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Article

Effect of accelerated stability study on characteristics of in situ nasal gel of mirtazapine loaded aquasomes

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Abstract

Drugs may lose their therapeutic efficacy and potentially develop harmful consequences due to physical and chemical degradation. Therefore, the aim of this work is to study the physical stability and degradation kinetics of Mirtazapine (MRT) loaded aquasomes in situ nasal gel and calculate the shelf life of the tested formula. International Conference on Harmonization (ICH) protocols were followed in conducting the stability tests. MRT was prepared as nanoparticles called aquasomes and incorporated into a nasal gel. Four gelling polymers were used in this study, namely HPMC K4M (F1), carbopol (F2), chitosan (F3), and carrageenan (F4). The formulations were kept in storage for six months at 4 ± 2 °C, 25 ± 2°C and 65 % ± 5 relative humidity (RH). As a consequence, each gel formulation is assessed for its physical stability, including its clarity, pH, gelation temperature, and gelling time. The drug content, degradation kinetics, and shelf life of the nasal gel formulation were also calculated. The study's results demonstrated that the tested formulae were physically stable during the period of testing with very slow degradation and were found to be of zero order, Variations in the characteristics were not statistically significant by using one-way ANOVA analysis. The shelf life of the Arrhenius plot ranged from 1.333 years (F3) to 2.2 years (F1). The results indicate that the stability of MRT-loaded aquasomes in situ nasal gel is acceptable and stable. Furthermore, HPMC K4M shows higher stability and longer shelf -life.

Keywords: Accelerated stability study, ICH guidelines, Mirtazapine, Aquasomes, *In-situ* nasal gel, Shelf-life.

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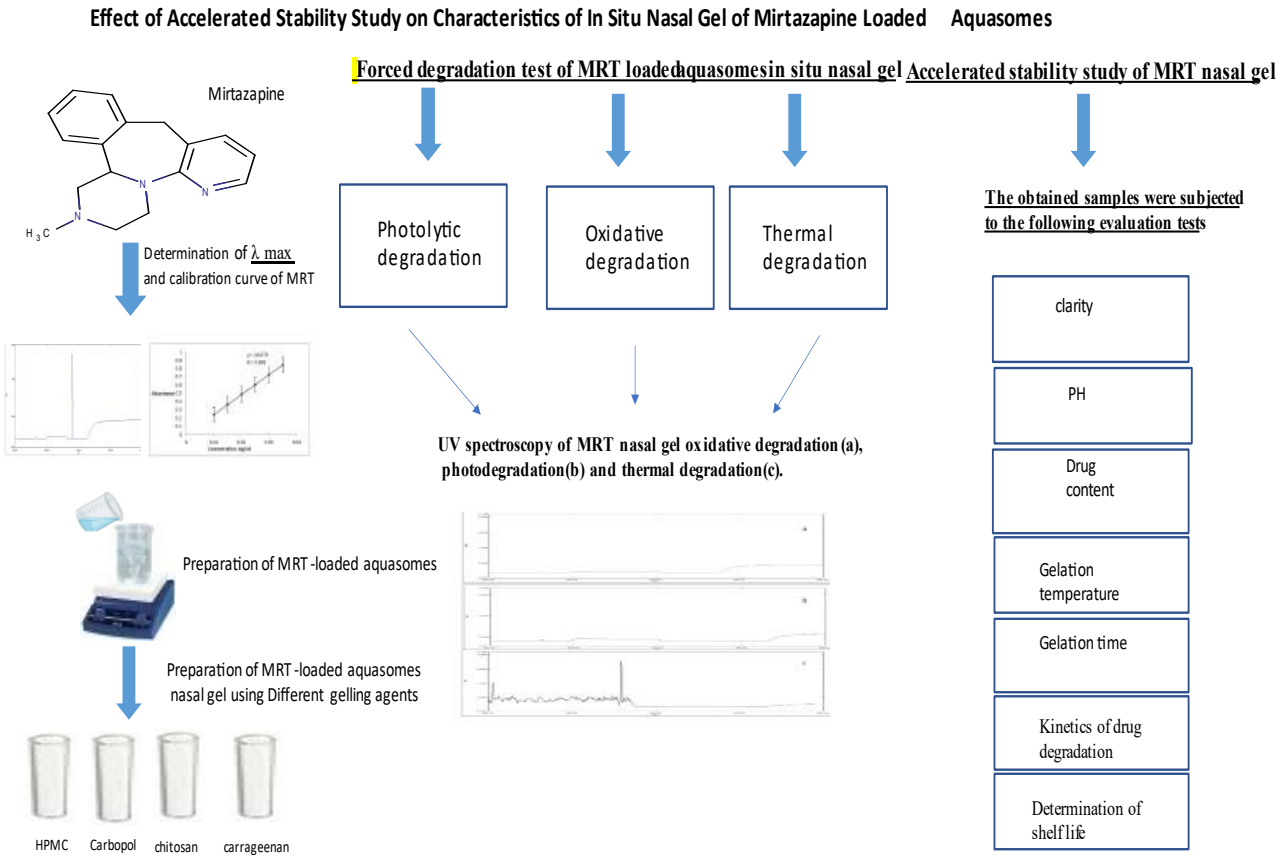
Graphical Abstract



