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Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for Gliclazide

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Abstract

Self-nanoemulsifying drug delivery systems (SNEDDS) were developed with the objective to overcome problems associated with the delivery of Gliclazide, a poorly bioavailable, anti-diabetic having pH dependant solubility. Solubility of Gliclazide in oily phases and surfactants were determined to identify components of SNEDDS. Various surfactants and co-surfactants were screened for their ability to emulsify selected oily phases. Ternary phase diagrams were constructed to identify area of nanoemulsification for the selected systems. The influence of Gliclazide and pH of dilution medium on the phase behavior of selected system were assessed. The globule size of optimized Gliclazide SNEDDS in various dissolution media was determined to check the effect of pH on its behavior. The optimized Gliclazide SNEDDS needed surfactant content less than 55% and yielded nanoemulsion of mean globule size 146 nm, which was not affected by the pH of dilution medium. The optimized SNEDDS released the Gliclazide drug completely within 20 min irrespective of the pH of dissolution medium.

Keywords: Gliclazide; SNEDDS; pH dependant solubility, co-surfactant

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INTRODUCTON

Most of the nanoparticle system have been developed for the delivery of poorly water soluble drugs for enhance there bioavailability in the GI-tract. Nanoemulsion are preferred drug delivery system because of there stability and possibility of easy oral administration to improve drug self-emulsification in the gut [1]. Gliclazide revealed that it is practically in soluble in water and, therefore absorb poorly with irritation in gastric lining and hence shows bioavailability just 40%. Thus in order to improve its bioavailability, it is necessary to enhance its solubility and

dissolution characteristics. Conventional tablet of gliclazide are available with the dose of 40mg and therefore keeping these finding in mind, it was decided to increase solubility of gliclazide by formulation of self-nanoemulsifying drug delivery system (SNEDDS), which may result in increase in solubility and dissolution with subsequent reduction in dose [2-3]. Development of lipid base drug delivery strategies that will retain all the bioavailability related advantages will be advantageous for optimizing gliclazide delivery. Self-nanoemulsifying system would be one such approach to achieve optimum gliclazide delivery. Self-nanoemulsifying drug delivery system (SNEDDS) are isotropic mixtures of oil, surfactant, co-surfactant and drug that form fine oil-in-water nanoemulsion when introduce into aqueous phase under agitation [4]. Self-nanoemulsifying system of gliclazide would be an efficient, convenient and more patient compliance [5]. Self-nanoemulsion SNEDDS can be directly filled in hard gelatin capsule size 0 due to there anhydrous nature enabling its administration as unit dosage form.

In this study, we developed an optimized formulation using a self-nanoemulsifying drug delivery system in order to improve the solubility and bioavailability of Gliclazide. Composition of SNEDDS was optimized using solubility, Ternary phase diagram, droplet size and drug release…etc.

MATERIALS and METHODS

Materials

Gliclazide was a generous gift from Bal Pharma (Bangalore, India).
CAPTEX 355, CAPTEX 300, CAPTEX 350, CAPMUL MCM, CAPMUL PG8 obtained as a gift sample from ABITEC CORPORATION, Ohio, USA. LABRAFILL M 2125 CS,LABRAFILL M 1944 CS, LABRAFACE CC, LAUROGLYCOL 90 obtained as a gift sample from Colorcon India, Goa (GATTEFOSSE, FRANCE). CREMOPHORE EL & CREMOPHORE RH obtained as a gift sample from BASF Ltd, All other chemicals were used of analytical reagent grade and double distilled water was used throughout the experiments.

Solubility studies

The solubility of Gliclazide in various modified oils, buffers and 10% (w/w) surfactant solutions was determined by using shake flask method. Briefly, an excess amount of Gliclazide was added to each vial containing 1 g of the selected vehicle, i.e., either oil, surfactant solution or buffer. After sealing, the mixture was vortexes using a cyclomixer for 10 min in order to facilitate proper mixing of Gliclazide with the vehicles. Mixtures were then shaken for 48 h in a water bath shaker (Remi, Mumbai, India) maintained at room temperature [6-7]. Mixtures were centrifuged at 5000 rpm for 5 min, followed by filtration through membrane filter 0.45 um. Filtrate was suitably diluted with ethanol and GLICLAZIDE dissolved in various vehicles was quantified by a validated HPLC method developed in house.

