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FORMULATION OF GLICLAZIDE USING SELF-NANO-EMULSIFICATION TECHNOLOGY

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ABSTRACT
Self-nano-emulsifying drugs delivery systems present an effective drug delivery system for the formulation of a hydrophobic drug with poor water-solubility. Gliclazide is practically insoluble in water and is amenable for self-nano-emulsifying drug delivery. This study aimed to develop a formulation of gliclazide using Cremophor RH 40 and Tween 20 with capryol 90 as oil vehicle. A solubility study, particle size analysis and ternary phase diagram were developed to select the optimal formulation to take forward to stability testing. Accelerated physical stability and self-emulsification studies were performed. They were also compared for globule size, transmittance, polydispersity index and zeta potential. The solubility was greatest in Capryol 90 64.86 mg/mL, Cremophor RH 40 44.98 mg/mL, PEG 400 105.66 mg/mL, and Tween 20 86.83 mg/mL. The construction of the ternary phase diagram in the formulation using cremophor 40 as surfactant has good characteristics and wide area in nano-emulsifying formation than Tween 20. Gliclazide can be formulated and exhibits good stability in a self-nano-emulsifying drug delivery system.

KEYWORDS: Gliclazide, SNEDDs, formulation, nano emulsifying.

INTRODUCTION
Self-nano-emulsifying drug delivery systems (SNEDDS) are defined as anhydrous homogenous liquid mixtures consisting of oil, surfactant, drug and co-emulsifier or solubiliser, which spontaneously form an oil-in-water nanoemulsion (NE) of approximately 200nm or less in size upon dilution with water, under gentle stirring.[1]

Desai et al.[1] reported that SNEDDS have potential to improve oral bioavailability and therapeutic efficacy of hydrophobic drugs via several mechanisms.

- Improving solubility of hydrophobic drugs.
- Improving permeability/transport for poorly permeable drugs.
- Modulating biodistribution and drug disposition.
- Preventing degradation of drugs in the physiological milieu.
- Enabling targeted delivery of the drugs to the site of action.

A NE improved the oral bioavailability, and CNS delivery of saquinavir[2] enhanced dissolution of griseofulvin[3] and improved the bioavailability of the alkaloid huperzine by lymphatic uptake.[4] The authors concluded that the nano-size of these formulations was responsible for the enhancement of drug dissolution and absorption, owing to the large surface area.[1] The lipophilic nature of these systems also facilitates delivery of drugs into difficult to penetrate tissues such as the central nervous system. However, such systems are subject to oxidation of the vegetable oils, which may raise safety issues.

Desai et al.[1] compared high- and low energy emulsification methods. High-energy methods require sophisticated equipment and extensive energy input which is associated with increased cost. This is significant in the pharmaceutical industry, leading researchers to focus on low-energy emulsification methods.[1]

The Biopharmaceutics Classification System[5] (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. There three major factors that influence the rate and extent of drug absorption from immediate release solid oral dosage forms; dissolution, solubility and intestinal permeability. The BCS classified the immediate release solid oral formulations into Class 1: High Solubility – High Permeability, Class 2: Low Solubility – High Permeability, Class 3: High Solubility – Low Permeability and Class 4: Low Solubility – Low Permeability (FDA, Biopharmaceutics Classification System, 22/12/2017). Compounds in BCS class II, with a log P ranging 2-3 can be improved in solubility and

bioavailability using self-lipid formulations.[6] Gliclazide is a sulfonylurea derivative, widely used in diabetes treatment around the world, which is only partially soluble in water. The absorption of gliclazide is influenced by the size of the particle.[7] The reformulation of the related sulphonylurea glipizide, to enhance solubility and dissolution, has been proposed.[8] (A self-emulsifying gliclazide was formulated in the micron size range[9], but to date no attempt to use a nano-size delivery system for this medication has been identified.

Nanoparticles as drug delivery systems

Wilczewska et al.[10] reviewed nanoparticles as a drug delivery system (DDS), specifically nanocarriers, and their interactions with drugs. The paper suggested that using nanoparticles as DDS can overcome some of the conventional limitations of drugs, such as limited effectiveness due to poor distribution and lack of receptor sensitivity.[10] Their review found that nanocarriers with optimized physicochemical and biological properties are taken up by cells more readily than larger molecules, meaning they can be used as delivery tools for currently available bioactive compounds.[10]

The article explains that the method of incorporating the drug with the nanocarrier, and the strategy of its targeting, are important considerations. A drug may be absorbed, or covalently attached to the nanocarriers' surface, or it can be encapsulated into it. Once the drug nanocarrier conjugate reaches the tissues, the therapeutic agent is released. Controlled release may be achieved through changes in temperature, pH, and osmolality or via enzymatic activity.[10] The authors concluded that nanocarriers as DDS are designed to improve the pharmacological and therapeutic properties of conventional drugs, and that the incorporation of drug molecules into nanocarriers can protect a drug against degradation, whilst in addition, offering possibilities of targeting and control of release characteristics.[10] In comparison with traditional formulations, nanocarrier-drug conjugates are more effective and selective. They have the potential to reduce the toxicity and other adverse side effects in normal tissues by accumulating the drug at target sites, resulting in a lower dosage requirement. Conversely, drawbacks of nanoparticle-based targeting systems include; process scale-up issues, low drug loading capacity, low loading efficiency and poor ability to control the size distribution of carriers.[10]

