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FORMULATION AND CHARACTERIZATION OF NIMODIPINE NANOEMULSION AS AMPOULE FOR ORAL ROUTE

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ABSTRACT: The aim of this study was to develop oil in water nanoemulsion of nimodipine intended for oral use. Nimodipine is a calcium channel blocker that has poor oral bioavailability (3-30%) due to its low solubility and first- pass metabolism. So, nanoemulsion of nimodipine increase its solubility and enhance its oral bioavailability. Pseudo -ternary phase diagrams were made by using aqueous titration method. Nanoemulsions of pseudo-ternary phase diagrams composed of S mix (mixture of surfactant and co- surfactant), oil and deionized water. These nanoemulsions subjected for thermodynamic stability tests in order to select optimized formulations. Nimodipine nanoemulsions were subjected for characterizations studies in order to select the optimized formula. The characterizations studies explain that the optimized formula NE5 composed of 0.6% of nimodipine, 25% of S mix (2:1) that mean (16.66% tween 20: 8.33% PEG400), 10% of oleic acid and 64.4% of deionized water. The optimized formula NE5 characterized by droplet size (50 – 81.2 nm), polydispersity index (0.01), pH value (6.51), higher zeta potential (- 45.6 mV), drug content (98.9%), low viscosity and *in vitro* release of nimodipine was significantly higher ($P<0.05$) for NE5. Scanning probe microscopy (SPM) study confirms that the droplet size of NE5 was in nanoscale. It can be concluded that the optimized formula (nimodipine NE5) was a promising formula of nanoemulsion to enhance the oral bioavailability of nimodipine.

INTRODUCTION: Oral conventional (immediate release) drug products (solid or liquid) such as tablets, capsules and suspensions are formulated to release the active drug immediately after oral administration. In the formulation of conventional drug products no effort was made to modify the drug release rate.

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Immediate-release products generally result in relatively rapid drug absorption and onset of action but in conventional oral drug products that containing poorly water- soluble drug (lipophilic drug), the drug absorption may be gradual due to slow dissolution rate or selective absorption across the gastrointestinal tract, that result in a delayed onset time [1]. According to the biopharmaceutics classification system (BCS), the class II includes the drugs that have low solubility and high permeability. Many drugs belong to this class show low bioavailability due to poor solubility and insufficient dissolution process [2]. In order to improve the bioavailability of drugs that belong for class II and obtain a good clinical efficacy, the

solubility of these drugs must be improved and this can be achieved by using several techniques such as particle size reduction, pH adjustment, solid dispersion, self-emulsification, co-solvency and modern technique which is nanotechnology [3]. Nimodipine is a 1,4-dihydropyridine calcium channel blocker, its chemical name was [2,6-Dimethyl-4-(3'-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-beta-methoxyethyl ester 5-isopropyl ester]. Used for the improvement of neurological outcome by reducing the incidence and severity of ischemic deficits in patients with subarachnoid hemorrhage from ruptured congenital aneurysms [4].

According to biopharmaceutics classification system (BSC), its belong for class II (low solubility – high permeability). Bioavailability is about (3-30%) following oral administration due to low water solubility and extensive first-pass metabolism via CYP 3A4 in the liver. Nanoemulsion defined as an advance drug delivery system that has a great attention in the delivery of drugs, biological active agents and genetic materials which have a problem in their release [5]. Nanoemulsion is a colloidal dispersions composed of an oil that emulsified in aqueous phase surfactant and co surfactant at appropriate ratio. Its isotrpically clear possess a good thermodynamic and kinetic stability. Nanoemulsion present as an oil in water (o/w) in which the oil droplets dispersed in external aqueous phase , water in oil (w/o) in which the water droplets dispersed in external oil phase and bicontinuous nanoemulsions, in which the micro domains of oil and water are inter dispersed within the system [6] .

Kunal Jain *et al.* Develop oral nanoemulsion of atorvastatin, pharmacokinetic studies showed that the absorption of atorvastatin from nanoemulsion resulted in 2.87 and 2.38- fold increase in relative bioavailability as compared to conventional tablet and pure drug suspension respectively [7] . Tran TH, Guo Y, Song D, Bruno RS, Lu X. Prepared self-nanoemulsifying drug delivery system of quercetin. They found that the maximum concentration of plasma quercetin for optimized SNEDDS increased by three fold compared with the quercetin control suspension [8]. So, the aim of this study is to develop oil in water (o/w) nanoemulsion of nimodipine employed for oral use in order to increase nimodipine solubility for enhancing its oral bioavailability.

MATERIALS AND METHODS: Nimodipine and isopropyl myristate were purchased from Hyper chem company, China. Oleic acid, tween 20 and tween 80 were purchased from Thomas baker (chemicals) Pvt Ltd, India. Castor oil obtained from Evans medical Ltd, United Kingdom. Olive oil supplied by Pomace olive oil, oilex, S.A. Spain. Liquid paraffin obtained from Merck, Germany. Poly ethylene glycol 400 supplied by M/s provizer pharma. India. Propylene glycol obtained from Avon chem, United Kingdom. Methanol supplied by Avantor performance materials, Norway. Hydrochloric acid from Grin land chemical comp, United Kingdom. Na_2HPO4, KH_2PO4 and deionized water were supplied by Janeen for chemical and laboratory materials, Baghdad, Iraq.

Methods:

Differential scanning calorimetry study: Differential scanning calorimeter (DSC) analysis was performed using DSC Shimadzu 60, Japan, at of 10ºC/min heating rate, under nitrogen environment. The temperature range used was 50 – 250ºC.

Solubility study for screening of components: The solubility of nimodipine was determined in various oils which are (castor oil, olive oil, liquid paraffin, oleic acid and isopropyl myristate), surfactants which are (tween 20 and tween 80) and co-surfactants which are (poly ethylene glycol 400 and propylene glycol). Excess amount of nimodipine powder was added to 2ml of each oil, surfactant and co- surfactant that contained in small plain tubes. Then these tubes were tightly closed and placed in an isothermal shaker water bath at 25 ± 0.5 ^{0}C for 72 hr. After this time the samples were centrifuged at 3000 rpm for 20 min, then the supernatant layer for each sample filtered by using filter membrane (0.45 µm). After filtration the samples diluted with methanol and the solubility determined at λ max 238nm by using UV- visible spectrophotometer and the measurement was done in triplicate [9].

