International Journal of Drug Delivery 5 (2013) 270-283

http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Dilution Phenomenon in Mixed Surfactant based Self Micro Emulsifying Formulations of Ginger Oleoresin: Ex *Vivo* and *In Vivo* Performances

Abhinav Garg[1], Anoop Kumar[2], Kanchan Kohli[*1]

***Corresponding author:**

Kanchan Kohli

[1]Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India.

[2]Department of Pharmaceutical Technology, Meerut Institute of Engineering & Technology, NH-58, Meerut Baghpat Bye Pass Road, Meerut-201205, Uttar Pradesh, India.

Abstract

Aqueous solubilization of Ginger Oleoresin (GO) in *pseudo* self micro emulsifying carrier and its influence on ex-vivo intestinal permeation and in-vivo performances was investigated. GO pre-concentrates was prepared using surfactants, Tween 80, Tween 20 and/or 1:1 mixture with a co-surfactant propylene glycol at S/Cos ratio 1:1. Aqueous dilutable region of GO in single or mixed surfactant systems was located from ternary phase diagram drawn between ternary components consisted of surfactant/co-surfactant ratio at 1:1, GO and aqueous phase. Various microstructures were characterized across the dilution line using conductometric and rheological method. Three formulations were selected across the dilution line from mixed surfactants phase diagram as microemulsion area was found to be larger in mixed surfactants over single surfactant based ternary system. GO SMEDDS formulations were physically characterized for refractive index, pH, droplet size and stability assessment. The permeability of GO in diluted pre-concentrate was determined across ex vivo rat intestinal method. Two fold enhancement (p<0.01) in intestinal permeability of GO was obtained from SMEDDS formulation when diluted upto 9.0ml in comparison to under diluted (2 ml) or over-diluted (25 ml) and control formulation (GO in Tween 80). These findings strongly suggested that SMEDDS diluted upto 9ml behave like a pseudo self emulsifying carrier which inherently had microemulsion characteristics (droplet size 122nm). Modulation of intestinal permeability upon dilution was found closely related with dynamics of microemulsion system. Dilution mediated transitions in microstructure of GO SMEDDS was associated with the changes in the orientation of surfactant molecules at the oil-water interface of microstructures during solubilization of GO. In vivo studies revealed that orally administered GO preconcentrate produced 1.6 folds enhancement in oral bioavailability of GO over control. Present study demonstrates that intestinal permeability and oral bioavailability can be modulated via exploration of fully dilutable pre-concentrate GO system which could be a possible carrier to enhance oral bioavailability of GO.

Keywords: Ginger oleoresin, Dilutable SMEDDS, Mixed surfactant, Ex vivo permeation, Bioavailability, Solubilization

Introduction

Ginger, a folklore medicine and has been used a common household remedy in Indian subcontinent since very long time. Ginger oleoresin (GO), is a bioactive fraction extracted from rhizhome of ginger (*Zingiber Officanale Roscoe*.) It has potential anti-inflammatory, anti oxidant, anti viral, anti colon cancer activity due to inhibition of cycloxygenase, 5-lipoxygenase and eicosanoid levels.[1-2] Its usefulness in GIT distresses e.g. diarrhea, stomach ache has been most widely in therapeutic practice. GO was found to effect the xenobiotic/drug metabolism in a concentration-dependent fashion and modulate the metabolizing enzymes (CYP2C19). Recently, GO interaction with p-glycoproteins substrates has increased its the interest in pharmaceutical formulations as it affects the pharmacokinetics of low bioavailaible drugs. [3]

The major impediment in formulations development of GO is its poor aqueous solubility which limits its oral bioavailability and pharmaceutical usage. [4] Major active components known for pungency are present in GO are series of gingerols specifically 6-gingerol and dehydrated gingerols. Following oral administration of GO (300mg/kg dose) in rats, 6-gingerol is the most abundantly present in GO, produces the plasma concentration of 4.23 mcg/ml owing to high tissue binding and high intestinal metabolism. [5]

Poor solubilization of drug substance is the major issue in development of pharmaceutical formulations. To overcome such characteristics, Lipid based self micro emulsifying delivery systems (SMEDDS) have emerged as drug carrier system gave promising results by improving the solubilization characteristics of drug and inturn bioavailability enhancement. of poorly soluble drugs e.g. carvidilol, itraconazole as well as natural products of therapeutic importance like curcumin and carotenoid lutein.[6-9]

SMEDDS has been shown to play an important role in modulating the ADME of poorly bioavailable drugs which has limited aqueous solubility. Double surfactant based SMEDDS formulations has been reported to modulate the intestinal permeation characteristics, inhibiting intestinal efflux pumps, p-glycoproteins inhibition, as well as stabilization of drug in gastrointestinal regions to increase the oral bioavailability of drugs [10-11]. In an example, tocopheryl polyethylene glycol succinate (TPGS) and Cremophor EL40 were used in double surfactants in SMEDDS, has been found to modulate the intestinal permeability of tacrolimus. The possible explaination to this finding was the effect of selected surfactants on p glycoprotein inhibition. [12]. Similarly, it has been investigated that intestinal CYP3A activity in rat was found to be significantly inhibited by PEG-400. [13]

In an optimized SMEDDS formulation prepared using either single or mixed surfactants (tween 80) has been reported to loosen up intracellular tight junctions of enterocytes which significantly increases the permeability of drug to several times, and thereby enhance the absorption of gancyclovir [14-15] In an another report, Tweens was found to inhibit the p-glycoproteins mediated drug efflux and increased the paracellular absorption of poorly permeable drug to many folds, e.g. carmazepine permeability was altered using tween based dilutable formulation. [16] Tween 80 significantly increased absorption by changing transport balance between absorptive transport and secretive transport of rhodamine 123 and digoxin. [17]

It is likely that Tween based dilutable SMEDDS formations of GO would be as an alternative carriers in the drug delivery of naturally occurring compounds e.g. gingerols as GO has poor aqueous solubility characteristics. On these basis, it has been proposed that the formulated system would facilitate the aqueous solubilization of GO, altered the intestinal permeability and may show interactions with drug transporters.

The aim of present investigation is (i) to assess the feasibility in formation of self micro emulsifying dilutable formulation of GO and its physical characterization (ii) to investigate the in vivo/ex vivo performances of self emulsifying diluted/ preconcentrate of GO and its interrelationship with microemulsion microstructures.

Material and Methods

GO was purchased from Synthite Chemical, Kerala, India (containing 30% w/w of total gingerols, devoid of any heavy metals, as analyzed by ICPMS, data not shown), Tween 80 (polyoxyethylene sorbitol mono-oleate) and Tween 20 (polyoxyethylene sorbitol mono-laurate) was procured from Sigma. Analytical grade propylene glycol (PG) was obtained from Merck, India. All other reagents and buffer solutions used in different studies were of analytical grade. Bi-distilled followed deionized water was used in all experiments and was prepared in department laboratory.

Construction of phase diagram and formulations of GO-SMEDDS

Pseudo-ternary phase diagram was dawn from oil phase consisting of GO, (specific gravity 1.023 gm/cm^3), surfactant and co-surfactant mixture at surfactant/cosurfactant mass ratio (k_m=1.0) and water by aqueous titration method. Surfactants either single or mixed consisted of Tween80 and Tween20 (1:0, 1:1, 0:1; HLB 16, 16.5 and 17 respectively) whereas propylene glycol (specific gravity=1.036gm/cm^3) was taken as co-surfactant. The temperature was maintained by RE 320 THERMOSTAT controlled at 30±0.5 ºC. Three GO SMEDDS formulations was prepared across the dilution line and characterized

Characterization of GO-SMEDDS

Conductivity measurement

The electrical conductance ( ) was measured using DELUXE conductivity meter (MS Electronics India Pvt Ltd, Harayana, India) operating at 50Hz. The temperature was kept at 30±0.5 C and maintained by thermostatic controlled. Conductance of microemulsion was measured by taking preconcentrate mix of oil and surfactant/co surfactant and titrated with aqueous phase.