Martin et al.[11] investigated the use of nano self-emulsifying systems for managing poorly water-soluble drugs. The study highlights the confusion on differentiating between self-micro-emulsifying drug delivery systems (SMEDDS) and self-nano-emulsifying drug delivery systems (SNEDDS). The author defines a microemulsion to be a thermodynamically stable fluid mixture of water, oil and surfactants. This definition differentiates microemulsions from NE, which may only be kinetically stable. It also highlights the importance of differentiating between SMEDDS and SNEDDS as it may have a biopharmaceutical relevance. The study found that theoretical considerations indicated that particle size alone is often not sufficient to assign categories; and polydispersity must be considered. They proposed stability categories to differentiate stable swollen micelles and consequently microemulsions from NE.[11]

The study of Bhattacharya and Prajapati[12], investigated the formulation approach for self-emulsifying drug delivery systems. They state that self-emulsifying drug delivery systems (SEDDS) offer a promising new approach. The authors highlight the value of SEDDS because 40% of new drug candidates exhibit poor aqueous solubility and nearly 50% are highly lipophilic in nature. The study found that surfactants having hydrophilic-lipophilic balance (HLB)>12 makes good candidates for SEDDS. They also highlighted the importance of using suitable excipients[12]. The table below outlines the types of oils used in marketed SEDDS (table 1).

Table 1: The types of oils used in marketed products based upon SEDD's (data from[12]).

Type of Oil	Marketed Product	Medication
Corn Oil	Depakene™ Capsules	Valproic Acid
Olive Oil	Sandimmune™ Oral Solution	Cyclosporine
Sesame Oil	Marinol™ Soft Gelatin Capsule	Dronabinol
Soya Bean Oil	Accutane™ Soft Gelatin Capsule	Isotretinoin
Peanut Oil	Prometrium™ Soft Gelatin Capsule	Progesterone
Hydrogenated Soya Bean Oil	Accutane™ Soft Gelatin Capsule	Isotretinoin
Bees Wax	Vesanoid™Soft Gelatin Capsule	Tretinoin

Bhattacharya and Prajapati[12] concluded that self-emulsifying drug delivery systems offer a promising approach towards projecting future generations of dosage formulations. Many studies reveal that the physical stability of the drug is enhanced when an SEDDS formulation is used. Most importantly, BCS 2 and 4 drugs have a wider application in SEDDS formulations. SEDDS are mainly prepared in liquid dosage form, but due to stability in solid SEDDS forms, more research is projected in this direction.[12]

The aim of this study was to pilot a formulation of gliclazide in a self nano-emulsifying delivery system that is physico-chemically stable.

METHOD

The project was conducted as part of a student exchange experience between the University of Wolverhampton UK and Universitas Islam Indonesia at the nanotechnology laboratory by a UK student supervised by local Faculty.

Gliclazide was a purchased from a local pharmaceutical company in Indonesia (origin, Zhejiang Jiuzhou Pharmaceutical Co. Ltd., Taizhou City, China), Capryol 90 (Gattefose, Saint-Priest, France), Chremopore RH 40 (Sigma-Aldrich, St Louis, MI, USA) Tween 20 and PEG 400 Kao, Tokyo, Japan), $Na_2HPO_2.2H_2O$, KH_2PO_4, HCl 37% analytical grade (Merck, Darmstadt, Germany), Methanol (HPLC Grade, J.T Baker, Fair Lawn, USA)

Chromatographic Analysis

The assay of gliclazide used a high-pressure liquid chromatography method (Waters e2695 with UV-Vis 24890)(Waters Corporation, Milford MI, USA). Sunfire C18 Column (250 mm X 4,6 mm, 0.5 µm)(Waters Corporation, Milford MI, USA) was utilized to obtain separation with a mobile phase of methanol and phosphate buffer pH 3 (70:10), 1 mL/min flow rate and wavelength 229 nm UV detection.

Solubility Test for Excipient

To test the solubility of gliclazide in different excipient oils, surfactant and co-surfactant, an excess amount of gliclazide was added to each of the Eppendorf™ tubes containing 1 ml of test substance. The potential excipients investigated were Capryol 90 (oil), Chremopor RH (Surfactant) and PEG 400 (Co-Surfactant). The mixture was vortexed on vortex mixer for 5 min to facilitate proper mixing and dissolution of gliclazide in that excipient. The mixtures were then allowed to equilibrate at 30°C for 72 hours in shaker (Memmert WNB29 with Shaker SV2945), (Memmert, Schwabach, Germany). The samples then centrifuged at 10,000 rpm (Hettich Mikro185), (Hettich, Pocklington, UK) for 13 min to separate the undissolved drug. Aliquots of supernatant were suitable diluted and the gliclazide present in each excipient quantified by HPLC. Standards were prepared by dissolving weighed aliquots of gliclazide in each diluent.

Construction of ternary phase diagrams

The ternary phase diagram was prepared from the mixture of selected various concentration of oil, surfactant and co-surfactant mixture with sonication (Biologics, Inc 300VT), (Biologics, Woburn, MA, USA). Ternary phase diagrams of oil, surfactant and co-surfactant were plotted; each of them representing an apex of the triangle using Triplot 4.1.2 software (Loughborough University, Loughborough, UK). All compositions were examined for NE formation after diluting each of the mixtures to 250 ml with double-distilled water. Thereafter, transmittance of the resulting dispersions were determined by using UV/Visible spectrophotometer 650 nm (Hitachi U-2900)(Hitachi, Tokyo, Japan), and globule size, polydisperse index (PDI) and zeta potential, determined by using particle size analyser (Horiba SZ-100), (Horiba, Fukuoka, Japan). The table of the ternary diagram mixture was used to select formulae with various concentrations (% v/v) of capryol 90, cremophor RH 40, tween 80 and PEG 400 to evaluate. The Capryol 90 solution had a high range concentration providing a suitable loading capacity to act as a vehicle for gliclazide (Table 2).