Construction of pseudo- ternary phase diagrams: The components of pseudo- ternary phase diagrams include oil , mixture of surfactant

and co- surfactant (S mix) and deionized water were developed using aqueous titration method. Surfactant and co- surfactant were mixed in different weight ratios (1:1, 1:2, 2:1, 3:1). For each phase diagram, the oil and S mix ratio were blended in different weight ratios until maximum ratio of oil and S mix was obtained. Twenty three different combinations of oil and S mix were prepared, these combinations were slowly titrated with aqueous phase (deionized water) and visual inspection was made for transparency. The titration of deionized water is stopped when transparent and clear oil in water (o/w) nanoemulsion was produced. No heating used during formulation. The nanoemulsions of pseudo- ternary phase diagram don't contain drug. These nanoemulsions were subjected for thermodynamic stability tests in order to obtain more stable formulations that employed in preparation of nimodipine nanoemulsions [10].

Screening of prepared nanoemulsion on the basis of thermodynamic stability tests: [11]
The thermodynamic stability tests used to assess the physical stability of formulations are:-

Centrifugation test: The formulations of nanoemulsions were centrifuged for 20 min at 3000 rpm and checked for phase separation, creaming or cracking.

Freezing–thawing test: This test was done by exposing the formulations for two different temperatures which are (21^{o}C) and (-21^{o}C) using refrigerator and the time for each temperature not less than 24 hours. This test used to indicate accelerated stability of formulations.

Heating-cooling test: Heating and cooling test was done by keeping the formulations at 40^{o}C and at 0^{o}C by refrigerator. The time for each temperature test should be not less than 48 hours. This test used to indicate the racking effect on the formulations stability.

Preparation of nimodipine loaded nanoemulsion: Nimodipine nanoemulsions was produced by dissolving the quantity of drug in specialized amount of oil. Then the determined quantity of S mix added for oil loaded drug, after that the whole mixture was blended together by vortex mixer. Then the aqueous phase (deionized water) titrated drop by drop to obtain transparent, clear (o/w) nanoemulsion [12].

Characterization of nimodipine nanoemulsions:
Droplet size measurement: After placing the samples in the sonicator for 20min at 35^{0}C, the droplet size measured by placing specimens of sample in particle size analyzer ABT-9000 nano laser. The droplet size and distribution plot of droplets were recorded.

Polydispersity index (PDI) measurement: The measurement of (PDI) gives an information about the uniformity of droplet distribution within the formulated nanoemulsion [13]. Its measured by particle size analyzer ABT-9000 nano laser for nimodipine loaded nanoemulsions. The measurement was done in triplicate.

pH measurement: The pH measurement regarded as important parameter for final product of drug loaded nanoemulsion because any alteration in pH will affect the stability of the formulated nanoemulsion [14] . pH measurement was done by using digital pH meter.

Percent of light transmittance (%T) measurement: The percent of light transmittance of nimodipine nanoemulsions was measured by using UV visible spectrophotometer at 650 nm [15]. Keeping distilled water as blank .The measurement was accomplished in triplicate.

Drug content estimation: Small volume was taken from the formula of nimodipine loaded nanoemulsion and diluted with methanol. Then the absorbance measured by using UV- visible spectrophotometer at λ_{max} 238nm and the concentration calculated by using the equation of methanol calibration curve [16] .

Zeta potential (ZP) measurement: Zeta potential describes the function of the charge on the surface of droplets. The measurement made by using zeta sizer instrument (nano brook zeta plus), the sample of nimodipine nanoemulsion putted in zeta cuvette and the reading was recorded.

Electrical conductivity measurement: Electrical conductivity gives an indication about the type of prepared nanoemulsion.

In (o/w) nanoemulsion, the continuous phase is water (aqueous phase) has electrical conductivity while (w/o) nanoemulsion, the continuous phase is oil and don't has electrical conductivity [17]. The conductivity measured by a conductivity meter. The measurement was done in triplicate.

Viscosity measurement: The viscosity measurement of nimodipine nanoemulsions was made by using NDJ-digital viscometer (spindle no. 1) at 25^0C. The viscosity measured without making dilution for the formulations. The measurement was done in triplicate.

***In vitro* release study:**

1- *In vitro* release of nimodipine loaded nanoemulsions: The *in vitro* release of nimodipine loaded nanoemulsion occurs by using dissolution apparatus USP-II (Copley dissolution tester DIS 8000, UK) and involving dialysis bag technique. The formula (5gm) of drug loaded nanoemulsion which contain 30mg of nimodipine putted in the dialysis bag. The dialysis bag immersed in 900 ml of dissolution medium. The dissolution medium was HCL buffer pH (1.2)+0.5%tween 20 for two hours , then replaced with phosphate buffer pH (6.8)+0.5%tween 20 for one hour [18].The dissolution apparatus set at 37 ± 0.5°C and velocity of rotation at 50 rpm for 3hours . 5ml samples were withdrawn at regular time intervals (15, 30, 45, 60, 75, 90, 105, 120 minutes) from dissolution medium HCL buffer pH(1.2)+0.5% tween 20 and the samples replenished by 5ml of fresh medium to maintain sink condition. Then the medium replaced with phosphate buffer pH (6.8)+0.5% tween 20 and 5ml samples were withdrawn at regular time intervals (135, 150, 165, 180 minutes) and the sample replenished by 5ml of fresh medium to maintain sink condition. All samples were withdrawn must be filtered by using filter membrane (0.45 µm). The samples were analyzed by UV- visible spectrophotometer at 238 for determining nimodipine content [19].

