

# GLIBENCLAMIDE LOADED TRANSDERMAL NANOEMULSION GEL: FORMULATION AND CHARACTERIZATION

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**Abstract:** The aim of present study was to design, develop and characterize transdermal nanoemulsion gel of the poorly water soluble drug, glibenclamide (GCL). Solubility of GCL was determined in various oils, surfactants and Co-surfactants. Phase diagrams were constructed to identify nanoemulsion region using oil, surfactant and co-surfactant in aqueous environment. Formulations were evaluated for drug content, optical clarity, droplet size, zeta-potential, pH, viscosity, transmission electron microscopy (TEM) and release studies. Among the formulations prepared and evaluated, optimized formulations showed mean particle size between 9.28 nm to 13.13 nm and 10.75 to 14.26 nm after 24 hour post dilution in distilled water and phosphate buffer pH 7.4. Infrared spectroscopy and differential scanning calorimetry indicated compatibility between drug, oil, surfactant and co-surfactant. Transmission electron microscopy of these formulations confirmed the spherical shape of globules with no signs coalescence of globules and precipitation of drug. Hence nanoemulsion gel formulations of GCL owing to nanosize have the potential to enhance their absorption without interaction or incompatibility between the ingredients

**Keywords:** Nanoemulsion gel, Glibenclamide, Transmission Electron Microscopy

## 1. INTRODUCTION

A mounting number of recently discovered drug substances exhibit poor water solubility and hence low absorption after oral administration. Technology Catalysts International reported in 2002 that approximately 35-40 % of all new chemical compounds suffer from deprived aqueous solubility which leads to poor oral bioavailability, high intra and inter subject variability and lack of dose proportionality<sup>[1-3]</sup>.

Various formulation strategies are reported in the literature including the use of surfactants, cyclodextrins, nanoparticles, solid dispersions, micronization, lipids, and permeation enhancers to enhance the solubility. These strategies are successful in the selected cases and have specific advantages and limitations<sup>[4-9]</sup>. In recent years, oral bioavailability of a poorly water soluble drug has been improved by a focused study on lipid-based formulations such as microemulsions, nanoemulsions, self-emulsifying formulations, self-microemulsifying formulations, emulsions and liposomes. Most of them increase surface area of the drug to improve solubilisation behaviour, as well as permeation<sup>[3]</sup>. Amongst the lipid-based systems, nanoemulsion is a promising technology to improve the rate and extent of absorption of poorly water-soluble drugs.

Nanoemulsions are isotropic, thermodynamically stable transparent (or translucent) systems of oil, water and surfactants with a droplet size usually in the range of

10-100 nm. Their long term stability, ease of preparation and high solubilisation of drug molecules make them promising as a drug delivery tool. Recently there has been search in the exploration of nanoemulsions for transdermal delivery<sup>[10-11]</sup>. Transdermal drug administration generally refers to topical applications of agents to healthy intact skin either for localized treatment of tissues under the skin or for systemic therapy. For transdermal product, the goal of dosage design is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin<sup>[12]</sup>. Transdermal drug delivery has main advantages over the oral route of administration such as improving patient compliance in long term therapy, by-passing first pass metabolism, sustaining drug delivery, maintaining a constant and prolonged drug level in plasma, minimizing the inter and intra-patient variability, and making it possible to interrupt or terminate when necessary<sup>[13-14]</sup>.

Glibenclamide (GCL) is a second-generation sulfonylurea frequently used as oral hypoglycaemic agent for the treatment of non insulin-dependent (type II) diabetes mellitus; but has been associated with severe and sometimes fatal hypoglycemia and gastric disturbances like nausea, vomiting, heartburn, and increased appetite after oral therapy<sup>[15]</sup>. The aqueous solubility of GCL is low and highly pH-dependent in the physiological range because of its pKa value of 5.3. Low aqueous solubility gives rise to unsatisfactory dissolution profile leading to

potential problem of poor bioavailability and bioequivalence of drug dosage form. Furthermore, micronized GCL has shown better absorption than nonmicronized form<sup>[7]</sup>. Hence, the absorption of drug can be enhanced using transdermal nanoemulsion gel technique. Transdermal route will eliminate the side effects associated with oral administration of GCL.

The aim of this study was to design, develop and evaluate transdermal nanoemulsion gel of GCL using non irritant, pharmaceutically acceptable ingredients without additional permeation enhancers (components of nanoemulsions themselves acts as a permeation enhancers). Lipid, surfactant, co-surfactant, oleylamine, carbopol 940, and triethanolamine were incorporated to design and develop a suitable transdermal nanoemulsion gel of GCL. Transdermal nanoemulsion gel of GCL was formulated in absence and presence of oleylamine, a positive charge inducer. With regard to dermal delivery the use of positively charged carriers is advantageous, because the positive charge promotes an intensive adsorption to the negatively charged skin. This increases the retention time and thus the bioavailability<sup>[16]</sup>.

