American Journal of
Nanoscience and Nanotechnology Research
http://www.ivyunion.org/index.php/ajnnr

Research Article

Evaluation of Accelerated Stability Testing of a Mirtazapine-loaded Nanoemulsion as per International Conference on Harmonization (ich) Protocols

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Abstract : Remarkable alteration in bio – therapeutic toxicity may occur as a result of physicochemical deterioration of active pharmaceutical ingredients or drugs hence the need for drug stability studies. The aim of this research was to investigate the stability of mirtazapine in nano-emulsion and to establish the shelf life of mirtazapine in the formulation. Nanoemulsion was prepared by spontaneous emulsification method (titration method). Thermodynamic stability studies on the nanoemulsions were performed at various temperatures. Particle sizes, polydispersity index, zeta potential were used to characterize the nanoemulsions. Physicochemical properties were determined on the optimized nanoemulsions using standard methods. Mirtazapine was incorporated into the oil phase in 1.5 % w/v to give an oil/water nanoemulsion formulation. The ICH protocols were observed in carrying out the stability studies for a period of three months. Determination of the shelf life of the nano-emulsion formulation was achieved following accelerated stability studies at 4 ± 0.7 °C, 25 ± 0.5 °C, 40 ± 0.5 °C, 50 ± 0.4 °C and 65 % ± 5 relative humidity (RH). Result showed that the droplet size, conductivity, refractive index were slightly increased while the pH and viscosity slightly decreased during the 3 months period. The observed slight changes in the parameters were not statistically significant (**p** 0.05). The shelf life was found to be 2.88 years at room temperature. The degradation (%) of the optimized mirtazapine nanoemulsion was determined. This research work confirmed that the physicochemical stability of mirtazapine was enhanced in the nanoemulsion.

Keywords: Nano-emulsion; Mirtazapine; Shelf-life; ICH; Conductivity; Viscosity

Received: January 12, 2018; **Accepted**: February 6, 2018; P**ublished**: February 21, 2018

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

The integrity and quality of drug preparation changes under the influence of certain environmental factors such as light, humidity and temperature. World Health Organization has been addressing the salient issues as it relates to drug stability and storage with particular attention to the developing countries most of which are located in the tropical climate zones where drug stability poses more serious problems which may lead to changes in the shelf-life of the pharmaceutical product [1]. Nanoemulsions are isotropic, thermodynamically stable, transparent system of oil, water and surfactants with a droplet size range of 20 – 200 nm [2]. Nanoemulsions may become unstable due to the effect of environmental factors on the globular size leading to coalescence, flocculation and Ostwalds ripening [3-6]. This calls for standard protocol on stability studies on the formulated products. An increase in the mean globular size with time coupled with a decrease in globular number could be attributed to coalescence in the nanoemulsion formulation [7], as such, a careful stability studies using microscopic examination or electronic particle counting device such as the Photon Correlation Microscope with Zetta- sizer [8] and the Transmission Electron Microscope [9] were conducted. Oswald ripening is an irreversible transfer of small globlets into a larger one so as to form entirely new ones with larger size. In flocculation, several smaller droplets come together to form floccules, this may result in creaming. The above three factors may lead to total breakdown of an emulsion formulation giving rise to an observed phase inversion of the dispersed system.

Accelerated stability studies are carried out to check the effect of the above factors on the overall integrity and quality of the mirtazapine loaded nanoemulsion formulation. Change in viscosity can equally be used as a study tool in determining the stability of nanoemulsions, any variation in globular size or number, or in the orientation or migration of emulsifier over a period of time may be detected by a change in apparent viscosity. In order to compare the relative stabilities of range of similar products, it is often necessary to speed up the process of creaming and coalescence. This can be achieved by means of exaggerating temperature fluctuations to which any product is subjected under normal storage condition and this is the basis of accelerated stability studies. Change in the droplet size as well as droplet count is measured by the use of Transmission Electron Microscope or the Scanning Electron Microscope, while the use of Photon Correlation Microscope with Zetta-sizer is essential for Zeta-potential determination. Mirtazapine, [±]-2-methyl-1,2,3,4,10,14b-hexahydropyrazino-[2,1-9]pyrido[2,3-e]benzazopine whose chemical structure is presented in Figure 1, is a tetracyclic antidepressant drug that exist as enantiomers and both enantiomers contributes to the antidepressant activity. Clinically, mirtazapine is used for the treatment of moderate to severe depression and anxiety.