Screening of surfactants for emulsifying ability

Emulsification ability of various surfactants was screened. Briefly, 300 mg of surfactant was added to 300 mg of the selected oily phase. The mixture was gently heated at 45–60^{0}C for homogenizing the components. The isotropic mixture, 50 mg, was accurately weighed and diluted with double distilled water to 50 ml to yield fine emulsion. The ease of formation of emulsions

was monitored by noting the number of volumetric flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and their transmittance was assessed at 638.2 nm by UV-160A double beam spectrophotometer (Shimadzu, Japan) using double distilled water as blank [2,8].

Screening of co-surfactants

The turbidimetric method was used to assess relative efficacy of the co-surfactant to improve the nanoemulsification ability of the surfactants and also to select best co-surfactant from the large pool of co-surfactants available for peroral delivery Surfactant, 0.2 g was mixed with 0.1 g of cosurfactant. Capryol 90 (CAE), 0.3 g, was added to this mixture and the mixture was homogenized with the aid of the gentle heat (45–60 ^{0}C) The isotropic mixture, 50 mg, was accurately weighed and diluted to 50 ml with double distilled water to yield fine emulsion. The ease of formation of emulsions was noted by noting the number of flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and their transmittance was measured at 638.2 nm by UV-160A double beam spectrophotometer (Shimadzu, Japan) using double distilled water as blank. As the ratio of co-surfactants to surfactant/s is the same, the turbidity of resulting nanoemulsions will help in assessing the relative efficacy of the co-surfactants to improve the nanoemulsification ability of surfactant/s [5].

Construction of ternary phase diagrams

Ternary diagrams of surfactant, co-surfactant and oil were plotted; each of them, representing an apex of the triangle Ternary mixtures with varying compositions of surfactant, co-surfactant and oil were prepared. The surfactant concentration was varied from 30 to 75% (w/w), oil concentration was varied from 25 to 75% and co-surfactant concentration was varied from 0 to 30% (w/w). For any mixture, the total of surfactant, co-surfactant and oil concentrations always added to 100%. For example, in the experiment, first mixture consisted of 75% of surfactant (either Cr-EL or SHS 15), 25% of the oily phase (CAE) and 0% of co-surfactant (Ak-MCM). In the further experiments, the co-surfactant was increased by 5% for each composition, oily phase concentration was kept constant and the surfactant concentration was adjusted to make total of 100%. Forty-two such mixtures with varying surfactant, co-surfactant and oil concentrations were prepared in this investigation. The percentage of surfactant, co-surfactant and oil used here in was decided on the basis of the requirements stated by Pouton (2000) for the spontaneously emulsifying systems [9-11]. Compositions were evaluated for nanoemulsion formation by diluting 50 mg of each of the 42 mixtures to 50 ml with double distilled water. Globule size of the resulting dipersions was determined by photon correlation spectroscopy (Beckman Coulter N-5, Wipro, Mumbai). Dispersions, having globule size 200 nm or below were considered desirable. The area of nanoemulsion formation (NE) was identified for the respective system in which nanoemulsions with desired globule size were obtained.

Effect of GLICLAZIDE and pH of the aqueous phase on ternary phase diagrams of the selected system

The drugs as well as pH of the vehicle have considerable influence on the phase behavior of the spontaneously emulsify. In view of this, the effect of Gliclazide and pH of the aqueous phase on

the phase behavior and area of nanoemulsion formation was studied. In these investigations, Gliclazide was dissolved in the CAE at ratio is 2:1 was treated as an oily phase and various compositions, 42 in number, were prepared in the similar fashion. The influence of the pH of aqueous phase on the phase behavior and area of nanoemulsion formation was investigated by diluting 50 mg of oily mix to 50 ml with various vehicles viz. water, buffer pH 1.2, buffer pH 3.0 and buffer pH 6.8. The mean globule size of the resulting dispersions was measured by using photon correlation spectroscopy (PCS) and the data obtained was used to identify the area of nanoemulsion formation.