Viscosity measurement

Rheological studies of samples were performed on Brookfield R/S (Model ps-p) cone and plate viscometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA; attached spindle # 50.3250; type C25-2 at shearing rates (*1/s*) values 25-400. The software used for the calculations was Rheocal V2.8. Apparent viscosities (eta value) were measured and an average of ten readings at the interval of ten seconds with total run time of 100 seconds was recorded at 30± 0.5ºC. Distance between the cone and plate was kept 0.01mm with cone angle 1

Analysis of globule size and surface charge

The globule size was determined using a photon correlation spectrometer (Zetasizer 1000 HAS, Malvern Instruments, Worcestershire, UK). Light scattering was monitored at 25 C at a 90 angle. Average particle diameter (dm), polydispersity index (p_i) and zeta potentials were recorded and shown in Table 3. The p_i reflects the uniformity of droplet diameter and can be used to depict the size distribution in the droplets population. The measurement time was 2 min and each run underwent 10 sub runs. The preconcentrate was dispersed with different concentration of water for which 1.0 ml aliquot was withdrawn and added into a sample cell for globule size measurement. Each size value reported was the average of at least three independent measurements. Zeta potential was measured simultaneously on the same diluted sample using the same equipment and operating conditions as measured in droplet size.

pH

pH of the samples was determined at room temperature by SIMTRONIC digital pH meter India ltd, model SE-962P.

Specific gravity

Specific gravity of samples was measured with calibrated BOROSIL specific gravity bottles at 30± 0.5 ºC. For each measurement, the specific gravity bottle was washed, rinsed and vacuum dried. The average limit of error in specific gravity measurement was ± 2%.

Refractive Index

Refractive index of samples was determined by using ABBE type refractometer at 30± 0.5 ºC.

Stability Assessment of GO-SMEDDS

Centrifugation

Mechanical stability of formulations was determined by placing the sample in a centrifuge (TARSON) operated at 3000 rpm for 30mins and visual inspection of any physical in-stabilities in sample was observed.

Thermal testing

Thermal stability of the samples was assessed by placing them at 30± 0.5 C maintained in an oven for six months. The samples were visually inspected for appearance, phase separation, loss of volume due to evaporation at the intervals of 1, 2, 3 and six months.

Permeability & Bioavailability assessment of GO-SMEDDS

Ex vivo Intestinal permeability

Intestinal permeability of GO (quantified as 6-gingerol) was determined from GO pre-concentrate and its control formulations using ex vivo intestinal perfusion method. [34,35]. Approval to carry out ex vivo intestinal permeation on rat ileum was taken from Institution Animal Ethical Committee, of Jamia Hamdard, New Delhi, India. These studies were followed prior approval under CPSEA guidelines and confirms to national guidelines on the care and use of laboratory animals (protocol proposal No: 566/2009). Male wistar rats weighing 250±20 g were provided by Jamia Hamdard, New Delhi, India. The animals were housed in standard conditions of temperature (25±2 C), in 12/12 h light and dark cycles and fed with standard pellet diet and water *ad libitum*. Animal handling was followed in accordance to Good Laboratory Practice (GLP).

Rats were kept under fasting condition prior 12 h before experiment but allowed to have free access of water. Rats were sacrificed by vertebral dislocation method and entire gastrointestinal segment from ileum was surgically removed. Isolated ileum (5.0cm) was washed and thoroughly rinsed and immediately immersed in phosphate buffer pH 7.4 chamber [29, 30], besides aspiration of carbogen gas was also maintained so that tissue viability remains till the end of experiment.

Ileum sac was filled with 1.0ml of sample and ligated at both ends. The receiver chamber was filled with phosphate buffer (pH-7.4) (50.0 ml volume) and stirred with a magnetic rotor at a speed of 100 rpm in a hot air oven maintained at a temperature of 37±0.5ºC. Samples (0.5 ml) were withdrawn at pre-determined intervals (0, 0.1, 0.2, 0.4, 0.8, 1.0 h) and filtered through 0.45 µm membrane filter and analyzed for GO content. Analytical methodology of GO consisted of HPLC method where 6-gingerol was determined at λ_{max} of 290.0 nm by reproducing official compendia method as given in Indian Pharmacocpoeia (IP) 2010 modified as per system suitability[18]. The apparent permeability (Papp) of GO was calculated using following equation.

$P_{(app)} = dQ/dt * (1/AC_0)$

where dQ/dt is the slope of line determined from graphical plot drawn between cumulated drug permeated vs. time and A is the effective area of the ileum portion taken under study, C_0 is the initial amount of oleoresin filled in the length of ileum sac.

Pharmacokinetic study

Male *wistar* rats (250±20g) were taken, provided by the Central Animal House, Jamia Hamdard, New Delhi, India. The rat conditions were maintained and described previously under ex-vivo permeation section. The rats were kept intentionally deprived of food but given free access to water 12 h before the experiment to begin.

GO SMEDDS formulation was orally administrated in undiluted form and control group was administered GO in Tween 80 at the dose level of 300 mg/kg of body weight. Each formulation was administered by oral gavage to rats using an animal feeding needle. Blood samples (0.75 ml) were collected through alternate retro orbital plexus at the intervals of 0, 15, 30, 45, 60, 120, 240 and 480 min after oral administration (n=6 for each time point) into heparinized microcentrifuge tubes.

Plasma was separated from collected blood samples followed centrifugation at 13,000 rpm at 20 C for 20 min, and were stored in minus 80 C deep freezer before analyzing. The samples were analyzed by reproducing LCMS-MS method as reported by Wang et al, 2009 [12] modified as per system suitability validated prior applying test samples.

Table-1 Composition of GO pre-concentrate, diluted GO-SMEDDS and control formulations from mixed surfactant system

	S/CoS (wt%)	GO (%wt)	Water (%wt)	Type
GOM_0	97.5	2.5	-	reverse micelle
GOM_2	94	1.25	4.7	water-in-oil
GOM_9	81	0.5	18	oil-in-water
GOM_{25}	68	0.2	27	oil-in-water
GOO_{twn}	-	05	-	
GOO_{pyl}	-	05	-	

GOM_0=GO undiluted pre-concentrate; GOM_2=GO microemulsion diluted to 2.0ml; GOM_9= GO microemulsion diluted to 9.0ml; GOM_{25}= GO coarse emulsion diluted to 25.0ml; GOO_{twn}= GO in tween 80; GOO_{pyl}= GO in propylene glycol.

Table-2 Physical characterization of GO-SMEDDS formulations

	S. gravity (gm/cm^3)	pH	Ref. index	Droplet size (nm)	Viscosity (mPas)	Conductivity (μS/cm)
GOM_0	1.113±0.01	-	1.324	-	40.42	0.946
GOM_2	1.095±0.01	7.05±0.02	1.125	22	92.45	12.14
GOM_9	1.045±0.02	6.49±0.03	1.355	122	42.84	614.2
GOM_{25}	1.010±0.03	6.41±0.02	1.224	a	55.31	1281.1
GOO_{twn}	1.012±0.01	6.23±0.02	1.230	-	49.85	941.6
GOO_{pyl}	1.034±0.02	6.12±0.03	1.235	-	53.76	843.2

*value determined at 400 sec^{-1} shearing rate a=formation of coarse emulsion; Data represent mean ± standard deviation (sd)

Table-3 Evaluation of short term thermal and mechanical stability of GO-SMEDDS formulations

	Centrifugation[a] (3000 rpm)	Thermal stability[b] (40 C)	Conductivity (μS/cm)	Viscosity (mPas)	Droplet size (nm)
GOM_0	stable	stable	1.024±1.21	41.34±1.23	-
GOM_2	stable	stable	12.52±2.22	93.56±2.34	22
GOM_9	stable	unstable	625.1± 3.35	44.34 ±3.91	135
GOM_{25}	unstable	unstable	1250.4±5.67	51.91±5.67	c
GOO_{twn}	stable	stable	929.2±5.67	50.55±2.48	-
GOO_{pyl}	stable	stable	845.7±7.56	55.21±3.54	-

Data represent mean ± standard deviation (SD), n=3; [a] stability assessed for a hour cycle [b] for six month storage [c] formation of coarse emulsion

Results and Discussion

In order to prepare GO SMEDDS, ternary components selected were GO, propylene glycol, and polyoxyethylene sorbitan mono-laurate (Tween-20) or polyoxyethylene sorbitan mono-oleate (Tween 80) or its mixture at 1:1 as oil phase, co-surfactant and surfactant/s respectively at surfactant/ cosurfactant ratio (Smix ratio; 1:1). Single surfactant based phase diagrams was represented from Fig 1a and 1b while phase diagram from mixed surfactant is shown in Fig 1c. Existence of isotropic regions from single or mixed surfactant system to be required for the formulation of GO SMEDDS was investigated in this paper.

