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NANOEMULSION AS A PLATFORM FOR PARENTERAL ADMINISTRATION OF CARBAMAZEPINE: IN VITRO AND IN VIVO STUDY

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ABSTRACT
The aim of this work was to formulate carbamazepine (CBZ) nanoemulsion for parenteral administration. A 2^3 full factorial design was employed to study the influence of type of co-surfactant (either propylene glycol (PG) or polyethylene glycol 400 (PEG400)), type of surfactant (either Polysorbate 80 or Poloxamer 188) and surfactant/co-surfactant ratio (Smix), on the physicochemical characteristics of the prepared nanosystems. Nanoemulsions were prepared by spontaneous emulsification technique. *In vitro* and *in vivo* evaluation were investigated. Stability under different temperature (5.0 and 25°C) for 6 months was also tested. Droplet size ranged from 20.0±1 to123±4 nm. Encapsulation efficiency was up to 84%. According to the statistical analysis using polynomial equations and response optimization, the optimized formula was F6 with PEG400 as a co-surfactant, Polysorbate 80 as a surfactant and the highest Smix. *In vitro* release showed sustained release of CBZ over time that best fitted to Higuchi kinetic modeling. *In vivo* study revealed the superiority of the optimized formula (F6) over the marketed drug suspension with delaying of the onset and reduction of the frequency of clonic convulsion. Regarding stability, most of the prepared formulae showed nonsignificant change in particle size and entrapment efficiency indicating good physical stability.

KEYWORDS: nanoemulsion, anticonvulsant, factorial design, epilepsy.

1. INTRODUCTION
Carbamazepine (CBZ) is a medication that have long been used to treat epilepsy. It can also be used to treat peripheral neuropathy (nerve pain caused by diabetes) and trigeminal neuralgia (a pain condition of the face). It is sometimes described in the management of bipolar syndrome, if other therapy is not effective.[1–3] Unfortunately, CBZ shows poor aqueous solubility that had resulted in the incomplete and slow absorption after oral administration.[4] Therefore, the preparation of parenteral formulation can overcome the problems encountered with the oral route. Parenteral route has the advantage of providing a drug form that could be helpful in the emergency cases. Many trials have been conducted to administer CBZ via intravenous administration. The early strategies based on enhancing CBZ solubility via, for example, complexation with hydroxypropyle-β-cyclodextrin[5,6] or employing co-solvency approach.[7] However, these trials were of limited applicability. Recently, the preparation of submicron/nano- emulsions was emerged as a promising way for CBZ parenteral administration.[8]

Nanoemulsions are colloidal dispersion systems formed by mixing two immiscible liquids in presence of surfactant, cosurfactant and/or cosolvent. The dispersion is stabilized by the interfacial film created by surfactant/cosurfactant or cosolvent system and usually having a droplet size range of 20–600 nm.[9] The major applications of nanoemulsions is to act as carrier for drug molecules with the aim of improving their bioavailability following oral administration[10] or sustaining drug release after parenteral injection.[8]

CBZ emulsion prepared by the traditional methods was reported.[11] To prepare CBZ nanoemulsion, high-pressure homogenization (SolEmuls®) technique was adopted.[12] Later on, nanoemulsion was prepared by the less sophisticated spontaneous emulsification technique that received increased attention due to simplicity and laboratory scalability.[8]

The aim of this work was to prepare CBZ nanoemulsion for parenteral administration**.** A 2^3 full factorial experimental design was employed to study the influence of three selected variables, each at two levels, on the physicochemical characteristics of the prepared nanosystems. The stability of the prepared formulations was studied under two storage conditions. The optimized formulation was evaluated in vivo for its anti-convulsion effect.

2. MATERIALS AND METHODS

2.1. Materials

Carbamazepine CBZ raw material, Castor oil, medium-chain triglyceride, soya bean oil, and CBZ reference standard (99%) were kindly supplied by Medizen Pharmaceutical Industry (Egypt). Propylene glycol (PG), polyethylene glycol 400 (PEG 400), Polysorbate 80 (Tween 80®), Polyoxyl 35 castor oil (Cremophor® El), Poloxamer 188 (Kolliphor®P188) were kindly supplied by AL Andalous Pharmaceutical industries (Egypt). Acetonitrile, HPLC grade, was purchased from JT Baker (NJ, USA). Formic acid, sodium chloride (NaCl), potassium chloride (KCl), Disodium phosphate (Na2HPO4), potassium dihydrogen phosphate (KH2PO4), and triethylamine were purchased from Scharlau Chemicals, Spain.