Optimization of formulae

SNEDDS were optimized for following parameters:

• Drug loading.
• Amount of oily phase.

Evaluation of GLICLAZIDE loaded SNEDDS

Optimized SNEDDS were evaluated for robustness to dilution, globule size, effect of Gliclazide loading and *in-vitro* dissolution profile.

Robustness to dilution

Robustness of Gliclazide SNEDDS to dilution was studied by diluting it 50, 100 and 1000 times with various dissolution media viz. water, SGF pH 1.2 and Phosphate buffer pH 7.4 The diluted nanoemulsions were stored for 12 h and observed for any signs of phase separation or drug precipitation.

Globule size analysis

The formulation, 50 mg, was diluted to 50 ml with media like double distilled water, water, SGF pH 1.2 and Phosphate buffer pH 7.4. Visual observations were made immediately after dilution for assessment for self-nanoemulsification efficiency, appearance (transparency), phase separation, and precipitation of drug. The mean globule size and polydispersity index (P.I.) of the resulting nanoemulsions were determined by PCS [12-13]. Measurements were obtained at an angle of 90. Nanoemulsions were diluted respective vehicles to ensure that the light scattering intensity (between 6E+004 to 1E+006), was within the instrument's sensitivity range. The resultant nanoemulsions were also allowed to stand for 6 h at room temperature to assess dilution stability.

Effect of GLICLAZIDE loading

The increase or decrease in the amount of Gliclazide would influence the globule size of the resultant nanoemulsions if Gliclazide were participating at interface of nanoemulsion. In order to investigate role of Gliclazide, various formulations were prepared containing varying amount of Gliclazide from 20 to 5% (w/w). SNEDDS, 50 mg, was diluted to 50 ml with different media viz. double distilled water, water, SGF pH 1.2 and Phosphate buffer pH 7.4 and the mean globule size of resulting nanoemulsions were determined by PCS [14].

In vitro dissolution profile

SNEDDS of Gliclazide was filled in size '0' hard gelatin capsules. *In vitro* release profile of

SNEDDS was studied using USP XXIII apparatus I at 37 ± 0.50^{0}C with a rotating speed of 100 rpm in dissolution media namely, SGF pH 1.2 and Phosphate buffer pH 7.4 so as to evaluate the effect of pH on *in vitro* dissolution. During the study, 1 ml of aliquots were removed at predetermined time intervals (10, 20, 30 and 45 min) from the dissolution medium and replaced with fresh buffer [15-17]. The amount of Gliclazide released in the dissolution medium (**Table 5**) was determined by UV spectrophotometer at 226.2λ

RESULTS AND DISCUSSION

Solubility studies

Solubility studies were aimed at identifying suitable oily phase and surfactant/s for the development of Gliclazide SNEDDS. Identifying the suitable oil, surfactant/co-surfactant having maximal solubilizing potential for drug under investigation is very important to achieve optimum drug loading [9, 11]. Solubility of Gliclazide in various buffers, oily phases and 10% (w/w) surfactant solutions is presented in **Figs. 1–3**, respectively.

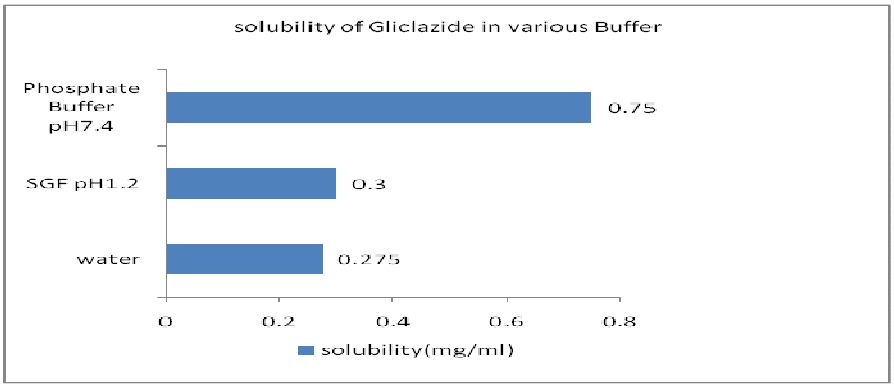

Figure 1: Solubility of GLICLAZIDE in various buffers.
Data are expressed as mean ± S.D(n=3)