Ternary system components selected in formulation designing of GO-SMEDDS are safe, biocompatible, non volatile with appreciable IEG limits and have generally regarded as safe (GRAS) status. The surfactants chosen in GO SMEDDS were mutual miscibile with GO and the selected co-surfactant. Tween 80 (HLB = 15) and Tween 20 (HLB = 18) are non-ionic surfactants employed in topical, oral and parenteral formulations. [19-21] Propylene glycol, a co-surfactant/cosolvent has good solublization capacity for lipophilic as well as hydrophilic drugs and exhibits mutual misicibility with wide variety of surfactant and oils components.

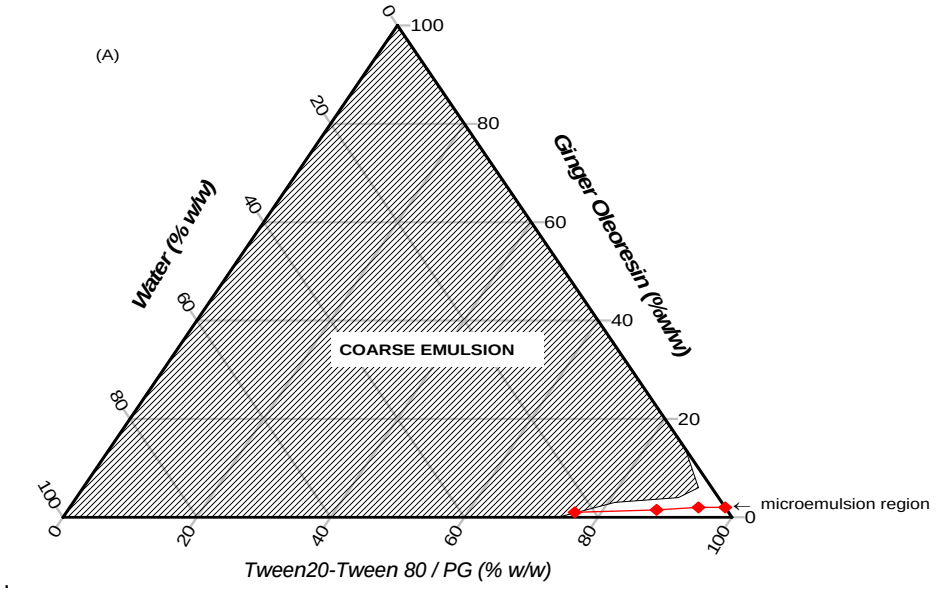
A possible explanation for the identification of dilutable region in phase diagram prepared from mixed surfactants over single surfactant was to increase the possibility to find more microemulsion region which may produce dilutable GO SMEDDS on aqueous phase dilution. Moreover, double surfactant has been shown to have excellent solubililization potential than single surfactant. More isotropic region with increased micro-emulsion area has been seen from mixed surfactants based ternary diagrams. Surfactant/cosurfactant mass ratio (k_m=1.0) was kept at unity. The role of cosurfactant was not produced much significant difference in the microemulsion phase region at higher surfactant proportions (data not shown).

Surfactants were selected on the basis of different hydrophilic lipophilic balance (HLB) values to form GO SMEDDS. Single surfactant did not produce significant affect on the microemulsion area or aqueous dilutable region. While using higher surfactants ratios at 2:1 and 1:2, did not provide any increase in isotropic region of microemulsion. It revealed that GO has poor solubilization characteristics in cosolvents also. Moreover, feasibility of larger higher surfactant ratios would result in poor rheological characteristics which may result in difficulty in aqueous dilution.

The extent of aqueous dilutable region in isotropic system was confined upto 9.0ml and beyond that region this system was broken down as GO SMEDDS turned into dull brown, eventually formation of coarse emulsion of GO took place. Therefore, dilution phenomenon may be explained in terms of transitioning from microstructures when GO SMEDDS was converted into microemulsions. Formation of GO SMEDDS was found to depend upon the limited dilution capacity (upto 9ml) which may be associated with existence of internal microstructures when GO SMEDDS was converted into microemulsion upon mixing with water (data not shown). Effect of dilution phenomenon on microstructures can be analogically described as the formation of pseudo SMEDDS when diluted upto 9.0ml where internalized GO in dispersed phase nanodroplets was partially stable. Further addition of water make pronounced changes in internal structure of nano-size dispersion leads to transformation into coarse emulsion.

Phase diagram

Poor misicibility of GO in oils, co-solvents is the major hindrance in the formulation of its conventional dosage forms. Ternary components was selected on the basis of miscibility of GO and found to be miscibile with propylene glycol, and mixture (1:1) of polyoxyethylene sorbitan-monooleate, polyoxyethylene sorbitan mono-laurate at S/Cos ratio 1.0. To identify the microemulsion region and to delineate it from coarse emulsion and liquid crystalline regions, pseudo ternary phase diagram was plotted. Phase diagrams from single vs. mixed surfactant based systems are represented in Fig 1. The existence of two distinct regions was appeared in single or mixed surfactants system as revealed from the phase boundary drawn in phase diagram. Microemulsion region are represented from single phase, isotropic area in the phase diagram where oil, water and surfactant/co-surfactants components spontaneously formed at room temperature. It is noteworthy to mention the area of microemulsion region from mixed surfactant system is more pronounced in comparison to single surfactants. The reason for selection of mixed surfactant system is obvious as it has more dilutable region in comparison to either tween 20 or tween 80. No difference in microemulsion area of ternary phase diagram drawn using tween 80 or tween 20 was observed. Despite the microemulsion areas was also identified at surfactants/cosurfactant ratios at 2:1 and 1:2 levels. Higher ratios of S_{mix} in microemulsion produced more viscous system which delayed mixing with water and the formation of thick gels resulted.



