Chitosan and Carrageenan). The drug-loaded dispersion was gradually infused with mucoadhesive gels before being mixed with Plx407 (solution), all stirring continuously.

3.5. Evaluation of MRT loading aquasomes IN Situ Nasal gel formulations

3.5.1. Physical investigations

As illustrated in Table 2 it was discovered that every formulation was between clear and very clear [29]. Moreover**,** pH values fell between 4.62 and 5.93, which is within the nasal mucosa's tolerance limit. The MRT content results were verified to be within acceptable ranges of 96.56 to 99.55%. The formulations' gelling temperatures varied from 29.5 to 32.1 °C and recorded a quick gelation time between 8.0 and 9.7 seconds.

3.5.2. Mucoadhesive strength

Results in Table 2 demonstrated that changes in mucoadhesive strength were correlated with variations in gelling agent concentration. As the amount of gelling agent increased, a progressive rise in mucoadhesive strength was seen in agreement with the study [44, 45].

Table 2. Formulations and Physical characterizations of thermoreversible mucoadhesive nasal gels that contain varying concentrations of chitosan and carrageenan polymers.

Formulae	Chitosan (%w/v)	Carrageenan (%w/v)	Clarity	PH	Drug content (%)	Gelation temperature (C)	Gelation time (seconds)	mucoadhesive strength (dyne/cm2)	Viscosity (cp)
F1	0.3	-	++	4.71±0.12	99.04±0.27	31.3±0.14	8.12±0.82	843.56±9	267.09
F2	0.5	-	++	4.62±0.18	98.11±0.78	30.8±0.22	8.0±0.19	1150.23±7.8	530.11
F3	0.7	-	++	4.67±0.14	99.38± 0.32	29.5±0.32	8.22±0.35	1513.43±12	1753.9
F4	-	0.5	+++	5.93±0.32	99.55±0.68	32.1±0.00	9.7±0.940	734±16	977.86
F5	-	1	+++	5.34±0.61	98.06±0.88	31.5±0.18	9.2±0.62	1700±10.6	1015.8
F6	-	1.5	+++	5.12±0.18	96.56±0.45	31.3±0.43	8.85±0.28	2046±11.9	2124.4

Each formula contains 18% w/v Plx407 and 150 mg of MRT-loaded aquasomes. The symbol (+) indicated to clear solutions, and (+++) indicated very clear solutions.

3.5.3. Viscosity and rheological study

From the results in Table (2), It is evident that the viscosity dramatically increased when the mucoadhesive polymer content was raised [46, 47]. When the concentration of chitosan increased from 0.3 to 0.7% w/v the viscosity increased from 267.09 cp to 1753.9 cp and when the concentration of carrageenan increased from 0.5 to 1.5 % w/v the viscosity increased from 977.86 cp to 2124.4 cp. All formulations displayed pseudoplastic behavior in the rheological investigation, which means that their viscosity decreased as the shear rate increased as shown in Figure 4.

3.5.4. In vitro release of MRT loaded aquasomes from in situ nasal gel.

According to the findings (Figure 5), the preparations with the lowest amount of each mucoadhesive polymer (chitosan and carrageenan) exhibited the largest MRT release when compared to preparations with higher amounts of the same polymer.

3.5.5. Kinetic evaluation of the in vitro release data

Table 1 (supplementary data) shows the computed kinetic data for the in vitro release of MRT from several preparations of thermoreversible mucoadhisive nasal gel. In vitro release of MRT from all preparations is found to follow Higuchi diffusion order owing to results of the coefficient of determination (R^2).

3.6. Stability study

Formulations F1 and F4 of in situ nasal gel were chosen for stability testing because they had the highest release, suitable gelation temperatures, pH levels, MRT contents, viscosities, and mucoadhesive strengths. Figure 2 (supplementary data) displays the percentage of MRT that was still present after three months of storage of formulations of in situ nasal gel at 4°C and 25°C. For formulations maintained at 4°C and 25°C, the pH ranges were, respectively, (4.50-6.34) and (4.96-5.17) (Table 2 supplementary data). After

three months of storage at 4°C, it was discovered that the gelation temperature had not changed according to the data.