## 2. MATERIAL AND METHODS

Glibenclamide (GCL) was a generous gift from USV (Mumbai, India). Acconon<sup>®</sup> 200 E6 (ACC 200 E6), Acconon<sup>®</sup> E (ACC E), Capmul MCM<sup>®</sup> (CPL), Capmul MCM C8<sup>®</sup> (CPL C8), Captex<sup>®</sup> 200P (CPX) and Captex<sup>®</sup> 355 (CPX 355) were kindly donated by Abitec Corporation (Janesville, USA). Peceol<sup>®</sup> (PCL), Labrasol<sup>®</sup> (LBS), Labrafac<sup>®</sup> CC (LBF) and Lauroglycol<sup>®</sup> (LRG) were supplied by Colorcon (Goa, India). Miglyol 829 (MGL), and lipoxol (LPX) were gifts from Sasol (Witten, Germany). Cremophor RH 40 (Cr RH 40) and Cremophor EL (Cr EL) were generous gifts from BASF (Ludwigshafen, Germany). Corn oil (CN) and Oleylamine (OA) was procured from Fluka (Steinheim, the Netherlands). Castor oil (CT), Span 20 (SP 20), Span 80 (SP 80), Tween 20 (T-20), Tween 80 (T-80), Propylene glycol (PG), and Tetraethylene glycol (TEG) were purchased from Merck Ltd. (Mumbai, India). All solvents and reagents were of analytical grade. All other chemicals were of AnalaR grade.

### 2.1 Screening of oils, surfactants and cosurfactants

One gram of each excipient was weighed in capped vials, and an excess amount of GCL (200 mg) was added to it. The mixture was subjected to vortex mixing for 5-10 minutes. The mixtures were kept in water bath shaker (Remi, India) and subjected to shaking at 50 °C for 24 hrs. The equilibrated samples were filtered using Whatman paper No 1 (Whatman, England) to separate the

undissolved drug. The filtrate was appropriately diluted with methanol. The concentration of GCL was quantified by measuring the absorbance at 300 nm using UV-Visible spectrophotometer (UV-1800 Pharmspec, Shimadzu, Japan). The content of GCL was calculated from the standard curve.

### 2.2 Phase diagram studies

Ternary phase diagrams were constructed by diluting the homogenous liquid mixture prepared at varying mass ratios of oil, surfactant and cosurfactant with water to definite volume (100 ml), at ambient temperature. Diluted samples were stirred vigorously for sufficient length of time for homogenization and the end product was visually monitored against dark background by illuminating the samples with white light, after 24 hrs post dilution. Total ninety nine formulations were developed, keeping the amount of GCL constant to identify the nanoemulsion region. After identification of the nanoemulsion region in the phase diagram, formulations were selected at desired component ratios.

### 2.3 Preparation of true nanoemulsion

A series of true nanoemulsions were prepared using varying mass ratios of oil, surfactant and cosurfactant (Table 1). In all the formulations, the level of GCL (0.012 gm) was constant. GCL was completely dissolved in the weighed amount of molten mixture of ACC 200E6, Cr RH40 and T-80. The homogenous mixture was diluted with 100 ml distilled water with stirring to get nanoemulsion.

### 2.4 Dispersion stability studies

To overcome the problems of metastable nanoemulsion formulations, dispersion stability tests were performed. Selected nanoemulsion formulations were centrifuged at 3500 rpm for 30 min. The formulations that showed no phase separation were taken for heating and cooling cycle. Six cycles between the refrigerator temperature (4 °C) and 45 °C with storage at each temperature for 48 h were done. Those formulations which were stable at these temperatures were subjected to freeze-thaw cycle test. Three freeze-thaw cycles were done for the formulations between -10°C and +25°C. The formulations that survived dispersion stability tests were selected for further studies.

### 2.5 Evaluation of true nanoemulsions

#### 2.5.1 Drug Content

Assay of weighed amount of isotropic mixture of oil, surfactant and cosurfactant were carried out to determine the GCL content. The weighed samples were

dissolved in 10 ml methanol and stirred by vortex mixing. The solutions were filtered, using whattman filter paper. The content was estimated spectrophotometrically (UV-1800, Shimadzu, Japan) at 300 nm using standard curve.

### 2.5.2 Spectroscopic Absorbance

The optical clarity of nanoemulsion formulations (F1-F6) was measured spectroscopically upon dilution. Each formulation containing 12 mg of GCL was diluted with 100 ml of phosphate buffer of pH 7.4 and distilled water respectively in glass beaker. Absorbance of each dispersion at 0, 8 and 24 hrs post dilutions was measured spectrophotometrically (UV-1800 Pharmaspec) at 400 nm.

### 2.5.3 Droplet Size Analysis

Formulations (F<sub>1</sub> to F<sub>6</sub>) each of 1 gm were diluted with 500 ml of phosphate buffer of pH 7.4 and distilled water in volumetric flask. The volumetric flask was inverted twice to ensure complete dispersion of the formulation. After ensuring complete dispersion of the formulation the droplet size of resultant nanoemulsion was determined by photon correlation spectroscopy that analyze the fluctuation in light scattering due to the brownian motion of the droplets as function of time using

a Zetasizer Nano Series (Malvern Instruments). Light scattering was monitored at 25°C at 90° angle.

### 2.5.4 (ζ)-Zeta potential measurement

Zeta potential of the formulations (F1 to F6) with oleylamine and without oleylamine was measured by using Malvern Zetasizer (Malvern Instruments) equipped with a 4.0 mW He-Ne red laser (633 nm). Zetasizer measures the potential ranged from -120 to 120 V. For measurement of zeta potential 1 gm of each formulation were diluted with milli Q water (100 ml).