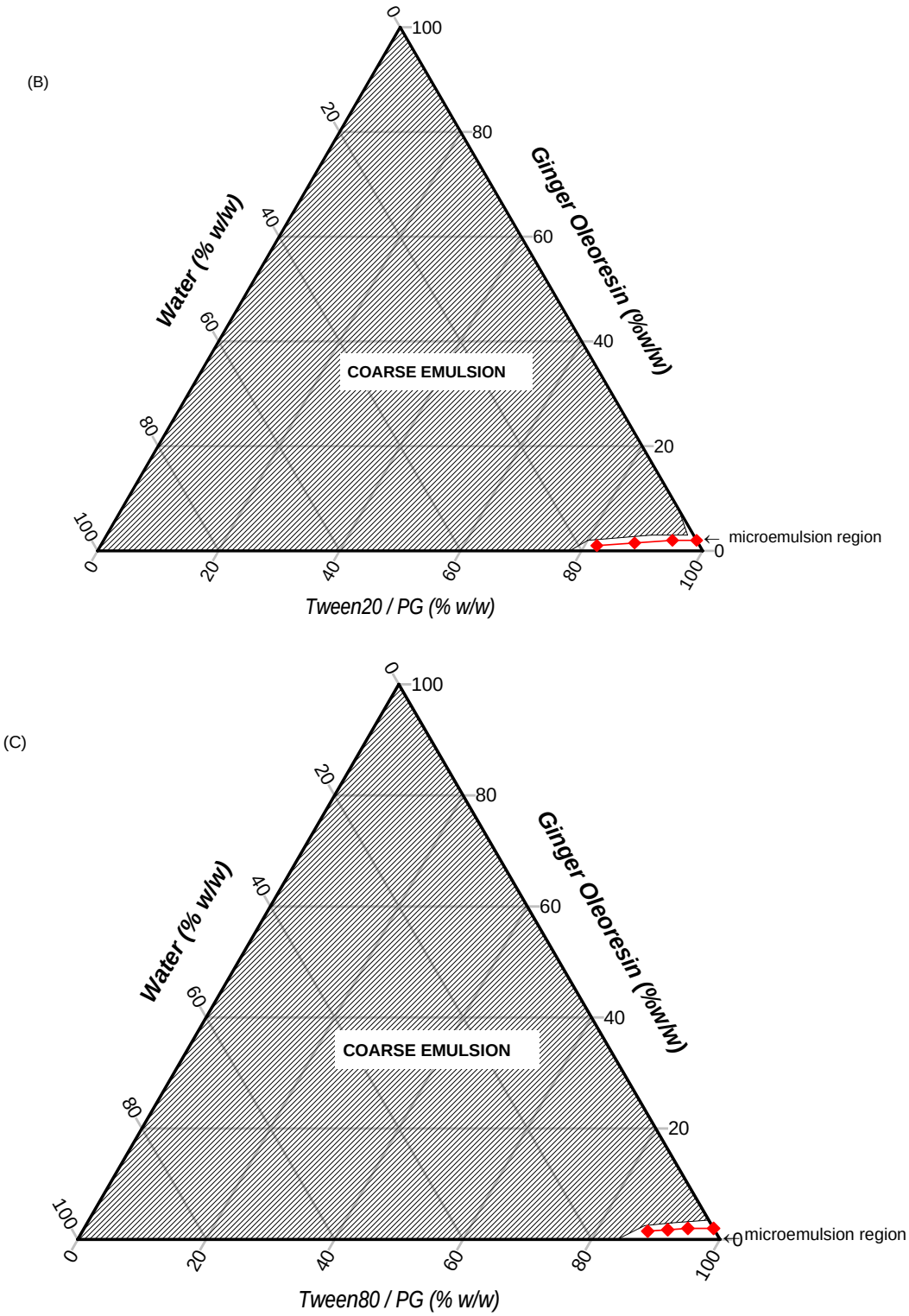


Figure 1 *Pseudo* ternary diagrams drawn at Smix 1:1 at different type of surfactant a) mixture of Tween80-Tween20 (1:1); b) Tween 20; c) Tween 80 alone. Ternary diagram shows phase boundary to delineate the microemulsion region (one phase, transparent region) from coarse (two phase, shaded region). Aqueous dilution line was drawn across microemulsion region to characterize microstructures domains.

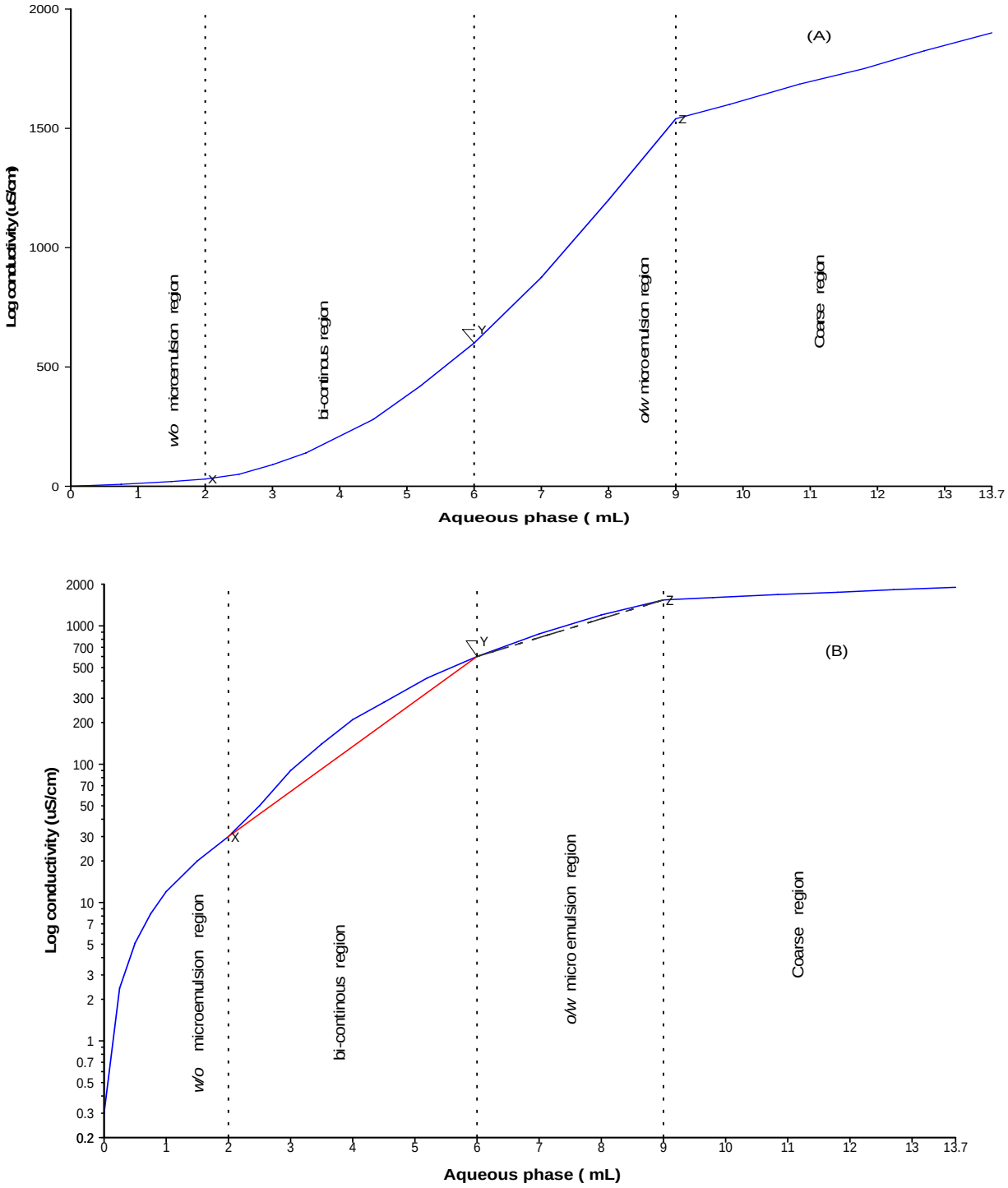


Figure 2: (a) Effect of aqueous dilution on the conductivity of ginger oleoresin pre-concentrate (GO 5% and S/CoS 95%) transformation from reverse micelle to coarse emulsion via formation of different microemulsion structures in the course of dilution. (b) Plot of logarithm transformed conductivity values vs. addition of aqueous phase volume showing percolation phenomenon.

Conductivity measurement

Conductance measurement is a precise characterization tool extensively used in dispersion systems and more specifically to quantifying the changes observed at microstructure level brought out by the addition of aqueous phase. The results obtained from electrical conductance ( ) also described the inherent characteristics of phase behavior during formation of microemulsion droplets from pre-concentrates/GO-SMEDDS. Microstructural transition from oil continuous to water continuous microemulsion systems were characterized from conductivity changes and percolation appeared with aqueous phase dilutions. In the present context, percolation phenomenon was also observed in GO SMEDDS during aqueous phase dilution as represented in figure 2.

The linear plot was drawn between conductance and the volume of aqueous phase added to the GO preconcentrate was represented in fig.2 (a). It showed that initially very small rise in conductance values was observed when its composition was represented from oil/surfactant/co-surfactant mixture containing 95% of S/CoS, 5% of oil phase. Electrical conductance of preconcentrate was changed slowly along the line OX but gradually increased with dilution represented from XZ line. As depicted in the fig 2a, linear conductivity was plotted with volume of aqueous phase in GO SMEDDS strongly suggest the formation of oil continuous system when preconcentrate of GO was diluted upto 2.0ml since no sharp increase in conductance of preconcentrate was observed. It suggests that undiluted preconcentrate existed in the form of reverse miceller mixture consisted of GO and S_{mix}, which upon aqueous phase dilution internalized the water molecules in its core to form w/o droplets. Formation of w/o microemulsion was continued till the system was diluted upto 2.0ml.

Much variation in conductance values has been observed when the volume of aqueous phase exceeds above 2.0ml. Conductance of GO SMEDDS was sharply raised to higher values with very little quantity of aqueous phase added. This incremental change in conductivity of GO SMEDDS was continued upto 9.0 ml of water addition and the system was appeared to be fully transparent. The first sign when GO integrity in SMEDDS changed in visual appearance of microemulsion was observed when volume of aqueous phase exceeds to 9.0ml Beyond 9.0ml the microemulsion formed from SMEDDS turned into dull in appearance and lost into brilliance.