After storage at 25°C, there was a small drop in the gelation temperature of the nasal gel under study.

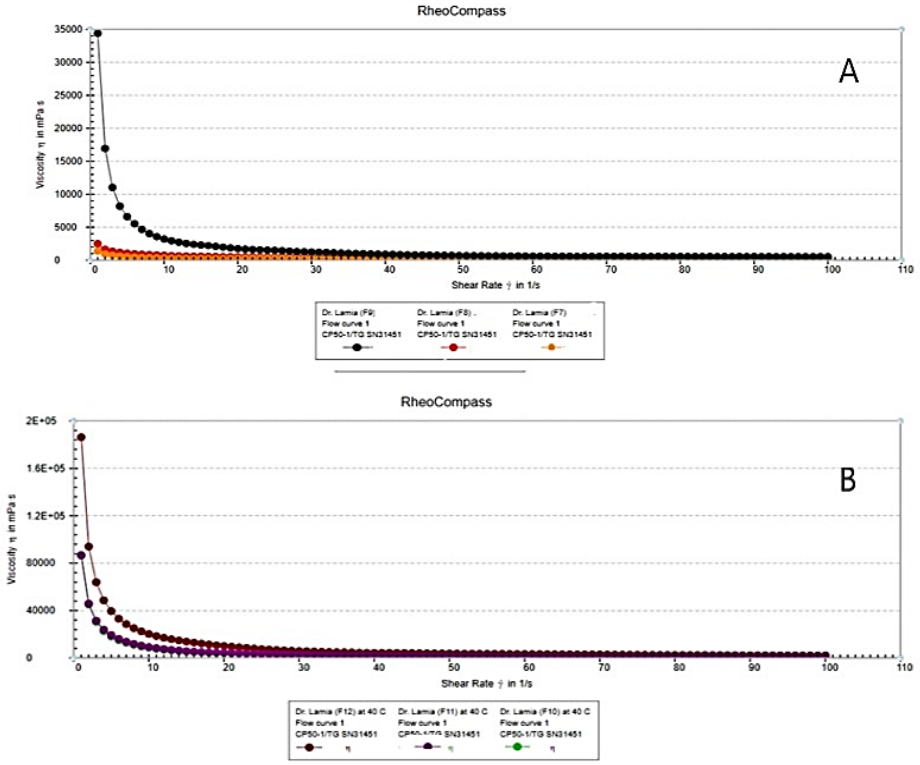

Figure 4. Relationship between MRT-loaded aquasomes thermos-reversible nasal gel viscosity and shear rate of F1, F2, and F3(A) and F4, F5, and F6 (B) at 35 ºC.