2.2. High-performance liquid chromatographic analysis (HPLC)

The HPLC apparatus consisted of Agilent LC - system (1260, USA) equipped with a model LC pump VL and a variable-wavelength detector (set at 230 nm), and injection volume with a 50 µL. The stationary and mobile phases used included Hyperclone CN (250 mm ×4.6 mm, 5µm particle size, 100 A˚ pore diameter). The mobile phase consisted of acetonitrile: water (20:80 v/v) containing 0.3 ml of formic acid and 0.5 ml of triethylamine per liter. The mobile phase was pumped in an isocratic flow for 10 min at a flow rate of 2 ml/min at a temperature of 35°C. The method was validated for CBZ assay, according to ICH guidelines (ICH, 2005), with respect to specificity, linearity, precision, and accuracy.

2.3. Determination of CBZ solubility in different oils

The solubility of CBZ in castor oil, medium-chain triglyceride (capric/ caprilic diglycerides), and soya bean oil was measured by adding extra amount of CBZ to oil and left for 24 h to reach equilibrium with moderate stirring. The supernatant sample was analyzed by HPLC method after centrifugation at 6000 rpm for half an hour.

2.4. Design and optimization of CBZ nanoemulsion

Experimental design used to evaluate the effect of different variables with lowest number of formulation trials. In this study, Polysorbate 80 and Poloxamer 188 were chosen as hydrophilic surfactants, while PG and PEG 400 were used as co-surfactants (to facilitate the stabilization process). Statistical analysis using polynomial equations for the required responses will indicate how significant the effect of each variable. A 2^3 full factorial experimental design was used to study the influence of three variables, with two levels each, on the physicochemical characteristics of the prepared nanoemulsions. These variables were as follow; (A) Type of co-surfactant (either PG or PEG400); (B) Type of hydrophilic surfactant (either Polysorbate 80 or Poloxamer 188); and (C) Smix ratio at two levels (2:1 and 1:1; Cremophor EL: to either PG or PEG, respectively). The selected parameters were droplet size, entrapment efficiency (EE) and T50 (time required for the release of 50% of the loaded drug). Using this design, eight trials were performed in three replicates, at least, to estimate experimental error (Tables 1 and 2).

Table 1: The factorial design 2^3 of carbamazepine nanoemulsions.

Independent variables	Levels	
	Low	High
A (type of co-surfactant)	PG	PEG 400
B (type of surfactant)	Polysorbate 80	Poloxamer 188
C (Smix ratio)	1:1	2:1
Coded values	-1	+1

Table 2: Full factorial design with coded values of carbamazepine nanoemulsions.

Formula	Coded values			Actual values		
	A	B	C	Co-surfactant (A)	Surfactant (B)	Smix ratio (C)
F1	-	-	-	PG	Polysorbate 80	1:1
F2	+	-	-	PEG 400	Polysorbate 80	1:1
F3	-	+	-	PG	Poloxamer 188	1:1
F4	+	+	-	PEG 400	Poloxamer 188	1:1
F5	-	-	+	PG	Polysorbate 80	2:1
F6	+	-	+	PEG 400	Polysorbate 80	2:1
F7	-	+	+	PG	Poloxamer 188	2:1
F8	+	+	+	PEG 400	Poloxamer 188	2:1

2.5. Preparation of nanoemulsion

Nanoemulsions were prepared by spontaneous emulsification process with slight modification.[13,8] Table 3 shows the composition of the prepared formulations. Briefly, to prepare the oily phase, exactly weighed 0.4 g of CBZ was dispersed in 5.0 g castor oil (the oil showed best drug solubility) using a magnetic stirrer at 500 rpm. The calculated amount of hydrophobic surfactant, Cremophor EL was dissolved in the co-surfactant (PG or PEG 400) using a magnetic stirrer

(Stuart®, UK) at moderate speed and then added to the oil phase. The aqueous phase was prepared by dissolving 0.4 g of benzyl alcohol (preservative) and the hydrophilic emulsifier (Polysorbate 80 or Poloxamer 188) in the calculated volume of phosphate buffer saline (PBS) pH 7.4 under stirring at 500 rpm (IKA electrical stirrer). The oily phase was then gradually added to the aqueous phase while stirring at 500 rpm to produce 100 ml of CBZ nanoemulsion (4.0 mg/mL). All formulations were stored in the refrigerator at about 2-8°C for 24 h. By visual inspection, all prepared systems were transparent one phase system with no apparent un-dissolved drug crystals.

Table 3: Compositions of the proposed formulations and processing parameters for carbamazepine nanoemulsions.