Table 2: Ternary diagram mixture of selected formula with various concentration.

Material	Low concentration (%)	High concentration (%)
Capryol 90	40	65
Cremopor RH 40	5	55
Tween 20	5	55
PEG 400	5	55

Centrifugation test

Gliclazide SNEDDS were diluted 100 times using Water for Injections, then centrifuged at 3,500 rpm for 30 minutes. It was then observed visually to check the phase separation.

Heating-cooling cycle test

The heating-cooling cycle test was conducted for six cycles across a temperature range from 4°C and 40°C with storage of the formula for not less than 48 hours. The formula would need to be stable for this test. The formula was centrifugated to 3,500 rpm for 15 minutes and then it was observed visually to check the phase separation.

Freeze-thaw cycle test

The freeze-thaw cycle test was conducted with six cycles across a temperature range from -20°C and 25°C with storage of the formula for not less than 48 hours. The formula must be stable at this temperature. The formula was centrifuged visually observed to check the phase separation.

Endurance test

The formula was diluted with the dilutions of 25, 50, 100, and 250 times with water for injections. Then, the change in transmittance, polydispersity index (PDI), and particle size of the formula was evaluated.[13]

Accelerated storage stability test

The accelerated storage test, which was conducted for 1 month with the storage condition of 40°C±2°C/ 75%

RH±5% RH. Then, the change of % transmittance, polydispersity index (PDI), and particle size of the formula was evaluated at weeks 0, 1, 2, 3, and 4.[14]

HPLC Method Development and Validation

a. Preparation in Phosphate buffer pH 3

Phosphate buffered solutions of gliclazide were prepared by dissolving 0,68 gram KH_2PO_4 in a 500 ml volumetric flask and Phosporic Acid 85% (An aliquot 0,613 ml of this solution was diluted with double-distilled water in a 10 ml volumetric flask).A sample of 0.7 ml from these solutions in a 500 ml volumetric flask with KH_2PO_4, the volume was made up to the mark with double-distilled water to achieve the pH 3.

b. Preparation Standard Stock Solutions

Standard stock solutions of gliclazide were prepared by dissolving 10 mg in 100 mL methanol to obtain concentration 100 µg/mL.

c. Determination of λ_{max}

An accurately weighed quantity of gliclazide (10 mg) each were transferred in 100 ml volumetric flask, dissolved in sufficient quantity of methanol. The volume was made up to the mark with methanol to achieve the concentration 100 µg/ml. An aliquot (1 ml) of this solution was diluted with methanol in a 10 ml volumetric flask up to mark to achieve a final concentration of 10µg/ml. The standard solution of gliclazide was scanned in the range of 200-400 nm using HPLC and was recorded to determine the λ_{max} of the drugs. The study of spectrum revealed that gliclazide showed a well-defined λ_{max} at 229 nm.

d. Linearity and Range

As per ICH guidelines, the linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity study for the proposed method was established by least-square linear regression analysis. Sample solutions of gliclazide were prepared in the concentration range of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50µg/ml by transferring appropriate volume of stock solution to a 10 ml of volumetric flask and making up the volume with methanol. All dilutions were scanned in λ_{max} at 229 nm.

RESULTS

Solubility Test for Excipient

A solubility test was conducted to select suitable excipients for the SNEDD formulation. The criteria for selection of the excipients were:(1) the formulation should be safe and biocompatible using small amount surfactant and co-surfactant to produce the NE. (2) the compositions should develop a large NE area formation in the ternary phase diagram. (3) It must have a high drug loading (4) The SNEDDS formulation should provide a small and homogeneous emulsion droplet size.[15] (The solubility of gliclazide in excipient was determined by HPLC analysis in surfactant, co-surfactant and oil. The solubility of gliclazide in the co-surfactant was highest in PEG 400 (Table 3).

Table 1: The solubility of gliclazide in different excipient including oil, surfactant and co-surfactant. Data are represented by mean value ± SD (n=6)

Vehicles	Solubility (mg/mL) Mean ± SD
Capryol 90 Oil	64.857 ±15.378
Surfactant - Cremophor RH 40	44.975 ±2.513
Tween 20 Co-surfactant	86.834 ±0.455
PEG 400	105.660 ±0.171

Gliclazide in PEG 400 had the highest solubility 105.66 mg/mL. The solubility each excipient of SNEDDs formulation impacts the formulation because lower surface tension can produce smaller globules in the emulsifying formation. The free energy of a regular emulsion formation is a direct function of the energy required to develop a new surface between the oli and water phase, the two-phases contribute to separate time-dependent decreases in the interfacial area and thus the free energy of the system, and will develop a droplet of an emulsion stabilized by the emulsifying agent. Spontaneous emulsification occurs due to lower free energy.[16] Self-emulsification results when the entropy favouring dispersion is greater than energy needed to increase the surface area of the dispersion.[17]

Ternary Phase Diagram

Tables 4-13 show the results for the gliclazide formulation into a NE using different surfactants in different oil: surfactant ratio combinations. The selection of co-surfactant of the SNEDDS formulations can be evaluated from the size of globule formation, % transmittance and polydispersity index. The criteria sought are size below 200nm, % transmittance 70-100% and Polydispersity index (P.I) below 0.7. The formation diagram constructed with surfactant cremophor RH40 showed a better area in NE formation than using tween

20 (Table 3). The formulation containing cremophor RH40 can produce NE with capryol 90 as oil up to 50% in those proportions. On the other hand using tween 80 as surfactant can develop a NE with oils ratio until 65% but lower % transmittance.