2- *In vitro* release of nimodipine conventional tablet: The *in vitro* release of conventional (immediate release) tablet of nimodipine occurs by using dissolution apparatus USP-I (basket method). The tablet putted in the basket and the basket immersed in 900ml of dissolution medium (HCL buffer pH (1.2) +0.5%tween 20). The apparatus set at 37 ± 0.5°C and rotation velocity at 50 rpm for 2 hours. 5ml samples were withdrawn from dissolution medium at regular time interval (15, 30, 45, 60, 75 minutes) and the samples replenished by fresh dissolution medium to maintain sink condition. After 75minutes the release was completed. The samples filtered by using filter membrane (0.45 µm), then analyzed by UV-visible spectrophotometer at 238 nm to determine nimodipine content [20].

Kinetics models and drug release mechanism: [21] Determination of release kinetic of drug was done by using various kinetics models. The results of dissolution must be fitted for these kinetics models which are (zero order kinetic, first order kinetic, higuchi model, korsmeyer and peppa's model).

Drug - excipient compatibility:

Fourier Transformed Infrared Spectroscopy (FTIR): One of the important aspects in prepared nanoemulsions was that no interaction between the components of formulation. The compatibility between drug and all excipients explained by (FTIR) spectroscopy. The study of compatibility by FTIR performed by making spectrums for nimodipine (pure powdered drug) using KBR disk, for nimodipine nanoemulsions using cuvette specialized for liquid samples [22].

Scanning probe microscopy (SPM) study: The shape, size and cumulative distribution of droplets within formulated nanoemulsion of nimodipine were detected by SPM (triple probe microscope). The detection was made by putting a drop of nimodipine nanoemulsion on a glass slide and then detect [23].

Statistical analysis: The results of the study were made as average of triplicate readings for each sample. Analysis of variance test (ANOVA) was used to investigate that the results of study considered as significant results when ($P< 0.05$) and the results considered not significant when ($P> 0.05$) [24].

RESULTS AND DISCUSSION:

Differential scanning calorimetry (DSC) study: DSC thermo gram of pure nimodipine was explained in **Fig. 1**. This thermo gram showed sharp peak at126.73 ^{0}C [25]. And corresponded with the reported melting point of nimodipine.

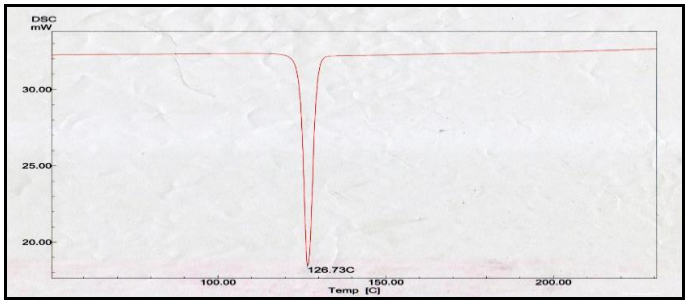

FIG. 1: DSC THERMO GRAM OF NIMODIPINE (PURE POWDER)

Screening of components on the basis of solubility study: [26] The selection of main components (oil, surfactant and co-surfactant) in preparation of nanoemulsion was regarded as important factor for producing stable nanoemulsion. The purpose from study the solubility of nimodipine in various oils, surfactants and co surfactants was to select the suitable oil phase that has higher solubility for nimodipine drug and this important for the formulation of nanoemulsion in order to maintain the nimodipine in solubilized form and no precipitation of drug will occur, also identifying the suitable surfactant and co- surfactant that aim in production of nanoemulsion with good stability.

The results of nimodipine solubility in various oils ensure the order; oleic acid > castor oil > isopropyl myristate > olive oil > liquid paraffin, the results of nimodipine solubility in various surfactants are tween 20 > tween 80 and the results of nimodipine solubility in co- surfactants are PEG400> propylene glycol. All the results of nimodipine solubility in oils, surfactants and co-surfactants was explained in the **Table 1**. So, the main components that were selected in the formulation of nanoemulsion are oleic acid as oil phase, tween 20 as surfactant and PEG400 as co- surfactant.

TABLE 1: SOLUBILITY STUDY OF NIMODIPINE IN VARIOUS OILS, SURFACTANTS AND CO-SURFACTANTS

S. no.	Oils	Solubility (mg/ml)
1	Oleic acid	48.2
2	Castor oil	21.31
3	Isopropyl myristate	12.13
4	Olive oil	7.23
5	Liquid paraffin	5.31
S.no.	**Surfactants**	**Solubility (mg/ml)**
1	Tween 20	81.64
2	Tween 80	62.14
S.no.	**Co-surfactants**	**Solubility (mg/ml)**
1	PEG400	73.42
2	Propylene glycol	33.13

Construction of pseudo-ternary phase diagrams: The components of pseudo-ternary phase plot are oil, water and S mix (surfactant /co surfactant) which considered as variable component due to that it presents in different ratio such as 1:1, 1:2, 2:1 and 3:1 as shown in **Fig.2-5**. The shaded area represent the area of nanoemulsion and the larger shaded area indicates a good nanoemulsifying activity.