Formula/ Parameters		F1	F2	F3	F4	F5	F6	F7	F8
Oily Phase	CBZ	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
	Castor oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	Cremophor EL	25.0	25.0	25.0	25.0	33.33	33.33	33.33	33.33
	PEG 400	-	25.0	-	25.0	-	16.66	16.6	16.66
	PG	25.0	-	25.0	-	16.66	-		-
Aqueous Phase	Poloxamer 188	-	-	0.6	0.6	-	-	0.6	0.6
	Tween 80	6.0	6.0	-	-	6.0	6.0	-	-
	Benzyl alcohol	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
	PBS pH 7.4 **To**	100	100	100	100	100	100	100	100
Particle size		123±4 (0.1)	44±1 (0.5)	37±1 (0.2)	28±1 (0.3)	70±2 (0.6)	20±1 (0.2)	24±1 (0.2)	20±0.5 (0.2)
Zeta potential		-1.0 ±0.1	-4 ±0.4	-5 ±0.6	-3± 0.7	-5 ±0.5	-3 ±1	-5 ±0.7	-6 ±1
Entrapment efficiency		62 ±3	73 ±4	84 ±3	83 ±3	81 ±3	84 ±2	83 ±2	79 ±1
Viscosity		156 ±9	126 ±7	47 ±3	50 ±4	160±8	49±4	71±4	134±8
Release Efficiency (%)		73.9	54.8	23.4	30.7	29.3	23.1	26.6	35.6

- All formulations were adjusted to 100 ml using PBS pH 7.4
- Values between brackets are polydispersity index.

2.6. Statistical Analysis

Statistical analysis for the selected parameters in the present study was performed using MINITAB® (State College, Pennsylvania, USA) statistical software (Minitab release 17). The selected responses were droplet size (minimize), T50 (maximize), and entrapment efficiency (maximize). The multiple regression analysis was used to analyze the factorial design. The following equation was used to statistically evaluate the response:

$$Y = b0 + b1\,A + b2\,B + b3\,C + b12\,AB + b13\,AC + b23\,BC + b123\,ABC \quad \text{Eq. (1)}$$

Where Y is the selected response, b0 is y intercept, bi is slope coefficient of the independent variables and their interactions A, B C, AB, AC, BC and ABC.

2.7. Characterization of CBZ Nanoemulsion

2.7.1. Determination of droplet size, polydispersity index (PDI) and zeta potential

The mean particle size and PDI were measured at 25◦C by laser diffraction spectroscopy using the Zetasizer (Nano-ZS, Malvern instrument, UK). Each 15 µL sample was diluted to 10 ml using ultra-filtered (0.22 m) purified water and Sodium chloride (1.0 mM) for droplet size and zeta potential measurements, respectively.[14]

2.7.2. Entrapment Efficiency

Entrapment efficiency (EE) was measured indirectly by the separation of the unentrapped drug using the dialysis method.[15] The dialysis cellulose tubing (visking®, molecular weight cut-off=12 kDa, diameter 16 mm, SERVA Electrophoresis GmbH, Germany) was hydrated overnight in PBS (pH 7.4). One end of the dialysis tubes was tightly closed by thread, and then 2ml of CBZ nanoemulsion was placed into the dialysis tube (8 cm length) then tightly closed using threads at the other end. The dialysis tube was dipped in a beaker containing 100 ml PBS (pH 7.4) without stirring at room temperature for 6 hours. At the end of the experiment, drug concentration in PBS was analyzed using HPLC. By subtracting the unentrapped drug (free drug calculated) from the total drug concentration in the formula at zero time, the percentage entrapped drug was calculated using the following equation:

$$EE\,(\%) = [(Ct - Cf)/Ct]\,100 \quad \text{Eq. 2}$$

Where Ct is the concentration of total drug and Cf is the concentration of the unentrapped drug.

2.7.3. Viscosity Measurement

The viscosity of the prepared systems were measured using Brookfield Viscometers DVELV (Adjusted to 25 ± 0.1 ◦C) employing spindle LV#2 (62).

2.7.4. In vitro drug release

The *in vitro* drug release profile for CBZ nanoemulsions was measured using the reported dialysis method.[8] About 8 cm of dialysis tubing (visking®, molecular weight cut-off 12 k Da, Germany) was soaked in phosphate buffer pH 7.4 for 24 h. One end of the dialysis tube was closed by thread, and then 2ml of CBZ nanoemulsion was placed in the formed bag and then

tightly closed from the other end using thread. The bag was then placed in the dissolution vessels of the dissolution tester (PharmaTest, PTWS 120D Germany). The study employed dissolution apparatus USP type II (paddle method) with an agitation speed of 75 rpm. Dissolution medium was 900 ml PBS pH 7.4 at a temperature of 37 ±0.5oC that was maintained throughout the experiment where the samples were replaced by fresh dissolution medium. Samples, 2 mL each, were withdrawn at selected intervals for 12 h and analyzed for drug concentration using HPLC.