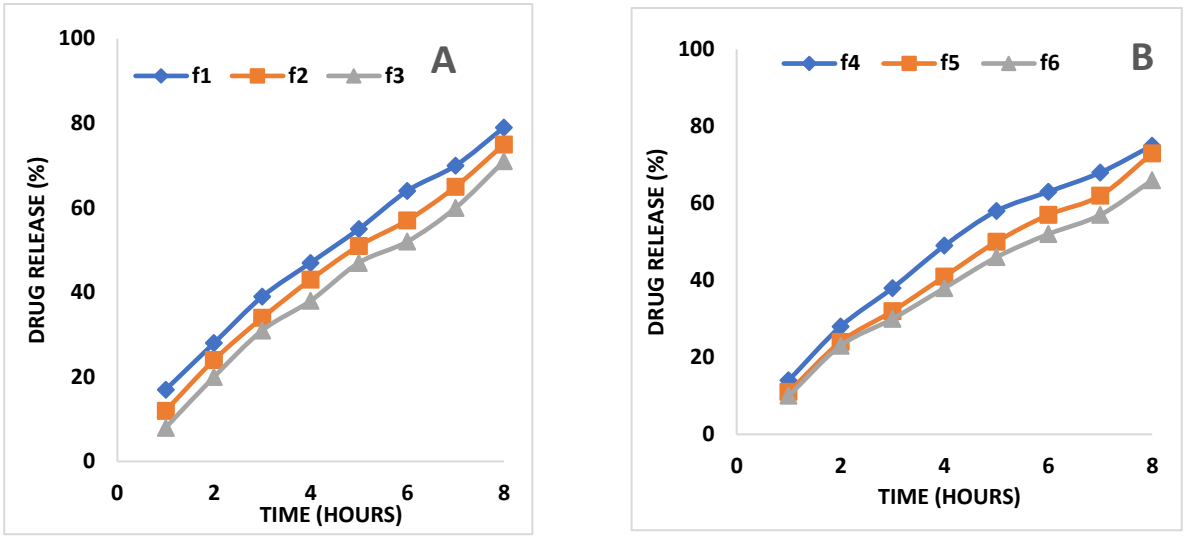

Figure 5. In vitro release of MRT loaded aquasomes from thermos-reversible nasal gel formulations of 18% w/v Plx407 using different concentrations of Chitosan(A), and Carrageenan(B) into PBS solution op PH 6.4.

4. DISCUSSION

To increase the antidepressant potential of MRT, a drug frequently used to treat depression, MRT-loaded aquasomes were created using the co-precipitation sonication method. First, a fine powder of calcium hydrogen phosphate (CaHPO4) was precipitated and used as a model for the creation of aquasomes [48]. During the co-precipitation sonication process, CaHPO4

ceramic core particles self-assemble under sonication, leading to an increase in surface free energy [48]. To prepare the aquasomes, a thin layer of polyhydroxy oligomer is coated onto the ceramic core particles, stabilizing them through ionic and non-covalent forces [49]. The biopharmaceutical characteristics of aquasomes and how successful they are as drug delivery vehicles are greatly influenced by the particle size of those formulations.

The particle size of aquasomes plays a crucial role in determining their biopharmaceutical properties and effectiveness as drug delivery systems. Our observations, as shown in Table 1, revealed that the particle size of aquasomes ranged from 219.3 ± 0.9 nm to 434.3 ± 0.49 nm, with F2 having the smallest particle size and F9 having the largest. The size and shape of the aquasomes were found to be influenced by various factors during the preparation process. We found that an increase in lactose concentration contributed to an increase in particle size. So, the size of the aquasomes varied from 219.3 ± 0.9 nm in F2 to 336.3 ± 0.89 nm in F8 when the coating ratio was increased from 100 to 300 mg, while the other variable, such as sonication time, was kept unchanged. Next, an increase in sonication time from 30 to 60 minutes decreased the particle size of aquasomes from 369.6±1.6 nm in F7 to 219.3±0.9 nm in F2 , though other variables, such as lactose concentration were kept steady [9]. However, at 90 minutes, aggregates of small particles were observed, resulting in an increase in particle size to be 434.3±0.49 in F9 [11].

All formulations showed efficient drug loading rates ranging from 61.2±1.5% in F9 to 82.6±2.8% in the formula (F2), which had the smallest particle size and showed the highest loading efficiency because the surface area will rise with decreasing coated core size, which raises the capacity for loading MRT in the formulation, which is in agreement with the study of Jagdale,2020 [7].

Zeta potential measures the electrostatic attraction or repulsion between particles. It is the best indicator for the stability of dispersions such as suspensions and emulsions. Experimental results showed that the zeta potential was found to be -15.9 mV, as shown in Figure 1. It specified that prepared aquasomes have sufficient charge to prevent the aggregation of vesicles. The presence of a negative charge on the surface of aquasomes is explained by the existence of a lot of electronegative atoms (-OH) in the chemical structure of lactose [11].

