Original Article

Tizanidine Intranasal Nanoemulsion In situ Gel: Formulation and In-Vivo Brain Study

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Abstract

In this work, intranasal nanoemulsion in situ gel (NIG) was developed as new dosage form for tizanidine. An o/w nanoemulsion (NE) was used, which composed from tween 80 as surfactant and ethanol as co-surfactant with ratio 1:2, oleic acid was used as oily phase. The prepared nanoemulsion formula was used to prepare nanoemulsion in situ gel (NIG) using poloxamer 407 as temperature dependent polymer. NIG formulas were evaluated for pH, drug content, sol-gel transition temperature, viscosity, spreadability, gelation time, in vitro drug release and in vivo brain study (using rabbits) in comparison with conventional in situ-gel (IG).

The pH, spreadability, % drug content, sol-gel transition temperature, and gelation capacity for the selected NIG were 6.2, 4.9 cm, 98.5%, 33 oC and ++ respectively. The in-vitro drug release study of the best NIG2 formula showed 100% after 4h.

In-vivo brain studies showed significant increase in Cmax and AUC ($P < 0.05$) in the cerebrospinal fluid of brain for the selected NIG2 formula in comparison to in situ gel (IG) prepared by the conventional method indicating the contribution of nanoemulsion insuito gel technique for enhancing drug permeability and hence increase in drug reach to cerebrospinal fluid.

Keywords: Nanoemulsion, Insuito gel, pH, Gelation time, Ethanol.

1. Introduction

In the early 1980s, the nasal route was promoted as an alternative to more conventional methods of administering medication that had a more positive outlook on the systemic delivery process. The nasal route is one that is straightforward, uncomplicated, and trustworthy. Because it possesses a porous endothelial membrane and a highly vascularized epithelium, it is able to quickly absorb drug into the systemic circulation. This is made possible by the combination of the two features. Because of this, it is possible to sidestep the hepatic first-pass elimination (1). The nasal lumen is separated from the thick blood artery network in the lamina propria cavity by only two cell layers.

The lamina propria cavity is lined with three different types of epithelia: squamous, respiratory, and olfactory epithelia. There are only two cell layers separating the nasal lumen and the extensive blood vessel network from the nasal epithelium, which has a permeability that is considered to be quite high (2). The transport of drugs through the nasal epithelium is affected by the physical and chemical characteristics of the drug molecules.

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In particular, the molecular weight, lipophilicity/hydrophilicity, degree of ionization, and rate of solubilization of the drug molecules all interfere with the transepithelial mechanisms of passage, which in turn affects the transport of drugs through the nasal epithelium. The properties of the drug molecules, both chemical and physical, have a role in determining whether or not they are able to be transported via the nasal epithelium (3).

There are many different drug transport mechanisms that have been described, such as the systemic pathway, in which the drug is absorbed directly into the systemic circulation through the nasal cavity, and then into the brain after crossing the blood brain barrier. Another example of a drug transport mechanism is the pulmonary pathway, in which the drug is absorbed directly into the pulmonary circulation through the lungs (4). In spite of the fact that the precise mechanism by which drugs are delivered to the central nervous system (CNS) is a mystery, it has been demonstrated that drugs that are given intranasally have the potential to reach the brain either directly or indirectly after they pass through the cribriform plate. This is the case even though it is a known fact that the precise mechanism by which drugs are delivered to the CNS is a mystery (5).

In recent years, it has become clear that the in situ gel system is one of the most successful and cutting-edge revolutionary drug delivery methods. One type of formulation is known as an in situ gelling system, and it is one that is in the form of a solution before it is absorbed by the body (6). A single stimulus or a combination of several different types of stimuli, such as a change in pH, a change in temperature, the exchange of solvents, exposure to ultraviolet light, or the presence of particular ions or molecules, can cause the sol-gel transition. Alternatively, the sol-gel transition can be caused by a single stimulus or a combination of several different types of stimuli (7).

Tizanidine is a myotonolytic drug that, when administered to the central nervous system, has the effect of an alpha-2 adrenoceptor agonist. Patients who have sustained an injury to their spinal cord or brain can benefit from its use as a spasticity treatment. It has a half-life that ranges from 2.1 to 4.2 hours, and its bioavailability is between 34 and 40% due to extensive fist pass metabolism. (8).

2. Materials

2.1 Construction of calibration curves

Calibration curves of TZN in ethanol and phosphate buffer pH 6.4 were obtained by preparing serial dilutions of the drug through transferring (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL) from the stock solution (1 mg/mL) to 10 mL volumetric flasks and diluted up to the sign (10mL). The absorbance of these diluted solutions were determined spectrophotometrically at the previously estimated λmax and plotted against concentration to get a calibration curve. The R^2 value and calibration curve equation were obtained.

2.2 Preparation of tizanidine nanoemulsion

TZN nanoemulsion was made by combining oleic acid as the oil phase with tween 80 as surfactants and ethanol as a co-surfactant in a ratios (surfactant: co-surfactant) (1:2) of surfactant to co-surfactant. TZN nanoemulsions were made by dissolving the required amount of TZN (4 mg) in 5% of oil that was specifically measured out. After that, the predetermined amount of Smix was added for the oil-loaded drug, and then the entire combination was mixed by using a vortex mixer for 5 min at a speed of 100 rpm. Then, deionized water was titrated one drop at a time in order to obtain a nanoemulsion that was translucent and clear (o/w) (9).