### 2.6 Preparation of nanoemulsion gel

Glibenclamide gel was prepared by dispersing 1% w/w of Carbopol-940 in sufficient quantity of distilled water. This dispersion was kept in dark for 24 h for complete swelling of carbopol-940. 12 mg of GCL was dissolved in specified quantities of ACC 200 E6, Cr RH 40 and T-80. GCL solution was added slowly to carbopol-940 dispersion. 0.5% of triethanolamine (TEA) was added in the mixture to neutralize carbopol-940. Then distilled water added to get the final preparation of 5 gm (F1G-F6G, Table III).

TABLE III  
Composition of nanoemulsion gel formulations

Ingredients (gm)	F1G	F2G	F3G	F4G	F5G	F6G
Glibenclamide	0.012	0.012	0.102	0.012	0.012	0.012
Carbopol 940	0.05	0.05	0.05	0.05	0.05	0.05
Acconon 200 E6	0.10	0.10	0.10	0.30	0.30	0.30
Cremophore RH 40	0.72	0.49	0.18	0.56	0.35	0.14
Tween 80	0.18	0.49	0.72	0.14	0.35	0.56
Oleylamine	0.05	0.05	0.05	0.05	0.05	0.05
Triethanolamine	0.025	0.025	0.025	0.025	0.025	0.025
DistilledWater	5.0	5.0	5.0	5.0	5.0	5.0

### 2.7 Evaluation of nanoemulsion gel

#### 2.7.1 Drug Content

Assay of weighed amount of nanoemulsion gel were carried out to determine the drug content. The samples were suitably diluted in methanol followed by vortex mixing. The filtered solutions were estimated for GCL content spectrophotometrically (UV-1800, Shimadzu, Japan) at 300 nm.

#### 2.7.2 pH Measurements

The pH of 10% w/v suspension of nanoemulsion gel in distilled water was determined using μ pH meter 361.

#### 2.7.3 Viscosity Determination

The viscosity of the formulations (0.5g) was determined as such without dilution using a Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, USA) using spindle #CPE40 at 25±3°C.

### 2.8 Transmission electron microscopy (TEM)

Morphological characterization of true nanoemulsion and nanoemulsion gel were investigated using transmission electron microscopy (TEM; Tecnail12, 120 KV, FEI Company, Eindhoven, the Netherlands). Selected nanoemulsion formulations (1gm) were diluted

with 500 ml of phosphate buffer of pH 7.4. A drop of emulsion was spread on a copper grid coated with carbon film and excess droplets were instantly removed using a filter paper. After a while, a drop of 2% (w/v) of phosphotungstic acid solution was dripped on the copper grid for about 60 seconds and excess solution was removed. Then the grid was dried in the air at room temperature before loading in the microscope.

## 2.9 In vitro dissolution studies

Cylindrical glass tubes open at both ends with an exposed surface area of 4.52 Cm<sup>2</sup> were used as diffusion cell. A dialysis membrane, (Hi Media, Mumbai) cut to suitable size was allowed to hydrate in distilled water for 24 hours. Dialysis membrane was fixed to one end of the cylinder with a rubber band.

Weighed amount of gel (~ 5 mg of GCL) was spread over the dialysis membrane. Precautions were taken to ensure uniform thickness of gel over the membrane and to remove any air bubbles between the membrane and the receptor media. The cell was immersed in a beaker containing 40 ml of receptor media maintained at 37±0.5 °C. Samples were withdrawn at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 hours. The withdrawn sample was replenished with 2 ml of fresh media. The withdrawn samples were analysed for GCL content by measuring the absorbance at 300 nm using UV-visible spectrophotometer (UV-1800 Pharmaspec). Three such determinations were carried out for each formulation. The in vitro release profiles namely, cumulative per cent drug release, release half life (t<sub>50%</sub>) and percent dissolution efficiency (% DE) were determined.

## 2.10 Compatibility study

### 2.10.1 Fourier transform infrared spectroscopic studies

An FTIR-8400S spectrophotometer (Shimadzu, Japan) equipped with attenuated total reflectance (ATR) accessory and DRS-KBR disc (Diffuse reflectance spectroscopy) was used to obtain the infra red spectra of pure drug, ACC 200E6, Cr RH40, T-80, physical admixtures of the drug with the individual excipients (in 1:2 ratio) and their co-melt (in 1:2 ratio). All the samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture. For

each the spectrum, 32 scans were obtained at a resolution of 4 cm<sup>-1</sup> from a frequency range of 4000-600 cm<sup>-1</sup>.