Precise composition in the microemulsion system of different w/o, bicontinous and o/w system was revealed and further on extrapolation, it delineated the phase boundary between micro emulsion and coarse emulsion.

Log conductance value of GO SMEDDS vs. volume of aqueous phase was plotted in fig 2(b). Graphical representation showed the existence of three distinct regions as appeared from percolation that the formation of w/o droplets took place from reverse micellar solution and on further addition of water, bi-continuous structures were formed upto 6.0ml which finally converted into o/w structures upto 9.0ml. No orientation of internal structures has been observed beyond this dilution point further. No decreasing pattern in the conductivity had been observed when o/w microstructure was turned into coarse emulsion. It showed that sharp incremental change in conductance values with water addition represents a transitioning in internal structure of microemulsion. When the microstructure system was broken down at the ends, mixed surfactants of GO SMEDDS could not retain the GO compound into microstructure and allowed the GO to escape into aqueous phase.

The conductivity of diluted SMEDDS formulations containing varied amount of oil and surfactant/co-surfactant were measured and their composition is shown in table-1.

Viscosity measurement

Rheological characterization has been employed in colloidal dispersion systems, transition between the internal structures or isotropic or mesophase existed in ternary phase diagram.

The viscosity of diluted GO SMEDDS was characterized along the dilution line at various shearing rates (1/s= 25, 50, 100, 200, 300 and 400 rpm) and represented in figure 3. Viscosity of GO SMEDDS was changed with addition of volume of aqueous phase to the physical mixture consisted of GO (3%) and S_{mix} (97%).

At the starting point, little variation in the viscosity of system was observed upto 2.0ml with addition of very small quantity of water. On further addition of water to 6ml, viscosity has been increased and reached its highest level at water phase added to 9.0 ml. Viscosity then showed declining trend on further addition of water.

It is well acknowledged that viscosity of dispersed phase was gradually increased upon aqueous phase dilution till formation of swollen micelles. These results indicated that weak entanglement between oil and water phases observed at w/o microemulsion system. At intermediate stage, water and oil phases obstruct mutually consequently increases the viscosity of the system. Dynamic viscosity of GO SMEDDS was higher when more entanglements between oil and water phases existed in presence of water molecules. Entanglements among the structures are probably due to the relative position of surfactant molecules at GO-water interface.

Oil continuous nanostructure was formed as a result of transition in microstructure from reverse miceller mixture consisted of S/CoS and GO diluted with aqueous phase. Therefore the viscosities of each microstructure varied with aqueous phase content.

Interpretation of viscosity behavior was almost comparable to that of electrical conductance measure of GO SMEDDS.

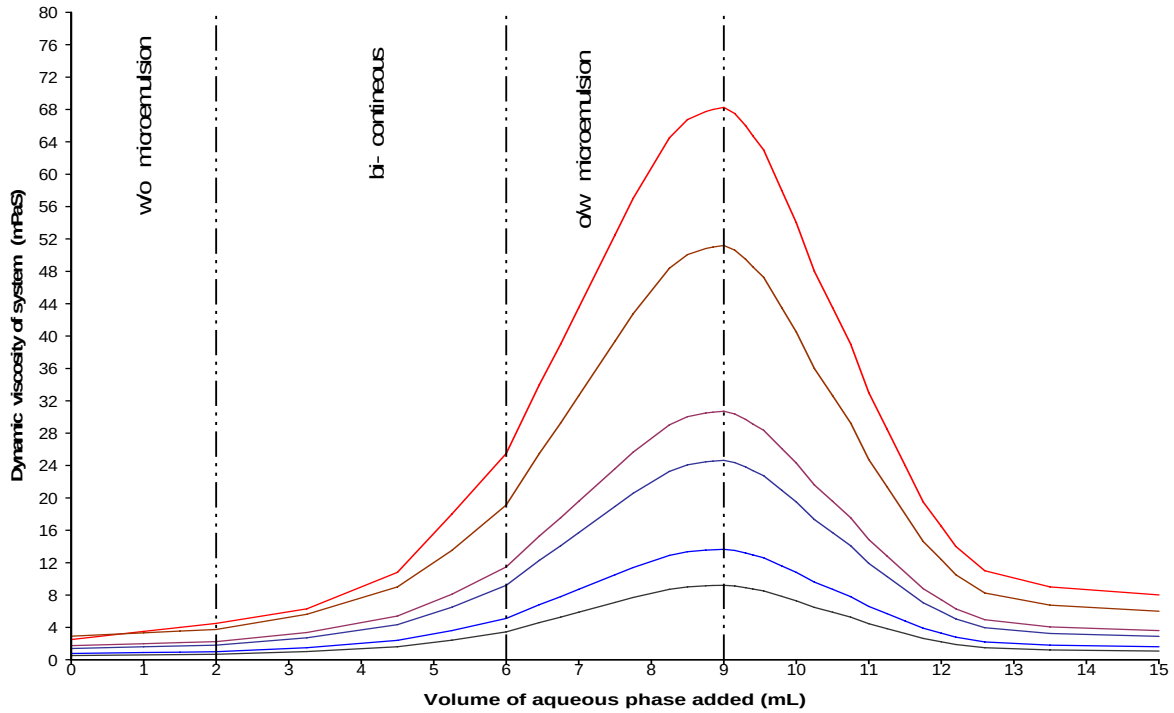


Figure 3: Effect of aqueous dilution on the dynamic viscosity of ginger oleoresin pre-concentrate (GO 5% and S/CoS 95%) measured at shearing rate (25-400 sec^{-1}) of mixed surfactant system.

Formulation characterization

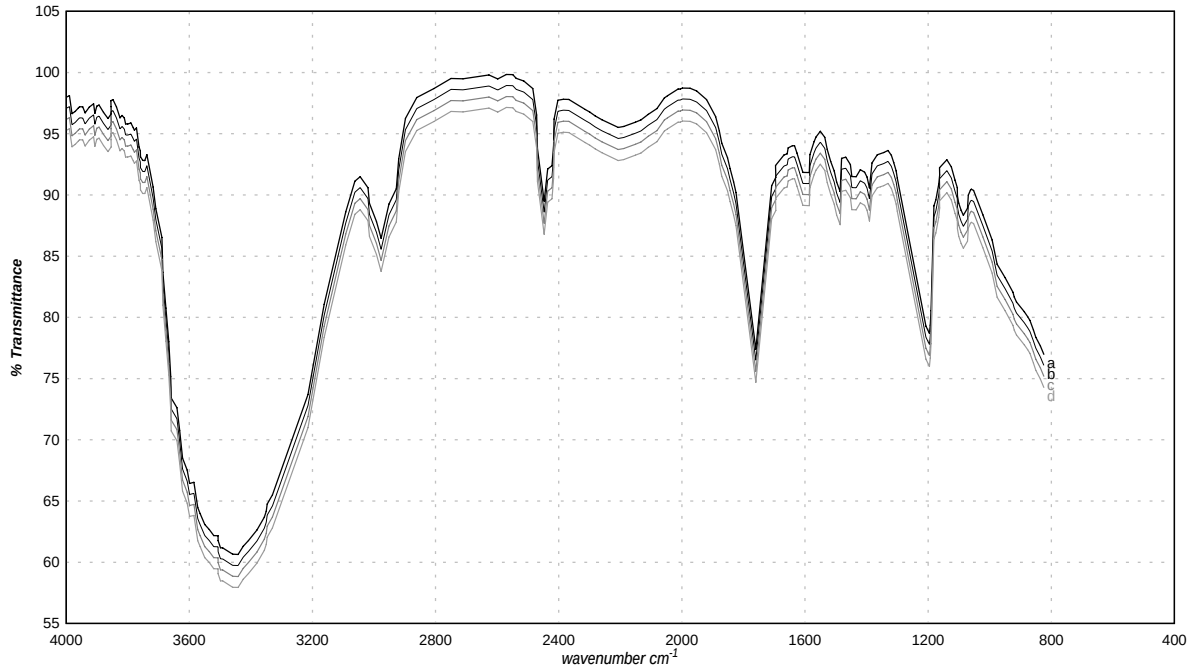


Figure 4: FTIR spectra of the GO SMEDDS diluted with varying proportion of aqueous phase and spectrum of GO. a, b, c, d indicated 0, 0.5, 2, 9ml addition of water to GO SMEDDS.