2.3 Preparation of nasal in situ gel for TZN (IG) using conventional method

poloxamer 407 in different weight ratios were weighed and dissolved in the aqueous phase of the nanoemulsion formula by stirring (500 rpm) at 4° C in a container for a period of two hours. The temperature of the water was kept at 4 oC during the preparation time. After that, the poloxamer solution was kept cold in the refrigerator for a one day. The previous prepared NE (a mixture of ethanol, tween 80 at a ratio of 1:2, and oleic acid oil at a concentration of 5%) was incorporated into the poloxamer solution, then add water up to 100 ml by add water (10). The contents of the NIG formula is shown in table 1.

Table 1: Composition of NE and NIG Formulas of TZN

NE-F	TZN %w/v	Oleic acid oil %v/v	Surfactant	Cosurfactant	Smix ratio	Smix %v/v	DDW %v/v	poloxamer 407 %
NE	0.04	5	Tween 80	Ethanol	1:2	55	40	-
NIG1	0.04	5	Tween 80	Ethanol	1:2	55	40	16
NIG2	0.04	5	Tween 80	Ethanol	1:2	55	40	18
NIG3	0.04	5	Tween 80	Ethanol	1:2	55	40	20

2.4 Characterization of nasal nanoemulsion in situ gelling formulas of tizanidine

2.4.1 pH measurement of NIG formulas

The pH of the nasal preparation is extremely significant for a number of reasons, the most essential of which are to inhibit the proliferation of disease-causing bacteria, to avoid irritation of the nasal mucosa, and to maintain normal physiological ciliary activity (11). The apparent pH of all of the generated NIG formulae (NIG1-NIG3) was determined by taking the measurements at room temperature (12).

2.4.2 Sol-gel transition temperature measurement

To determine the temperature at which gelation occurs, 2 ml of the refrigerated NIG formula (NIG1-NIG3) were poured into a test tube with a capacity of 10 ml and a diameter of 1 cm. The tube was then covered with parafilm and placed in a water bath maintained at a temperature of approximately 15 oC. The temperature of the water bath was raised gradually by 2 oC and equilibration was permitted for 5 minutes after each temperature increase. This process continued until gelation took place and the preparation did not move when the test tube was slanted at an angle of 90 o (13).

2.4.3 Viscosity measurements of optimized nanoemulsion and NIG formulas

The samples were not diluted in any way before their viscosity was determined. Rheological property of prepared nanoemulsion insuito gel formulas was determined using NDJ-5S digital viscometer and spindle number 3 at 25±1 °C. A graduated cylinder containing 40 ml of the prepared sample was fitted with a viscosity spindle, which was then revolved at a range of speeds, including 6, 12, 30, 50, 60, and 100 rpm. The experiment was performed three times for each sample, and the findings were reported as the mean ± SD (14).

2.4.4 Spreadability test

After the NIG formulae (NIG1-NIG3) had completely gelled, the spreadability of each NIG formula was evaluated by depositing roughly 1 gram of each NIG formula in the center of a glass plate with a square area of 400 cm2. Other glass plate of the same size used in order to cover the glass plate that contained the NIG formula. After that, a 1 kg scale was carefully applied to the upper side of the plate; as a result, the NIG expanded out in the space in between the plates. The 1 kg weight was then removed after 1 minute, and the diameter of the spread region was measured in centimeters (15).

2.4.5 Gelation time

10 mL of each of the prepared NIG formulations (NIG1-NIG3) were added to 100 mL of phosphate buffer with a pH of 6.4 at 32.2 oC in a beaker with a moderate stirrer (50 rpm) to prevent breaking of formed NIG (16). Gelling capacity was calibrated into three groups according to the gel stiffness, gelation time, and duration as such: (+) gelation happens after a few minutes, remains for a few minutes, and rapidly disperses; (++) gelation happens immediately and continues for a few hours; (+++) gelation happens immediately and continues for an extended period of time (17).

2.4.6 Drug release

The use of a dialysis membrane (Mwt 12000 Da) was used for the release of TZN. In order to determine the in vitro drug release from each of the NIG formulations (NIG1- NIG3) that were developed, a rotating paddle dissolving equipment type II was utilized. With a speed of 50 rpm, sealed dialysis bag that contained 6 grams of NIG (equal to 30 grams of nasal gel) was submerged in 300 milliliters of phosphate buffered (pH 6.4) dissolving media. The temperature of the medium was kept at 34.5 oC throughout the conditioning process (18). 2 mL aliquots outgoing at time periods (5, 10, 15, 30, 45, 60, 90,120 , 180 and 240 minutes) and immediately replaced with fresh dissolution medium (19). The drug content in the withdrawn sample was determined spectrophotometrically using a UV-Vis spectrophotometer at the selected λmax.

2.4.7 Comparison between the selected NIG formula (NIG 2) and conventional IG formula containing same drug and composition

The in situ gel formula (IG) prepared by the conventional method (without using nanoemulsion) containing the same content and ratios of the polymers. It is evaluated in vitro (viscosity, gelation temperature, spreadability, pH, gelation study and drug release) in comparison with NIG formula (in situ gel prepared by nanoemulsion technology).