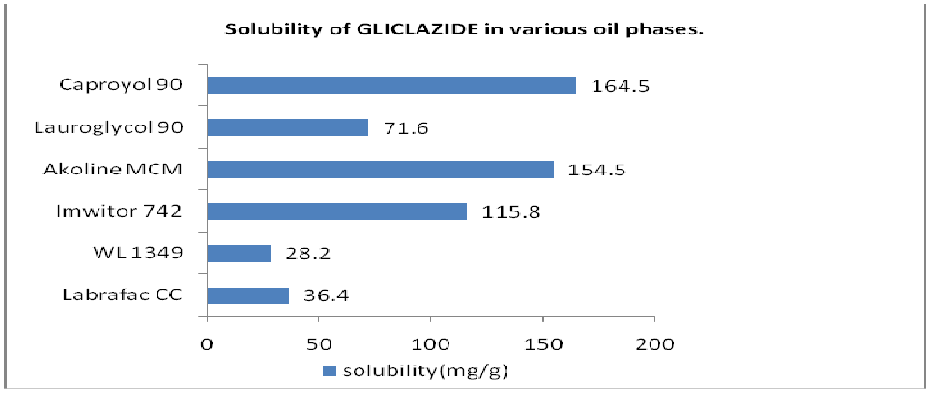

Figure 2: Solubility of GLICLAZIDE in various oil phases.
Data are expressed as mean ± S.D(n=3)

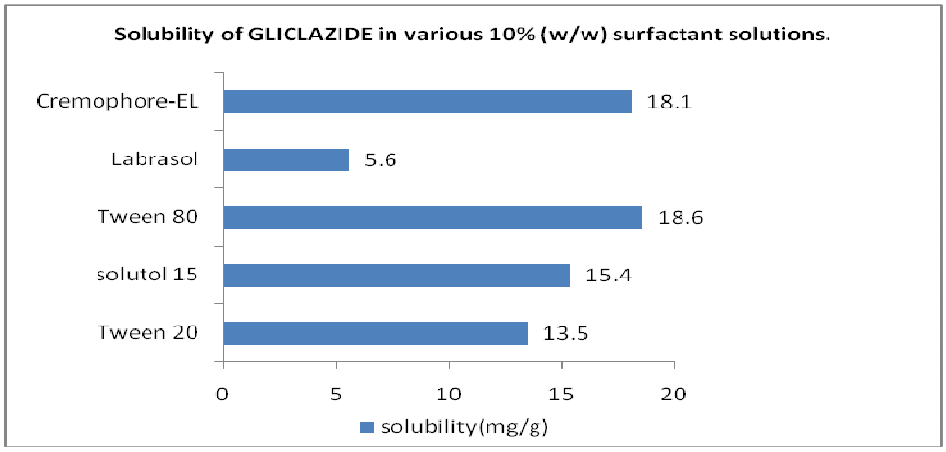

Figure 3: Solubility of GLICLAZIDE in various 10% (w/w) surfactant solutions.
Data are expressed as mean ± S.D (n=3)

Solubility studies (**Fig. 1**) clearly indicated that Gliclazide has pH dependant solubility. Amongst the various oily phases that were screened, Capryol 90 (CAE) (**Fig. 2**) could solubilize target amount of Gliclazide (40 mg) at relatively small concentration of 300 mg. The selection of surfactant or co-surfactant in the further study was governed by their emulsification efficiency rather than their ability to solubilize Gliclazide.

Screening of surfactants for emulsifying ability

The %transmittance values of various dispersions are given in **Table 1.** Emulsification studies clearly distinguished the ability of various surfactants to emulsify CAE. These studies indicated that Cr-EL and SHS-15 had very good ability to emulsify CAE. These studies indicated that Cr-EL and SHS-15 had very good ability to emulsify CAE followed by Tween 20 and Tween 80, whereas, Labrasol appeared to be poor emulsifier for CAE. Although, the HLB values of the surfactants used in the investigation were in the range of 13 to 16 except for Poloxamers and Tween 20, there was a great difference in their emulsification ability. This observation is in line with the investigations reported by Malcolmson et al. (1998) and Warisnoicharoen et al. (2000) [14, 16-17]. who concluded that microemulsification is also influenced by the structure and chain length of the surfactant. Cr-EL and SHS-15 rendered very good nanoemulsions requiring short time for nanoemulsification and were selected for further investigation.