### 2.10.2 Differential Scanning Calorimetry (DSC) Analysis

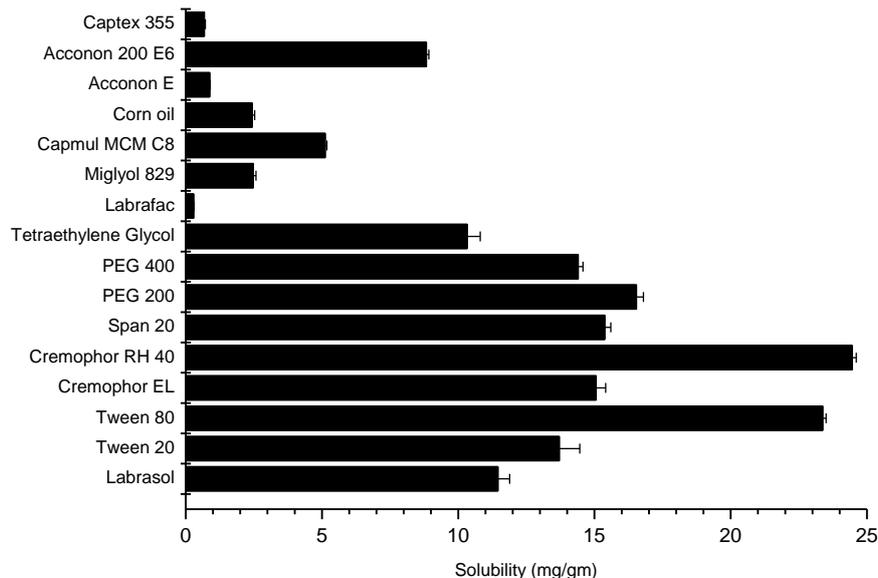
Thermal analysis was carried out using differential scanning calorimeter (DSC) (Q10; TA instruments, Waters Inc., New Castle, DE, USA) with a liquid nitrogen cooling accessory. The analysis was performed under purge of dry nitrogen gas (50 cc/min). High purity indium was used to calibrate the heat flow and heat capacity of the instruments. Sample (5-10 mg) placed in flat bottom aluminium pan was firmly crimped with lid to provide an adequate seal. Sample was heated from ambient temperature to 200 °C at preprogrammed heating rate of 5 °C/min. All samples, namely, glibenclamide (GCL), excipients (ACC 200E6, Cr RH40, and T-80), Physical mixtures (1:2, drug excipients) and Co-melt mixtures (1:2, drug excipients) were analysed in a similar manner.

## 3. RESULTS AND DISCUSSION

Nanoemulsion formulations of GCL were prepared using varying ratios of oil, surfactant and cosurfactant containing fixed amount of GCL and OA. Among the various formulations made, nine formulations (F1-F9) were selected on the basis of various assessed parameters.

### 3.1 Screening of oils, surfactants and cosurfactants

A spectrophotometric method was developed for the estimation of GCL in methanol and its  $\lambda_{\max}$  was found to be 300 nm. The nanoemulsion formulation should be clear, transparent, monophasic liquid at ambient temperature and should have good solvent properties to allow presentation of the drug in solution. The solubility of GCL in various oils, surfactants and cosurfactants are presented in Figure 1. Upon scanning the  $\lambda_{\max}$  of GCL in presence of various oils, surfactants and cosurfactants, it was observed that the  $\lambda_{\max}$  of GCL was retained. It can be inferred that selected oil, surfactant and cosurfactant will not interfere with the developed analytical method of the drug. Furthermore, the results confirm that there is compatibility between the drug and oil, surfactant and cosurfactant used in this study.



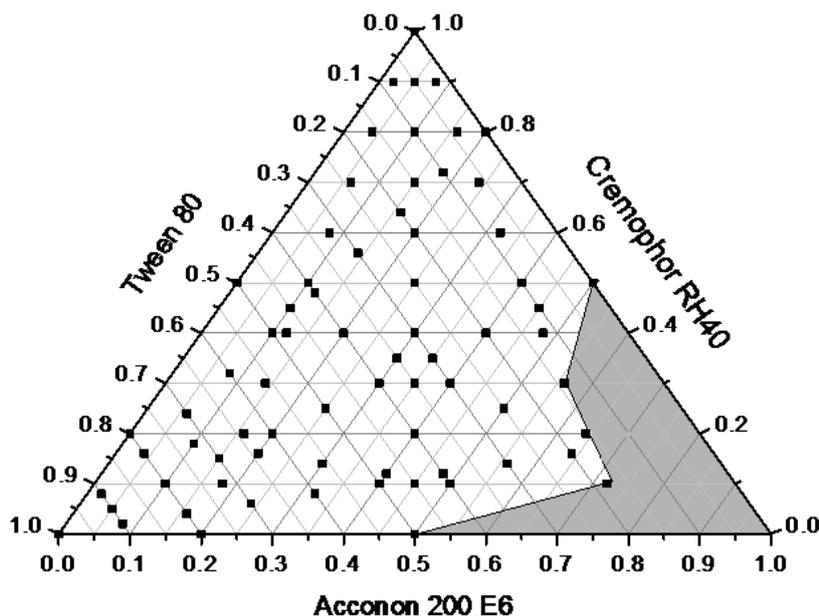
**Fig.1.** Solubility profile of Glibenclamide in various excipients (n=3).

Among the various oils screened, the maximum solubility of GCL was found in ACC 200 E6 and was selected as oil. GCL also showed good solubility in Cr RH40 and T-80 and was selected as surfactant and co-surfactant respectively.

### 3.2 Phase diagram studies

The relationship between the phase behaviour of a mixture and its composition can be captured with the aid

of a phase diagram. Ternary phase diagram were constructed for different mass ratios of oil, surfactant and cosurfactant. Care was taken to ensure that observations were not made on metastable systems; although the free energy required to for an emulsion is very low. After identification of the microemulsion region (clear and transparent area) in the phase diagram (Figure 2), nine formulations were selected at desired component ratios, keeping the concentration of the drug constant.



**Fig. 2.** Ternary phase diagram of ACC 200 E6, Cr RH40 and T 80. Clear area represents monophasic region shaded area represents biphasic zone.

### 3.3 Dispersion stability studies

Nanoemulsions are thermodynamically and physically stable systems and are formed at particular

concentrations of oil, surfactant and water making them stable to phase separation, creaming or cracking. It is the thermostability that differentiates nanoemulsions from

emulsions with kinetic stability and eventually phase separation. Thus, the formulations were tested for their physical (dispersion) stability by using centrifugation, heating-cooling cycle and freeze-thaw cycle. Only those formulations which survived dispersion stability tests

were selected for further study. Formulations F7, F8 and F9 become turbid during heating-cooling cycle which indicates that these formulations were unstable and screened out for further study (Table 1). F1 to F6 were evaluated further.