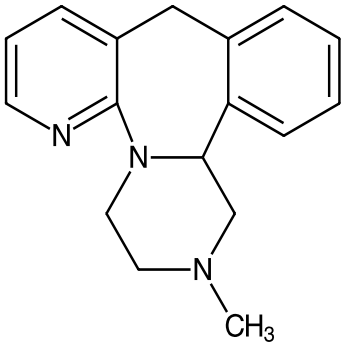

Figure 1 Chemical structure of mirtazapine.

Mirtazapine is available only as tablets in doses of 15, 30 and 45 mg respectively [10]. The drug is practically insoluble in water and its logarithm partition coefficient (Otanol-water) is 2.9, indicating high hydrophobicity hence the nanoemulsion is oil in water type. In this work, the stability of mirtazapine loaded nano-emulsion was investigated and the shelf-life of such nano-emulsion formulation was established.

Materials and methods

Materials

Mirtazapine was purchased from Mylan Pharmaceuticals, (USA), Propylene glycol, Sodium lauryl sulphate (Sigma- Aldrich, USA). Sunflower oil of analytical grade was used without further purification.

Preparation of Mirtazapine loaded Nanoemulsion

The water titration method was employed [11] in this study. Mirtazapine (1.5 % w/v) was dissolved into co-surfactant or Smix in a beaker and warmed at 37 °C in a water-bath and mixed. The drug was dissolved; this was followed by slow titration with aqueous phase to get a clear final preparation of 100 % (v/v). Nanoemulsion was prepared by subjecting the sample to homogenization using Virtis 6-105 Homogenizer at 50 Hz for 10 minutes.

Characterization of Nanoemulsions (Droplet Size and Morphology)

Size and size distribution

The Photon Correlation Microscope with Zeta-Sizer (Malvern Nano ZS.ZS 290.UK) and Transmission Electron Microscope (VEGAimuGmbH,Germany), were used to characterize the nanoemulsions. Droplet size and morphology of the nano-emulsions were studied using TEM (operating at 200 KV) at increasing magnification and diffraction modes. Sample preparation involved placing a drop of 1 in 100 ml dilution of nanoemulsion on a copper grid, allowed to dry and further sealed with a monolayer of gold. After drying, the sample was photographed by transmission electron microscopy.

Viscosity Measurement

The viscosity of nano-emulsion was measured using the small sample adaptor of a Brookfield rheometer (Model DV-III, Brookfield Eng. Labs, Inc., Stoughton, MA, USA) at 25 °C. An average of three data points was obtained to determine the viscosity at a shear rate of 7.34s [12].

Refractive Index and pH Measurement

The refractive index (RI) of the nano-emulsion was determined using an Abbe-refractometer [13] whereas, the pH of the samples was done using a pre-calibrated pH meter. A 5 ml of the sample was transferred into a beaker and pH meter probe was immersed into the container and the pH reading recorded. The pH of the freshly prepared formulation was measured and compared with the changes in pH of the formulation after specified intervals at the different studied temperatures.

Conductivity Measurement [14]

Conductivity of nano-emulsion sample was measured using a conductometer (Cyberscan, Eutech Instrument, Singapore). A 2 ml sample was transferred into a beaker and the conductivity measured and recorded. The conductivity of the freshly prepared formulation was measured and the result was compared with the change in conductivity of the formulation after a period of 90 days at 30 days interval at different temperatures under study.

Statistical Analysis

Statistical analysis was performed using one-way Anova test to determine the difference of all the parameters studied initially and after 90 days of observation at all storage conditions. A significant difference was considered at a p-value of less than 0.05.

Stability Studies in line with ICH Protocols.