2.5 In-vivo nasal brain study

2.5.1 Study design

Six rabbits weighing between 1.75 - 2.5 kilograms were purchased from the college of veterinary medicine at Tikrit university. These rabbits were kept in the animal house at Tikrit University, where the temperature was kept at approximately 25 oC and the humidity was kept at a range of between (60-65%). Intraperitoneal injections of ketamine at a dose of 100 mg/kg and xylazine at a dose of 10 mg/kg were used to put each rat under anesthesia for the experiment (20). After determining the appropriate nasal dose for the rabbits, an equivalent quantity of tizanidine was given to each of the test rabbit. The body surface area (BSA) normalization method and the human equivalent dose (HED) of pharmaceuticals involving the species (Km) factor (body weight in k divided by BSA in m2) were used in the calculation of animal nasal (rabbit) doses. The formula for this calculation is as follows: body weight in k divided by BSA in m2 (21).

$$\text{HED (mg)} = \text{Animal dose (mg)} \times \frac{\text{Aminal km}}{\text{Human km}} \quad \text{…….. Eq 3}$$

Measured dose of tizanidine is administer deep inside the right nostril of each rabbits of selected NIG formula and conventional intranasal in situ gel (IG).

The rabbit was placed in a lateral recumbency position with

the head flexed at an angle of 90 o to the spine. With a needle measuring 25 millimeters and 22 gauges, samples of cerebrospinal fluid were obtained from the cisterna magna (22) as in figure 1. CSF were placed in the refrigerator almost immediately after being frozen for further study (23).

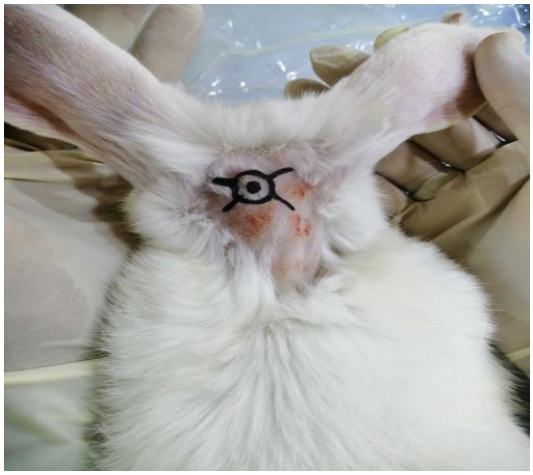

Figure 1: the needle entry point for CSF aspiration in rabbit

2.5.3 HPLC analysis for tizanidine in cerebrospinal fluid sample

Before conducting the analysis, the cerebrospinal fluid sample was allowed to thaw at room temperature before being combined with an aliquot (200 µL) of the internal standard working solution; the pH value of the sample was adjusted to 1.0 by adding 5.5 µL of 6.0 M hydrochloric acid, and then the sample was centrifuged at 12,000 rpm for 5 minutes. Under the influence of the nitrogen in the hood, the supernatant was evaporated. After dissolving the residue in 150 µL of mobile phase, the mixture was vortexed for one minute before being centrifuged at 12,000 rpm for 20 min. After being transferred into an autosampler vial with a sealed cap, the supernatant was placed in a refrigerator at 4 oC for one day prior to undergoing an HPLC examination (24).

2.5.4 Chromatographic conditions

A 50 µL of extracted samples were injected into a Knauer HPLC system (Germany) which consist of a Wellchrom K-1001 pump, a Rheodyne 7125 injector and a K 2501 UV detector with a thermostatic column compartment connected to a Eurochrom 2000 injector. HPLC was performed on an analytical C18 column (Knaure;150 ×4.6 mm; 5 µm particle size; 25cm length) supported by guard column C18 - 4 mm diameter (Germany). The wave length was adjust at the selected λmax 230nm.

The mobile phase (acetonitrile– water (20:80, v/v) with 0.25 mM at a flow-rate of 1mL/min was used and the retention time was 1.5 min for tizanidine. The auto sampler was maintained at 35° C and the total run time was 3.0 min (25).

2.5.5 Pharmacokinetic parameters

Analyses of the pharmacokinetics of tizanidine were carried out using a non-compartmental approach. After nasal administration, the maximum CSF drug concentration, denoted by the letter "C max," as well as the amount of time needed to achieve the maximum SCF concentration, denoted by "T max," were directly measured from the CSF concentration time curve (26). For each individual rabbit, the area under the curve of the CSF drug concentration against time curve from 0 to 60 min (AUC 0 to 60), as well as the area under the curve of the CSF drug concentration versus time curve from 0 to infinity (AUC 0 to infinity), were determined and calculated. The trapezoidal rule was utilized in the determination of the AUC0-24, and the following equation was utilized in the determination of the AUC0.01- α (27):

$AUC\mathbf{0}-\boldsymbol{\alpha}$= AUC 0-60+ (C last)/K …. Eq 4

2.5.6 Statistical Analysis of bioavailability study

Comparison between the in vivo parameters of tizanidine following the administration of prepared NIG2 and conventional prepared IG formulations was carried out using one way analysis of varience (ANOVA) followed by tukey-multiple comparison test. Statistical calculation were carried out using GraphPad Prism 7.0 software.

3. Result and discussion

3.1 pH measurement of selected NE and NIG formulas

The pH of all NE formula and NIG formulas (NIG1-NIG3) was measured using pH meter. The pH values were ranged (6.2-5.6) as shown in table (2). The formulations have acceptable drug content values and pH values compatible with the nasal pH (5.6– 6.2) which is required to prevent the nasal damage or irritation of the nasal mucosa (28).