Table 2: Oil: Surfactant mix (Smix) (40:60).

No.	Capryol 90	Cremophor RH	PEG 400	Size (nm)	% Transmittance	P.I.
1	40	5	55	336.90 ± 1.40	5.570 ±0.009	0.534 ± 0.038
2	40	10	50	372.90 ± 4.90	3.108 ±0.004	0.659 ± 0.041
3	40	15	45	200.90 ±4.10	1.718 ±0.001	0.473 ± 0.040
4	40	20	40	117.10 ± 0.50	31.183 ±0.065	0.393 ± 0.029
5	40	25	35	85.80 ± 0.70	70.780 ±0.042	0.400 ± 0.001
6	40	30	30	54.50 ± 0.20	83.593 ±0.042	0.389 ± 0.025
7	40	35	25	37.50 ± 0.60	91.475 ±0.029	0.406 ± 0.022
8	40	40	20	77.40 ±2.00	93.670 ± 0.073	0.535 ± 0.001
9	40	45	15	119.70 ± 0.20	89.504 ± 0.045	0.668 ± 0.029
10	40	50	10	170.30 ± 0.50	87.312 ±0.008	0.698 ± 0.011
11	40	55	5	184.60 ± 1.30	84.348 ±0.016	0.627 ± 0.040

Table 3: Oil: Smix (45:55).

No.	Capryol 90	Cremophor RH	PEG 400	Size (nm)	% Transmittance	P.I.
1	45	5	50	351.10 ±26.00	8.931 ±0.018	0.470 ±0.780
2	45	10	45	372.90 ± 4.90	3.231 ±0.002	0.659 ± 0.041
3	45	15	40	212.50 ± 1.80	1.096 ± 0.000	0.383 ± 0.006
4	45	20	35	179.50 ± 13.20	21.173 ±0.006	0.404 ± 0.053
5	45	25	30	70.20 ± 0.50	69.123 ± 0.061	0.438 ± 0.023
6	45	30	25	43.70 ± 0.20	89.130 ± 4.078	0.352 ± 0.039
7	45	35	20	45.60 ± 0.30	86.505 ± 0.005	0.439 ± 0.033

Table 4: Oil: Smix (50:50)

No.	Capryol 90	Cremophor RH	PEG 400	Size (nm)	% Transmittance	P.I.
1	50	45	5	35.60 ± 1.20	87.297 ±0.188	0.546 ± 0.024
2	50	40	10	60.60 ± 1.40	82.974 ±0.012	0.422 ± 0.019
3	50	35	15	75.10 ± 0.50	70.520 ±0.039	0.447 ± 0.036
4	50	30	20	89.60 ± 0.40	35.132 ±0.021	0.387 ± 0.010
5	50	25	25	186.00 ± 0.50	0.880 ± 0.000	0.420 ± 0.037
6	50	20	30	262.70 ± 2.70	1.122 ± 0.000	0.690 ± 0.014
7	50	15	35	324.30 ±12.70	0.937 ± 0.000	0.711 ± 0.031

Table 5: Oil: Smix (60:40)

No.	Capryol 90	Cremophor RH	PEG 400	Size (nm)	% Transmittance	P.I.
1	60	35	5	2914.90 ± 919.30	7.301 ±0.045	2.996 ± 0.628
2	60	30	10	2325.30 ± 163.60	4.899 ±0.148	1.463 ± 0.298
3	60	25	15	n.d	1.332 ±0.002	n.d
4	60	20	20	n.d	34.244 ±0.033	n.d
5	60	15	25	n.d	58.632 ±5.680	n.d
6	60	10	30	n.d	61.133 ±0.006	n.d
7	60	5	35	n.d	54.666 ±0.033	n.d

Table 6: Oil: Smix (65:35)

No.	Capryol 90	Cremophor RH	PEG 400	Size (nm)	% Transmittance	P.I.
1	65	2,5	32,5	n.d	7.772 ±0.001	n.d
2	65	5	30	n.d	1.227 ± 0.004	n.d
3	65	10	25	n.d	0.692 ±0.001	n.d
4	65	15	20	n.d	0.404 ± 0.001	n.d
5	65	20	15	n.d	7.304 ±0.000	n.d
6	65	25	10	n.d	19.711 ± 0.001	n.d

Table 7: Oil: Smix (40:60).