Accelerated stability studies were performed on optimized mirtazapine nano-emulsions in line with International Conference on Harmonization (ICH) protocols [15]. Replicates of the prepared samples were kept at refrigerator temperature (4 ± 0.7 °C), room temperature (25 ± 0.5 °C/60 ± 5 % RH.) and 40 ± 0.5 °C /75 ± 5 % RH. Samples were withdrawn at 0, 30, 60 and 90 days. The samples were evaluated for droplet size, viscosity, pH, conductivity and refractive index. Three batches of the optimized mirtazapine nano-emulsion were equally taken in a glass vial and kept at accelerated temperatures of 30, 40, 50 and 60 °C at ambient humidity, samples were taken and analyzed for mirtazapine content using UV-Spectrophotometer at 290 nm.

Determination of Shelf-Life [16]

Withdrawn samples were extracted by dissolving in methanol. Drug content in the solvent extracted was analysed spectrophotometrically against standard solvent solution of mirtazapine. The concentration of mirtazapine in the nano-emulsion formulation at different elevated temperatures were established and recorded. Samples of pure sunflower oil and surfactant-co-surfactant mixture without mirtazapine were also ran separately to check the interference of excipients used. The amount of drug decomposed and the amount remaining at each time interval was calculated. The degradation order was determined by a graphical method, while degradation rate constant (K) was determined at each temperature. An Arrhenius plot was constructed between log K and 1/T to determine the Shelf-Life of the optimized nano-emulsion formulation. The degradation rate constant at 25 °C (K_{25}) was determined by extrapolating the value at 25 °C from the Arrhenius plot. The Shelf-Life ($T_{0.9}$) of formulation was determined using (equation 1):

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

Where: K is the degradation rate constant. (Dimensionless)

Results

The results obtained from the experiments and measurements are presented in Tables 1-4 and Figures 2-5.

Table 1 Droplet-size, viscosity, refractive-index and conductivity of optimized nano-emulsion, during the period of 90 days of storage

Time (days)	Temp.(°C)	Mean droplet size(nm)±SD (n=3)	Mean viscosity (mp)±SD (n=3)	RI±SD (n=3)	pH±SD (n=3)	Conductivity (µs) ±SD, (n=3)
0	4 ± 0.7	128.2 ± 0.7	33.2 ± 1.1	1.416 ± 0.02	8.4 ± 0.01	250 ± 3.2
30	4 ± 0.7	128.9 ± 0.3	32.8 ± 1.2	1.512 ± 0.01	8.4 ± 0.01	251 ± 2.2
60	4 ± 0.7	128.5 ± 0.7	32.8 ± 1.1	1.514 ± 0.02	8.4 ± 0.03	250 ± 3.2
90	4 ± 0.7	128.4 ± 0.8	32.9 ± 1.0	1.520 ± 0.01	8.4 ± 0.02	250 ± 2.2
0	25 ± 0.5	128.1 ± 0.3	32.4 ± 1.0	1.500 ± 0.02	8.3 ± 0.02	251 ± 3.4
30	25 ± 0.5	128.4 ± 0.2	32.4 ± 1.2	1.510 ± 0.01	8.2 ± 0.01	251 ± 1.0
60	25 ± 0.5	129.2 ± 0.1	32.3 ± 1.0	1.512 ± 0.02	8.1 ± 0.03	252 ± 2.0
90	25 ± 0.5	129.8 ± 0.3	32.2 ± 1.2	1.513 ± 0.03	8.1 ± 0.01	252 ± 1.0
0	40 ± 0.5	128.6 ± 0.2	32.1 ± 2.0	1.512 ± 0.08	8.1 ± 0.02	252 ± 4.0
30	40 ± 0.5	129.4 ± 0.4	31.8 ± 1.2	1.513 ± 0.09	8.0 ± 0.08	253 ± 2.0
60	40 ± 0.5	129.8 ± 0.8	31.6 ± 2.0	1.514 ± 0.06	8.0 ± 0.05	253 ± 4.0
90	40 ± 0.5	129.9 ± 0.7	31.0 ± 1.0	1.514 ± 0.08	8.0 ± 0.02	254 ± 2.0

Table 2 Degradation of optimized nano-emulsions.