Introduction

Mirtazapine (MRT) is a tetracyclic anti-depressant drug authorized by the U.S. Food and Drug Administration (USFDA) to treat depression (Musallam *et al.*, 2022). It is a Biopharmaceutics Classification System (BCS) class II medication with a poor oral bioavailability of 50% due to first pass metabolism; thus, MRT was prepared as nanoparticles (aquasomes) because aquasomes have characteristics that are similar to those of water, and can retain and protect pharmaceutical drugs (Jagdale & Karekar, 2020). Aquasomes provide a water-like environment due to the presence of a carbohydrate coating, which preserves the conformational stability of the biochemically active molecule. Polysaccharide film stabilizes the ceramic core through ionic, non-covalent, and entropic forces (Banerjee & Sen, 2018). MRT loaded aquasomes were incorporated in in situ nasal gel by using a variety of gelling agents to prevent extensive first-pass metabolism, provide rapid drug delivery to the brain, increase therapeutic efficacy, and lessen side effects (Naresh *et al.*, 2020).

It is crucial that MRT-loaded aquasomes nasal gel formulations are safe and effective (Hamrapurkar *et al.*, 2011). Due to dose reductions brought on by the unstable drug formulations, under medication may result. Moreover, when the MRT breaks down, hazardous byproducts may be produced. The drug has the potential to modify its physical characteristics while being transported from one location to another. When forecasting stability, kinetic concepts can be utilized to account for changes in physical appearance (Aashigari *et al.*, 2018) Also, stability testing data is a crucial necessity to help judge the quality and control approval of new novel MRT formulations. A drug's ability to keep its physical, chemical, microbiological, toxicological, protective, and informational requirements while being stored in a specific container or sealing system is known as its "stability" (Bajaj *et al.*, 2012). The stability of finished formulations is affected by environmental variables such as temperature, humidity, light, and aspects of the product, including the active ingredient's (API) and pharmaceutical excipients' physical and chemical properties, the dosage form's makeup, and the production process (Narayan *et al.*, 2017). The World

Health Organization (WHO) has been working to address the major issues with drug stability and storage, with a focus on poor countries. They are particularly prevalent in tropical climatic regions where drug stability presents more significant problems, that could affect the drug shelf life (Bajaj *et al.*, 2012).

There are certain guidelines for this stability testing supplied by the International Conference on Harmonization (ICH Q1A-R2). The availability of approved stability-indicating testing techniques that identify characteristics as changing during storage and have an effect on quality, safety, or efficacy is crucial. The ICH recommendations stress that these analytical techniques are reliable and appropriate for identifying degradation products both qualitatively and quantitatively (Ahmed *et al.*, 2012). One of the ICH recommendations, known as accelerated stability, calls for studies to determine the stability and degradation reactions of medicinal products or drug substances at high temperatures and then use modelling to determine the reactions performed at lower temperatures during long-term storage. During drug substance and drug product development, this method offers considerable advantages for science-based decision-making (Clancy *et al.*, 2017). Due to the production of predictive data for the calculation of the shelf life of drugs, it is essential to estimate the kinetic behaviour of these reactions (Bhangare *et al.*, 2022a).