Table-4 FTIR characterization of GO-SMEDDS formulations

	OH stretching ($3000\text{-}3600\ cm^{-1}$)	CH stretching, Intensity ($2800\text{-}3000\ cm^{-1}$)	Ester Linkage, Intensity ($1200\text{-}1300\ cm^{-1}$)
GOM_0	3410	2919, 41	1295, 72
$GOM_{0.5}$	3412	2920, 23	1296, 63
GOM_2	3414	2920, 52	1296, 75
GOM_9	3417	2920, 45	1296, 73
GOM_{25}	3437	2924, 85	1281, -
GO	3433	2929, 72	-

Figure 4 shows the IR spectra of GO SMEDDS and GO and their characteristic group frequencies are given in the table-4. In reverse micelle, GOM $_{0.5}$, GOM_2 and GOM_9 systems, group frequency representing -OH group were slightly changed from 3410 to 3415 cm^{-1} regions as well absorption intensities. Slight change in group frequencies in GO SMEDDS with addition of water to reverse micelles indicates relatively strong interaction between polar head groups of surfactants with water molecules. This interaction was weakened with addition of water upto 9.0ml of water but GO molecules were strongly bound in the lipophilic side chain of the surfactant. Beyond 9.0ml or at higher water content, polar head group of surfactant molecules has least affinity towards water molecules also it freed the GO molecules from its core as its group frequency matched with pure GO.

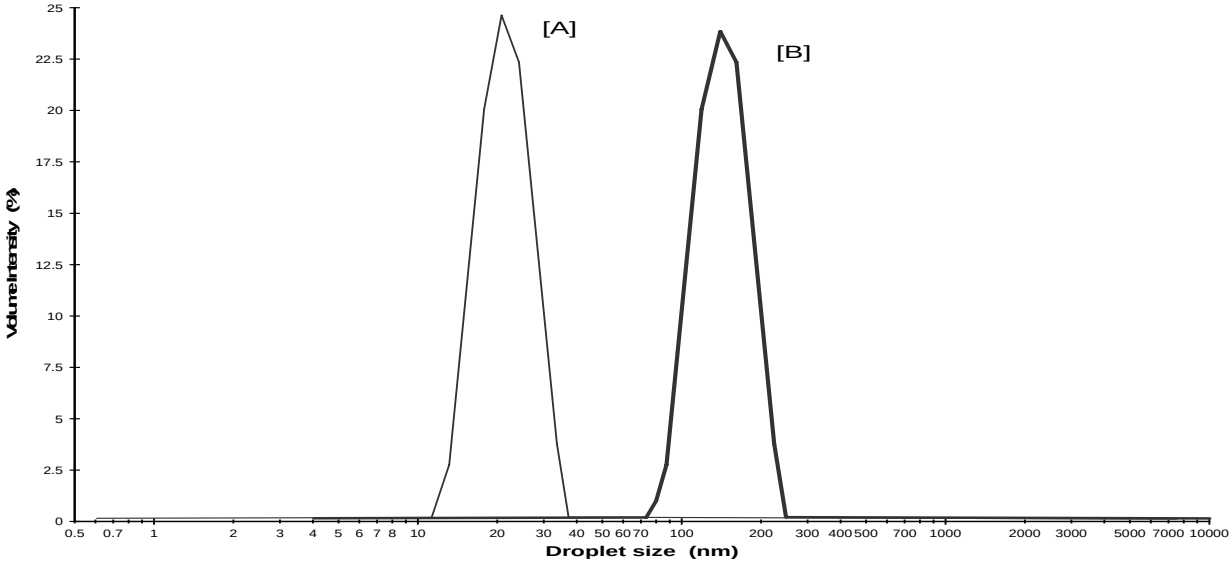


Figure 5 GO SMEDDS droplet size and droplet size distribution changed under the effect of aqueous dilution. A) GO SMEDDS diluted to 2.0ml aqueous phase volume; B) diluted to 9.0ml with aqueous phase volume.

Droplet size measurement & polydispersity index

Droplet size and its distribution of GO-SMEDDS diluted upto 9.0ml was shown in the figure 4 and again it was further confirmed by droplet size measurement. Droplet size and polydispersity of different formulations diluted from 2.0ml to 9.0ml were shown in figure 4 (a) and (b) respectively. GO droplet size was changed from 22 to 122nm when volume of aqueous phase was increased from 2.0ml to 9.0ml in the SMEDDS. These results obtained from droplet size measurement confirmed that addition of aqueous phase volume to pre-concentrate mixture increases the size of microemulsion droplets due to transformation of reverse micelle to swollen reverse micelles. [22] It also suggested that the structural variation initiated when surfactant/co surfactant mixture with GO mixed with aqueous phase, droplet size of diluted preconcentrate formulation of GO was in nano size range with low polydispersity index. As the formulation composed of non ionic surfactants, its zeta potential values remained unaffected with addition of water.

Specific gravity Refractive Index & pH

GO SMEDDS found to be safe as its pH values were in neutral range between 6.8 to 7.2. Ternary components chosen are lipidic / non ionic in nature and could not be effected with the pH. Other characteristics of GO SMEDDS like specific gravity and refractive index were shown in the table -2.

Ex vivo intestinal permeation

Ex vivo permeation from GO-SMEDDS as well as its control formulations were determined using rat intestinal perfusion method. Calculated P_{eff} values of GO from GO SMEDDS diluted with aqueous phase and controlled groups were represented in figure 5. From the permeation study, it has been demonstrated that dilution played a significant role in intestinal permeation of GO from undiluted GO-SMEDDS. No significant difference in GO permeability was observed from GO-SMEDDS or from GO formulation control. Experimental data suggests that no significant difference in GO permeability was observed from undiluted or over diluted GO-SMEDDS. Statistical difference in GO permeability was observed from GO-SMEDDS diluted upto 9.0 ml. (p<0.01)

The observation of permeability enhancement from GO SMEDDS was found to be closely associated with dilution effect on SMEDDS. During dilution,of surfactant/cosurfactant and oil premix, various nanostructures was formed when it was diluted with water.[23] It has been proposed that existence of microstructures altered the nature of dispersed phase globules with aqueous dilution. In the present case, when the preconcentrate system was over diluted, GO in dispersed phase occupy large droplet size, possibly in the coarse emulsion range and limit the solubilization of GO in SMEDDS. Lesser diluted SMEDDS transformed from of reverse micelles to w/o. Eventually in both the cases systems could not give significant difference in GO permeation. These results were in accordance with the results obtained from conductivity, viscosity and droplet size studies that nature of globules and globule size distribution of GO in SMEDDS was significantly varied with dilution. Incorporate amount of surfactant present in the GO SMEDDS may be regarded as another contributing factor for permeability enhancement.

Tween 80 alone has been shown to modify the permeation characteristics of compounds across intestinal membrane. [24], It is also observed that the control formulations comprised of tween 80, and PG did not show significant differences in intestinal permeability of GO. This phenomenon could be attributed to poor solubilization of GO in these vehicles, however its solubilization was improved in GO SMEDDS.