Table 2: pH of the Selected Tizanidine NE and NIG Formulas

NE – F	pH
NE	**6.2**
NIG1	**6.1**
NIG2	**5.8**
NIG3	**5.6**

3.4.2 Sol-gel transition temperature measurement

In situ preparation was designed to be in solution state at 25º C and changed to gel near nasal temperature. Table 3 showed gelation temperature of the prepared NIG formulas (NIG1-NIG3).

Effect of poloxamer 407 concentration was studied on the gelation temperature and it was found that the NIG3 formulas containing 20 %w/v of poloxamer 407 had sol-gel transition temperature range 31° C, while the NIG2 formula containing 18 %w/v of poloxamer 407 had sol-gel transition temperature range 33° and NIG1 formulas containing 16 %w/v of poloxamer 407 had sol-gel transition temperature range 37° C.

Poloxamer is polyethylene oxide–polypropylene oxide–polyethylene oxide (PEO–PPO–PEO) tri-block copolymer. In a dilute aqueous solution at low temperature, poloxamer molecules exist as unimers (29). Higher poloxamer 407 concentration resulted in gel formation at lower temperatures (30).

3.4.3 Spreadability test

From the results (Table 3), it has been shown that as the concentration of the poloxamer 407 polymer increased the viscosity of the solution increased and the spreadabilty decreased NIG1> NIG2>NIG3. The term "spreadability" refers to the extent of the area across which the gel can be easily spread when it is applied. Therefore, it is a characteristic that is connected to the viscosity of the mucoadhesive polymer. The higher the viscosity, the lower the spreadability, and the bigger the amount of gel that is retained in the nasal cavity (31).

3.4.4 Gelation time

Gelling capacity is coded as describe in table (3). NIG3 showed immediate gelation remained for an extended period (+++) due to high concentration of poloxamer 407(20 %w/v). NIG1 showed gelation happened after few minutes and disperse rapidly (+) due to low concentration of poloxamer 407 (16 %w/v). NIG2 showed immediate gelation remained for few hours (++) due to good concentration of poloxamer 407 (18 %w/v) (32) as in figure 28.

Table 3: Gelation Temperature, Spreadability and Gelling Capacity of NIG Formulas

NE - F	Gelation temperature °c	Spreadability cm	Gelling capacity
NIG1	37	6.2	+
NIG2	33	4.9	++
NIG3	31	4.2	+++

3.4.5 Viscosity measurements of NIG formulas

The values of viscosity for each of the NIG formulae (NIG1-NIG3) at their respective gelation temperatures are presented in Table 4. At temperatures below the point of gelation, the viscosity of each of the preparations was found to be fairly low, but a significant rise in viscosity was observed at temperatures above that threshold. When the concentration of poloxamer 407 was increased from 16% to 20%, however, there was an increase in viscosity. This increase in viscosity can be attributed to an increase in the micelle size and number, which in turn led to a higher level of interaction between the micelles, forming a viscous gel (33).

The results also showed that as the rotation speed rose (shear rate), the viscosity dropped, which indicated that the preparation had a pseudoplastic flow (shear thinning liquids) (34) (35), as shown in figure 2.

Table 4: Viscosities of NIG Formulas at their Own Gelation Temperature (n = 3)

Shear stress rpm / NE – F	6	10	12	20	30	50	60	100
NIG1	1500	1275	1105	955	875	760	630	540
NIG2	2100	1790	1620	1430	1290	1150	1020	840
NIG3	2800	2600	2220	2030	1760	1520	1320	1140