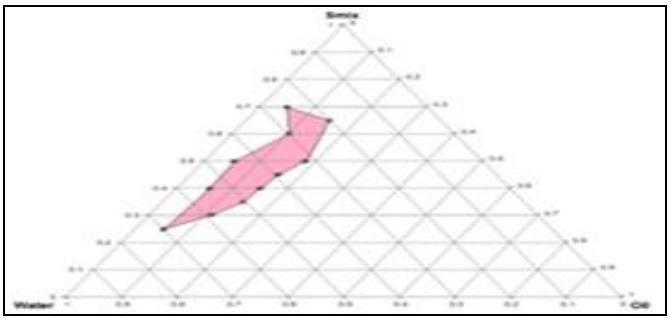

FIG.2: PSEUDO-TERNARY PHASE DIAGRAM OF OLEIC ACID, SMIX (1:1) AND DEIONIZED WATER

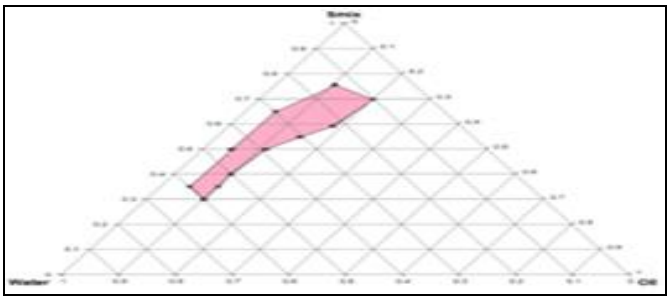

FIG.3: PSEUDO-TERNARY PHASE DIAGRAM OF OLEIC ACID, SMIX (1:2) AND DEIONIZED WATER

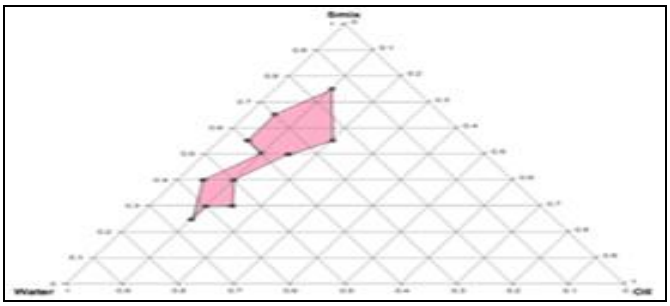

FIG.4: PSEUDO-TERNARY PHASE DIAGRAM OF OLEIC ACID, SMIX (2:1) AND DEIONIZED WATER

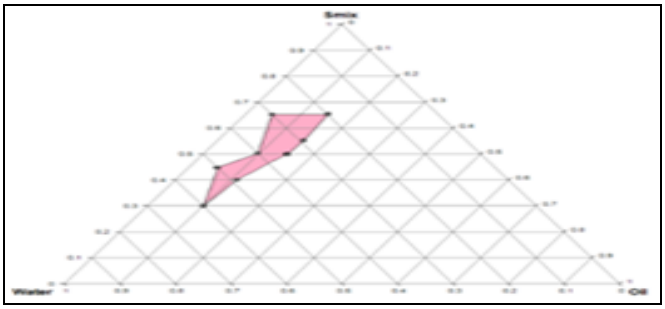

FIG.5: PSEUDO-TERNARY PHASE DIAGRAM OF OLEIC ACID, SMIX (3:1) AND DEIONIZED WATER

Screening of prepared nanoemulsion on the basis of thermodynamic stability tests: The results of thermodynamic stability tests for formulations that obtained from pseudo- ternary phase diagram were explained in the **Table 2.**

TABLE 2: THERMODYNAMIC STABILITY TESTS FOR FORMULATIONS OF PSEUDO-TERNARY PHASE DIAGRAM

S mix ratio	Formula NO.	%W/W of component nanoemulsions (without drug)			Thermodynamic stability tests			Results
		S mix (S:CoS)	Oil	Water	Centrifuge	Freeze-thawing	Heating – cooling	
1:1	F-1	30(15:15)	13	57	√	√	√	Pass
	F-2	40(20:20)	15	45	√	√	√	Pass
	F-3	45(22.5:22.5)	16	39	√	√	√	Pass
	F-4	50(25:25)	17	33	√	√	√	Pass
	F-5	45(22.5:22.5)	5	50	√	√	√	Pass
1:2	F-6	30(10:20)	10	60	√	√	√	Pass
	F-7	40(13.33:26.66)	10	50	√	√	√	Pass
	F-8	45(15:30)	5	50	√	√	√	Pass
	F-9	50(16.66:33.33)	11	39	√	√	√	Pass
	F-10	55(18.33:36.66)	14	31	√	√	√	Pass
2:1	F-11	25(16.66:8.33)	10	65	√	√	√	Pass
	F-12	30(20:10)	10	60	√	√	√	Pass
	F-13	30(20:10)	15	55	√	√	√	Pass
	F-14	40(26.66:13.33)	10	50	√	√	√	Pass
	F-15	45(30:15)	5	50	√	√	√	Pass
	F-16	50(33.33:16.66)	15	35	√	√	√	Pass
	F-17	55(36.66:18.33)	20	25	√	√	√	Pass
3:1	F-18	30(22.5:7.5)	10	60	√	√	√	Pass
	F-19	40(30:10)	12	48	√	√	√	Pass
	F-20	45(33.75:11.25)	5	50	√	√	√	Pass
	F-21	50(37.5:12.5)	14	36	√	√	√	Pass
	F-22	55(41.25:13.75)	16	29	√	√	√	Pass
	F-23	65(48.75:16.25)	15	20	√	√	√	Pass

Depending on the results of thermodynamic stability tests that explained in the **Table 2**. And keeping in mind high percentage of water, low percentage of S mix and different S mix ratio of formulations, nine formulas were selected which are F-1(NE1) , F-2(NE2) , F-6(NE3), F-7 (NE4), F-11(NE5), F-12(NE6), F-13(NE7), F-18(NE8), F-19(NE9) for preparation of nimodipine loaded nanoemulsions and these nanoemulsions were subjected for characterization.

Preparation of nimodipine loaded nanoemulsions: Nimodipine loaded nanoemulsions prepared by dissolving 0.6 gram of nimodipine in the specialized quantities of oil and S mix of selected formulations to prepare a formula of 100 gram. These nanoemulsions were stored in tight glass containers for characterization study.

Characterization of nimodipine nanoemulsions: Several tests were performed for studying the characterization of prepared nanoemulsions. The results of droplet size, PDI, pH and %T measurements for nimodipine loaded nanoemulsions (NE1-NE9) were explained in the **Table 3**.