Time (days)	Temp.(^{o}C)	Drug content (mg)	Drug conc.degraded (mg)	% drug remaining	Log (%) drug remaining.
0	4 ± 0.7	15.000	0	100	2
30	4 ± 0.7	14.960	0.040	99.733	1.9988
60	4 ± 0.7	14.900	0.100	99.333	1.9970
90	4 ± 0.7	14.835	0.165	98.900	1.9950
0	25 ± 0.5	15.000	0	100	2
30	25 ± 0.5	14.915	0.085	99.433	1.9975
60	25 ± 0.5	14.830	0.170	98.867	1.9951
90	25 ± 0.5	14.755	0.245	98.367	1.9926
0	40 ± 0.5	15.000	0	100	2
30	40 ± 0.5	14.900	0.100	98.333	1.9970
60	40 ± 0.5	14.810	0.190	98.733	1.9945
90	40 ± 0.5	14.715	0.285	98.200	1.9821
0	50 ± 0.4	15.000	0	100	2
30	50 ± 0.4	14.860	0.140	99.066	1.9959
60	50 ± 0.4	14.750	0.250	98.333	1.9926
90	50 ± 0.4	14.675	0.325	97.833	1.9912

The correlation coefficient when compared for Figure 1 and Figure 2 shows that the coefficients for first-order is more reliable as compared to the zero-order, hence it was confirmed that the degradation of mirtazapine follows a first-order kinetics. As such, the log % of drug remaining was plotted against time (Fig.1) and K was calculated from the slope of the curve at each temperature (equation 2). The results are presented in Table 3.

Slope = - 2.303K.....equation 2

Where: K is the degradation rate constant.

Table 3 Temperature, K (day-1) and Absolute temperature.

Temp.(°C)	Slope	K (day^{-1})	log K	Absolute Temp.(T)	1/T x 10^{-3}
4	-0.000056	0.000129	-3.88849	277	3.610108
25	-0.000008	0.000184	-3.73462	298	3.355705
40	-0.000087	0.000201	-3.69653	313	3.194888
50	-0.000108	0.000248	-3.60562	323	3.095975