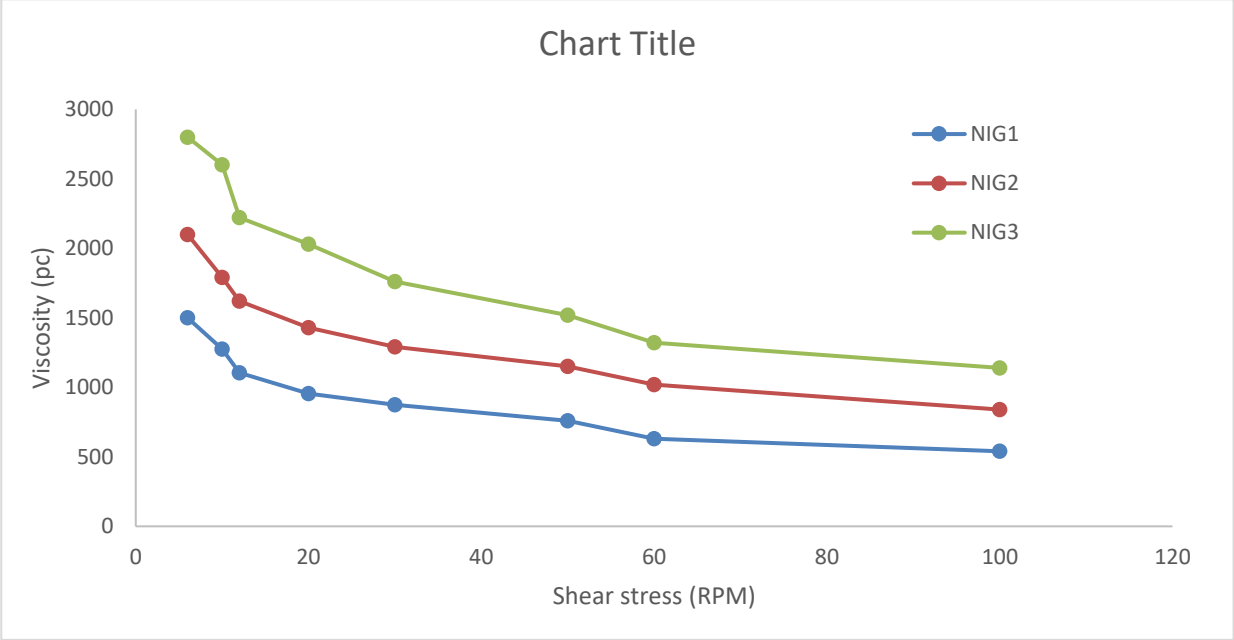

Figure 2: Viscosities of tizanidine NIG formulas at gelating temperature of each formula (n = 3)

3.4.6 Drug release by in vitro study

The release profiles for tizanidine from different NIG formulas (NIG1- NIG3) were shown in figure (3). The NIG1 formula containing 16 %w/v poloxamer 407 showed higher % release after 4 h than the NIG2 (18 %w/v poloxamer 407) and NIG3 (20 %w/v poloxamer 407) formulas (although no differences in drug release in NIG1, NIG3 and NIG6 ($p > 0.05$), this is because the poloxamer 407 delays the drug release.

When increase in poloxamer 407 concentration lead to the increase in gel viscosity and also decrease in the rate of release with an increase in the poloxamer 407 concentration, this delay in drug release result from increase in the number and size of the micelles formed at higher polymer concentrations which could cause a greater tortuosity in the aqueous phase of the gel structure and a slower rate of dissolution (36).

The NIG2 was selected as the optimum formula since it shows 100% drug release after 4 h, immediate gelation occurred for few hours, good spreadability and gelation temperature.

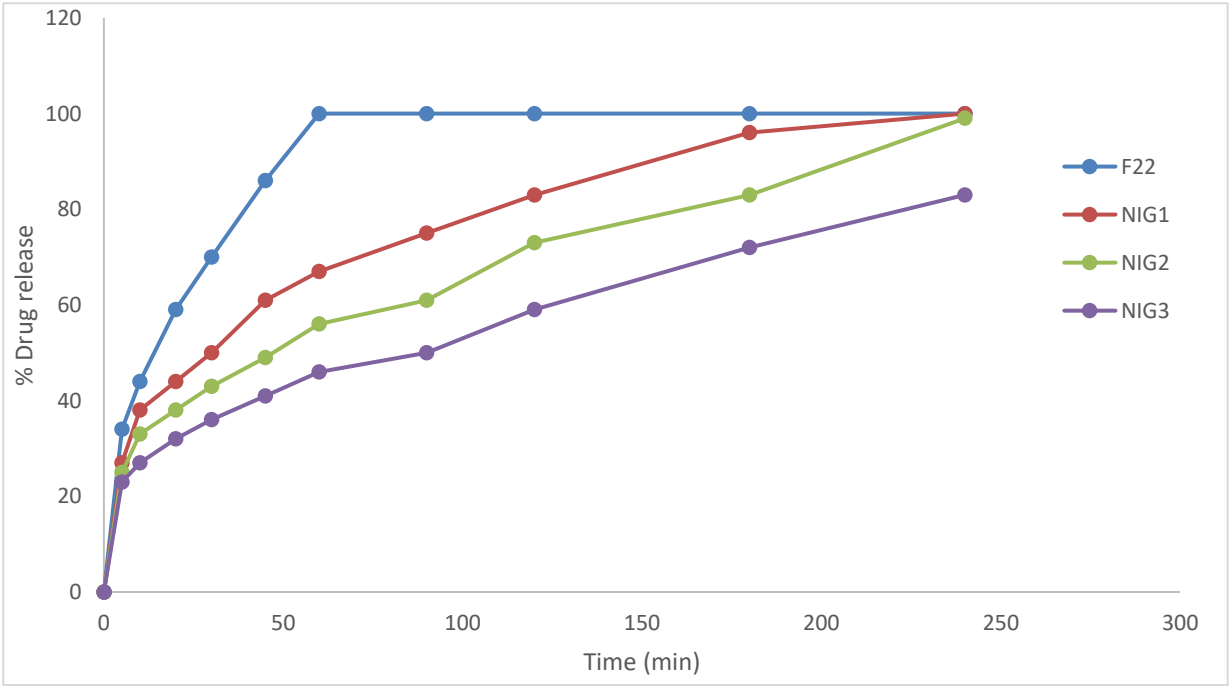

Figure 3: In-vitro drug release profile of tizanidine from NIG formulas (n= 3) in phosphate buffer solution (pH 6.4) at 34±1° C.