Table 1: Emulsification efficiency of various non-ionic surfactants

Surfactant	% Transmittance
Tween 20	94.6
Tween 80	93.3
Cremphore EL	99.4
Solutol HS 15	97.9
Labrasol	59.9
Poloxamer 407	97
Poloxamer 188	65.1

Data expressed as mean (*n*= 3).

Screening of co-surfactants

The investigations clearly distinguished the ability of various co-surfactants, both hydrophilic and lipophilic, to improve the nanoemulsification of selected surfactant/s. All the cosurfactants increased the spontaneity of the nanoemulsion formation. Interestingly, all the hydrophilicco-surfactants appeared to be equivalent in improving nano-emulsification ability of Cr- EL and SHS 15. In case of lipophilic co-surfactants, good correlation was observed between the structure and chain length of co-surfactant (or molecular volume) of co-surfactant and the transmittance values of resulting dispersions. Larger the chain length or structure (or molecular volume) of the co-surfactant lesser was the transmittance value. This correlation was applicable to Ak-MCM, Imwitor 742, Lauroglycol 90, Lauroglycol FCC and Plurol oleique CC 497 (Table 2) However, Akomed E and Labrafil 1944 CS did not follow this behavior. Among Akoline MCM, Imwitor 742, Lauroglycol 90, Lauroglycol FCC and Plurol oleique CC 497, Ak-MCM, a mixture of capric/caprylic acid mono-, di- and tri- glycerides, due to its smallest molecular volume appeared to be the best co-surfactant. Imwitor 742 and Ak-MCM were almost equivalent which is attributed to similarity in their mono-, di-, and tri-glyceride proportions of capric/caprylic acids. However, Lauroglycol 90, Lauroglycol FCC were less effective as co-surfactants. This was attributed to the presence of lauric acid backbone, which is longer in chain length than capric/caprylic acid. But they were more efficient than Plurol oleique, which has oleic acid backbone, which is longer in chain length than lauric acid.

Table 2: Emulsification studies on surfactant/co-surfactant combinations

Co-surfactant	% Transmittance	
	Cremophore EL	Solutol HS 15
Transcutol	99.7	98.7
Propylene glycol	99.6	98.6
Polyethylene glycol	99.5	98.3
Labrafil 1944 CS	99.2	97.9
Plurol Dioleique CC 497	91.2	73.7
Lauroglycol FCC	98.6	85.5
Lauroglycol 90	98	63.3
Imwitor 742	99	95.5
Akoline MCM	99.2	95.1
Akomed E	99.7	99.1

Data expressed as mean (n= 3).

These observations are in line with the investigations reported by Malcolmson et al. (1998) and Warisnoicharoen et al. (2000) [14,16] Surprisingly, Akomed E, despite of its larger content of diglycerides and triglycerides of capric/caprylic acid as com- pared to Ak-MCM and Imwitor 742, appeared to be best among all lipophilic co-surfactants which can further be validated with the help of globule size analysis. Labrafil 1944 CS (PEG-8- oleate/linoleate), which has oleic and linoleic acid backbone showed superior performance over Plurol oleique, Lauroglycol FCC and Lauroglycol 90 probably due to more hydrophilicity and surfactant like properties. In conclusion,

emulsification studies gave good insight into the efficiency of various cosurfactants. Among lipophilic co-surfactants, Akomed E, Ak-MCM and Imwitor 742 exhibited superior profile with Akomed E showing the best performance. However due to its less solubilizing potential for Gliclazide. it was not used for further studies. Ak-MCM a lipophilic co-surfactants with good solubilizing potential for Gliclazide was selected and Cremophore EL, Akoline MCM-CAE and Solutol HS 15-Akoline MCM-CAE systems were developed for further studies.