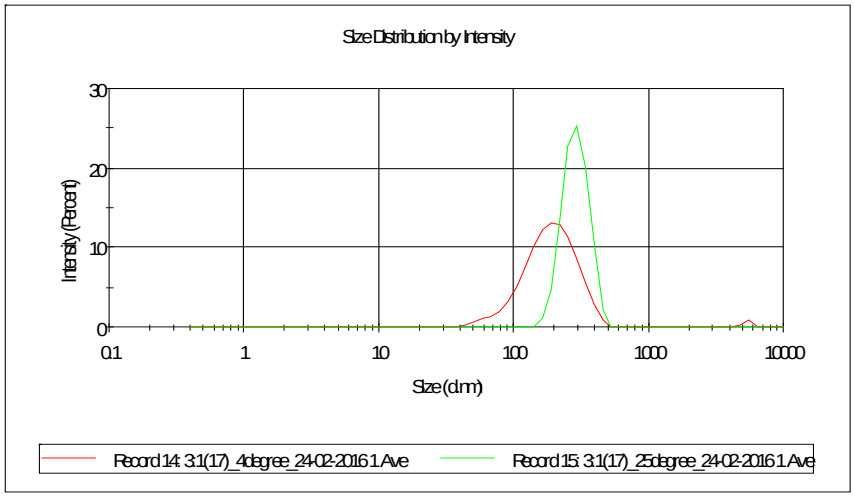

Figure 2 Size distribution by intensity

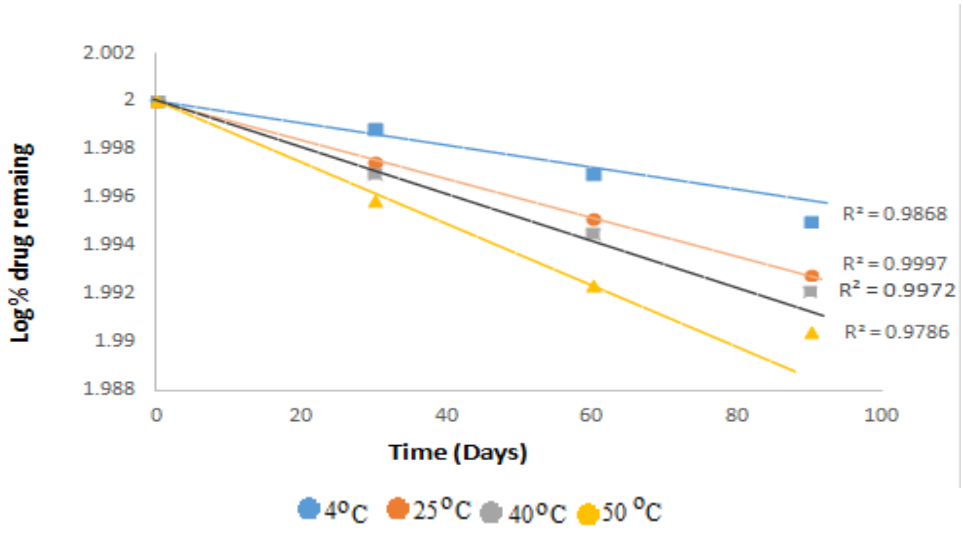

Figure 3 First-order degradation kinetics of mirtazapine from optimised nano- emulsions, at different temperatures.

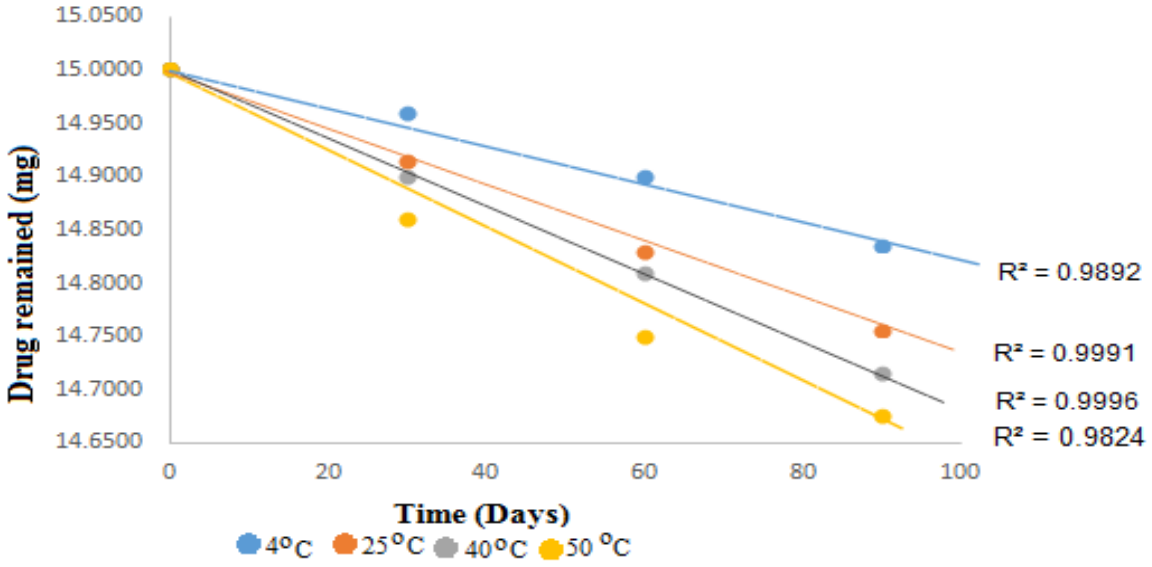

Figure 4 Zero-order degradation kinetics of mirtazapine from the optimized nano-emulsions at different temperatures