TABLE I

Composition of nanoemulsion formulations

Code	GCL (gm)	ACC 200 E6 (gm)	Cr RH40 (gm)	T-80 (gm)	Cr RH40: T-80	OA (gm)
F1	0.012	0.10	0.72	0.18	4:1	0.01
F2	0.012	0.10	0.495	0.495	1:1	0.01
F3	0.012	0.10	0.18	0.72	1:4	0.01
F4	0.012	0.30	0.56	0.14	4:1	0.01
F5	0.012	0.30	0.35	0.35	1:1	0.01
F6	0.012	0.30	0.14	0.56	1:4	0.01
F7	0.012	0.50	0.40	0.10	4:1	0.01
F8	0.012	0.50	0.25	0.25	1:1	0.01
F9	0.012	0.50	0.10	0.40	1:4	0.01

### 3.4 Evaluation of true nanoemulsion

#### 3.4.1 Drug Content

Irrespective of difference in composition the drug

content of formulation F1 to F6 was found in range of 99.36- 100.56% indicating uniform distribution of GCL in formulation (Table II).

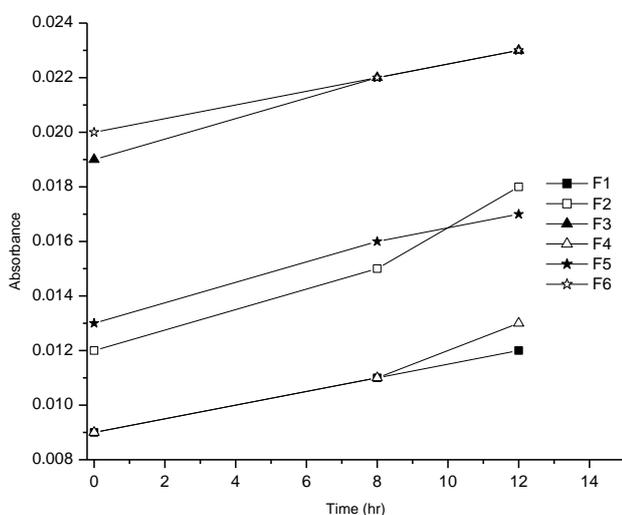
TABLE II

Evaluation of true nanoemulsions

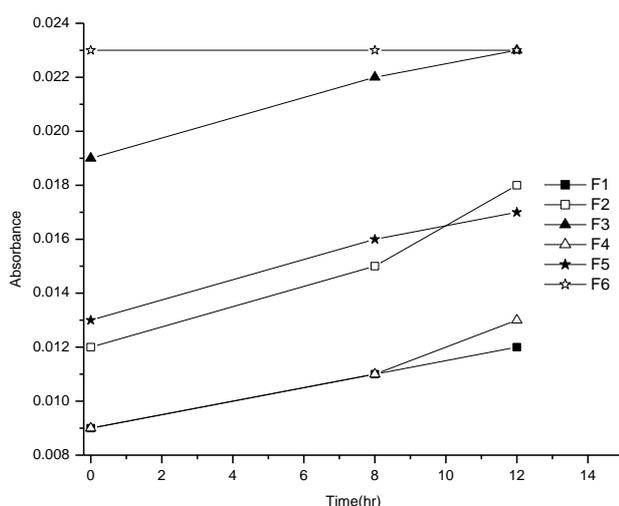
Code	Drug Content	Mean Particle Size (nm)				Zeta Potential (mV)	
		Distilled Water		PB (pH 7.4)		Without OA	With OA
		0 hours	24 hours	0 hours	24 hours		
F1	99.73 (0.46)	11.60 (0.45)	12.65 (0.15)	13.58 (0.01)	13.89 (0.18)	5.30 (1.30)	30 (0.28)
F2	100.56 (1.33)	10.08 (0.58)	11.47 (0.77)	11.46 (0.23)	12.56 (0.18)	5.26 (0.40)	25 (0.49)
F3	99.36 (1.42)	9.25 (0.13)	9.28 (0.33)	10.63 (0.23)	10.98 (0.40)	-10.75 (0.78)	25 (0.54)
F4	100.12 (1.32)	11.68 (0.32)	12.70 (0.94)	13.19 (0.08)	14.26 (0.18)	1.00 (0.42)	80 (0.85)
F5	99.81 (1.03)	11.33 (0.05)	13.13 (0.30)	11.26 (0.59)	12.82 (0.24)	3.19 (1.29)	15 (0.07)
F6	100.32 (1.94)	9.60 (0.14)	10.18 (0.56)	10.43 (0.16)	10.75 (0.06)	4.35 (0.34)	90 (0.08)

### 3.4.2 Spectroscopic Absorbance

Lower absorbance should be obtained with optically clear solutions because cloudier solution will scatter more of incident radiation, resulting in higher absorbance. Aqueous dispersions with small absorbance are optically clear and oil droplets are thought to be in a state of finer dispersions. To assess the optical clarity quantitatively, UV-VIS spectrophotometer was used to measure the amount of light of a given wavelength transmitted by the solution. Absorbances of formulations F1-F6 upon dilution with distilled water and PB of pH 7.4 at different time intervals are presented in Figure 3 and Figure 4. The result indicated that all the formulations F1-F6 were well stable till 24 hrs as their absorbance values didn't changed at the end of 24 hrs.



