Effect of Accelerated Stability Study on Characteristics of In Situ Nasal Gel of Mirtazapine Loaded Aquasomes

Abstract

background

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.

Method

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.

Results

The study's results demonstrated that the tested formulae were physically stable during the period of testing with very slow degradation and found to be zero order, Variations in the characteristics were not statistically significant by using one-way ANOVA analysis. The shelf life from the Arrhenius plot ranged from 1.333 years in (F3) to 2.2 years in (F1).

Conclusion

The results indicate 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.

1- Introduction

Mirtazapine (MRT) is a tetracyclic anti-depressant drug authorized by U.S. Food and Drug Administration (USFDA) to treat depression**(Musallam, Mahdy, Elnahas, & Aldeeb, 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) and 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, Dilip, & Sunil, 2020).**

It is crucial that MRT-loaded aquasomes nasal gel formulations are safe and effective **(Hamrapurkar, Patil, Desai, Phale, & Pawar, 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, kinetics concepts can be utilized to account for changes in physical appearance **(Aashigari, Goud, Sneha, Vykuntam, & Potnuri, 2018)** Also, the 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, Singla, & Sakhuja, 2012).** The stability of finished formulations is affected by environmental variables such the 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, Choudhary, & Res, 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 change 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, El-Say, Mahmoud, Samy, & Badawi, 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 modeling 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, Hodnett, Orr, Owen, & Peterson, 2017)**. Due to producing predictive data for the calculation of the shelf life of drugs, it is essential to estimate the kinetic behavior of these reactions **(Bhangare et al., 2022a)**.

The analysis of the rate of drug breakdown is referred to as degradation kinetic. 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 as HPMC K4M (F1), Carbopol (F2),

Chitosan (F3), and Carrageenan (F4). These four formulations were designed for studying the change in the physical properties and drug content over storage period as well as calculate its shelf life.

2- Material and Method

2.1Materials

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.

2.2 Methodology

2.2.1 Determination of λmax 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, Ramana, Reddy, Kumar, & Research, 2012). Using a suitable measuring flask and one milliliter of the prior solution, 10 milliliters of the PBS medium were 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 λmax at maximum absorbance**(N Karaşen, Altinöz, & analysis, 2000) (N. Karaşen & Altinöz, 2000)**.

2.2.2 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, Rajan, & Sahana, 2021).

2.2.3 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, Dintakurthi, & Subrahmanyam, 2013)**.

2.2.4 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.

2.2.5 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 of the safety, efficiency, and superior degradation studies as a legal necessity **(Singh et al., 2013)**. For assurance quality of the evaluated nasal gel, the ICH Guidelines include regulations pertaining to the procedures for establishing and implementing the 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, Patel, Prajapati, & Agrawal, 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 & 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 & Manasa, 2018).**

2.2.6 Accelerated stability study of MRT nasal gel

In situ, 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, Xavier, John, Patience, & Krause, 2018).**

The obtained samples were subjected to the following evaluation tests:

1- Clarity

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, Gurav, Bolmal, & Technology, 2020).**

2- Determination of PH

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).**

3- 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 in order to measure the gelation temperature. The water bath's temperature was raised from 20 to 40 °C over a period of 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 minutes 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, Badr-Eldin, Ahmed, Aldawsari, & Technology, 2018).**

4- Gelling time

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.

5- 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. It is essential that the drug (MRT-loaded aquasomes) 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. **(.A* & 2019).** 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).**

6- 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).**

7- 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).**

8- Determination of shelf life

The Arrhenius equation is a popular model for the impact of temperature on the rate of drug degradation **(Peleg, Normand, Corradini, & nutrition, 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 & Technology, 2013)**

RT E a k Ae− = (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 & Technology, 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 (equation1):

Shelf Life = 0.1052/K.....equation 1

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

3-Results

3.1 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 **Prabhat, S., S. Rajan, and S.J.J.A.P.R. Sahana**, **2021(Prabhat et al., 2021)**