2.8. Drug release kinetic studies

DD-Solver software was used to analyze the release data of CBZ from nanoemulsions and the mechanism of release was detected by fitting the release data to various mathematical kinetic models: zero-order model, first-order model and Higuchi model. The highest degree of correlation coefficient (R^2) determines the suitable mathematical model that follows drug release kinetics.

2.9. In vivo anticonvulsant studies

Experiments were performed according to the National Regulations of Animal Welfare and Institutional Animal Ethical Committee. The study protocol and animal treatment procedure were approved by the ethical committee of college of pharmacy, Tanta University. Swiss Albino mice (25± 5.0 g body weight) were provided by the Medical Technology Center, Alexandria University in Egypt. The mice were kept in rodents' cages at room temperature with free access to water and rodents' food.

The animal dose was calculated according to the human dose and conversion factor.[16] The animals were divided into three groups, five animals each. The first test group was injected intravenously via the tail vein with 0.25 ml (40mg/kg) of nanoemulsion formulations F6. The second group was injected with placebo nanoemulsion (F6 without drug) and was taken as a negative control group. The third group was treated by oral administration of an equivalent amount of CBZ from the commercially available CBZ suspension (2% w/w) via oral feeding tube and was taken as the positive control group.

To induce convulsions in mice, 0.2 ml of Strychnine hydrochloride in saline solution (0.25mg/ml) was injected by intraperitoneal route after five minutes of IV injection or 30 minutes in case of oral administration.[17] Mice were then immediately placed in a glass box (internal diameter 30 X 60 cm) to observe the expression of convulsions. Parameters to be recorded were the onset time of the first clonic convulsion, frequency of convulsions per minute, time of death, and survival number.

2.10. Stability Studies

The stability study of the prepared CBZ nanoemulsions was performed at different storage conditions; at refrigeration (5 ± 3 °C) and room temperature (25 ± 2 °C) for a period of 6 months. The average droplet size and EE were evaluated immediately after preparation (zero time), and after six months.

3. RESULTS AND DISCUSSION

The standard curve of CBZ in PBS, pH 7.4, illustrated a linear relationship between concentrations in the range of (1.6-16 µg/ml). The regression equation was y = 92.42x - 12.031 and R^2 = 0.999. The limit of detection (LOD) was 0.259 µg/ml and limit of quantitation (LOQ) was 0.784 µg/ml, which indicate high sensitivity of the implemented HPLC method. The percent recovery ranged from 98% to 102% with RSD less than 2% indicating high accuracy of the adopted method. Both intra-day and inter-day RSD were less than 2%, reflecting precision of the adopted method and accuracy.

3.1. CBZ solubility in different oils

Drug solubility in the oil phase is very important in the development stage of nanoemulsion.[14] CBZ presented the highest solubility in castor oil (7mg/ml), whereas solubility was found to be 3.5mg/ml and 2.0mg/ml in soybean oil and MCT, respectively. This result is in good agreement with other finding.[8] Therefore, castor oil was used to prepare the nanosystems.

3.2. Optimization of CBZ Nanoemulsion

The prepared CBZ nanoemulsion trials were optimized using 23 full factorial design which assess the effect of independent variables like the type of co-surfactant (A) (PG and PEG 400), the type of surfactant (B) (Polysorbate 80 and Poloxamer 188) and Smix (surfactant: cosolvent) ratio (C) and their interactions (AB) (AC) (BC) (ABC) on the physicochemical parameters of CBZ nanoemulsions. The selected parameters were particle size (Y1), entrapment efficiency (Y2) and T50 of the *in vitro* drug release through application of one-way ANOVA at 0.05 level.

3.3. Droplet Size

The droplet size and polydispersity index (PDI) are very important physicochemical parameters for parenteral emulsion. Large particle size is clinically unacceptable due to possible formation of emboli.[17,18] Polydispersity index (PDI) reflects how far the size distribution deviate from the average value, where values between 0.25- 0.5 are satisfactory for parenteral colloidal dispersion.[20] Therefore, the effect of formulation variables on the droplet/particle size of the prepared nanosystems was evaluated. The obtained results are shown in Table 3.

Droplet size ranged from 20.0 nm (F6 and F8) to 122nm (F1). Such small size could be due to the relatively high concentration of the surfactant cocktail. Increase in emulsifier concentration decreases the interfacial tension and reduces the droplet size.[21] The effect of independent variables on particle size of the designed nanoemulsions is illustrated by contour plot (Figure 1).

Statistical regression analysis using polynomial equation for particle size is shown in Equation 3.
Particle Size = 45.267- 18.464A- 18.411B- 12.823 C + 14.675 AB+ 3.741 AC + 7.364 BC - 2.789 ABC Eq. 3

Where A is the co-surfactant, B is the surfactant, C is the Smix ratio and AB, AC, BC, AC, and ABC are the interactions between the three factors.