**Fig. 3.** Plot of absorbance versus time for formulations F1-F6 post-dilution with water.



**Fig. 4.** Plot of absorbance versus time for formulations F1-F6 post-dilution with PB of pH 7.4

### 3.4.3 Photon correlation spectroscopic studies

Nanoemulsions are characterized by the droplet size in nanometer size range; the droplet size of the emulsion is a crucial factor because it determines the rate and extent of drug release as well as drug absorption. Also, it has been reported that the smaller droplet size of the emulsion may lead to more rapid absorption and improve the bioavailability. Therefore droplet size analysis was performed to see whether the resultant emulsions are indeed nanoemulsions. Monitoring of change in the size distribution can provide valuable information for optimizing the formulation. Irrespective of the different ratios of oil, surfactant and co-surfactants, no apparent change in mean particle size was observed in different dilution media namely distilled water and phosphate buffer of pH 7.4 (Table II). Moreover, no significant increase in mean particle size was observed even after 24 hr post-dilution in different dilution media for the formulation F1-F6.

### 3.4.4 ( $\zeta$ )-Zeta Potential Determination

Many physiological studies have proved that apical potential of absorptive cells, as well as that of all other cells in the body, is negatively charged with respect to the epidermal cells<sup>[5]</sup>. A nanoemulsion which results in the positively charged dispersed oil droplets upon dilutions with an aqueous phase, leads to adhesion to the epidermal cell. Zeta potentials of nanoemulsion formulation with and without OA are tabulated in Table II. Data reveals that zeta potential ranges from -6.26 to -14.35 mV for the formulation F1-F6 without OA. The emulsion droplets of these formulations possess negative zeta potential because of the presence of free fatty acids. However in the presence of OA, as a positive charge inducer, all formulations acquire positive zeta potential, which varied between +15.90 and +24.80 mV, suggesting increased adhesion of the droplets to the cell surface because of electrostatic attraction. Similar observations were made by Singh et al, 2010<sup>[7-9]</sup>.

## 3.5 Evaluation of nanoemulsion gel

### 3.5.1 Drug content

Drug content of transdermal nanoemulsion gel (F1-F6) was in the range 99.12- 100.32 % (Table IV) indicating uniform distribution of GCL in formulation. Further it can be inferred that gelling process didn't affect the uniform distribution of GCL.

TABLE IV  
Evaluation of nanoemulsions gel formulation

Code	Drug Content	pH	Viscosity (mPa)	t <sub>50%</sub> (hour)	% DE
F1G	99.64 (0.43)	5.48 (0.015)	745.14 (2.318)	6.44	45.78
F2G	100.32 (1.24)	5.40 (0.021)	721.13 (2.237)	5.24	48.82
F3G	99.16 (1.38)	5.18 (0.010)	563.27 (1.879)	4.21	51.57
F4G	99.12 (0.86)	5.52 (0.010)	610.19 (2.023)	3.36	54.05
F5G	99.12 (0.86)	5.38 (0.020)	570.89 (2.011)	2.86	56.29
F6G	100.24 (1.94)	5.20 (0.015)	520.58 (1.660)	2.43	59.33

### 3.5.2 pH Measurements

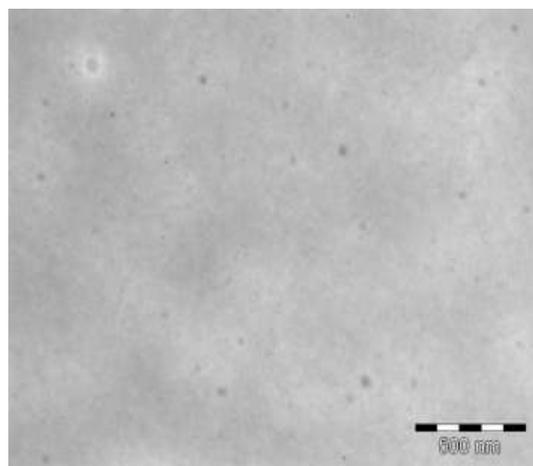
pH of 10% w/v of nanoemulsion gel ranged between 5.18 to 5.48 (Table IV). The result indicates that pH of all the nanoemulsion gel (F1-F6) is in close approximation to the skin pH (5.5-6.0), inferring the compatibility of formulation with skin.

### 3.5.3 Viscosity Determination

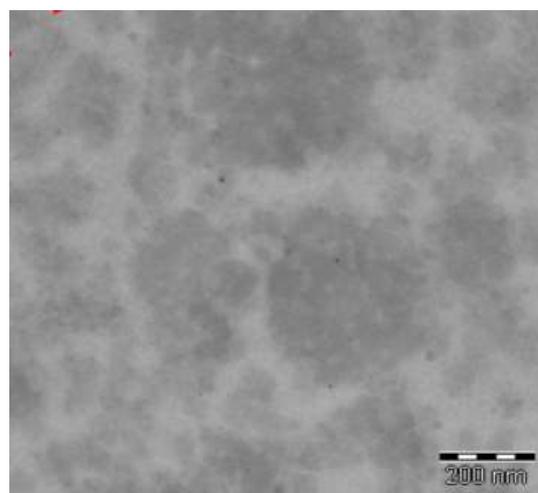
The viscosity of the nanoemulsion gel formulations (F1G-F6G) was determined as such without dilution and tabulated in Table 4. Data indicates that at fixed levels of ACC 200 E6, the viscosity decreases from 745.14 to 563.27 as the concentration of Cr RH40 decreases (Table IV). Further, it was observed that as the concentration of ACC 200E6 increases, the viscosity decreases.