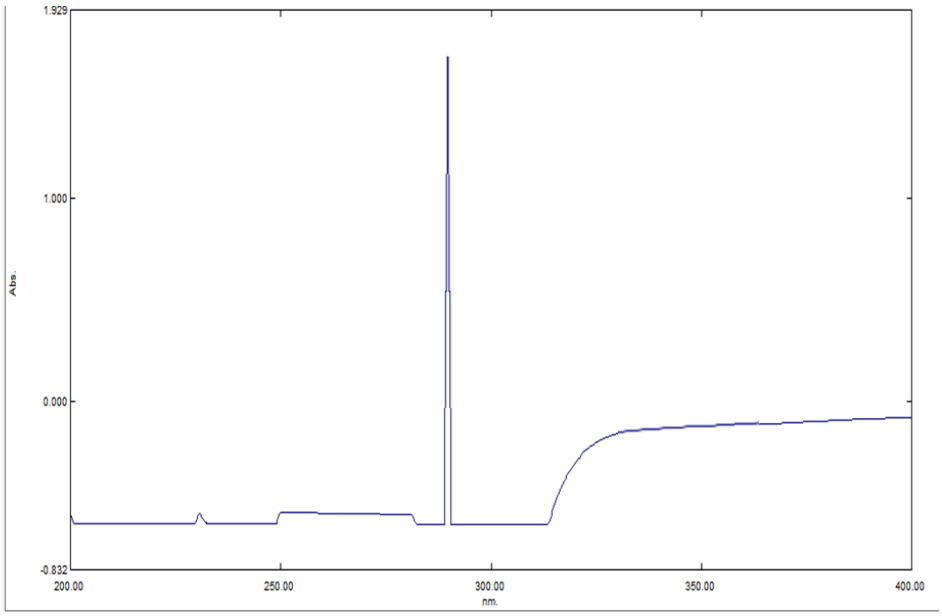

Figure (1): The maximum wavelength of measuring MRT absorbance (λmax) was 289 nm in phosphate buffer PH 6.8.

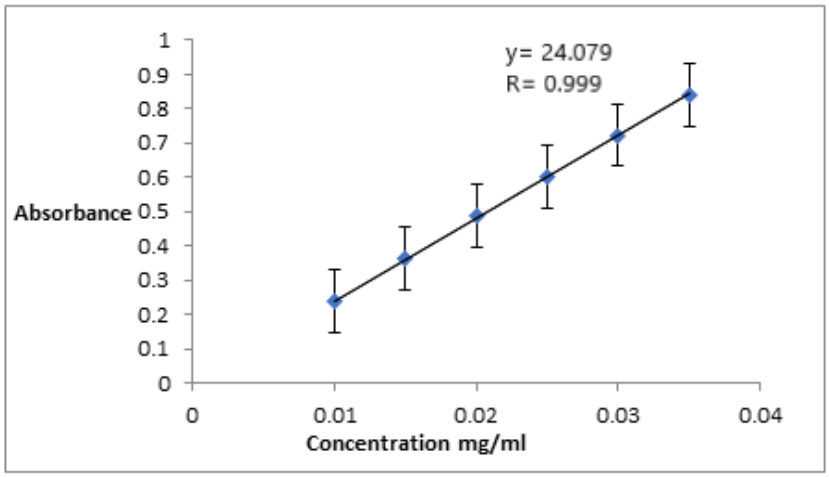

Figure (2): Standard calibration curve of MRT in (PBS) PH 6.8.

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

By graphing absorbance v/s concentration, MRT standard calibration curve was created as in Figure 2. A 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, Mbah, Osadebe, & Krause, 2017)**

3.3 Assaying the validity of the assay method

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, Abbas, El-Khateeb, & analysis, 2001),** forced degradation of Guanabenz **(Shearer & Deangelis, 1979)**, and degradation of Tolmetin sodium**(Bakshi, Singh, & analysis, 2002).** As shown in Figure (3 a) 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 (3 b).