3.4.7 Comparison between the selected NIG formula (NIG 2) and conventional IG formula containing same drug and composition

Comparison between NIG2 (nanoemulsion in situ gel prepared by using nanoemulsion technology) and IG (in situ gel prepared by conventional method without using nanoemulsion) in order to investigate the effect of introducing NE on the in vitro and in vivo behavior of the prepared formula. Table 5 showed the in vitro evaluation of the NIG2 and IG, all the parameters for NIG2 were better than that of IG. As well as the viscosity of the IG is significantly higher than that of NIG2 (Figure 4) which affect the flow property. The in vitro dissolution of NIG showed faster and high burst effect followed by higher prolonged release for 4 h than that of IG in phosphate buffer (pH 6.4) (Figure 5), since the drug be as nanosized emulsion droplet in the prepared formula (NIG2) which will increase total surface area for drug release and transfer, which improves drug permeability and diffusion through dialysis bag and may improve drug bioavailability. Therefore, introducing nanoemulsion technology in the preparation of nasal insitu gel for tizanidine gave better physical properties (including pH, spreadability, gelation temperature, in vitro release as well as color, viscosity and gelation capacity) than nasal in situ gel containing the same content prepared by the conventional method. So nasal nanoemulsion insitu gel (NIG2) may improve bioavailability of the drug through improving its permeability through nasal cavity.

Table 5: Comparison between Physical Properties of NIG2 and IG Formulas

In vitro evaluation data	NIG2	IG
pH	6.2	5.3
Spreadability (cm)	4.9	3.8
% drug content	98.5	97.6
T sol-gel ºC	33	34
Gelation capacity	++	+++

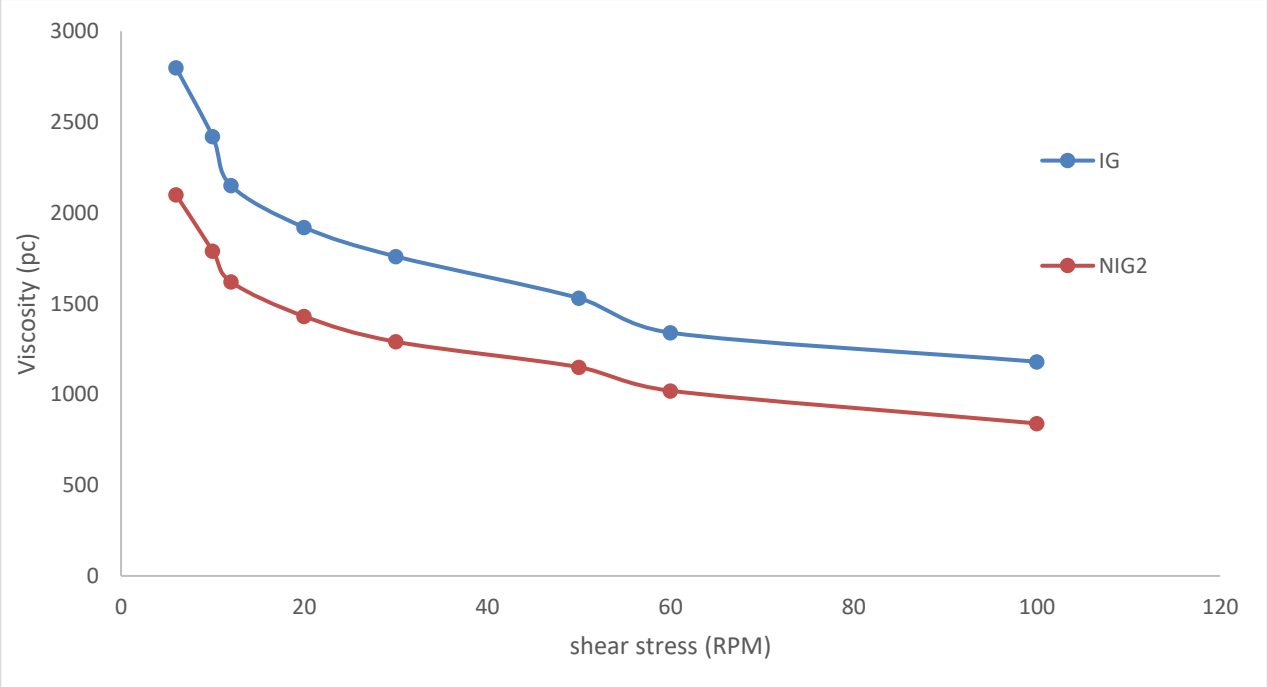

Figure 4: Viscosity of the selected NIG2 and conventional IG at gelation temperature (n = 3)