The analysis of the rate of drug breakdown is referred to as degradation kinetics. Improved knowledge of the process underlying drug degradation may be produced using the data from the degradation kinetic research. Also, it assists in making recommendations for better pharmaceutical packaging and storage practices to lengthen the shelf life of these products (Bhangare *et al.*, 2022b). Studying the kinetics of degradation is important because it has an impact on how stable medicines are during the design and testing phases of a product. The rate of their degrading response can be slowed down with the aid of suitable storage settings. This guarantees drugs' stability for the course of their shelf-life (Bhangare *et al.*, 2022b).

In the current work, stability studies were performed on four formulations of in situ nasal gel containing MRT-loaded aquasomes formulated by different gelling agents: HPMC K4M (F1), Carbopol (F2), Chitosan (F3), and Carrageenan (F4). These four formulations were designed for studying the change in physical properties and drug content over the storage period as well as calculating its shelf-life.

Materials and Methods

Materials

Mirtazapine was given as a gift from Al- Debeiky© Pharma (DBK) (Cairo, Egypt). Disodium Hydrogen Phosphate, Calcium chloride, and lactose were bought from El-Nasr Pharmaceutical Chemicals Co© (ADWIC), Cairo, Egypt. Poloxamer 707, HPMC K4M, Carbopol, Chitosan, and Carrageenan were kindly sent as a trial gift from EIPICO Pharma©, (Cairo, Egypt). Hydrogen peroxide, Ethanol, and Dihydrogen potassium orthophosphate were purchased from EL-Gomhouria Pharmaceutical Co©, Cairo, Egypt.

Determination of λ_{max} of MRT

Scanning of MRT in phosphate buffer solution (PBS) at pH 6.8. We produced a stock solution with a concentration of 0.1 mg/ml by precisely dissolving 10 mg of MRT in 100 ml of PBS (Benajeer *et al.*, 2012). Using a suitable measuring flask and 1 ml of the prior solution, 10 ml of the PBS medium was created at a concentration of 0.01 mg/ml. By using PBS as a blank, the stock solution was evaluated by spectrophotometric scanning at various wavelengths between 225 and 360 nm, and we calculated λ_{max} at maximum absorbance (N Karaşen & S Altinöz, 2000) (N. Karaşen & S. Altinöz, 2000).

Calibration curve in PBS PH 6.8

Fresh aliquots of the standard stock solution were pipetted out at the rates of 1, 1.5, 2, 2.5, 3, and 3.5 before being appropriately diluted with PBS (pH 6.8) to achieve a final concentration of between 0.01 and 0.035 mg/ml. Using PBS (PH 6.8) as a blank, the solutions were scanned in spectrum mode at 289 nm (Prabhat et al., 2021b).

Preparation of MRT-loaded aquasomes

Three steps were used to create MRT-loaded aquasomes: first, an inorganic ceramic core was created using calcium phosphate, which was created by reacting calcium chloride with disodium hydrogen phosphate; second, the core was coated with sugars (polyhydroxy oligomers), such as lactose; and third, the MRT was loaded onto this assembly by incubating the MRT in coated core solution for 24 hours at 4°C (Patel *et al.*, 2016). Formulas were prepared using the Vengala, et al. 2013 approach (Vengala *et al.*, 2013).

Incorporation of MRT loaded aquasomes in thermoreversible mucoadhesive nasal gel

Using a thermosensitive hydrogel called Poloxamer 407 (Plx407) and several types of gelling agents such as (Hydroxy Propyl Methyl Cellulose) HPMC K4M (F1), Carbopol (F2), Chitosan (F3), and Carrageenan (F4), MRT-loaded aquasomes were integrated in thermoreversible mucoadhesive nasal gel. (Abou Youssef *et al.*, 2018; Vengala *et al.*, 2013).