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, Khodke, Patole, & Damle, 2012).**

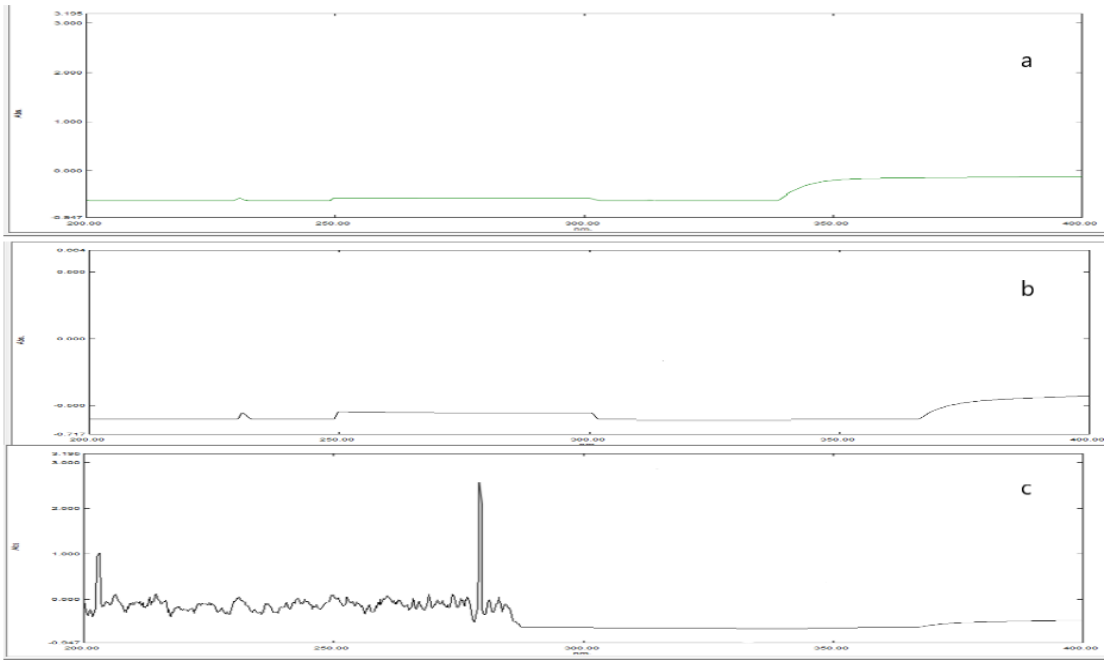

Figure (3) UV spectroscopy of MRT nasal gel oxidative degradation (a), photodegradation(b) and thermal degradation(c).

3.4 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 (4.63-6.04) 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) show 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) show 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 formula containing Chitosan as gelling agent to 2.2 years in formula containing HPMC K4M as gelling agent. ANOVA was carried out for the calculated t_{90} by Turkey-Kramer Multiple Comparison Test as shown in Table 6 and Figure (4).

Table (1) In situ gel's physical stability under two distinct temperatures before and after six months of storage