No.	Capryol 90	Tween 20	PEG 400	Size (nm)	% Transmittance	P.I.
1	40	5	55	n.d	7.376 ±0.044	n.d
2	40	10	50	n.d	3.290 ±0.002	n.d
3	40	15	45	n.d	2.901 ±0.003	n.d
4	40	20	40	n.d	2.382 ±0.002	n.d
5	40	25	35	264.30 ± 29.00	1.405 ±0.000	0.473 ± 0.018
6	40	30	30	188.60 ± 3.30	1.072 ±0.001	0.356 ± 0.031
7	40	35	25	240.80 ± 13.00	1.265 ±0.000	0.488 ± 0.059
8	40	40	20	386.90 ± 140.80	2.425 ± 0.002	0.580 ± 0.174
9	40	45	15	206.60 ± 1.30	3.107 ±0.000	0.403 ± 0.047
10	40	50	10	235.40 ± 6.40	3.137 ±0.002	0.540 ± 0.086
11	40	55	5	371.60 ± 112.40	2.386 ± 0.002	0.479 ± 0.108

Table 8: Oil: Smix (45:55).

No.	Capryol 90	Tween 20	PEG 400	Size (nm)	% Transmittance	P.I.
1	45	5	50	4729.00 ± 8.60	2.020 ±0.001	7.535 ± 1.722
2	45	10	45	n.d	2.821 ±0.006	n.d
3	45	15	40	404.10 ± 45.00	3.199 ±0.002	3.090 ± 0.148
4	45	20	35	97.80 ± 0.00	3.226 ±0.005	1.369 ± 0.000
5	45	25	30	145.60 ± 4.80	1.504 ±0.001	0.386 ± 0.224
6	45	30	25	186.70 ± 3.00	1.431 ± 0.001	0.514 ± 0.125
7	45	35	20	175.60 ±0.00	1.098 ± 0.001	0.853 ± 0.000

Table 9: Oil: Smix (50:50).

No.	Capryol 90	Tween 20	PEG 400	Size (nm)	% Transmittance	P.I.
1	50	5	45	158.90 ±34.90	11.338 ±0.073	0.314 ±0.073
2	50	10	40	187.60 ± 5.20	6.258 ±0.011	0.360 ± 0.183
3	50	15	35	1637.50 ± 310.00	6.605 ±0.032	0.702 ± 0.002
4	50	20	30	229.00 ± 0.00	4.884 ± 0.014	0.427 ± 0.000
5	50	25	25	187.80 ± 5.50	2.522 ± 0.002	0.412 ± 0.045
6	50	30	20	314.80 ±6.60	1.678 ± 0.002	0.485 ± 0.078
7	50	35	15	467.40 ± 41.50	1.344 ±0.001	0.510 ± 0.098

Table 10: Oil: Smix (60:40).

No.	Capryol 90	Tween 20	PEG 400	Size (nm)	% Transmittance	P.I.
1	60	5	35	2723.00 ± 0.00	3.407 ±0.029	1.472 ± 0.000
2	60	10	30	n.d	3.168 ±0.014	n.d
3	60	15	25	n.d	1.419 ±0.007	n.d
4	60	20	20	838.80 ± 14.20	1.430 ±0.004	5.678 ± 6.579
5	60	25	15	842.50 ± 1013.80	1.843 ±0.000	1.250 ± 0.540
6	60	30	10	113.10 ± 26.40	1.476 ±0.002	0.777 ± 0.607
7	60	35	5	184.30 ± 12.50	1.504 ±0.001	0.591 ± 0.145

Table 11: Oil: Smix (65:35).

No.	Capryol 90	Tween 20	PEG 400	Size (nm)	% Transmittance	P.I.
1	65	2,5	32,5	4669.90 ± 362.10	4.141 ±0.019	5.762 ± 1.934
2	65	5	30	n.d	1.927 ±5.194	n.d
3	65	10	25	n.d	2.029 ±0.003	n.d
4	65	15	20	505.90 ± 31.60	1.619 ±0.002	5.132 ± 0.336
5	65	20	15	465.80 ± 38.20	1.274 ±0.000	3.120 ± 0.078
6	65	25	10	131.50 ± 5.80	1.467 ±0.001	0.524 ± 0.143

From the tables above, the SNEDDS gliclazide formulation in various concentrations of capryol 90 using cremophor RH40 and PEG 400 as co-surfactant possess good characteristics in size formation below 200nm and provide the largest self-nano emulsifying region with high oil proportion, providing optimal loading capacity.

Based on the ternary diagram area formation (Figure 1), the composition of surfactant, co-surfactant and oil can

be developed from cremophor RH 40, PEG 400 and Capryol 90. Surfactant cremophor RH 40 can develop the larger area in NE formation (system A). The largest area formation will produce good proportion and stability of NE dispersed system.