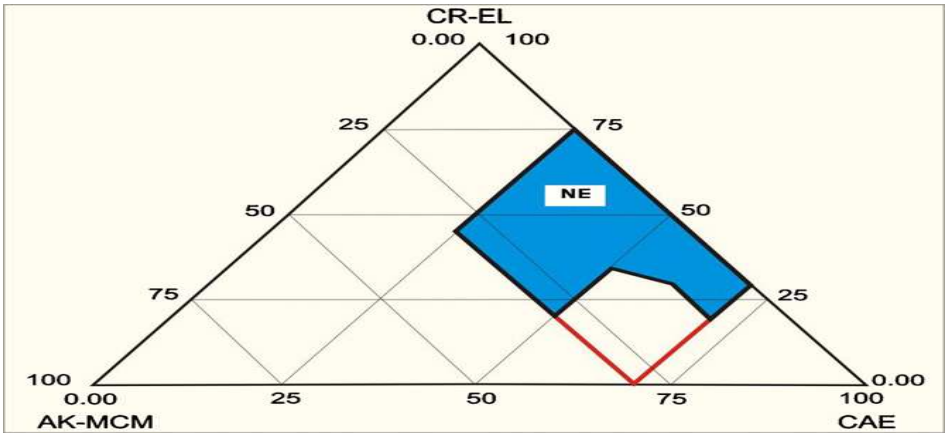

Figure 4. Ternary diagram of CR-EL, AK-MCM and Capryol 90 (CAE)

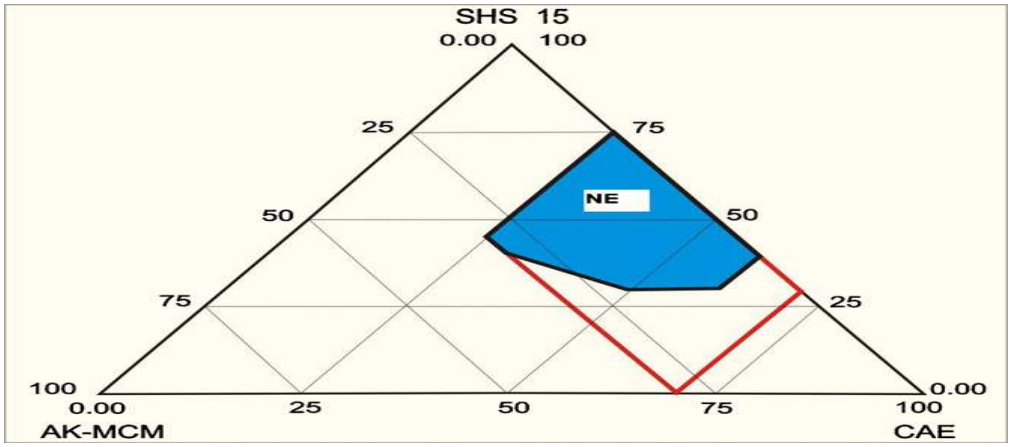

Figure 5. Ternary diagram of SHS-15, AK-MCM and Capryol 90 (CAE).

Construction of phase diagrams

The phase diagrams of Cremophore EL-Akoline MCM-CAE and Solutol HS 15-Akoline MCM-CAE systems are shown in **Figs. 4 and 5.** The outer parallelogram indicates the area, which was explored for locating nanoemulsification region. The filled region indicated with NE indicates the region in which nanoemulsions of desired size were obtained. From **Figs. 4 and 5**, it is evident that Cr-EL-Akoline MCM-CAE system has larger nanoemulsification region as compared to

Solutol HS 15-Akoline MCM-CAE system. Cremophore EL- Akoline MCM-CAE system yielded nanoemulsions for the compositions that had as high as 70% (w/w) of oily phase comprising of oil + lipophilic co-surfactant concentration, whereas, Solutol HS 15-Akoline MCM-CAE system yielded nanoemulsions for compositions having about 60% (w/w) of oily phase. These compositions had ability to solubilize various hydrophobic drugs and have potential to become plat form systems. In view of current investigation, due to larger nanoemulsion region and greater capacity for incorporation of oily phase, which is most desirable for Gliclazide, Cremophore EL-Akoline MCM-CAE system was selected for further studies.