The cold procedure described by Schmolka IR.12 was used to create Plx407 gel. Plx407 (18% w/v) was dissolved in cold water while being constantly stirred

(RQ-122, Remi, India), and it was left at 4°C overnight (Naresh *et al.*, 2020).

The mucoadhesive bases were then made using Plx407 (18% w/v) at room temperature, using a magnetic stirrer, precise quantities of the mucoadhesive hydrogels HPMC K4M (F1), Carbopol (F2), Chitosan (F3), and Carrageenan (F4) were dissolved. The produced gels were chilled in a refrigerator to 4°C. Then, 18%w/v Plx407 was gradually added while being continuously stirred with a magnetic stirrer in an ice jacket with a thermostat. After that, dispersions were chilled overnight to produce transparent sols for further characterization (Abou Youssef *et al.*, 2018)

Under magnetic stirring, MRT-loaded aquasomes (enough to contain 40 mg of MRT) were added to the chosen mucoadhesive Plx407-based in-situ gel formulations. The produced gels were placed in glass vials and kept chilled at 4° C for future analysis.

Forced degradation test of MRT loaded aquasomes in situ nasal gel ICH quality guideline

For both pharmacological substances and products, forced degradation studies are a crucial component of a stability program that complies with regulations. In 1993, ICH Guideline Q1A formally established forced degradation studies as a legal necessity (Singh *et al.*, 2013). For assurance of the safety, efficiency, and superior quality of the evaluated nasal gel, the ICH Guidelines include regulations about to the procedures for establishing and implementing stability studies. These regulations outline the nasal gel stability tests conducted over time and under various environmental storage circumstances (pH, temperature, light, air, and humidity). The ICH Q1A-R2 test identifies photolytic, oxidative, and thermal degradation as the main routes for degradation of these. (Blessy *et al.*, 2014; Ioele *et al.*, 2021). Stress tests are run to show the method's specificity in measuring changes in drug ingredient concentration in the absence of such data on probable degradation products. The UV spectroscopic approach is the most popular analytical technique for measuring changes in drug concentration (Blessy *et al.*, 2014).

Photolytic degradation

A sufficient volume of nasal gel (20 ml) was put into glass vials for daytime or UV light degradation. The vials were left in the sun for 12 hours or were exposed to UV light at 254 and 366 nm (Ultraviolet spectrophotometer, Jenway LTD, UK, ty, Dunmow, Essex, CM63LB, Model 6405 UV/VIS (England) in a wooden cabinet with a 15 cm gap between the source and the sample solution. Finally, aliquots of the solution (3 ml) had their drug concentration measured spectrophotometrically at 289 nm (Tolba & El-Gamal, 2016).

Oxidative degradation

A 20-ml solution of the nasal gel containing MRT-loaded aquasomes was diluted with a comparable amount of 3% hydrogen peroxide. A full day was given for the diluted solutions to stand. Ultimately, spectrophotometric testing at 289 nm was used to determine the drug concentration in solutions. (Sharma and Murugesan, 2017).

Thermal degradation

Many drugs are known to have thermolabile properties. The velocity of the reaction tends to increase as the temperature rises, which in turn causes the product to degrade. The appropriate amount of nasal gel (20 ml) is subjected to dry heat for varying periods throughout these investigations, and the solution (3 ml) is then spectrophotometrically measured at 289 nm to determine the drug concentration (Venkataraman and Manasa, 2018).

Accelerated stability study of MRT nasal gel

In-situ, the gel's physical and chemical stability was assessed under two distinct temperature and humidity settings. Following accelerated stability investigation as ICH(Q1A-R2), sufficient amounts of each chosen formulation (50ml) were kept in tightly closed amber glass bottles (were purchased from El-Hikma Pharmaceutical Chemicals Co©, Cairo, Egypt) and kept in refrigerators at 4°C and thermostatically controlled ovens at 25°C with 65% relative humidity (Guideline, 2003; Wu *et al.*, 2016). At the start of the experiment and intervals of 7, 14, 30, 45, 60, 75, 90, and 180 days, enough samples of these nasal gel at each temperature were acquired. Using a UV-Spectrophotometer at 289 nm, the samples' clarity, pH, gelation temperature, gelling time, and drug concentration were assessed. (Kenneth *et al.*, 2018).