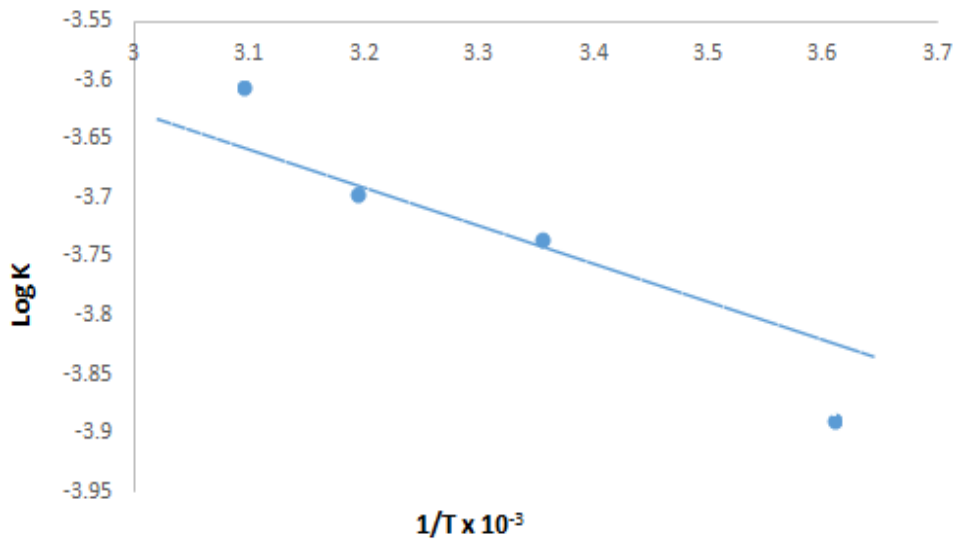

Figure 5 A graph of log K against 1/T x 10^{-3}

The shelf-life of mirtazapine-loaded nanoemulsion was observed from calculation using equation 1 to be 2.88 years.

Discussion

Accelerated stability studies are particularly useful for assessing how the quality of a drug varies over time under the influence of a variety of factors such as humidity, temperature and light. A drug proto-type must be fully assessed with regards to its physical, chemical and microbiological characteristics at the start of study and throughout the intended shelf life period. Optimised nano-emulsion formulation subjected to accelerated stability studies were evaluated on the basis of droplet size, viscosity, pH, conductivity and refractive index for the period of 90 days. The study showed that there was no significant change in all the parameters after 90 days. The refractive index, conductivity and droplet size were slightly increased while the pH and viscosity decreased. The stability of the mirtazapine nano-emulsion was also checked at refrigerator (4 °C) and room temperature (25 °C). The study showed that there was no significant change in all the parameters during the 90 days period of storage.

Statistically, the changes in these parameters were not significant (p • 0.05). These results justified the fact that the optimized formulations were stable as there were no significant changes in the physical parameters (droplet size, viscosity, pH, conductivity and refractive index). The degradation of mirtazapine was very slow at each temperature which indicated the chemical stability of mirtazapine in the nano-emulsion formulation below room temperature. The optimized nano-emulsion was found to be physicochemically stable.

The order of degradation of active pharmaceutical ingredient (mirtazapine) was determined at each temperature by a graphical method. The degraded and remaining concentrations of mirtazapine at different temperature are shown in Table 3 and the degradation partern was found to be first-order (Figure 3.)

Conclusion

The simple water titration method was effective in achieving reproducible mirtazapine nano-emulsion with the desired physicho-chemical and safety characteristics. The shelf life from the Arhenius plot was found to be 2.88 years at room temperature and the degradation (%) of the optimized mirtazapine nano-emulsion was determined (Table 2), thus establishing that the physicochemical stability of mirtazapine was enhanced in the nanoemulsion.

Acknowledgements

X. Siwe Noundou is grateful for an NRF Postdoctoral Fellowship (88559) and a Rhodes University Post-Doctoral Research Fellowship.

Author Contributions

Author Name	Research Conception/ Design	Data Acquisition	Data Analysis/ Interpretation	Manuscript Preparation	Final Approval
K. M. EZEALISIJI[1]	x	x	x	x	x
X. SIWE NOUNDOU[2]	x	x	x	x	x
C. J. MBAH[3]	x		x		x
P. O. OSADEBE[3]	x		x		x
R. KRAUSE[2]	x	x	x		x

Disclosure

The authors declare no conflict of interest.

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