The clarity and PH of several MRT-loaded aquasomes in situ nasal gel kept for six months.						
Formula	**Clarity**			**PH ± SD**		
	At time=0	**After kept for 6 months**		**At time=0**	**After kept for 6 months**	
		(4 ± 2 °C)	**(25 ± 2°C)**		**(4 ± 2 °C)**	**(25 ± 2°C)**
F1	+++	+++	+++	5.71±0.08	5.65±0.04	5.45±0.09
F2	++	++	++	5.77±0.03	5.70±0.43	5.83±0.38
F3	++	++	++	4.71±0.12	4.65±0.06	4.23±0.02
F4	+++	+++	+++	5.93±0.32	6.04±0.12	5.85±0.09
Gelation time and gelation temperature of several MRT loaded aquasomes in situ nasal gel kept for six months						
Formula	**Gelation temperature ± SD**			**Gelation time(s) ± SD**		
	At time=0	**After kept for 6 months**		**0=At time**	**After kept for 6 months**	
		(4 ± 2 °C)	**(25 ± 2°C)**		**(4 ± 2 °C)**	**(25 ± 2°C)**
F1	30.5±0.34	30.17±0.26	28.65±0.31	8.65±0.38	8.45±0.14	9.45±0.49
F2	30.3±0.89	30.1±0.37	27.88±0.45	8.33±0.06	9.34±0.73	10.03±0.38
F3	31.3±0.14	31,56±0.13	26.02±0.05	8.12±0.82	9.07±0.02	10.49±1.32
F4	32.5±0	32.1±0.34	27.5±0.29	9.7±0.94	10.04±0.65	10.76±2.09
(++) means clear solution, (+++) means very clear solution.						

Table (2) Percent MRT preserved in formulations of in situ nasal gels kept for six months at 4 ºC and 25 ºC.

Percent MRT preserved in formulations of in situ nasal gels kept for six months at 4 ºC				
Time (days)	**%MRT retained at specified time intervals (days) ±SD for the following formu**			
	F1	**F2**	**F3**	**F4**
0	100.32±0.30	99.28±0.12	99.04±0.27	99.55±0.23
7	100.32±0.15	99.28±0.20	99.04±0.25	99.47±0.29
14	100.15±0.25	99.25±0.43	98.94±0.15	99.13±0.85
30	100.05±0.14	99.15±0.76	98.15±0.28	99.13±0.17
45	100.05±0.46	98.77±0.15	98.15±0.46	99.08±0.18
60	99.70±0.40	98.65±0.17	98.03±0.43	98.45±0.40
75	99.20±0.15	98.65±0.34	97.47±0.56	98.32±0.43
90	99.20±0.15	98.30±0.28	97.40±0.15	98.20±0.29
180	98.08±0.41	97.32±0.33	95.76±0.54	96.85±0.16
Percent MRT preserved in formulations of in situ nasal gels kept for six months at 25 ºC.				
Time (days)	**%MRT retained at specified time intervals (days) ±SD for the following formu**			
	F1	**F2**	**F3**	**F4**
0	100.32±0.30	99.28±0.12	99.04±0.27	99.55±0.23
7	100.12±0.45	99.28±0.80	99.04±0.98	99.37±0.21
14	100.12±0.25	99.12±0.23	98.55±0.55	99.37±0.32
30	100.03±0.84	99.10±0.46	98.00±0.38	99.03±0.82
45	99.95±0.49	98.70±0.10	98.00±0.46	98.78±0.64
60	99.50±0.40	98.43±0.57	97.93±0.13	98.25±0.48
75	99.06±0.43	98.15±0.13	97.15±0.32	98.08±0.83
90	98.96±0.25	97.36±0.48	97.10±0.21	98.00±0.42
180	97.6±0.17	97.38±0.23	96.16±0.09	96.45±0.65

Table (3): Kinetic parameters and calculated correlation coefficients of the degradation profile of MRT in situ nasal gel formulations at 4 ºC according to zero, first and second order kinetics.