Experimentally, the TEM images used to study the surface morphology of aquasomes revealed that aquasomes were spherical and within the nanosize range, and no drug crystals were visible, as shown in Figure1.

The FTIR spectrum and DSC thermogram of MRT-loaded aquasomes and suggested polymers revealed that the characteristic stretching vibrations in MRT-loaded aquasomes were also present in the final gel formula without any significant shift in the wave number of peaks or changes in intensity of the peaks, indicating no interaction between MRT-loaded aquasomes and the excipient, so aquasomes and other ingredients were compatible.

The selected formulation F2 was further incorporated into a thermoreversible in situ gelling system using 18% w/v of Plx407 and different concentrations of chitosan and carrageenan gelling agents. Experimental results showed that 18% w/v of Plx407gel with MRT exhibited the capacity to create a gel at the temperature of the nasal mucosa (34°C). However, it was employed for additional research to see whether adding mucoadhesive polymers would enhance their ability to adhere to mucous membranes [27, 50].

It was discovered that all of the gel compositions ranged from clear to extremely clear. The gel compositions, as shown in Table (2), did not include any residual material or grit. The pH of all the formulations varied from 4.62±0.18 in formula F2 to 5.93±0.32 in formula F4, i.e., which is within the range of the nasal mucosa (4.5–6.5) and can be easily tolerated and thus causes no irritation to it. The drug content of all the MRT-loaded aquasomes in situ gel formulations was found to be between 96.56 in F6 and 99.55% in F4 (Table 2). The MRT content for each formulation was verified to be within acceptable limits. The drug was dispersed uniformly, according to the MRT content.

The gelling temperature of the formulations ranged from 29.5±0.32 to 32.1±0.00°C (Table 2). Our findings show that increasing the amount of mucoadhesive substances lowers the gelling temperature because these compounds can attach to the poly (- ethylene) oxide chains in Plx407 molecules, promoting dehydration and increasing the entanglement of adjacent molecules with more intermolecular H bonds [51].

For in-situ formulations, a short gelation time of 8.0 to 9.7 seconds was observed (Table 2). Additionally, the formula with a greater carrageenan concentration (F6) showed a quicker gelation time of 8.85±0.28 seconds, while the formula with a low concentration (F4) showed a slower gelation time of 9.7±0.940 seconds. This finding suggests that when mucoadhesive compound concentration increases, effective sol-gel transition temperature decreases and gelation time decreases. As the chitosan content

rose from 0.3 to 0.7% w/v, the gelation time was not considerably impacted [31].

Mucoadhesive strength assessment is important since it has such a big impact on the lengthening of the residence period and decreasing nasal gel leakage. Mucoadhesive strength is the measure of how much formulation sticks to the mucous membrane at 34°C, or nasal temperature. The intranasal formulation's mucoadhesive power must be sufficient to withstand nasal clearance. In addition, if the mucoadhesive strength is too high, the mucosal membrane may be harmed [52].

Results in Table 2 demonstrated that the mucoadhesive strength of the formulations containing chitosan was found to range from 834.56 to 1513.43 dyne/cm^2, and the mucoadhesive strength of the formulations containing carrageenan was found to range from 734 to 2046 dyne/cm^2. The changes in mucoadhesive strength were correlated with variations in the concentration of gelling agents. As the amount of gelling agent increased, a progressive rise in mucoadhesive strength was seen. The nasal gel's increased gel strength may be caused by hydrogen bonding between glycoproteins in the nasal mucosa and bond-forming groups (such as hydroxy, ethoxy, and amine) of gelling polymers [44,45].