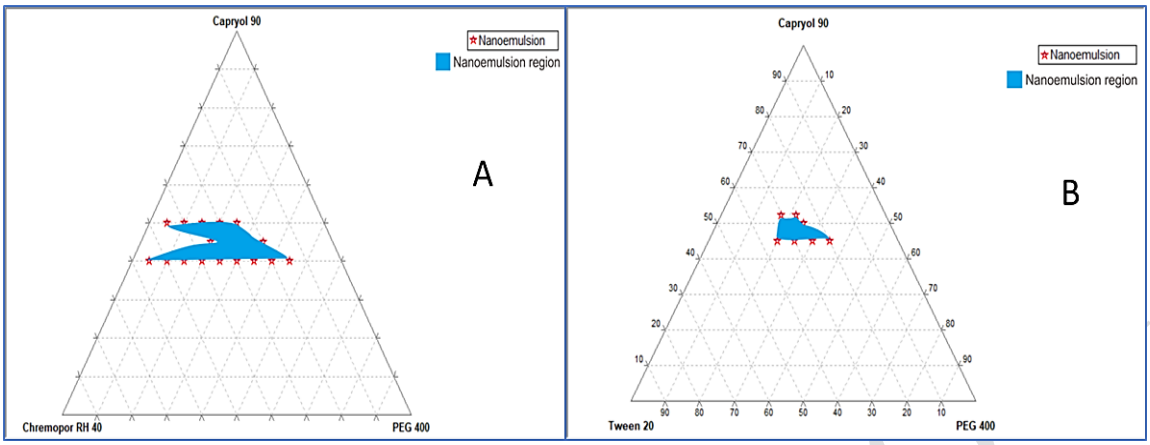


Figure 1: phase diagram of system A (Capryol 90/Cremophor RH/PEG 400); system B (Capryol 90/Tween 20/PEG 400. Blue areas represent the region of self-nanoemulsifying formation region.

Formulation of Gliclazide SNEDDs

The selected excipients for the gliclazide SNEDDs formulation was taken from larger ternary diagram region. Gliclazide was weighed and up to 40 mg added to Cremophor RH40 as surfactant and then sonication was applied to facilitate homogenization. This mix was then added PEG 400 as the co-surfactant and sonicated and finally this mix was added to Capryol 90 as the oil phase. The final mixture was sonicated for 2 minutes.

The formulation for the stability study was prepared from capryol 90, cremophor RH40 and PEG 400, the ratio of Oils/Surfactan and Co-Surfactant (S_{mix}) was 4:6; 4.5:5.5 and 5:5. Based on table 14 the characteristics for the gliclazide have a small globul size (below 200 nm), Polydispersity index lower than 0.7 and % transmittance almost 100%, it showed this formula has characteristics;, clear and with a small globul size.

Table 12: Formulation of SNEDDs contains 40 mg gliclazide in 2.5 mL. Data are represented by mean value ± SD (n=3).

Oil:S_{mix}	Capryol 90 (oil)	Chremphor RH 40 (surfactant)	PEG 400 (Co-surfactant)	Particle size (nm)	P.I	Transmittance (%)
4:6	40	30	30	55.7±1.9	0.457±0.02	99.80
	40	35	25	55.1±1.5	0.397±0.01	99.69
4.5:5.5	45	20	35	127,4±0,3	0,318±0,01	96,47
	45	25	30	109,8±1	0,378±0,02	97,98
5:5	50	30	20	87,6±0,8	0,477±0,00	98,95
	50	25	25	122,1±2,9	0,340±0,03	97,15

Centrifugation Test

The Gliclazide SNEDDS was diluted 100 times using water for injections, then centrifuged at 3,500 rpm for 30 minutes. It was then evaluated to check the phase separation (Table 15). The formula exhibited good stability against phase separation.

Table 13: Centrifugation test for the formulation, centrifuge at 3.500 rpm for 30 minutes (n=2).

Oil:S_{mix}	Capryol 90 (oil)	Chremphor RH 40 (surfactant)	PEG 400 (Co-surfactant)	Replication 1	Replication 2
4:6	40	30	30	No separation	No separation
	40	35	25	No separation	No separation
4.5:5.5	45	20	35	No separation	No separation
	45	25	30	No separation	No separation
5:5	50	30	20	No separation	No separation
	50	25	25	No separation	No separation

Heating-cooling cycle test

The stable formulae from the centrifugation test was used to conduct the heating-cooling cycle test. Six cycles across a temperature range of 4°C and 40°C with storage of the formula for not less than 48 hours. The formula must be stable at this temperature. Then, the formula was centrifugated with speed of 3,500 rpm for 15 minutes. No phase separation occurs (Table 16).

Table 14: Heating and cooling test provide absent phase separation during heating-cooling at the 4oC and 40oC.

Oil:S_{mix}	Capryol 90 (oil)	Chremphor RH 40 (surfactant)	PEG 400 (Co-surfactant)	Replication 1	Replication 2
4:6	40	30	30	No separation	No separation
	40	35	25	No separation	No separation
4.5:5.5	45	20	35	No separation	No separation
	45	25	30	No separation	No separation
5:5	50	30	20	No separation	No separation
	50	25	25	No separation	No separation

Freeze-thaw cycle test

The stable formulae resulting from the heating-cooling cycle test was used to conduct the freeze-thaw cycle test that was conducted with six cycles at the temperature of -20°C and 25°C with storage of the formula was not less than 48 hours. The formula must be stable at this temperature. Then, the formula was centrifugated with speed of 3,500 rpm for 15 minutes, and then it was observed visually to check the phase separation (Table 17).

Table 15: The Freeze-thaw cycle test for the formula in six cycles at the -20oC and 25oC, the formula have good stability from freeze condition.

Oil:S_{mix}	Capryol 90 (oil)	Chremphor RH 40 (surfactant)	PEG 400 (Co-surfactant)	Replication 1	Replication 2
4:6	40	30	30	No separation	No separation
	40	35	25	No separation	No separation
4.5:5.5	45	20	35	Separation	Separation
	45	25	30	No separation	No separation
5:5	50	30	20	Separation	Separation
	50	25	25	No separation	No separation

Endurance test

The stable formulae from the freeze-thaw cycle test was used to conduct the endurance test. The formula was diluted with the dilutions of 25, 50, 100, and 250 times with water for injections. Then, the change of % transmittance, P.I., and particle size of the formula was evaluated (Table 18).