Kinetic parameters of the degradation profile of MRT in situ nasal gel formulations at 4 ºC according to zero order kinetics.					
Formula	**Intercep (a)**	**Slope (b)**	**Correlation Coefficient (r)**	**Rate Constant (k) mg%/day**	**$t_{0.5}$ (days)**
F1	100.407	0.01331	0.9566	0.0133	3755.48
F2	99.35	-0.01	-0.978	0.011	545.85
F3	99.06	-0.01961	-0.96924	-0.01961	2549.55
F4	99.51	0.01513	-0.96536	0.01513-	3305.61
Kinetic parameters of the degradation profile of MRT in situ nasal gel formulations at 4 ºC according to first order kinetics.					
Formula	**Intercept (a)**	**Slope (b)**	**Correlation Coefficient (r)**	**Rate Constant (k) mg%/day**	**$t_{0.5}$ (days)**
F1	-0.1097	0.01324	0.5513	0.03065	22.41
F2	-0.16398	0.004399	0.974346	0.010131	68.40681
F3	0.00535	0.005232	0.950845	0.01205	57.51088
F4	-0.26463	0.006355	0.951147	0.014635	47.35327
Kinetic parameters of the degradation profile of MRT in situ nasal gel formulations at 4 ºC according to second order kinetics.					
Formula	**Intercept (a)**	**Slope (b)**	**Correlation Coefficient (r)**	**Rate Constant (k) mg%/day**	**$t_{0.5}$ (days)**
F1	-9.46991	0.089333	0.317957	0.089333	0.111
F2	1.414504	-0.0098	-0.96825	-0.96825	1.02043
F3	0.994416	-0.00798	-0.92368	-0.00798	-1.25389
F4	-0.26463	0.006355	0.951147	0.014635	47.35327

The calculated correlation coefficients of stability of MRT in situ nasal gel formulations at 4 ºC according to zero, first and second order systems.				
Formula	***Correlation Coefficient (r)***			***Order***
	Zero order	***First order***	***Second order***	
F1	***0.9566***	*0.8813*	*0.317957*	*Zero*
F2	***-0.97874***	*0.974346*	*-0.96825*	*zero*
F3	***-0.96924***	*0.950845*	*-0.92368*	*Zero*
F4	***-0.96536***	*0.951147*	*0.951147*	*Zero*

Table (4): Kinetic parameters and calculated correlation coefficients of the degradation profile of MRT in situ nasal gel formulations at 25 ºC according to zero, first and second order kinetics

Kinetic parameters of the degradation profile of MRT in situ nasal gel formulations at 25 ºC. according to zero order kinetics.

Formula	Intercept (a)	Slope (b)	Correlation Coefficient (r)	Rate Constant (k) mg%/day	$t_{0.5}$ (days)
F1	100.3639	-0.01511	-0.96204	-0.01511	3308
F2	99.462	-0.01955	-0.9853	-0.0195	2557
F3	98.97844	-0.02186	-0.9634	-0.02186	2278
F4	99.55322	-0.01868	-0.98714	-0.01868	2676

Kinetic parameters of the degradation profile of MRT in situ nasal gel formulations at 25 ºC. according to first order kinetics.

Formula	Intercept (a)	Slope (b)	Correlation Coefficient (r)	Rate Constant (k) mg%/day	$t_{0.5}$ (days)
F1	-0.199	0.0045	0.943	0.0134	67
F2	-0.1719	0.00619	0.9573	0.1426	48
F3	0.03065	0.005346	0.937374	0.012311	56
F4	-0.27647	0.007365	0.973923	0.016962	40

Kinetic parameters of the degradation profile of MRT in situ nasal gel formulations at 25 ºC. according to second order kinetics.

Formula	Intercept (a)	Slope (b)	Correlation Coefficient (r)	Rate Constant (k) mg%/day	$t_{0.5}$ (days)
F1	-9.84968	0.154822	0.342062	0.154822	0.064
F2	1.385	-0.0116	-0.9839	-0.01162	0.86
F3	0.924106	-0.0076	-0.88984	-0.0076	1.315
F4	1.817358	-0.01775	-0.92717	-0.01775	0.56

The calculated correlation coefficients of stability of MRT in situ nasal gel formulations at 25 ºC. according to zero, first and second order systems.