Effect of GLICLAZIDE and pH of the aqueous phase on ternary phase diagrams of the selected system

The phase diagrams indicating effect of Gliclazide and pH of the aqueous phase on phase behavior and area of nanoemulsion existence are shown in **Figs. 6.** It was expected that Gliclazide would influence the phase behavior and the area of nanoemulsion formation as in these formulae, Gliclazide substituted one-third amount of CAE as compared to the systems without Gliclazide. Phase diagrams studies indicated that there was remarkable influence of Gliclazide and also the pH of dilution medium on the area of nanoemulsion formation of the Cremophore based system. Incorporation of Gliclazide in CAE led to a considerable reduction in the area of nanoemulsion formation of Cremophore based SNEDDS when compared to the area in **Fig. 4.** Gliclazide, due to its low aqueous solubility, is likely to participate in the nanoemulsion by orienting at the interface. The reduction in the area of nanoemulsion formation could be due to Gliclazide influenced interaction of surfactant and co-surfactant with oil.

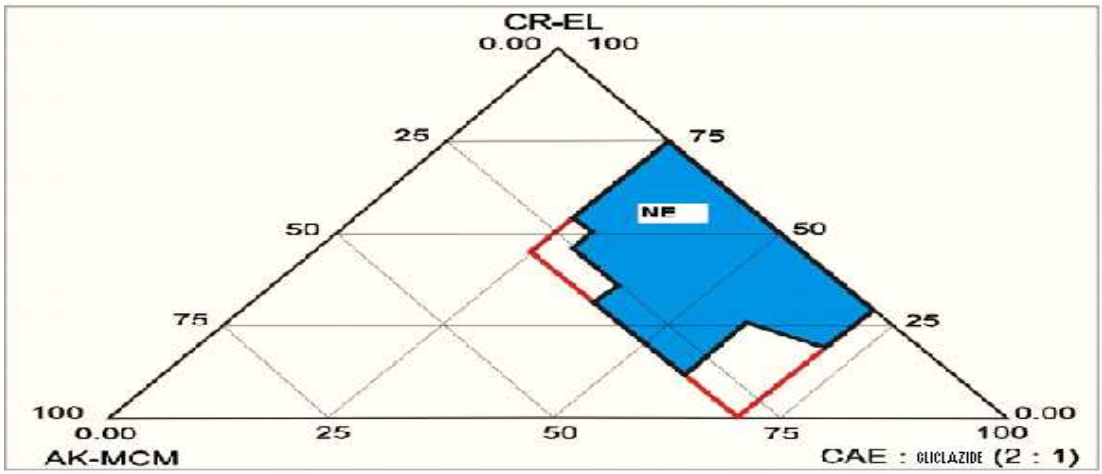

Figure 6. Pseudo-ternary diagram of CR-EL, AK-MCMand CAE +Gliclazide using pH 1.2 SGF as dilution medium.

Selection of optimized formulation

The optimized formulation was selected based on the drug loading efficiency and consistency in mean globule size at varying pH. The composition is given in **Table 3**

Table 3: Composition of optimized GLICLAZIDE SNEDDS

Ingredient	Quantity (mg/capsule)
Cremophore EL	195
Akoline MCM	65
Capryol 90	260
GLICLAZIDE	40
Total	560

Robustness to dilution

Nanoemulsions resulting from dilution of Gliclazide SNEDDS with various dissolution media were robust to all dilutions and did not show any separation even after 24 h of storage.

Globule size analysis

The mean globule size of Gliclazide SNEDDS after dilution with various dissolution media is given in **Table 4.** The Gliclazide SNEDDS showed fairly similar mean globule size within range of 140–150 nm when diluted with various dissolution media differing in pH. The time required for formation of nanoemulsions after dilution with various dissolution media was less than 1 min. The resulting nano-emulsions were translucent in appearance and they did not show any signs of phase separation and drug precipitation even after 6 h.