### 3.6 Transmission electron microscopy

TEM was used to explore the structure and morphology of true nanoemulsion (F6) as well as nanoemulsion gel (F6G) upon dilution with distilled water. TEM images of true nanoemulsion and nanoemulsion gel are depicted in Figure 5 and Figure 6 respectively. Figure 5 clearly indicates the spherical nature of true nanoemulsion with no sign of coalescence. Furthermore, no signs of drug precipitation were observed inferring the stable nature of nanoemulsion. Figure 6 depicts the presence of nanoemulsions which are embedded in gel network of carbopol 940. This study clearly indicates that the solubility and droplet size didn't changed while incorporating in gel.



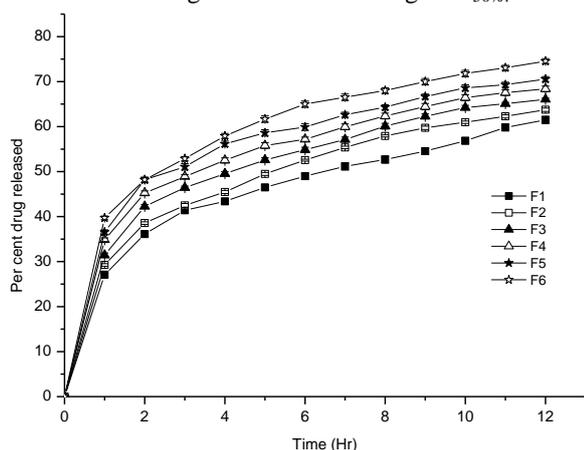
**Fig 5.** TEM image of true nanoemulsion (F6).



**Fig 6.** TEM image of nanoemulsion gel (F6G).

### 3.7 In Vitro release studies

In vitro release studies were performed to compare the release of drug from six nanoemulsion gel formulations (F1G-F6G), and their profiles are depicted in Figure 7. Furthermore, release was also characterized by  $t_{50\%}$  (dissolution half life) and percent DE (Table 4). Pharmacopoeias very frequently use these parameters as an acceptance limit of the dissolution test. Under this pretext, an ideal formulation should be optimized on the basis of maximizing DE and minimizing the  $t_{50\%}$ .



**Fig. 7.** Release profile of Glibenclamide nanoemulsion gel, n=3.

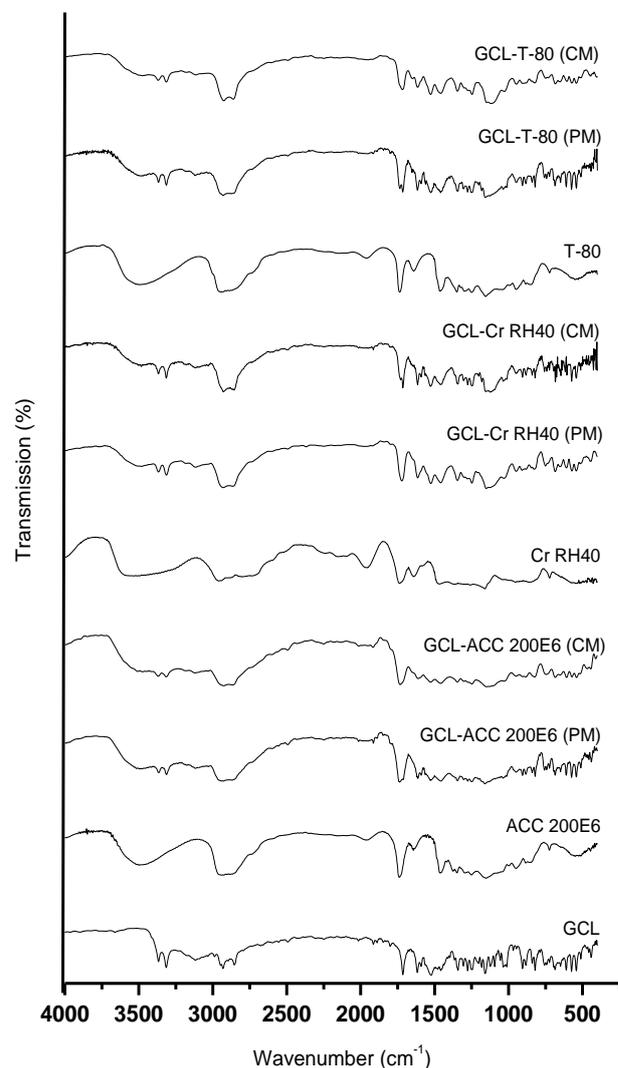
DE of the nanoemulsion gel (F1G-F6G) varied within 45.78- 59.33%, whereas  $t_{50\%}$  of these formulations varied between 2.43 to 6.44 hr. It was found that at fixed level of ACC 200E6 (F1G-F3G),  $t_{50\%}$  decreases from 6.44 to 4.21 hr, while DE values increases from 45.78 to 51.57 % (Table 4). This might be because of decrease in viscosity from F1 to F3 (Table 4). Similar trend was observed in F4G-F6G possibly because of decreasing consistency in these transdermal nanoemulsion gels.