The obtained R^2 value was 0.9987, all tested factors (A, B, and C), and their interactions were statistically significant ($P < 0.05$). According to equation 3, the most effective factor that resulted in increasing the particle size was -A (co-surfactant) and -B (surfactant) followed by +AB (co-surfactant and surfactant interaction) and -C (Smix ratio). The optimization required for droplet size is to minimize it. Consequently, the optimized formula according to the regression equation is F8 with +A (PEG 400), +B (Poloxamer 188), and +C (2:1) which has almost similar globule size with F6 (+A (PEG 400), -B (Polysorbate 80), and +C (2:1)).

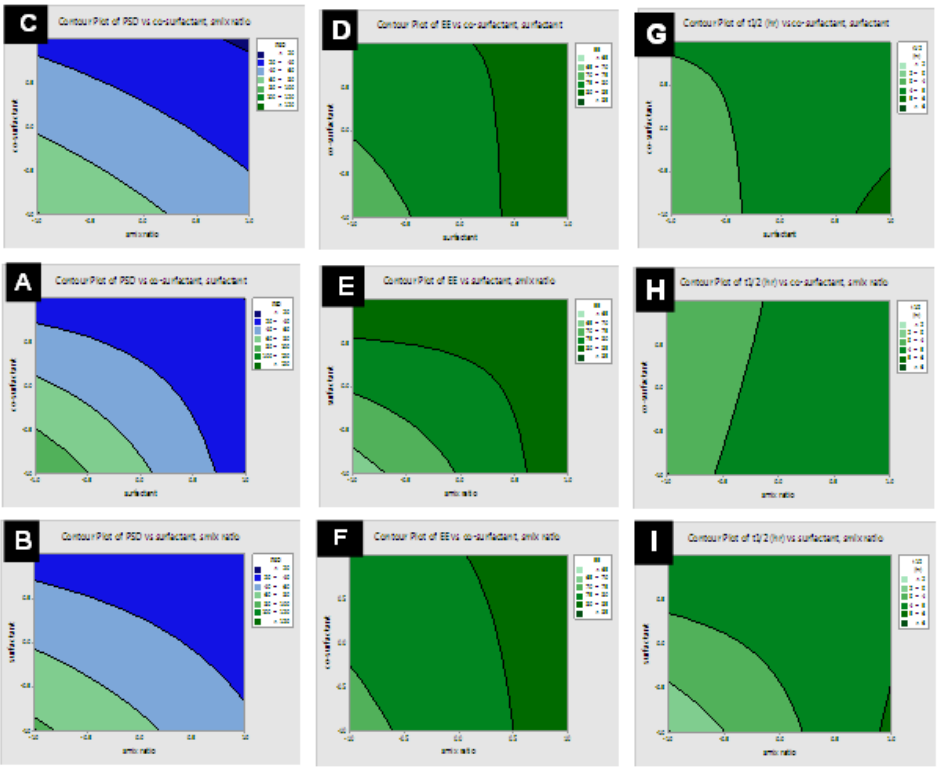

Figure 1: Contour plots illustrate the effect of independent variables (surfactant, co-surfactant and Smix ratio) on particle size (A-C), entrapment efficiency (D-F) and T50 (G-I) of carbamazepine nanoemulsions.

The type of surfactant and co-surfactant were the most effective factors regarding droplet size. For co-surfactant, the use of PEG 400 resulted in nanoemulsion with smaller droplet size compared to those prepared using PG. This may be due to higher hydrophilicity of PEG 400 compared to PG.[22]

Concerning the type of surfactant, the use of Poloxamer 188 as a surfactant significantly reduced the size compared to Polysorbate 80, this may be due to its high HLB value (HLB= 29) compared to Polysorbate 80 (HLB 15). Surfactants with higher HLB are known to be better o/w emulsifier and stabilizes the aqueous phase more along with reducing the particle size.[23] Additionally, Poloxamer 188 is a triblock copolymer and is usually employed as adsorbing molecules to reduce the aggregation of particles in colloidal state. Therefore, it is expected that some of the free Poloxamer molecules would adsorb to the globule surface forming barrier and impart some steric stabilization to the finished product against coalescence.[24]

Regarding the effect of surfactant/cosurfactant (Smix) on droplet size, it was found that the use of Smix (2:1) resulted in smaller droplet size compared to 1:1 ratio. This may be explained by the higher concentration of surfactant used, which allowed more interfacial tension reduction.