TABLE 3: CHARACTERISTICS OF NIMODIPINE LOADED NANOEMULSIONS WHERE, PDI, pH AND %T (MEAN ± SD, N= 3)

Formula Code	Droplets Size (nm)	Polydispersity Index (PDI)	pH	% Transmittance (%T)
NE 1	44.5- 66.1	0.078±0.001	5.40±0.016	96.50±0.08
NE 2	39.7- 56.1	0.098±0.001	4.83±0.012	95.98±0.08
NE 3	45.5- 62.9	0.151±0.002	6.22±0.016	97.20±0.06
NE 4	50- 67.1	0.113±0.002	4.98±0.018	96.10±0.04
NE 5	50- 81.2	0.010±0.001	6.51±0.016	97.96±0.12
NE 6	31.5- 44.5	0.020±0.001	5.61±0.009	96.93±0.16
NE7	28.1- 39.6	0.047±0.001	5.33±0.016	96.10±0.04
NE 8	14- 24.6	0.022±0.001	6.12±0.012	96.90±0.12
NE 9	11.1- 14	0.036±0.001	4.94±0.008	95.94±0.06

Analysis of results of characteristics (droplet size, PDI, pH and %T) for nimodipine loaded nanoemulsions explains that the droplets size was decrease with increase the S mix ratio specially for 1:1 (NE1, NE 2), 2:1 (NE 5, NE 6, NE 7) and 3:1(NE 8, NE 9). Analysis of variance indicates that there was significant correlations between the droplet size of these nanoemulsions and % (w/w) of S mix ($P<0.05$). But in S mix ratio 1:2 , the droplets size don't decrease due to low amount of surfactant (tween20) that presents in the formulations [27].

The results of PDI indicates the uniformity of droplets distribution within the formulations. All formulations have PDI value less than (1.0). The lower value of PDI was (0.01) for NE5, this explains that NE5 has higher uniformity of droplets distribution within formulation.

The results of pH measurement for both formulations (drug free and drug loaded) explain that the pH values of drug free nanoemulsions was slightly acidic due to presence of an acid which is oleic acid that used as oil phase in the formulations. When nimodipine drug that has strongest basic properties incorporated in drug free nanoemulsions, the pH values of the resulted nimodipine loaded nanoemulsions were increased. So, the results of pH measurement were significant ($P<0.05$). The higher pH value in the formulations of nimodipine nanoemulsions was (6.51) for NE5 and this pH value regarded as good candidate for oral administration [28]. The results of %T explain that the formulated nanoemulsions were clear and transparent, and the transparency of nanoemulsions indicates that the droplets size was in nano-scale [29]. The higher value of %T in nimodipine loaded nanoemulsions was (97.96%) for NE5.

Drug content estimation: The results of drug content in nine formulations of nimodipine loaded nanoemulsions were in range (92.1- 98.9%). The higher percent of drug content (98.9%) was found in NE5 that has S mix (2:1), and the lowest percent of drug content (92.1%) was found in NE4 that has S mix (1:2).

Zeta potential (ZP) Measurement: Zeta potential is the term that used to explain the electro kinetic potential in colloidal dispersions. The results of zeta potential of nimodipine nanoemulsions were in range (- 17.8mV to – 45.6 mV). Rule of thumb explains the values of zeta potential which are: range -5 mV to +5 mV indicate fast aggregation, about -20 mV or +20 mV provide short term stability, above +30 mV or below -30 mV indicate a good stability and above +60 mV or below -60 mV offers excellent stability[30]. This applied for electric stabilization and surfactant with low molecular weight, but in case of surfactant with large molecular weight such as non ionic surfactant (tween 20) provides additional steric stability for the formulations.

Electrical Conductivity Measurement: The results of electrical conductivity of nimodipine loaded nanoemulsions were found to be in range (0.18 - 0.28 m S/cm). This explains that the continuous phase of system was water and the type of formulated nanoemulsion was oil in water (o/w) nanoemulsion.

Viscosity Measurement: The results of viscosity of nimodipine nanoemulsions were found to be in range (33.026 – 107.782 mPa.sec.). It was noticed that all nimodipine nanoemulsions have low viscosity and this important to ensure the smoothen formulations, packing and convenient using specially, if the nanoemulsion dedicated for oral use [31].

***In vitro* release analysis:**

1-*In vitro* release of nimodipine nanoemulsions analysis: The release of nimodipine nanoemulsions (NE1-NE9) ensures the order: NE5 > NE6 > NE7> NE3> NE4> NE1> NE8>NE2>NE9. Agreeing to the analysis of variance, there was a significant difference ($P<0.05$) between the release of nimodipine in all nanoemulsions and time. The drug release profile for nimodipine nanoemulsions with S mix (1:1), S mix (1:2), S mix (2:1) and S mix (3:1) ensures the order : NE1> NE2 , NE3> NE4, NE5> NE6> NE7 and NE8> NE9 respectively as shown in **Fig.6.** The release of drug from nimodipine nanoemulsions (NE1-NE9) in dissolution media explains the influence of surfactant concentration (%w/w) on the release of nimodipine for each ratio of S mix. In each S mix ratio, as the concentration of tween 20 increase, the release of nimodipine decrease.

This occurs due to that increasing the concentration of tween 20 results in increasing the diffusion of nimodipine molecules from dialysis bag for the dissolution medium [32]. The higher release of nimodipine was found in NE5 that had S mix (2:1) due to low concentration of tween 20 in the formula that lead for low diffusion of nimodipine molecules from dialysis bag for dissolution medium. The lower release of nimodipine was found in NE9 that had S mix (3:1) due to high concentration of tween 20 in the formula that lead for high diffusion of nimodipine from dialysis bag for dissolution medium.