### 3.8 Compatibility studies

Apart from physical characteristics, compatibility between drug and excipient is a factor in determining the effectiveness of delivery system. Herein to consider compatibility between drug and excipient, we refer to solubility and/or interaction with no alteration in the chemical nature of the drug or the excipient. Because each drug has its own characteristic chemical and physical properties, no delivery prepared from a particular excipient will serve as a universal carrier for all the drugs [17]. The possible drug-excipient interaction was studied by FTIR and DSC analysis of pure drug, pure excipient, and their PMs and CMs.

#### 3.8.1 Fourier transform infrared spectroscopic studies

To characterize possible interactions between the drug and excipients, infrared spectra was recorded. IR spectra of glibenclamide, individual excipients, physical mixtures (PMs) and co-melts (CMs) of the drug with individual excipients are given in Figure 8. The spectral data shown in the Figure 8 showed the retention of the characteristic absorption of the drug in the 1:2 physical mixtures (PMs) and co-melts (CMs) with each individual excipient. FTIR spectrum of pure GCL showed characteristic amide peaks at 3367.82, 3315.74 and 1716.70  $\text{cm}^{-1}$  urea carbonyl stretching (urea N-H stretching) vibrations at 1618.33 and 1525.74  $\text{cm}^{-1}$ ;  $\text{SO}_2$  stretching vibrations at 1161.19 and 1342.50  $\text{cm}^{-1}$ .



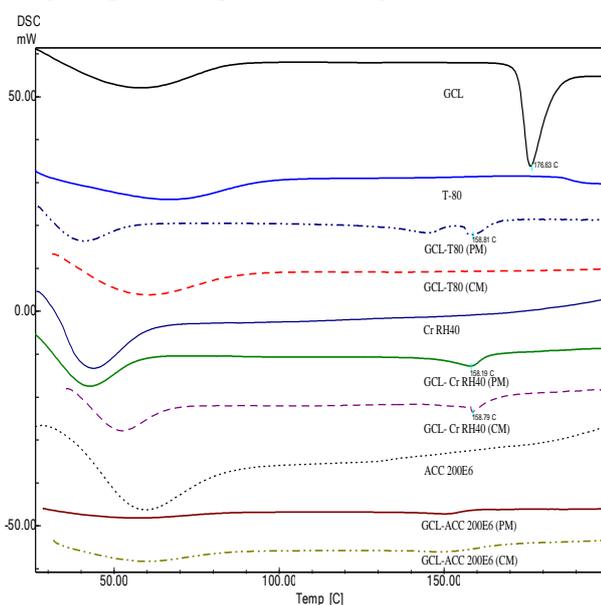
**Fig. 8** FTIR spectra of pure Glibenclamide, Acconnon 200E6, Cremophor RH40, Tween 80, physical mixtures and co-melts of glibenclamide with various excipients at 1:2 ratio.

These characteristic peaks of glibenclamide appearing at the above wavenumbers are also observed in the spectra of PMs and CMs, indicating retention of chemical identity

of glibenclamide. However, intensity of peaks corresponding to the drug were sometimes reduced or the peaks were broadened in PMs and CMs, possibly due to the mixing or loss of crystallinity. The FTIR spectrum data confirms that all the excipients do not alter the performance characteristic of the drug indicating their compatibility.

### 3.8.2 Differential scanning calorimetric Studies

The DSC was used to detect formulation incompatibilities resulting from drug-excipient interactions. The DSC thermograms of the pure glibenclamide, excipients (ACC 200 E6, Cr RH40, and T-80), their physical mixture and co-melt at the ratio of 1:2 (drug:excipient) are presented in Figure 9.



**Fig. 9** DSC thermogram of GCL, ACC 200E6, Cr RH40, T80, their PM and CM at 1:2 ratio

The sharp endothermic peak of GCL appeared at 176.63<sup>o</sup>C that corresponds to the drug melting point because of its crystalline nature. In thermograms of PM of GCL-T-80, the endothermic peak has shifted to lower melting point (158.81 <sup>o</sup>C). However in case of CM of GCL-T-80, no endothermic peak corresponding to GCL was present, possibly due to progressive dissolution of GCL during DSC measurement. Similar observations were observed in case of PM and CM of GCL-ACC 200E6. Peaks corresponding to PM and CM of GCL-Cr RH40, have shifted towards lower temperature (158.19 <sup>o</sup>C and 158.79 <sup>o</sup>C, respectively) because of melting point depression. Similar observation were reported by Singh et al, 2010 [7].

## 4. CONCLUSIONS

Among the six formulations (F1G-F6G) prepared and evaluated, F6G was found best for GCL loaded transdermal nanoemulsion gel. FTIR and DSC studies indicated no interaction between drug, oil, surfactant and cosurfactants. Formulation F6 showed mean particle size between 10.18 and 10.75 nm after 24 hours postdilution in distilled water and phosphate buffer of pH 7.4. TEM of nanoemulsion formulations confirmed the spherical shape of globules with no signs of coalescence of globules and precipitation of drug. DE and t<sub>50%</sub> of F6G was 59.33% and 2.43 respectively. The results of this study indicates that nanoemulsion gel formulations of GCL owing to nanosize have the potential to enhance their absorption without interaction or incompatibility between the ingredients. Furthermore, the selected formulations may be evaluated for their pharmacokinetic and pharmacodynamic profile in humans to the best of their advantages.

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