3.4. Entrapment Efficiency

The amount of drug encapsulated within the oil droplets was determined. The results are in Table 3. All formulations, except F1 and F2, showed a good percentage drug entrapment with values ranging from 79% (F8) to 84.4% (F3 and F6) indicating the ability of the prepared nanoemulsion to encapsulate a considerable amount of CBZ. System F1 and F2 showed a relatively low drug entrapping ability. The effect of independent variables on the entrapment efficiency of nanoemulsions

is illustrated by contour plot (Figure 1). The resultant regression equation for entrapment efficiency (EE %) was as following

EE = 78.808 + 1.075 A + 3.483 B + 3.192 C - 2.483 AB - 1.308 AC - 4.417 BC + 0.783 ABC Eq. 4

The obtained R^2 value was 0.8835 and all tested factors and their interactions were statistically significant ($P< 0.05$) except for factor A ($P = 0.061$) and factor ABC ($P = 0.161$). According to equation 4, the most effective factor affecting entrapment efficiency was -BC (surfactant and Smix interaction) with the highest coefficient value of 4.417 followed by +B, +C and -AB.

The optimization required for entrapment efficiency is to maximize it. Consequently, the optimized formulations according to the regression equation are those having -BC and -AB either with +B (Poloxamer 188) as for F3 or -B (Polysorbate 80) as for F6.

3.5. Viscosity of the prepared formulations

Though there is no defined value for the viscosity of parenteral preparations, the most important property in prepared liquid is that it should exhibit good syringeability (ease of withdrawing into a syringe) and injectability (ease of ejection from the syringe). The results of viscosity measurements are listed in Table 3. Viscosity of CBZ nanoemulsion ranged from 47 cP to 160 cP. There was a trend of increased viscosity of systems containing Smix 2:1 to those prepared at 1:1 level. This result is in good agreement with the published reports that stated that the viscosity of the nanoemulsion increases by increasing the concentration of the internal phase.[25] As low viscosity is preferred for parenteral preparations, formulations F3, F4 and F6 are best systems regarding viscosity.

3.6. In vitro release kinetics and half-life of the in-vitro CBZ nanoemulsion release

The cumulative amount of drug released versus time plots were constructed and are presented in Figure 2. The release efficiency (RE) of CBZ from different nanosystems were calculated and are presented in Table 3. All formulations showed similar release patterns of gradual liberation of CBZ over time. The total amount released after 12 hours ranged from 79% (F6) to 99% (F2). Similar trend was noticed for the release efficiency values (Table 3). Higher drug loading resulted in extending the release of CBZ, which reflected in the higher value of T50.

Surprisingly, formulations F1 and F2 showed a relatively high drug release compared to other formulations. This could be explained by the low entrapment efficiency value and the presence of a considerable amount of un-entrapped drug in the continuous aqueous phase of the nanoemulsion.

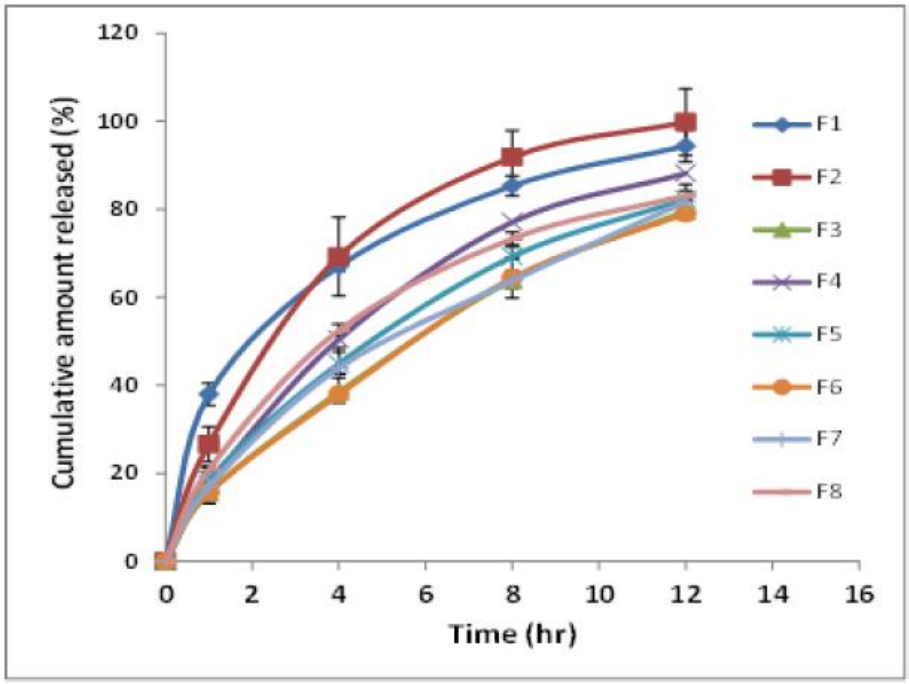

Figure 2: In vitro release of carbamazepine from different nanoemulsion formulations. For detailed formulations, refer to Table 3.