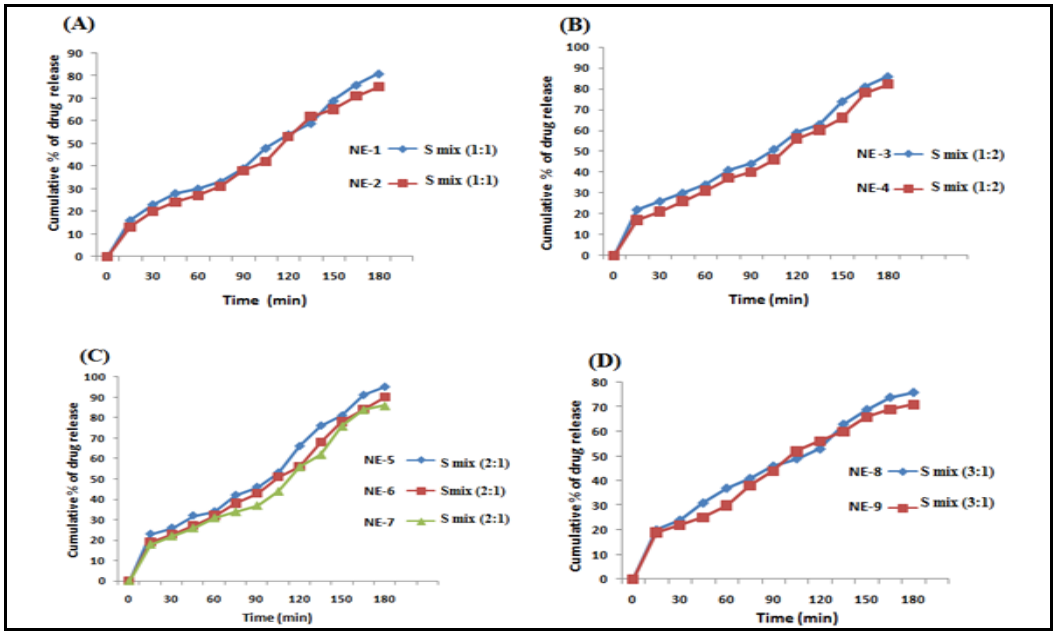

FIG.6: *IN VITRO* RELEASE OF NIMODIPINE NANOEMULSIONS, WHERE (A) RELEASE OF NE1 AND NE2, (B) RELEASE OF NE3 AND NE4, (C) RELEASE OF NE5, NE6 AND NE7, (D) RELEASE OF NE8 AND NE9

2- *In vitro* release of nimodipine conventional tablet analysis: The release of nimodipine from conventional tablet was completed at 75 min as shown in **Fig.7**. This lower release of nimodipine indicates that only small amount of drug dissolves in dissolution medium HCL buffer (pH1.2) +0.5%tween 20 due to the strongest basic properties of nimodipine. According to the analysis of variance, the nimodipine release profile was significantly lower (P> 0.05) for conventional tablet.

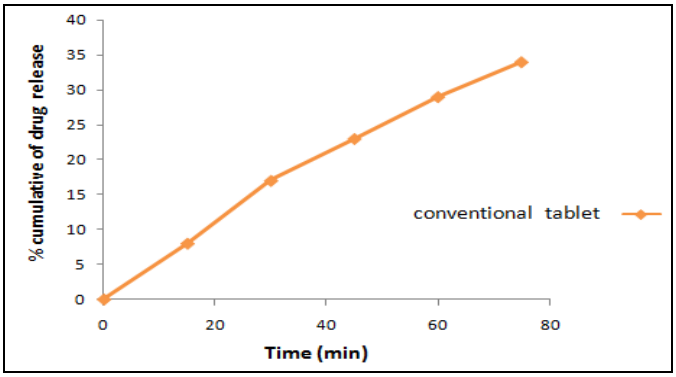

FIG. 7: *IN VITRO* RELEASE OF NIMODIPINE CONVENTIONAL TABLET (IMMEDIATE RELEASE)

Kinetic release of drug analysis: The data of dissolution were fitted for various kinetic models which are zero order kinetic (cumulative percentage of drug release against time), first order kinetic (cumulative percentage of drug remained against time), higuchi model (percentage of drug released against square root of time) and korsmeyer and peppa's model (log percentage of drug released against log of time). It was found that the higher regression coefficient (R^2) values in the zero order kinetic.

So, the kinetic of drug release in all nanoemulsions (NE1-NE9) and conventional tablet was zero- order kinetic and the values of diffusion exponent (n) for all nanoemulsions (NE1-NE9) and conventional tablet of nimodipine was significantly lower than 0.43 ($P<0.05$), this explains that the mechanism of release of nimodipine from nanoemulsions and conventional tablet was Fikian release (diffusion) [33]. As in **Table 4**.

TABLE 4: VALUES OF REGRESSION COEFFICIENT (R^2) AND THE VALUES OF DIFFUSION EXPONENT (n).

NE - code	Zero-order kinetic	First- order Kinetic	Higuchi-model	Korsemyer-peppas model	Diffusion Exponent
	R^2	R^2	R^2	R^2	N
NE-1	0.983	0.915	0.923	0.950	0.33
NE-2	0.989	0.963	0.924	0.967	0.41
NE-3	0.975	0.912	0.973	0.970	0.27
NE-4	0.973	0.930	0.967	0.971	0.14
NE-5	0.976	0.837	0.895	0.912	0.25
NE-6	0.982	0.874	0.910	0.905	0.38
NE-7	0.967	0.868	0.885	0.931	0.35
NE-8	0.970	0.960	0.965	0.968	0.42
NE-9	0.973	0.971	0.970	0.948	0.23
Conventional tablet	0.991	0.988	0.951	0.990	0.40

Selection of optimized formula of nimodipine nanoemulsions: After study the characterization of prepared nimodipine nanoemulsions. There was an indication that the (NE5) is the optimized formula, because its characterized by good droplet size range (50-81.2 nm) as in the **Fig. 8**. Low PDI (0.010), best pH value for oral use (6.51), good percent transmittance (97.96%), percent of drug content was higher (98.9), higher zeta- potential (-45.6) as shown in the figure 9**.** Effective electrical conductivity (0.28mS/cm), accepted viscosity range for oral use (40.012- 40.834 m Pa.s) and higher release of nimodipine from the formula.