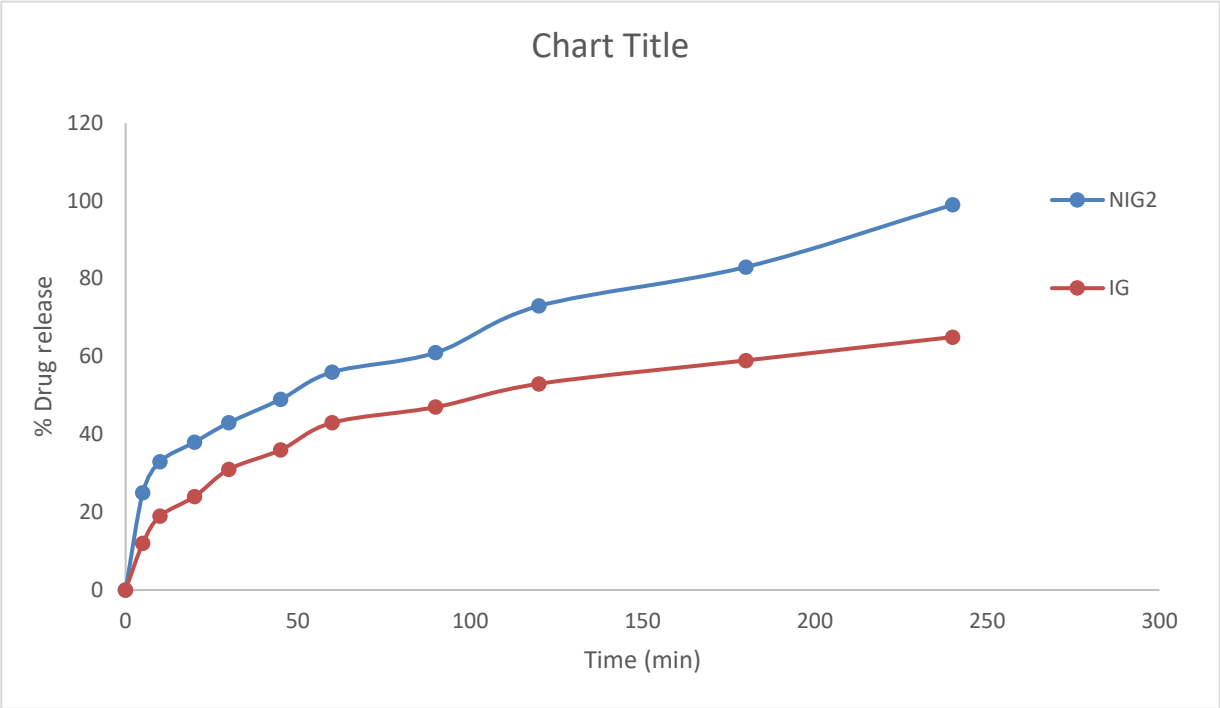

Figure 5: Dissolution profile of selected NIG2 and conventional IG at and temperature 34±1° C in phosphate buffer (pH 6.4)

3.5 In-vivo nasal brain study

The concentration of TZN in rabbit's CSF was determined using a reproducible, sensitive and rapid HPLC method. Figure (6) showed peak chromatograms for pure TZN of the CSF sample the chromatographic conditions and extraction procedure yielded a clear chromatogram for analysis.

The retention time of pure drug (TZN) were detected in ethanol and in CSF samples and were recorded to be 5 ± 0.5 min. The lower limit of detection of TZN in CSF by HPLC was measured by preparing different concentrations of OND-HCl (100-8000 ng/mL) in CSF and it was found to be 120 ng/mL.

The calibration curve of TZN in CSF showed in figure (7). A straight line with high regression coefficient (r2 = 1) was obtained by plotting the area under the peak versus the concentration, this proves that the TZN calibration curve obey (Beer's law) within the concentration range used.

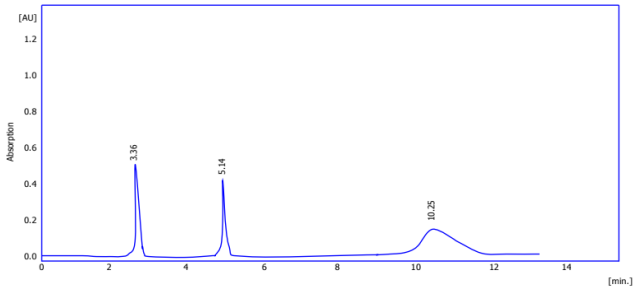

Figure 6: HPLC chromatograms for TZN in CSF

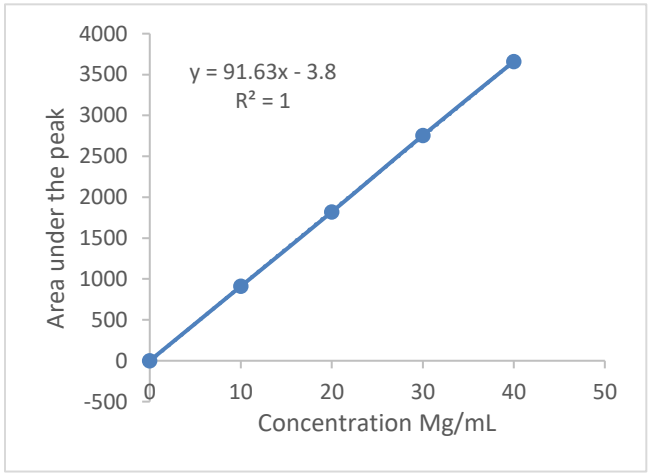

Figure 7: Calibration curve of TZN in rabbit CSF by HPLC.

Table (6) illustrated the arithmetic mean and standard deviation values of CSF drug concentration (ng/mL) of TZN at the selected time intervals for 6 rabbits having different weights (1.75-2.5 kg) and receiving single dose of TZN (2.5 mg) of the selected NIG2 and conventional prepared IG with one week washing period between them.

Figure (8) depicts the mean CSF profile as a function of time for the selected NIG 2 and prepared conventional IG using cross over design with one week washout period.

Table (7) shows the pharmacokinetic parameters for TZN in the prepared NIG2 and conventional prepared IG, from the result show the Cmax (18520.6 ±165 ng/ml) and AUC0–60 (513071.5 ± 96 ng/ml.min) of NIG2 was significantly higher (P<0.05) than that for IG (Cmax 5312.7 ±119ng/ml and AUC0–60 159042.2 ± 104ng/ml.min), and also the less time

required to reach the 20 min in NIG2 while in IG 30 min, which rapid absorption of drug NIG2, these results were good agreement with previous report by GU Fugen, which prepare in situ nasal gel of donepezil hydrochloride showed significantly increased AUC in rats (37).