To identify the type of CBZ release model, the correlation coefficient values (R^2) were calculated employing different release modeling; zero order, first order and Higuchi equations. The model showing the highest R^2 was taken as the model that most explain CBZ release. The release profiles fitted better to Higuchi kinetic model indicating diffusion mechanism. Table 4 shows the correlation coefficient values (R^2), the release rate constant (k), and the time of 50% release (T50) after fitting to Higuchi model.

Table 4: Carbamazepine release kinetics adopting Higuchi model for different formulations.

formula	order	R^2	K (mg/hr1/2)	T50 (hr.)
F1	Higuchi	0.980 ± 0.011	23.134 ± 0.483	1.988 ± 0.234
F2	Higuchi	0.948 ± 0.028	30.083 ± 4.059	2.561 ± 0.159
F3	Higuchi	0.994 ± 0.005	26.386 ± 1.458	5.505 ± 0.137
F4	Higuchi	0.985 ± 0.009	29.385 ± 1.633	4.175 ± 0.162
F5	Higuchi	0.996 ± 0.003	26.322 ± 1.215	4.752 ± 0.411
F6	Higuchi	0.993 ± 0.003	26.33 ± 0.298	5.546 ± 0.628
F7	Higuchi	0.999 ± 0.002	26.20 ± 1.938	5.132 ± 0.352
F8	Higuchi	0.982 ± 0.005	25.591 ± 0.886	4.125 ± 0.127

The values for T50 ranged from 1.9 (F1) to 5.5 (F6) hours. For release constant, values between 23.1 (F1) to 30.0 (F2) mg/hr1/2 were obtained. Regarding T50, the regression equation was as follow:

T50 = 4.2230 - 0.1213 A + 0.5112 B + 0.6657 C - 0.4630 AB + 0.0681 AC- 0.7712 BC + 0.0128 ABC Eq. 5

R^2 value obtained for T50 regression equation was 0.957 and all tested factors and their interactions were statistically significant ($p < 0.05$) except factor A (P values = 0.083), factor AC (P values = 0.316), and factor ABC (P values = 0.849).

According to equation 5, the most effective factor concerning T50 was -BC (surfactant and Smix interaction) followed by +C (Smix ratio), and +B (surfactant).

The interaction (-BC) found in formulations F3, F4, F5, and F6. The second and third effective factors were +C and +B followed by the interaction -AB. As a result, the optimized formulations concerning the T50 were F3 and F6 with -BC and -AB. This could be due to the existence of cremophor EL at its higher concentration that may resulted in increased drug retardation and enhanced CBZ solubility in lipid. This expected to increase the amount of drug entrapped in nanoemulsion leading to higher drug release half-life.[26]

3.7. Optimized formula for CBZ Nanoemulsion

To choose the optimized formula among all eight formulations, response optimization using Minitab for PSD, EE, and T50 was employed. According to the obtained results, formulation F6 was the optimized formula due to its optimum combination effects of high entrapment efficiency (84.40 % ± 2.4), besides its small particle size (20.0 nm ± 0.7) and higher T50 (T50 = 5.55±0.6 h).

3.8. In vivo anticonvulsant effect of CBZ Nanoemulsions

The efficiency of the optimized nanoemulsion system to reduce the incidence of convulsions was evaluated. One-test groups of Swiss Albino mice were injected intravenously via the tail vein with 0.25 ml of 0.4% w/w CBZ nanoemulsion F6. For comparison, negative control group was injected 0.25 ml placebo nanoemulsion (same compositions of F6 but without drug), other group was treated with the commercially available CBZ oral suspension (positive control). The recorded Parameters were the onset time of the first clonic convulsion, frequency of convulsions per minute, time of death, and survival number (Table 5).

Table 5: In vivo study parameters of carbamazepine after intravenous administration of nanoemulsion formula F6, in comparison to commercial carbamazepine oral suspension and placebo nanoemulsion of formula F6.

Formula / Parameters	CBZ nanoemulsion (F6)	Oral suspension (Tegretol)	Placebo
Onset of convulsion/min	13.8 ± 2.7	9 ± 2.5	6.2 ± 0.84
Frequency of convulsion/min	3.4 ± 0.54	5.4 ± 0.89	Continuous
onset of death/min	16±	11±	10.4 ± 0.89
% death	20%	20%	100%
% survival	80%	80%	0%

For negative control (group receiving placebo nanoemulsion), administration of strychnine to mice induced clonic convulsion with a mean onset time of 6.2 ± 0.8 minutes. The convulsions were continuous and resulted in the death of all mice (mean death time of 10 min). For positive control (group receiving commercial suspension), the onset was significantly ($P< 0.01$) delayed to 9.0 min with interrupted convulsions with mean frequency of 5.4 convulsion/min. Only one mouse died after about 11 min. For test group treated with CBZ nanoemulsion (formula F6), there was a marked delay ($P< 0.01$) in the onset time for the convulsions with reduced frequency compared to suspension. The onset of death was similarly delayed. The onset of death was significantly delayed to 16 min with the death of one mouse. These results indicate the superiority of CBZ in the nanoemulsion form and proof its efficiency in controlling epileptic seizers.