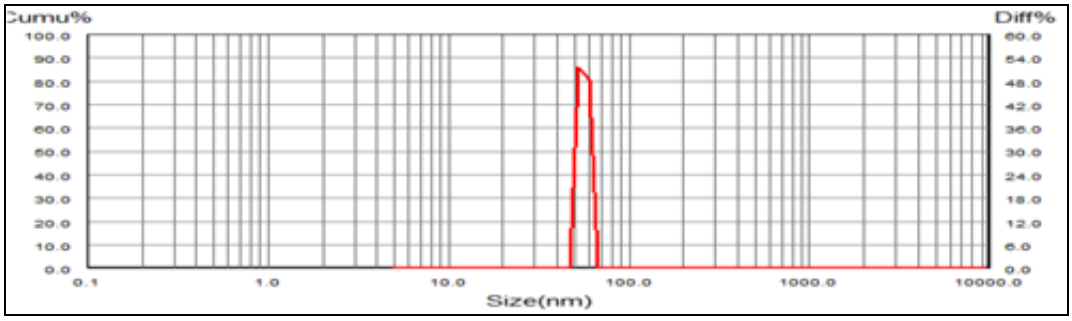

FIG.8: DROPLET SIZE RANGE OF OPTIMIZED FORMULA (NIMODIPINE NE5)

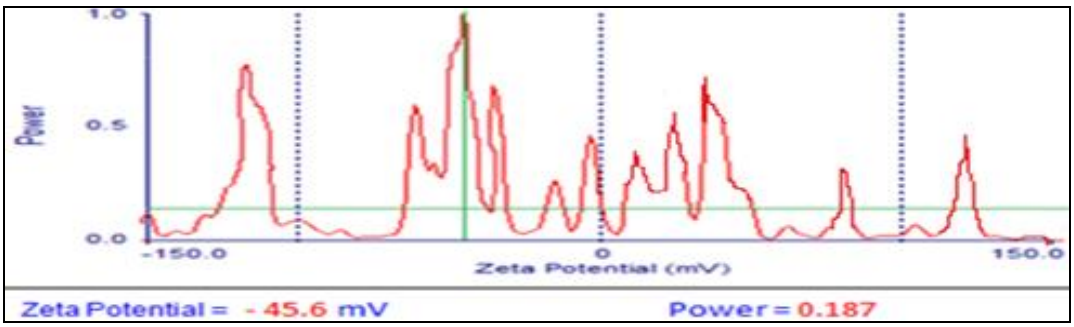

FIG. 9: ZETA POTENTIAL VALUE OF OPTIMIZED FORMULA (NIMODIPINE NE5).

Studies of Drug – Excipient Compatibility:

Fourier Transformed Infrared Spectroscopy (FTIR): FTIR spectra were made for pure nimodipine drug and for nimodipine nanoemulsion (NE5). The results of FTIR spectroscopy show the characteristic peaks of pure nimodipine drug as in **Fig. 10**, which are (NH stretching of aliphatic secondary amine) at 3298.2cm^{-1}, (NH bending of aliphatic secondary amine) at 1647.2 cm^{-1}, (C-H stretching of benzene ring) at 3097.6 cm^{-1}, (C-H asymmetric stretching of methyl) at 2981.2cm^{-1}, (C-H asymmetric stretching of methylene) at 2933.7 cm^{-1} , (C-H symmetric stretching of methyl) at 2899 cm^{-1}, (C-H symmetric stretching of methylene) at 2845cm^{-1}, (carbonyl stretching of ester) at 1691.5 cm^{-1}, ((C=C) stretching of benzene ring) at 1622.1cm^{-1},1523.7cm^{-1} and 1496cm^{-1}, (NO_2 asymmetric stretching) at 1454.3 cm^{-1}, (NO_2 symmetric stretching) at 1309.6 cm^{-1}.

These peaks of pure nimodipine appear in spectrum of nimodipine nanoemulsion as in **Fig. 11,** in which also there was broad peak from 2650cm^{-1} to 3380cm^{-1}, this broad peak belongs to the (OH-stretching of oleic acid) that result from hydrogen bond formation between oleic acid and water. This indicates that there was drug – excipient compatibility between all components of prepared nanoemulsions.