Clarity test of samples

Each produced formulation was evaluated visually against a white and black background, they must be found to be clear, free from any gritty particles and free-flowing solutions, they were categorized as turbid (+), clear (+ +), and extremely clear (+++) (Verekar *et al.*, 2020).

Determination of P^H

Using pH buffers 4 and 7, a digital pH meter (PH. Meter, Model 420, ORION) was calibrated to monitor the pH of the nasal gel. In a volumetric flask, a definite amount of gel formulations (5ml) was introduced, diluted with distilled water, and then an electrode was submerged in the gel. This technique was carried out three times (Naresh *et al.*, 2020).

Gelation temperature

A test tube with 2 ml of each formulation was put in a thermostatically controlled shaking water bath (Gallent Kamp, UK) and covered with parafilm to measure the gelation temperature. The water bath's temperature was raised from 20 to 40 °C over some time in steps of 0.5 °C. The test tubes were tilted 90 degrees to check for gelation after being shaken slowly for about 10 mi 15 at a rate of 20 strokes per minute to allow the test tubes to adapt quickly to the water bath temperature at each temperature rise (Ahmed *et al.*, 2018).

Gelling time

The gelling time of nasal gel preparations was determined using the methods outlined by Sherafudeen and Vasantha **(Sherafudeen & Vasantha, 2015).** Before administration, nasal gels are in sol form, but once they are, they go through gelation to become a gel. The time for the initial detection of gelation was noted as the gelling time. The gelling temperature (T sol-gel) of the formulated *in situ* gel preparations was determined by adding 2 ml of the formulated nasal gel to a test tube (10 ml), with a diameter of 1.0 cm. The tube was maintained in a 37 °C circulating water bath after being parafilm-sealed. Equilibration was allowed to occur after each temperature setting for 10 minutes. To inspect the sample's condition and the gelation, the test tube was lastly positioned horizontally.

Drug content assay

At intervals of 7, 14, 30, 45, 60, 75, 90, and 180 days, the collected samples of each formula were examined for drug concentration. For each formula, the process was performed three times, and the mean was determined. The drug (MRT-loaded aquasomes) must be evenly dispersed throughout the nasal gel. The presence of drugs was determined in samples taken from several sites inside the container. The vesicles were lysed by adding ethanol. Absorbance was detected at 289 nm against a blank after diluting 1 mL of the preparation in 100 mL of phosphate buffer with a pH of 6.4**.** Further, % drug concentration was determined using the following equation:

% Drug content= (Concentration of drug in gel solution/equivalent conc. of drug taken) ×100

As stated by zero, first, and second-order equations, the correlation coefficient (r) was calculated, and the rate of breakdown constant was calculated using the most appropriate correlation coefficient. It also estimated the kinetic parameters (a, b, r, k, and t 0.5). K20 and t_{90}calculations were also performed (Ahmed *et al.*, 2012).

Kinetics of drug degradation

Studying the kinetics of degradation is important because it has an impact on how stable MRT is during the design and testing phases of a nasal gel. The rate of their degrading response can be slowed down with the aid of suitable storage settings. This preserves the stability of nasal gel formulations during their shelf life (Bhangare *et al.*, 2022a).

The three orders of pharmaceutical degradation processes are zero, first, and second. By knowing the order of drug degradation, pharmaceutical specialists may predict the shelf life and appropriate storage environment for pharmacological ingredients and pharmaceutical dosage forms (Bhangare *et al.*, 2022b).

Determination of shelf-life

The Arrhenius equation is a popular model for the impact of temperature on the rate of drug degradation (Peleg *et al.*, 2012). Using the Arrhenius relation, it is possible to deduce the stability at room temperature or any lower temperature using accelerated data. Mathematical representations of the Arrhenius equation exist as:(Sungthongjeen, 2013)

$K = Ae^{-Ea/RT}$ (1)

ln k = ln A − E a / RT (2)

where k is the reaction rate constant of any order, R denotes the gas constant (1.987 calories degree-1 mole-1), A is the frequency factor, Ea is the activation energy and T is the absolute temperature (Sungthongjeen, 2013).