3.9. Stability Study

The prepared formulations were evaluated regarding their stability during storage. All nanosystems were tested under two storage conditions; at 5.0 ± 3.0 °C (by keeping them in the refrigerator) and 25 ± 2 °C (room temperature) for 6 months. Samples were tested regarding their droplet size and entrapment efficiency at zero time (immediately after preparation), after 6 months.

Figure 3 illustrates the effect of storage on the droplet size of the prepared nanosystems. All systems showed no precipitation or phase separation during the study period. For those kept at 25°C temperature, all nanoemulsions showed a significant ($p < 0.05$) increase in the droplet size, with the exception of F8. The extent of this increment was less in case of those kept in the refrigerator, with formulations F6 and F8 showing non-significant ($P > 0.05$) increase. This is due to the very fine droplet size and low interfacial tension between oil and water molecules.[27] Meantime, formulations F1, F2 and F5 who showed the largest droplet size exhibited the highest tendency for aggregation and coalescence to bigger droplets. The results also reflected that despite the low charge density on the dispersed droplets, physical stability was maintained.

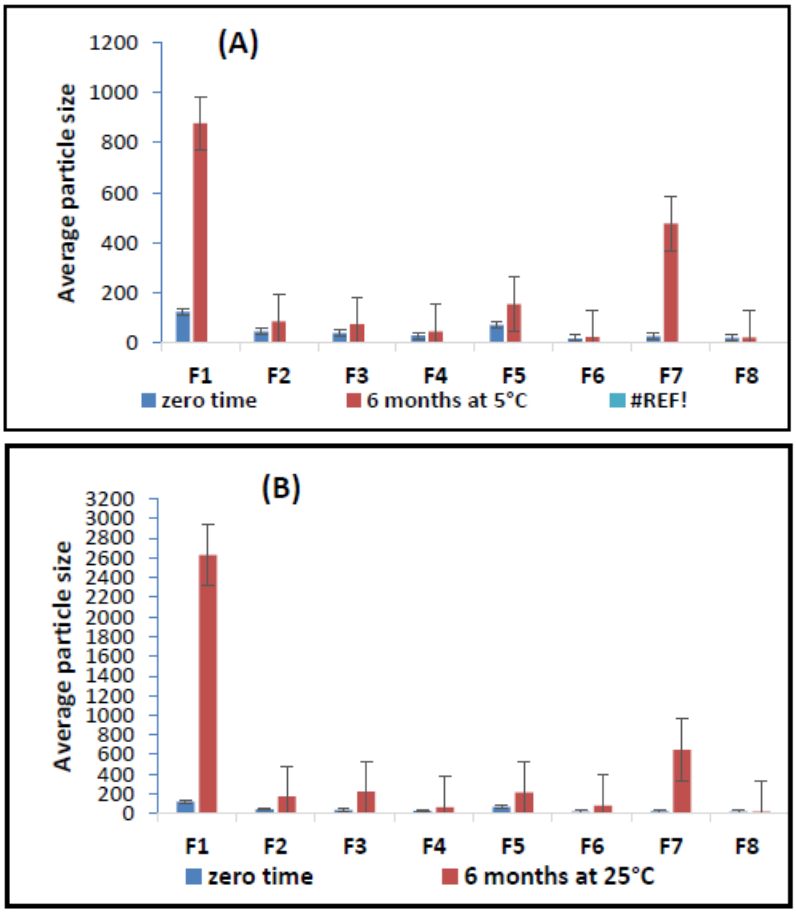

Figure 3: Effect of storage on the droplet size of the prepared carbamazepine nanoemulsion formulations kept at 5°C (A) and at 25°C (B). For detailed formulations, refer to Table 3.

Concerning the effect of storage on the entrapment efficiency, the results revealed that there was a reduced entrapment efficiency by time, under the two storage conditions. However, such reductions were found to be non-significantly differ from those measured immediately after preparation ($P>0.05$). This would indicate high integrity and stability of the nanoemulsion structure.

4. CONCLUSION

Carbamazepine nanoemulsion for parenteral administration was feasible using experimental design approach. The release study showed sustained drug release with Higuchi kinetic modeling. The physicochemical characteristics maintained for 6 months at different storage conditions. The *in vivo* study in experimental animals (mice) showed the superiority of the optimized nanoemulsion formulation over that of the marketed product with delayed onset of convulsion and reduced frequency.

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