Table 16: Formulation endurance test.

Oil:S_{mix}	Capryol 90	Chremphor RH 40	PEG 400	Globul size (nm) mean ± SD (n=3)			
				25 x dilution	50 x dilution	100 x dilution	250 x dilution
4:6	40	30	30	113±0,9	72.9±0.7	63.7±0,4	24.3±0.3
	40	35	25	116.5±0.3	22.5±0.3	21.6±0.2	22.3±0.5
4.5:5.5	45	20	35	n.d	n.d	n.d	n.d
	45	25	30	129.4±1.3	127.7±0.5	86.4±0.4	55.2±0.3
5:5	50	30	20	n.d	n.d	n.d	n.d
	50	25	25	108.5±0.3	115.4±0.2	83.4±0.4	64.8±1.3
Oil:S_{mix}	Capryol 90	Chremphor RH 40	PEG 400	Polidispersity Index mean ± SD (n=3)			
				25 x dilution	50 x dilution	100 x dilution	250 x dilution
4:6	40	30	30	0.287±0.03	0.273±0.02	0.308±0.4	0.162±0.04
	40	35	25	0.347±0.05	0.034±0.02	0.026±0.0	0.025±0.02
4.5:5.5	45	20	35	n.d	n.d	n.d	n.d
	45	25	30	0.273±0.00	0.344±0.03	0.317±0.01	0.384±0.01
5:5	50	30	20	n.d	n.d	n.d	n.d
	50	25	25	0.282±0.03	0.375±0.03	0.356±0.04	0.367±0.02
Oil:S_{mix}	Capryol	Chremphor	PEG	% transmittance mean ± SD (n=3)			

	90	RH 40	400	25 x dilution	50 x dilution	100 x dilution	250 x dilution
4:6	40	30	30	32.83±0.3	71.80±0.7	89.61±0.2	99.72 ±0.1
	40	35	25	0.37±0.05	97.59±0.3	99.35 ±0.6	100.01 ±0.2
4.5:5.5	45	20	35	n.d	n.d	n.d	n.d
	45	25	30	0.64 ±0.07	28.77±0.4	67.68±1.1	98.27 ±1.2
5:5	50	30	20	n.d	n.d	n.d	n.d
	50	25	25	5.23±0.01	31.99±0.2	60.46±0,4	97.37 ±0,5

Linearity and Range

Six different concentrations (2, 4, 6, 8, 10, 12 µg/mL) were obtained from the stock solution and diluted with methanol followed by a calculation of the limit of regression coefficient (r), slope, and intercept. The regression test (Figures 2 and 3) indicated that the concentration series gliclazide showed 0.9998 regression coefficient with the equation y=18017x-1670.1. The ICH (International Committee on Harmonization) recommends that a good linearity value for the analysis should be more than 0.998. The obtained linearity value has followed the defined criteria.

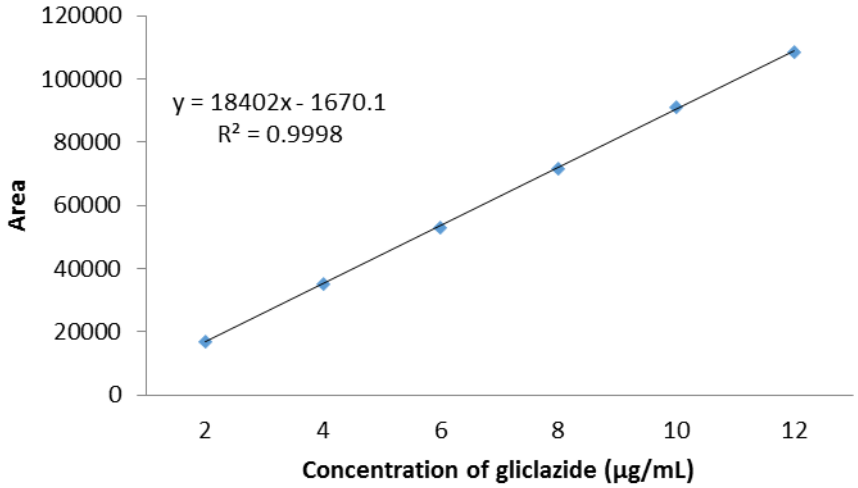
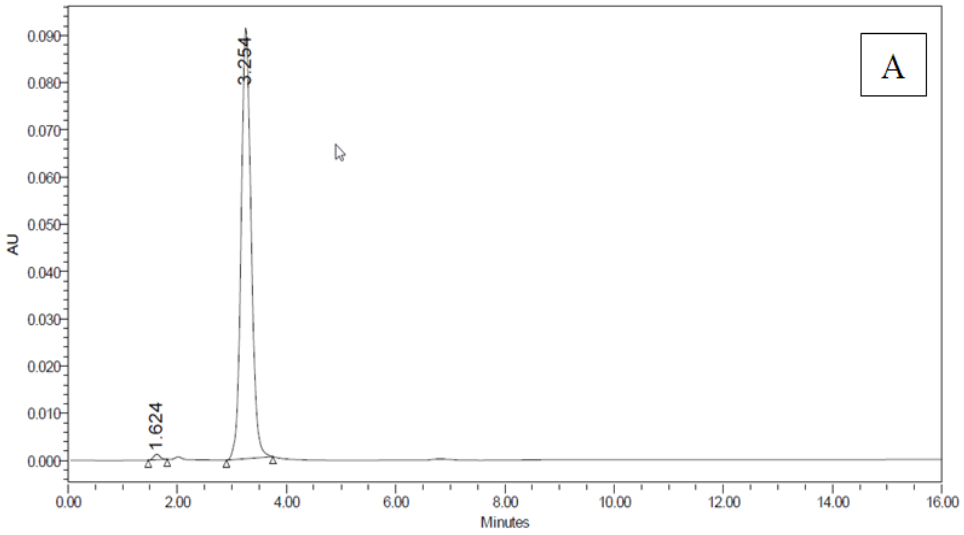