These results might be due to the presence of lipid in NIG2 which increased the lipophilicity and the affinity to permeate the blood brain barrier as well as presence of poloxamer 407 which acts as permeation enhancer. This result reveals that drug uptake into the brain from the nasal mucosa mainly occurs via two different pathways. One is the systemic pathway by which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing the blood brain barrier. The other is the olfactory pathway by which the drug partly travels from the nasal cavity to CSF and/or brain tissue (38) (39).

Table 6: Mean (± SD) CSF Concentration ng/mL of TZN at the Time Intervals Following Administration of Single Dose of the Prepared NIG2, and Conventional Prepared IG (n = 6).

	Mean (± SD) CSF concentration ng/mL	
Time (min)	**NIG2**	**IG**
5	8240.23 ±122	1253.5 ±128
10	14150.3 ±105	2647.4 ±116
20	18520.6 ±165	4324.3 ±136
30	10835.5 ±95	5312.7 ±119
45	6012.56 ±132	3102.5 ±125
60	3012.36 ±114	1322.4 ±109

Table 7: Pharmacokinetics Parameters TZN at the Time Intervals Following Administration of Single Dose of the Prepared NIG2, and Conventional Prepared IG (n = 6).

Pharmacokinetics parameters	**NIG2**	**IG**
T max (min)	20	30
C max (ng/mL)	18520.6 ±165	5312.7 ±119
AUC_{0-60} (ng/mL.min)	513071.5 ± 96	159042.2 ± 104
AUC0→α(ng/mL.min)	588380.5	196825
Ke_{brain} min^{-1}	0.040	0.035

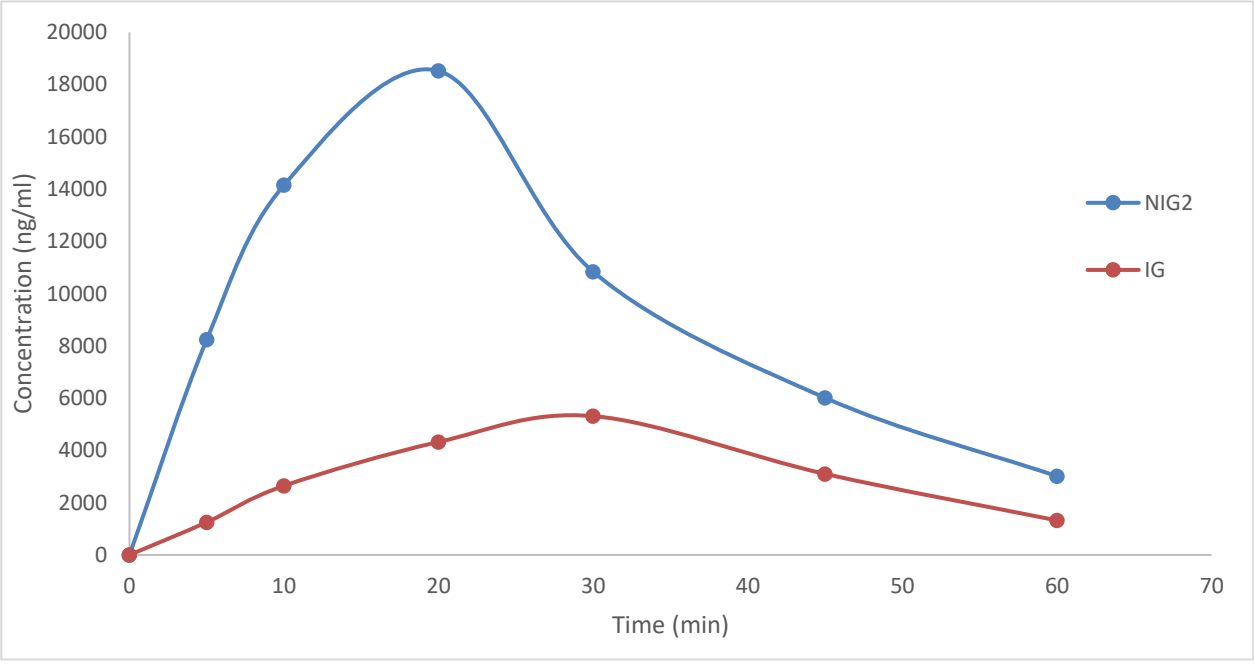

Figure 8: Mean CSF (n = 6) profile versus time obtained during the in vivo brain studies carried out in six rabbits receiving single dose of the prepared NIG2 and conventional prepared IG.

4. Conclusion

The study results indicated the possibility of application of nanoemulsion technology with in the formulation of the intranasal nanoemulsion in situ gel (NIG) for tizanidine (muscle relaxant drug), which is slightly water-soluble drugs class II and undergo highly first-pass metabolism, so prepare it as nasal nanoemulsion in suito gel.

upon applications in nasal cavity increment of drug permeability and absorption and finally increase concentration of drug within the cerebrospinal fluid.

Characterization and in vitro evaluation for all the prepared formulas had been applied and showed higher and faster release of the drug from NIG2 formula than that from conventional gel formula.

The in-vivo brain study for both NIG in comparison to conventional gel (IG) formula showed that the nasal in situ preparation containing nanoemulsion (NIG2) has higher and faster absorption with higher absolute cerebrospinal concentration than that IG that proves the contribution of nanoemulsion technology when incorporating in situ gel.

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