A graphical technique was used to establish the degradation order, and the degradation rate constant (K) was calculated for each temperature. By extrapolating the value at 25 °C from the Arrhenius figure, the degradation rate constant at that temperature (K25) was found. The Shelf-Life (t_{90}) of the formulation was determined using (equation3):

Shelf Life = 0.1052/K.....equation

Where: K is the degradation rate constant (Kenneth *et al.*, 2018).

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare various physical and chemical characteristics across nasal formulations before and after storage. ANOVA was also used to analyze the calculated t_{90}, and the Turkey-Kramer test was used for multiple comparisons. These statistical tests were conducted using the GraphPad Prism program, version 5 (GraphPad Software, La Jolla, CA). Each analysis was performed in duplicate, and the MRT formulations were tested in triplicate. The difference between the groups was significant at $p < 0.05$ (Dantas *et al.*, 2016).

Results

Determination of λ max of MRT

The maximum wavelength of measuring MRT absorbance (λmax) was 289 nm in phosphate buffer (PH 6.8) as illustrated in Figure (1). This absorbance peak

was in agreement with previous study conducted by Prabhat et al (Prabhat *et al.*, 2021a).

Calibration curve of MRT in phosphate buffer solution (PBS) PH 6.8

By graphing absorbance vs concentration, MRT standard calibration curve was created as in Figure 16 289 nm the λmax was found for MRT in at phosphate buffer (PBS) solution with a pH of 6.8. MRT standard calibration curve complies with Beer's Lambert rule between 0.01-0.035mg/ml (Ezealisiji *et al.*, 2017).

Evaluation of the validity of the assay methods

The results of the UV spectroscopic approach used to test the nasal gel containing MRT-loaded aquasomes are highly consistent and reproducible. Almost 100% of MRT was recovered from the nasal gel, demonstrating the method's precision and reliability as well as the excipients' absence of interference. The technique is also simple, quick, generally precise, and robust (Blessy *et al.*, 2014). UV spectroscopy method in our study provides information on the homogeneity of the spectral peak but it is not applicable for the degradants that have the same UV spectrum and we used it only to confirm the absence of drug peak that indicated degradation of the drug in forced stress conditions as in study (Blessy *et al.*, 2014), There were different studies used UV assay in forced degradation tests as oxidative degradation of Nortriptyline hydrochloride (El Ragehy *et al.*, 2001), forced degradation of Guanabenz (Shearer & Deangelis, 1979), and degradation of Tolmetin sodium(Bakshi & Singh, 2002). As shown in Figure (3a) upon treatment of MRT with 3% H2O2 at normal conditions no peak was detected at 289 nm wavelength indicating that MRT is broken down (Hamrapurkar *et al.*, 2011). MRT also degraded under the Photolytic conditions in Figure (3b). When MRT was exposed to dry heat the area of the MRT peak remained unchanged as shown in Figure (3 c) indicating the stability of the drug to dry heat (Potale *et al.*, 2012).

Accelerated stability study of MRT nasal gel

Physical stability for the in situ nasal gel containing MRT-loaded aquasomes formulations were evaluated at the time of manufacture and after six months of storage at (4 °C and 25 °C), incorporating clarity, PH, gelation temperature, and gelation time values. During the storage period, the clarity of all formulations remained constant between extremely clear (F1 and F4) and clear (F2 and F3). Moreover, there were no residue materials or gritty particles present in the gel as shown in Table (1). Considering the PH readings, the alterations were slight and did not differ from physiologically appropriate nasal PH. The PH range for all formulations was found to be (4.71-5.93). The PH range for all preparations stored at 4°C and 25 °C was (4.65-6.04) and (4.23-5.85) respectively as shown in Table (1).