Figure 2: Gliclazide calibration curve with six variation concentration series gliclazide showed 0.9998 regression coefficient with the equation y=18017x-1670.1



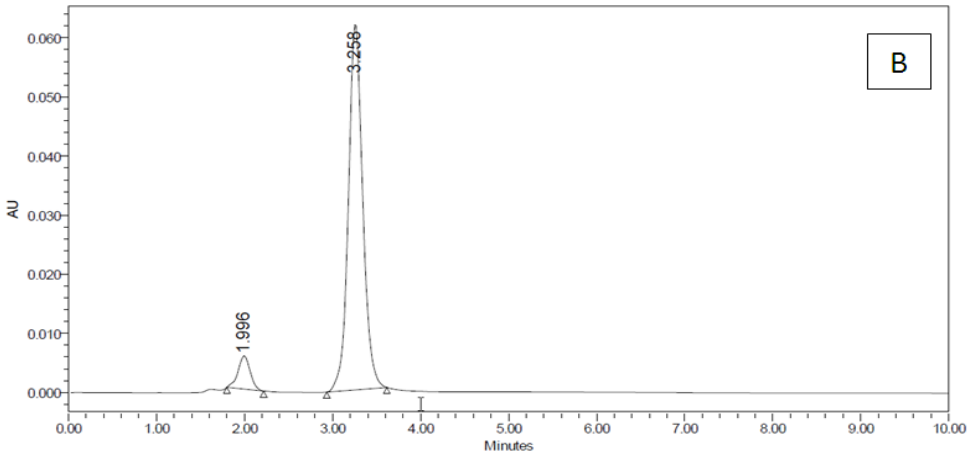


Figure 3: Gliclazide chromatogram (A) and gliclazide in PEG 400 sample of solubility test.

DISCUSSION

This pilot study was a resource-limited student project which nevertheless suggests good potential for the use of SNEDDS as a delivery system for this widely used drug, using well established approaches.[18]

The structures in NEs are much smaller than visible light wavelengths; so many NEs appear transparent.[19] The average droplet size of NEs ranges from 20 to 500 nm.[20] Consequently, NEs are transparent or translucent with a bluish colouration.[21] They are, by nature, sensitive systems because they are usually very fluid; at and signs of destabilization readily appear. Their very small droplet size causes a large reduction in gravity force, and Brownian motion may be sufficient for overcoming gravity.[22] Brownian motion prevents sedimentation or creaming, thus offering increased physical stability.[23] They may have high kinetic stability because their small droplet size makes them stable against sedimentation and creaming.[24] The small droplet size also prevents any flocculation of the droplet.

Choosing the optimal formulation generally requires trading between optimal particle size, mean dissolution time, emulsification time and maximisation of drug release.[18] Further work would be required to develop this into a viable delivery system.

A solubility test was used to select suitable excipient in SNEDDS. The solubility of gliclazide in excipient was determined by HPLC analysis in surfactant, co-surfactant and oil was highest in PEG 400. It was found that for loading drug capacity, high oils will increase capacity of drug incorporated into the formulation in drug delivery system. The NE formulation prepared was selected for globule size below 200nm, transmittance percentage of 70-100% and P.I. below 0.7. The composition of surfactant, co-surfactant and oil was achieved by using Cremophor RH 40, PEG 400 and Capryol 90 resulted in the largest area formation which produced stability of the NE disperse system.

The gliclazide SNEDDs formulation has shown a good stability, no phase separation.

The regression test indicated that the concentration series gliclazide showed 0.9998 regression coefficient with the equation y=18017x-1670.1. The ICH (International committee on Harmonization) recommends that a good linearity value for the analysis should be more than 0.998. The obtained linearity value has followed the defined criteria.

The solubility in Capryol 90 64.86 mg/mL, Cremophor RH 40 44.98 mg/mL, PEG 400 105.66 mg/mL, and Tween 20 86.83 mg/mL. The construction of ternary phase diagram in the formulation using Cremophor 40 as surfactant has a good characteristic and wide area in nano emulsifying formation than Tween 20. The gliclazide can be formulated and good stability in SNEDDs using Cremophor RH 40, PEG 400 and Capryol 90.

CONCLUSION

The application of a SNEDDS formulation of gliclazide offers the potential for improved delivery and a possible reduction in side effects for this very widely used agent.

Benefit to practice

When a medication is newly marketed and under patent protection there is little enthusiasm at that stage for adopting novel formulations. Advances in formulation science however offer opportunities to re-visit a number of established medicines to improve the formulations. Diabetes mellitus type 2 is a global epidemic that society is currently failing to control, and better tools may assist.

Limitation

This was a pilot project undertaken by an undergraduate student with limited resources and further development would be required.

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Conflict of interest: Nil known.

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