Table 4: Globule size and polydispersity index of Gliclazide SNEDDS at different pH conditions

Dissolution medium	Water	SGF pH1.2	Buffer pH7.4
Globule size (nm)	145.8	148.0	146.2
Polydispersity index	0.746	0.914	0.712

Globule size expressed as mean (n=3) where relative standard deviation was <10%, Data expressed as mean (n = 3).

Effect of GLICLAZIDE loading

The amount of Gliclazide influenced the globule size of nanoemulsions obtained after diluting Gliclazide SNEDDS with various dissolution media. The globule size decreased with the decrease in the % Gliclazide loading. **Fig. 7**

In vitro dissolution profile

In vitro dissolution profile of optimized Gliclazide SNEDDS in various dissolution media is given in **Table 5**. The dissolution profile of Gliclazide SNEDDS in various dissolution mediums showed that 100% of Gliclazide was released within 20 min irrespective of the pH of dissolution medium.

Table 5: *In vitro* dissolution profile of Gliclazide SNEDDS

In vitro dissolution profile of GLICLAZIDE SNEDDS

Time (min)	%Cumulative release	
	SGF pH 1.2	Phosphate Buffer pH 7.4
05	22.65 ± 0.42	23.2 ± 0.651
10	58.58 ± 0.810	60.50 ± 0.765
15	87.51 ± 0.650	87.20 ± 0.452
20	99.53 ± 1.6	100.1 ± 0.49
25	100.2 ± 0.860	101.0± 0.956

Data expressed as mean (*n*= 3).

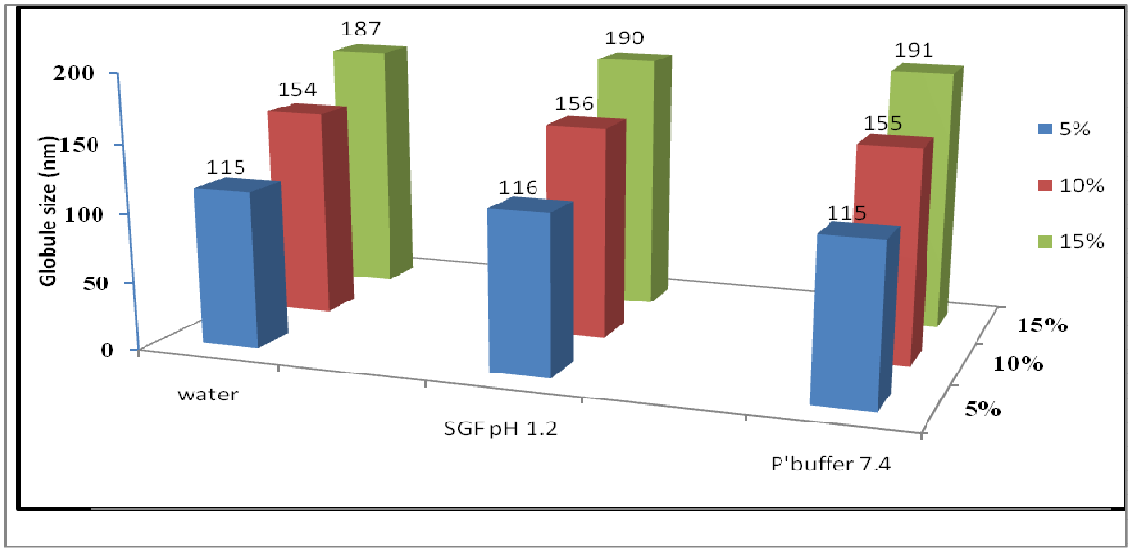

Figure.7: Effect of Gliclazide loading on mean globule size of SNEDDS.

Data are expressed as mean (n=3)

CONCLUSION

The method employed in the investigation for screening of SNEDDS excipients helped in understanding the emulsification efficiency of various surfactants for selected oily phase. It also helped in rapid screening of large pool of co-surfactants available for the peroral delivery. The potential of Ak-MCM, to act as a co-surfactant was established in the present investigation. Studies on ternary phase diagrams indicated that Gliclazide and the pH of dilution medium significantly affect the area of the nanoemulsion formation for the selected system. SNEDDS of Gliclazide exhibited rapid release independent of pH of dissolution media.

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