Table 2 demonstrated that the viscosity rose dramatically as the mucoadhesive polymer concentration increased [46]. The viscosity of formulations containing chitosan and carrageenan was in the range of 267.09 cp to 1753.9 cp and 977.86 cp to 2124.4 cp, respectively**.** Increasing the polymer concentration leads to shorter inter-micellar distances and more numerous micelles within the gel structure. According to El-Kamel (2002) [47]. This causes more crosslinks between surrounding micelles, increasing viscosity.

Additionally, in the rheological tests, all formulations had pseudoplastic behavior, which implies that their viscosity reduced as the shear rate rose, as shown in Figure 4. This shear-thinning behavior is responsible for ensuring that the drug is dispersed uniformly throughout the nasal cavity and will enhance the gel's spreadability. Benefits of the high viscosity at a low shear rate include the solution's propensity to remain in place after production and its capacity to prolong the period that in situ gel is in contact with the nasal cavity [53].

According to the findings of the study on the influence of gelling polymer amount on MRT release from nasal gel, the formulation with the lowest concentrations of each mucoadhesive polymer, including chitosan and carrageenan, demonstrated the highest MRT release in comparison to formulations with higher concentrations of the same polymer. It was apparent that MRT was likely confined to smaller polymer cells at higher polymer concentrations since the drug was frequently released via channels or gaps in the gel network. As a result, the concentration of gelling polymer increases along with the gel's viscosity. Additionally, the gelling polymer restricts the aqueous channels of the pluronic micelles, which stops drugs from diffusing through them [24].

The limited release may result from the mucoadhesive polymer's capacity to both raise the gel's viscosity and decrease or compress the poloxamer micelle's excess micellar aqueous channels, which allow MRT to diffuse. The increased polymer content is delaying this release even further [50]. In vitro MRT release was observed for all MRT formulations in accordance with the Higuchi diffusion model.

From the stability research and calculation of the percentage of MRT preserved after three months of storage of formulations of in situ nasal gels at 4°C and 25°C. The degrading process was discovered to come after the zero-order reaction. The pH measurements showed very minor alterations and stayed within the range of the normal physiological nasal PH. After three months of storage at 4°C, it was discovered that the gelation temperature had not changed according to the data. After being stored at 25°C, there was a modest drop in the formulation's gelation temperature, which may be attributed to the gel formulation's dehydration. These findings demonstrated that thermoreversible mucoadhesive nasal gel formulations may be stored under refrigeration in a temperature range of 2 to 8 °C [54].

5. CONCLUSIONS

The present study has succeeded in formulating MRT-loaded aquasomes in situ nasal gel as a promising intranasal delivery system. MRT-loading aquasomes were successfully prepared using the co-precipitation sonication technique. The best formula (F2) showed a considerably small vesicle size (219.3nm), high drug loading (82.6%), and adequate stability represented by zeta potential (-15.9 mV) were successfully loaded into in situ gel using poloxamer 407 (18%), with chitosan and carrageenan as the mucoadhesive agents. The developed MRT-loaded aquasomes nasal gel formulations showed suitable pH, % drug content, gelling time, gelling temperature, mucoadhesive strength, viscosity, and sustained drug release. According to stability studies, refrigeration conditions (2°C to 8°C) were adequate for the storage of formulations for thermoreversible nasal

gels since they resulted in the formulation gelling at the temperature that was tested. Thus, the current study investigates MRT-loaded aquasomes nasal gel, which could therefore be considered a good candidate for MRT delivery through the nasal mucosa which represents an efficient alternative to commercial oral route. The achieved outcomes are encouraging for further clinical trials of the MRT-loaded aquasomes nasal gel in animals and humans in future research.

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Ethical Statement: The protocol for using nasal goat mucosa was reviewed and approved by the ethical committee, Faculty of Pharmacy, Al-Azhar University (Approval number: 352).

Author Contribution: LH performed the experiment, collected the data, performed the graphical and statistical analysis, and wrote the manuscript. AD designed the research idea, supervised the data analysis, writing, and revised the manuscript. SE revised the manuscript.

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