Formula	Correlation Coefficient (r)			Order
	Zero order	First order	Second order	
F1	**-0.96204**	0.943	0.342062	**Zero**
F2	**-0.9853**	0.9573	-0.9839	**zero**
F3	**-0.9634**	0.937374	-0.88984	**Zero**
F4	**-0.98714**	0.973923	-0.92717	**Zero**

Table (5) Kinetic data for MRT loaded aquasomes in situ nasal gel formulations based on stability study according to zero order kinetics and shelf life of MRT loaded aquasomes according to Arrhenius plot.

formulae	K4 (mg%day-1	K25 (mg%.day-	Average Ea Cal/mol	Calculated K20 (mg%.day-	$t_{0.5}$ at 20 c (days)	t_{90} at 20 c (days)	Shelf life (years)
F1	0.01331	0.01511	2313.68	0.01245	4015.55	803.11	2.200 years
F2	0.0101	0.0142	2661.54	0.01315	3801.78	760.35	2.083 years
F3	0.01961	0.02186	884.51	0.02133	2343.89	486.77	1.333 years
F4	0.01513	0.01868	1646.58	0.01781	2806.71	561.34	1.537 years

Table (6): ANOVA for the calculated t 90 of MRT loaded aquasomes in situ nasal gel formulations based on stability testing.

Turkey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?
F1 vs F2	56.37	2.514	No
F1 vs F3	317.7	14.17	Yes
F1 vs F4	266.0	11.86	Yes
F2 vs F3	261.3	11.65	Yes
F2 vs F4	209.7	9.349	Yes
F3 vs F4	-51.67	2.304	No

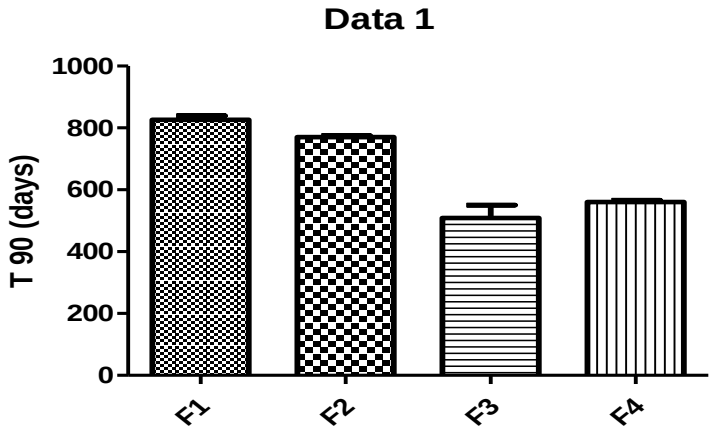

Figure (4): ANOVA for the calculated t_{90} of MRT loaded aquasomes in situ nasal gel formulations based on stability testing.

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 %J EMEA, 2002)**. The physical and chemical features of a drug prototype must be thoroughly evaluated both at the beginning of the manufacturing and during the planned shelf-life duration **(Schuh & Funk, 2019).**

It is clear from the forced degradation study that the in situ nasal gel containing MRT-loaded aquasomes was susceptible to the 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 mechanism 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, Tajne, & Ahmed, 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 the 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 the PH measurements of investigated MRT nasal gel formulation before and after storage were found to fall within the physiological nasal PH range and is well-tolerated, causing no discomfort to it.

The temperature at which liquid turns into a clear gel is known as the gel transition temperature **(Mohamed, Nasr, Salama, & Refai, 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 to 37 °C, it is seen to be 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 still present in the range of requirements. These findings showed that in situ nasal gel compositions may be stored under refrigeration in temperatures between 2 and 8 °C **(John, Nair, & Anoop, 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 min) 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, Osman, & Abd El-Fattah, 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 pace of the degradation reaction in the zero-order kinetic model is unaffected by the reactant concentrations (such as the concentration of freshly made nasal gel)**(Rehman, Akash, Rasool, Rehman, & Kinetics, 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 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 $\boldsymbol{t_{90}}$values (days) and shelf life **(Marzouk et al., 2018).**

5-Conclusion

According to the study's findings, each nasal gel dosage form formula showed good physical and chemical stability over the course of 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 t_{90} values (days), and shelf life was calculated to be 2.2 years at room temperature.

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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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