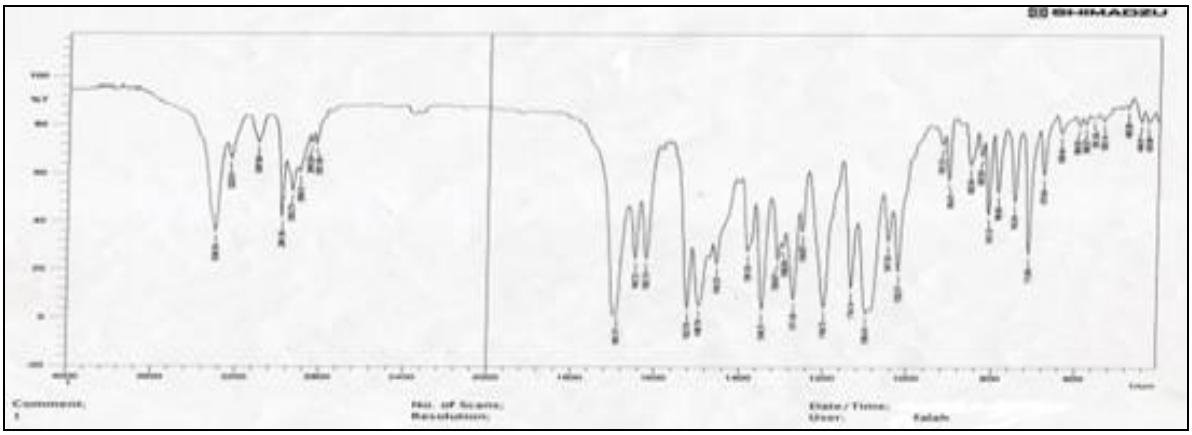

FIG. 10: FTIR SPECTRUM OF PURE NIMODIPINE POWDER

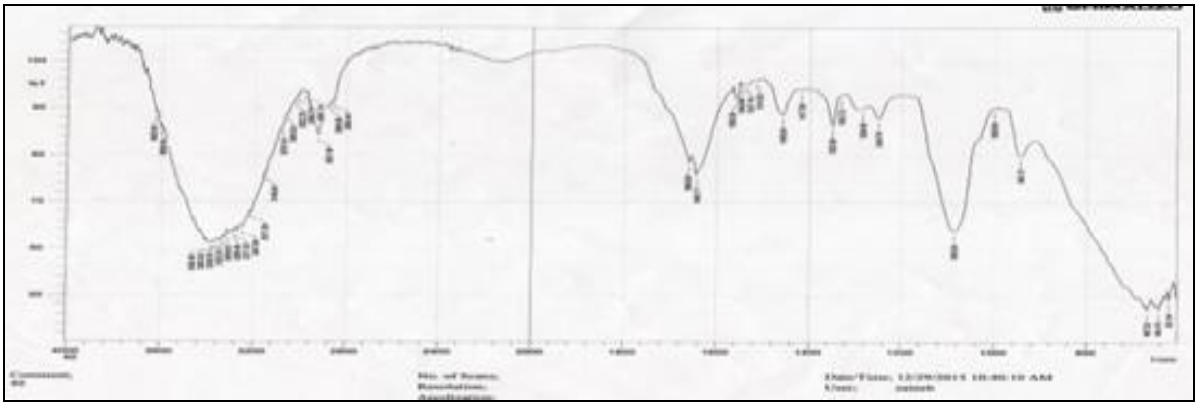

FIG. 11: FTIR SPECTRUM OF NIMODIPINE NANOEMULSION (NE5)

Scanning probe microscopy (SM) study: The results of (SPM) study indicate that the droplets morphology of nimodipine nanoemulsion (NE5) was spherical in shape and the droplets don't present in aggregation state as in **Fig.12.** The droplets size was in nano-scale and similar to the size range that obtained by particle size analyzer ABT-9000 nano laser as in **Fig.13**.

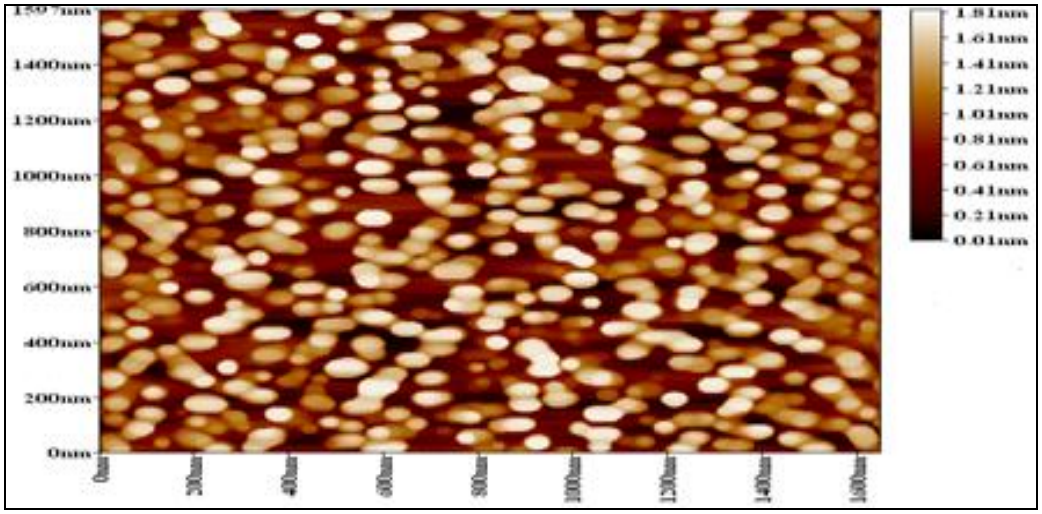

FIG. 12: SPM IMAGE OF NIMODIPINE NANOEMULSION (NE5)

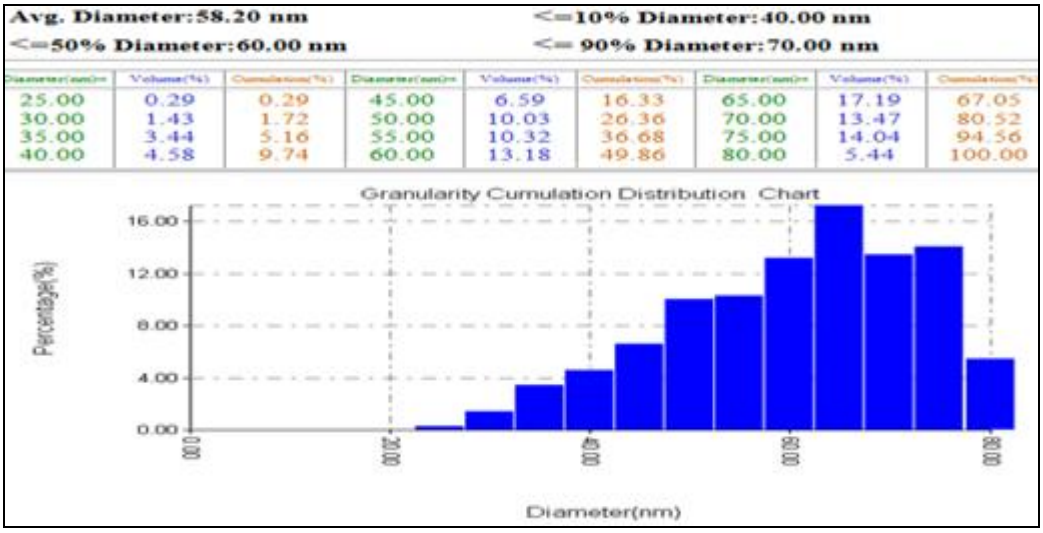

FIG. 13: DISTRIBUTION CHART EXPLAIN DROPLETS SIZE RANGE OF NIMODIPINE (NE5).

CONCLUSION: Nanoemulsion considered as an advance technique for improving the bioavailability of poorly water - soluble drugs (lipophilic drugs) that belongs for class II of biopharmaceutics classification system (BCS) by enhancing the solubility and minimizing the first pass metabolism for these drugs. The prepared formula of (NE5) as ampoule (5gram) that contain 30mg of nimodipine can be administrated via oral route for conscious patient as one ampoule every six hours. If the patient unconscious, the drug can be administrated by syringe through nasogastric tube (NG tube).

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