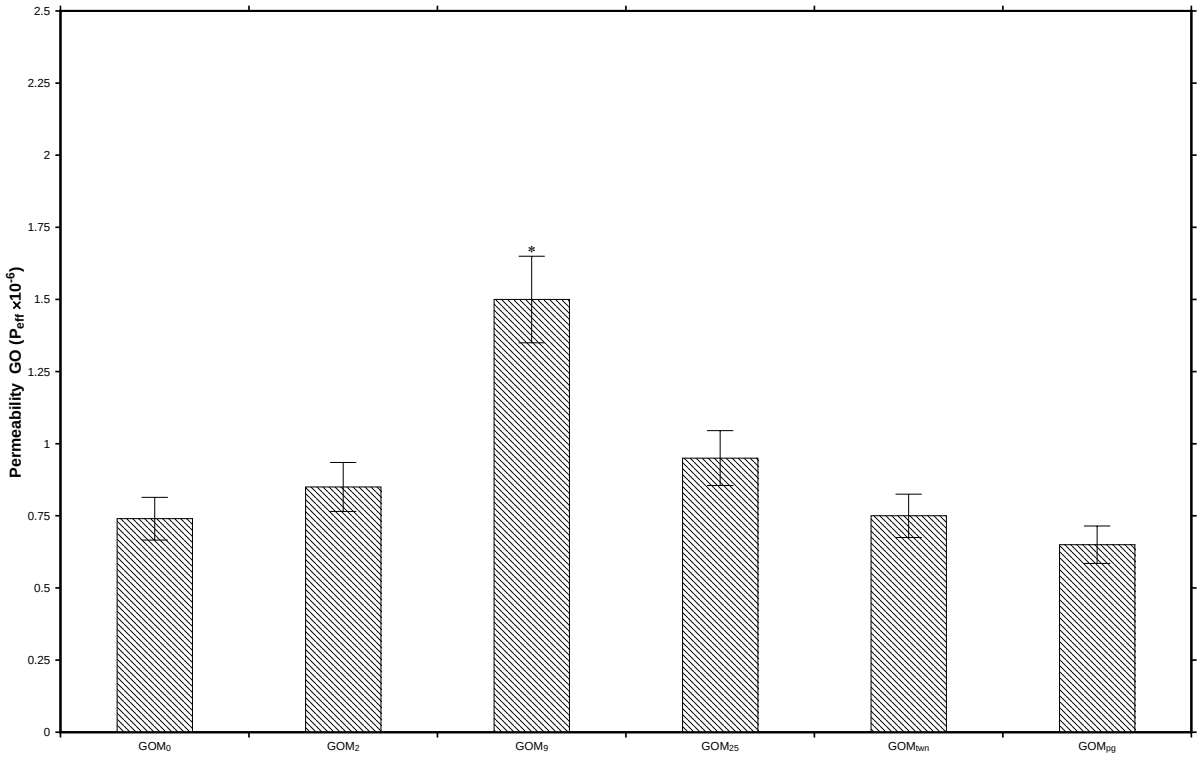


Figure 6: Effect of aqueous phase dilution on the intestinal permeability of GO (as quantified from 6-gingerol). Permeability of GO was also determined from selected ternary components like tween80 and PG. Data represent mean ± standard deviation (sd) n=6, Data was analyzed at significance level (p<0.01) using one way ANOVA with Dunnett test.

Pharmacokinetics studies

Table 5: Pharmacokinetic parameters of GO after oral administration of GO SMEDDS and control formulation

	GO CONTROL	GO SMEDDS
$AUC_{(0\text{-}8hrs)}$ (µg/ml hr)	1.46±0.24	2.37*±0.21
$MRT_{(0\text{-}8hrs)}$ (hr)	1.98±0.02	2.01± 0.02
Cmax (µg/ml)	0.64±0.03	1.55±0.01
Tmax (min)	0.50±0.01	0.39±0.02

Data represent mean ± standard deviation (sd) * p <0.01 by student t-test.

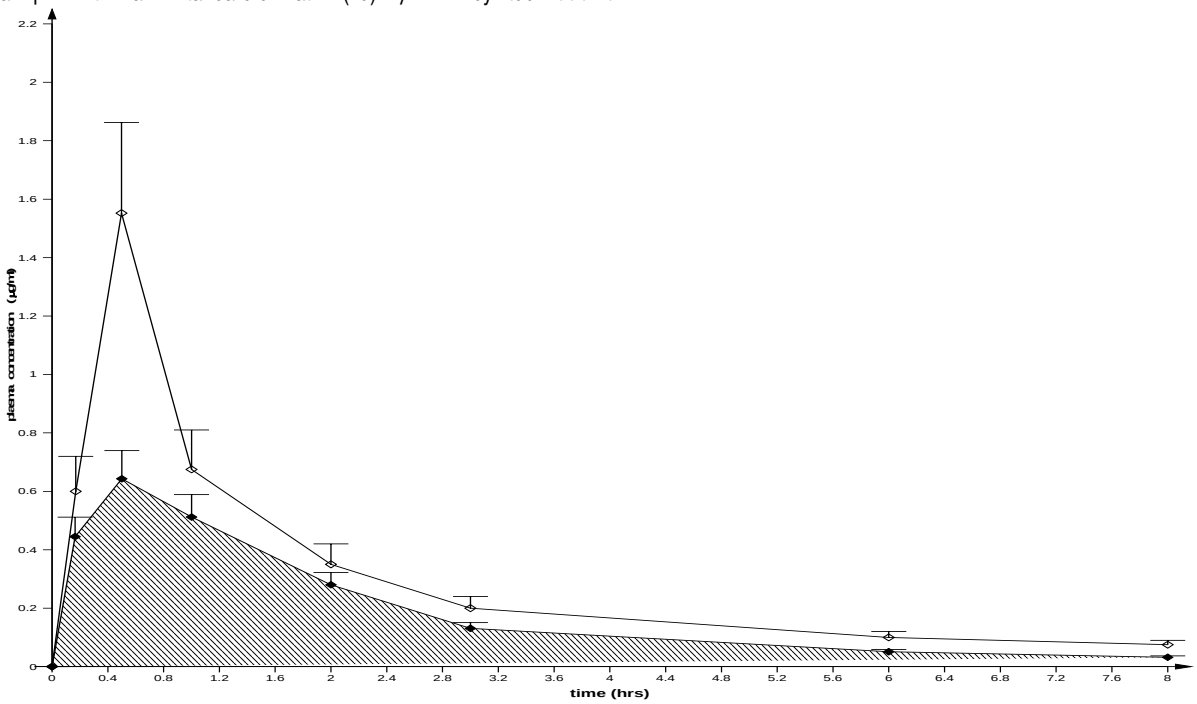


Figure 7: Mean plasma conc. vs. time profile of GO followed oral administration of SMEDDS vs. Control (GO in tween80 base). GO was orally administered at the dose of 300mg/kg in SMEDDS to Wistar rats. Data represent mean ± standard deviation (sd), n=6.

Pharmacokinetics studies

GO SMEDDS in preconcentrate form was orally administered to rats with minimal quantity of water (2.0ml). The purpose behind this assumption was the possibility of GO SMEDDS in pseudo self microemulsifible formulation may turned into coarse emulsion in contact with gastrointestinal secretions. Absorption profile of GO from control as well as SMEDDS formulation was shown from plasma concentration vs. time profile in the figure 7. Pharmacokinetic parameters were computed from absorption data as described in Section 2.8 and tabulated in Table 5. The results from in vivo study showed that the $AUC_{(0–8\ hrs)}$ and C_{max} of GO (quantified by 6-gingerol as most abundantly present in the GO) from GO SMEDDS formulation were increased by 1.6-fold and 2.4-fold respectively in comparison to the control group ($p<0.01$). 6-gingerol was rapidly absorbed from GO SMEDDS with a t_{max} of 30 mins while its control has same time point. No lag time during absorption of GO from entire region of gastro intestinal tract was observed.

Several contributing factors may be associated with bioavailability enhancement of GO formulated with SMEDDS. Aqueous dispersion of the active components from GO in the gastrointestinal environment being the rate-limiting step in GO absorption. GO-SMEDDS dispersed spontaneously into microemulsion when comes in contact with gastric secretions. An ultra fine dispersion of nano droplet size may be formed which facilitate the rapid and extensive absorption of GO due to availability of large surface area. [25] Followed by gastric emptying, microemulsion formed in stomach region undergoes dilution in intestinal region and eventually turned into coarse emulsion upon mixing with intestinal fluids which increases GO droplet size.