The gelling temperature of the formulations ranged from 30.3±0.29°C in F2 to 32.5±0.1 °C in F4 (Table (1)). Concerning the gelation temperature measurements after storage for six months, after being stored at 4°C for six months, it was discovered that it remained constant and ranged from 30.170.26 °C to 32.10.34 °C. After being stored at 25 °C, the formulation under study's gelation temperature slightly decreased and ranged from 26.02±0.05°C to 28.65±0.31°C. Gelling time (s) of the formulation ranged between 8.12± 0.82s and 9.7±0.94s, where the gelling time range for all preparations stored at 4°C and 25 °C was (8.45±0.14-10.04±0.65) and (9.45±0.49-10.76±2.09) respectively as shown in Table (1).

Drug content was analyzed before and during storage. The drug content of all formulations was observed in the range of100.32±0.30–99.04±0.27% before storage. Table (2) shows the percent MRT maintained following storage of in situ nasal gel formulations at 4 °C and 25 °C for six months. Tables (3 and 4) illustrate the kinetic parameters (intercept, slope, r, k, and t 1/2) for accelerated stability testing of MRT in situ nasal gel formulations at 4 °C and 25 °C according to different order reaction systems.

Accelerated stability testing was used to study the degradation of MRT, and the correlation coefficient (r) was found using zero, first, and second-order equations. The degradation rate constant was then calculated using the most appropriate correlation coefficient (r). Calculations were also made for other kinetics parameters (a, b, r, k, and $t_{0.5}$). Tables (3 and 4) show a comparison of the calculated correlation coefficient based on the three kinetic orders used. It was discovered that the decomposition reaction proceeded in zero order reaction in all formulations based on the values of the correlation coefficient. Table (5) shows the calculated E_a, K20, $t_{0.5}$at 20 ºC and t_{90}at 20 ºC for MRT-loaded aquasomes nasal gel formulations subjected to stability testing.

Table (5) shows the shelf life from the Arrhenius plot of the formulations ranging from 1.333 years in the formula containing Chitosan as a gelling agent to 2.2 years in the formula containing HPMC K4M as a gelling agent. ANOVA was carried out for the calculated t_{90} by theTurkey-Kramer Multiple Comparison Test as shown in Table 6 and Figure (4).

Discussion

Accelerated stability tests are very helpful for determining how a drug's quality changes over time as a result of a range of variables, including humidity, temperature, and light (Committee for Proprietary Medicinal Products % JEMEA, 2002). The physical and

chemical properties of a drug prototype must be thoroughly evaluated both at the start of manufacturing and throughout the intended shelf life (Schuh & Funk, 2019).

It is clear from the forced degradation study that the in situ nasal gel containing MRT-loaded aquasomes was susceptible to decomposition under oxidative and photolytic stress conditions because there was no absorbance peak at 289nm using spectrophotometric methods indicating decomposition of MRT and ensuring that the decomposition products did not give absorbance at 289 nm, as shown in figure 1. Hence, the MRT stability investigation may be done using spectrophotometric methods (Blessy *et al.*, 2014). Hydrogen peroxide is widely used for oxidation of drug substances in forced degradation studies because subjecting nasal gel solution to 0.1–3% hydrogen peroxide at neutral pH and room temperature produced relevant degradation of MRT and produced degradation products due to electron transfer mechanisms to form reactive anions and cations (Blessy *et al.*, 2014). The photostability testing of MRT-loaded aquasomes nasal gel must be evaluated to demonstrate that light exposure does not result in unacceptable change. Photostability studies exhibited that MRT decomposed due to exposure to UV (KP *et al.*, 2011). MRT was relatively stable under dry heat conditions for the time it was exposed because the area of the MRT peak remained unchanged, indicating the stability of MRT to dry heat (Potale *et al.*, 2012).

The *in-situ* nasal gel containing MRT-loaded aquasomes formulations was tested for stability at room temperature (25 °C) and in the refrigerator (4 °C). During 180 days, the nasal gel that had undergone accelerated stability experiments was assessed for clarity, pH, gelation temperature, gelation time, and real drug content (Kenneth *et al.*, 2018).