Breakdown of internal microstructure domain of microemulsion from SMEDDS, decreased the oral absorption of GO, which may either took place from lymphatic transport or through transcellular

pathways. In addition, modulation of intestinal permeation of GO formulated in SMEDDS using mixed surfactants could be corroborated with bioavailability enhancement as observed in the *ex vivo* intestinal permeation studies. [26-27]. It was seen from the absorption profile that following absorption phase immediate fall in plasma concentration of GO took place where GO was metabolized by intestinal microsomal enzymes. [28]

Conclusions

In the present investigation, the mechanistic approach to elucidate the physical behaviour of GO SMEDDS and its influence on preclinical bio-interfaces was explored. Dilution phenomenon was observed in mixed surfactant based GO SMEDDS and existence of different microstructures was located using rheology, conductometry and infra red spectroscopy. Different microstructures were taken as base formulations of ginger oleoresin (GO SMEDDS) which significantly modulated and enhanced the intestinal permeability and absorption of GO. However, over diluted GO SMEDDS compromised the microstructural integrity and hence challenged biopharmaceutical attributes of GO. Hence, it has been shown that the aqueous dilutability of a pseudo carrier system of GO hampered aqueous solubilization of GO in SMEDDS. Extent of absorption and improved solubilization of GO could be increased many folds to the current 1.6 times enhancement of GO if it was made fully dilutable and remained in range of nano droplet size.

Acknowledgements

Abhinav Garg is thankful to Indian Council of Medical Research (I.C.M.R.), Ansari Nagar, Delhi, India towards financial assistance as Senior Research Fellowship (S.R.F.)

References

[1]. Grzanna R, Lindmark L, Frondoza CG. Ginger-an herbal medicinal product with broad anti-inflammatory actions. J Med Food. 2005; 8: 125-132.

[2]. Zick SM, Turgeon DK, Vareed SK, Ruffin MT, Litzinger AJ, Wright BD, Alrawi S, Normolle DP, Djuric Z, Brenner DE, Phase II study of the effects of ginger root extract on eicosanoids in colon mucosa in people at normal risk for colorectal cancer. Cancer Prev Res (Phila) 2011; 4: 1929-1937.

[3]. Kim IS, Kim SY, Yoo HH. Effects of an aqueous-ethanolic extract of ginger on cytochrome P450 enzyme-mediated drug metabolism. Pharmazie. 2012; 67: 1007-1009.

[4]. Singh PK, Kaur IP. Development and evaluation of a gastro-retentive delivery system for improved antiulcer activity of ginger extract (Zingiber officinale). J Drug Target 2011; 19: 741-751.

[5]. Jiang SZ, Wang NS, Mi SQ. Plasma pharmacokinetics and tissue distribution of [6]-gingerol in rats. Biopharm Drug Dispos 2008; 29: 529-537.

[6]. Wei L, Sun P, Nie S, Pan W. Preparation and evaluation of SEDDS and SMEDDS containing carvedilol. Drug Dev Ind Pharm. 2005; 31: 78

[7]. Hong JY, et al., *A* new self-emulsifying formulation of itraconazole with improved dissolution and oral absorption. J Control Rel. 2006. 110: 332-8.

[8]. Joshi RP, Negi G, Kumar A, Pawar YB, Munjal B, Bansal AK, Sharma SS. SNEDDS curcumin formulation leads to enhanced protection from pain and functional deficits associated with diabetic neuropathy: An insight into its mechanism for neuroprotection. Nanomedicine. 2013.

[9]. Shanmugam S, Baskaran R, Balakrishnan P, Thapa P, Yong CS, Yoo BK., (2011). Solid self-nanoemulsifying drug delivery system (S-SNEDDS) containing phosphatidylcholine for enhanced bioavailability of highly lipophilic bioactive carotenoid lutein. Eur J Pharm Biopharm 79(2): 250-257.

[10]. Qi X, Wang L Zhu J, Hu Z, Zhang J., (2011). Self-double-emulsifying drug delivery system (SDEDDS): a new way for oral delivery of drugs with high solubility and low permeability. Int J Pharm. 409(1-2): 245-251.

[11]. Yin YM, Cui FD, Mu CF, Choi MK, Kim JS, Chung SJ, Shim CK, Kim DD. Docetaxel microemulsion for enhanced oral bioavailability: preparation and in vitro and in vivo evaluation. J Control Rel. 2009 Dec 3;140(2):86-94

[12]. Wang Y, Sun J, Zhang T, Liu H, He F, He Z. Enhanced oral bioavailability of tacrolimus in rats by self-microemulsifying drug delivery systems. Drug Dev Ind Pharm. 2011; 37: 1225-1230.

[13]. Mudra DR, Borchardt RT. Absorption barriers in the rat intestinal mucosa. 3: Effects of polyethoxylated solubilizing agents on drug permeation and metabolism. J Pharm Sci. 2010; 99: 1016-1027.

[14]. Li M, Si L, Pan H, Rabba AK, Yan F, Qiu J, Li G. Excipients enhance intestinal absorption of ganciclovir by P-gp inhibition: assessed in vitro by everted gut sac and in situ by improved intestinal perfusion. Int J Pharm. 2011; 403: 37-45.

[15]. Buyukozturk F, Benneyan JC, Carrier RL. Impact of emulsion-based drug delivery systems on intestinal permeability and drug release kinetics. J Control Rel. 2010; 142: 22-30.

[16]. Kogan A, Kesselman E, Danino D, Aserin A, Garti N. Viability and permeability across Caco-2 cells of CBZ solubilized in fully dilutable microemulsions. Colloids Surf B Biointerfaces. 2008; 66: 1-12.

[17]. Katneni K, Charman SA, Porter CJ. Impact of cremophor-EL and polysorbate-80 on digoxin permeability

across rat jejunum: delineation of thermodynamic and transporter related events using the reciprocal permeability approach. J Pharm Sci. (2007); 96: 280-293.

[18]. Secretary, Govt of India, Ministry of Health ,Indian pharmacopoeia 2010, VIth Ed, New Delhi, India 2010

[19]. Tavano L, Muzzalupo R, Trombino S, Cassano R, Pingitore A, Picci N. Effect of formulations variables on the in vitro percutaneous permeation of Sodium Diclofenac from new vesicular systems obtained from Pluronic triblock copolymers. Colloids Surf B Biointerfaces. 2010; 79: 227-234.

[20]. Mehta SK, Kaur G, Bhasin KK. Analysis of Tween based microemulsion in the presence of TB drug rifampicin. Colloids Surf B Biointerfaces. 2007; 60: 95-104.

[21]. Kim SK, Lee EH, Vaishali B, Lee S, Lee YK, Kim CY, Moon HT, Byun Y. Tricaprylin microemulsion for oral delivery of low molecular weight heparin conjugates. J Control Rel. 2005; 105: 32-42.

[22]. Chaiyana W, Rades T, Okonogi S. Characterization and in vitro permeation study of microemulsions and liquid crystalline systems containing the anticholinesterase alkaloidal extract from Tabernaemontana divaricata. Int J Pharm. 2013; 452: 201-210.

[23]. Fernandez S, Jannin V, Chevrier S, Chavant Y, Demarne F, Carriere F. In Vitro Digestion of the Self-Emulsifying Lipid Excipient Labrasol by Gastrointestinal Lipases and Influence of its Colloidal Structure on Lipolysis Rate. Pharm Res. 2013;

[24]. Jiang L, Long X, Meng Q. Rhamnolipids enhance epithelial permeability in Caco-2 monolayers. Int J Pharm. 2013; 446: 130-135.

[25]. Larsen AT, Ohlsson, AG, Polentarutti B, Barker RA, Phillips AR, Abu-Rmaileh R, Dickinson PA, Abrahamsson B, Ostergaard J. et al. Oral bioavailability of cinnarizine in dogs: relation to SNEDDS droplet size, drug solubility and in vitro precipitation. Eur J Pharm Sci. 2013; 48: 339-350.

[26]. Wang W, Li CY, Wen XD, Li P, Qi LW. Simultaneous determination of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol in rat plasma by liquid chromatography-mass spectrometry: Application to pharmacokinetics. J Chromatograpy B Analyt Technol Biomed Life Sci. 2009; 877: 671-679.

[27]. Kim MG, Shin BS, Choi Y, Ryu JK, Shin SW, Choo HW, Yoo SD Determination and pharmacokinetics of [6]-gingerol in mouse plasma by liquid chromatography-tandem mass spectrometry. Biomed Chromatogr. 2012; 26: 660-665.

[28]. Wahlang B, Kabra D, Pawar YB, Tikoo K, Bansal AK. Contribution of formulation and excipients towards enhanced permeation of curcumin. Arzneimittelforschung. 2012; 62: 88-93.