According to the study, there was no significant change in all the parameters during the 180 days of storage. There was no change in clarity throughout the storage period, where all preparations were between clear and very clear. Moreover, there were no residue materials or gritty particles present in the gel. For nasal formulations, PH is an important factor to take into account. The nasal mucosa's physiological pH typically ranges from 4.5 to 6.5. However, the nasal mucosa can withstand pH levels between 3 and 10 (Kumar *et al.*, 2019). All of the PH measurements of the investigated MRT nasal gel formulation before and after storage were found to be within the physiological nasal PH range and were well tolerated, causing no discomfort.

The temperature at which liquid turns into a clear gel is known as the gel transition temperature (Mohamed, 2020). For the evaluation of in situ gelling preparations, the gelation temperature is regarded as a crucial parameter because if the gelling temperature falls between 28 and 37 °C, it is seen as appropriate. Because if the gelling temperature is below 25 °C, a gel may form at room temperature, making it difficult to manufacture, handle, and administer, and if it is above 37 °C, a gel would not form at the temperature of the nasal cavity, leading to rapid nasal clearance of the administered drug (Kumar *et al.*, 2019). The investigated formulations' gelation temperatures did not change after being stored at 4 °C, however, there was a slight drop in those temperatures after being stored at 25 °C, which may have been caused by the dehydration of the gel formulation, but the gelling temperature of the formula containing HPMC K4M decreased but was still present in the range of requirements. These findings demonstrated that in situ nasal gel compositions can be stored under refrigeration at temperatures ranging from 2 to 8 °C (John *et al.*, 2013)

The amount of time needed for a polymeric system to transition from a sol to a gel at its gelation temperature is known as the gelation time. In addition to the physiological temperature of the nasal cavity, mucociliary clearance half-life (21 minutes) also has an impact on the performance parameters of the nasal-delivery formulations (Soane *et al.*, 1999). Before storage, all in situ formulations exhibited a rapid gelation time; however, after storage, the gelling time showed a little increase in value but remained within the acceptable range.

The chemical stability of MRT-loaded aquasomes in situ nasal gel formulations at room temperature and in the refrigerator was demonstrated by the extremely slow rate of MRT degradation at each temperature, which indicated the chemical stability of MRT-loaded aquasomes in gel formulations. The degradation of tested formulations was found to be a zero-order reaction based on the calculated values of the correlation coefficient (Marzouk *et al.*, 2018). To ascertain their contribution to the stability of nasal gel, kinetic models are investigated. A suitable model is chosen once data has been collected using various experimental techniques in order to assess their stability behavior. The zero-order kinetic model is the most commonly utilized kinetic model in drug stability. This model then uses their corresponding equations to show the outcomes of the data fitting into it. The rate of degradation in the zero-order kinetic model is unaffected by reactant concentrations (for example, the concentration of freshly made nasal gel) (Rehman *et al.*, 2020).

Statistically, the changes in these parameters after storage were not significant ($p > 0.05$). These findings supported the formulations' stability because there were no significant changes in the physical characteristics (clarity, pH, gelation temperature, and gelation time) (Naresh *et al.*, 2020). It was found that the formulations were physicochemically stable. We observed that the preparation using HPMC K4M as a

gelling agent had the highest shelf-life of 2.2 years while chitosan had the lowest shelf life of 1.33 years at room temperature according to the Arrhenius plot (Table 5), indicating that HPMC K4M had greater stability outcomes as seen by their higher t_{90}values (days) and shelf-life (Marzouk *et al.*, 2018).

Conclusion

According to the study's findings, each nasal gel dosage form formula showed good physical and chemical stability throughout a six-month observation period. Before and after six months, the nasal gel containing MRT-loaded aquasomes demonstrated the desired clarity, pH value, gelation temperature, gelation duration, and medication content which indicate the suitability of the tested polymers HPMC K4M, Carbopol, chitosan, and carrageenan as gelling agents for not affecting the quality and efficacy of MRT-loaded aquasomes in situ nasal gel. However, the nasal gel containing HPMC K4M as a gelling polymer showed the highest stability findings due to their higher $\boldsymbol{t_{90}}$ values (days), and the shelf life was calculated to be 2.2 years at room temperature.

Data Availability Statement

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors Contributions

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. All the authors agrees to publish the article.

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