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OLMESARTAN MEDOXOMIL-LOADED SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS: DESIGN, *IN-VITRO* CHARACTERIZATION, AND PHARMACOKINETIC ASSESSMENTS IN RABBITS *VIA* LC - MS/MS

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Keywords:

Olmesartan medoxomil, Olmesartan, Self-nanoemulsifying drug delivery systems, Reverse dialysis, Oral bioavailability, LC-MS/MS

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ABSTRACT: Olmesartan medoxomil (OLM) is a lipophilic (log P = 4.31) antihypertensive drug suffering from limited oral bioavailability in humans (26%) due to its low aqueous solubility, uncontrolled enzymatic conversion to the active metabolite (Olmesartan; OL) and efflux by drug resistance pumps. Surmounting such limitations *via* incorporation of OLM into self-nanoemulsifying drug delivery systems (SNEDDS). Based on OLM-equilibrium solubility studies in various oils, surfactants and co-surfactants, Capmul® MCM, Tween® 20, Cremophor® EL and polyethylene glycol - 400 (PEG) were combined in different ratios to plot ternary phase diagrams. OLM-loaded SENDDS were developed and evaluated forparticle size, polydispersity index (PDI), zeta potential, self-emulsification time, morphology, drug released percentages after 5-min (Q_{5min}%), 1 hour (Q_{1h}%) and dissolution efficiency percentages (DE_{1h}%). The OL pharmacokinetics from SNEDDS (F6) and Benicar® tablets were evaluated (LC-MS/MS) in rabbits. Spherical OLM-loaded SNEDDS were developed. The best-achieved SNEDDS (F6) showed short emulsification time (13 s), fine droplet size (60.00nm), low PDI (0.25), negative zeta potential (-14.4mV), promising dissolution parameters; Q_{5min}% (29.78%), Q_{1h}% (66.69%) and DE_{1h}% (47.96%) and enhanced *in vivo* absorption characteristics; shorter T_{max}, higher C_{max} and larger $AUC_{(0-48h}$; suggesting its potential for the enhancement of the oral absorption of practically insoluble drugs; like OLM.

INTRODUCTION: Olmesartan medoxomil (OLM) is a selective angiotensin II receptor antagonist with actions similar to those of losartan [1]. It is given in oral doses of 10mg to 20mg once daily for the management of hypertension [2]. Clinical trials on hypertensive patients revealed that OLM has a good tolerance without any serious side effects [3].

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Recently, the concomitant use of OLM with amlodipine was well tolerated and effectively lowered blood in patients with hypertension and type II diabetes [4]. OLM is an ester prodrug which is quickly de-esterified to olmesartan (active metabolite; OL) upon oral administration by the action of aryl esterase situated in plasma and intestine [5].

Unfortunately, OLM suffers from low oral bioavailability (26%) in healthy humans; possibly due to its highly lipophilic nature (log P = 4.31) and poor aqueous solubility, uncontrolled enzymatic conversion of OLM to the poorly

permeable OL in the gastrointestinal fluids as well as the efflux of hydrophobic drugs by the drug resistance pump in the gastrointestinal tract [6, 7]. Several approaches were investigated to improve the oral bioavailability of OLM, including the development of solid lipid nanoparticles [8], freeze-dried solid dispersions [9], nanosuspensions [10], nanoemulsions [11 - 13], and self-micro emulsifying drug delivery systems [14], and solid self-nanoemulsifying drug delivery systems [15].

According to Gursoy and Benita [16], the self-emulsifying drug delivery systems could be defined as isotropic mixtures of oils, surfactants, or alternatively, one or more hydrophilic solvents or surfactants. These systems can form fine O/W microemulsion droplets (less than 100nm) upon mild agitation and dilution in aqueous media like gastrointestinal fluids. The importance of these systems results from the resultant small droplet size and large surface area; allowing for the enhanced absorption of lipophilic drugs, like OLM, by opening tight junctions to allow Para cellular transport, increasing membrane fluidity to facilitate transcellular absorption, and inhibiting efflux pumps like P-glycoprotein [14, 16, 17]. Like Nano emulsions, self-nanoemulsifying drug delivery systems (SNEDDS) can be formulated with little energy input (heat or mixing) [18]. In fact, several SNEDDS - based products, *e.g.*, Fortovase® (saquinavir), Norvir® (ritonavir) and Sandimmune Neoral® (cyclosporine) were successfully commercialized due to the simplicity of scale up of manufacturing process [16, 19].

P-glycoprotein (P-gp) is a multidrug resistance (MDR) protein encoded by the MDR1 gene in humans. It serves as a biochemical barrier for the efflux of structurally diverse drugs [20]. Drugs that are substrates for P-gp bind and are transported back to the apical surface of the tissue in an ATP-dependent manner, thereby restricting the overall permeability of drugs. Cremophor® EL, and Tween® 80 were used to inhibit P-gp efflux transporter activity in Caco-2 cell monolayers of the intestinal mucosa [12, 20].

Herein, Cremophor® EL-, and Tween® 80-based SNEDDS could improve the oral bioavailability of OLM due to the dual improved effect of solubility/dissolution and P-gp efflux transporter inhibition. To confirm these assumptions, the pharmacokinetics of OL following oral administration of the best achieved OLM - loaded SNEDDS and the commercial Benicar® tablets were evaluated in rabbits using LC MS/MS. It could be hypothesized that improving the oral bioavailability of OL can increase clinical efficacy and / or reduce the oral dosage required to achieve the same effect with possible reduction in side effects.

MATERIALS AND METHODS:

Materials: Olmesartan Medoxomil (OLM) was purchased from GVK Biosciences, Hyderabad, India. Capmul® MCM EP (glycerol monocaprylocaprate; Capmul®) and Captex® 355 EP/NF (triglycerides of caprylic / capric acid; Captex®) were kindly donated by ABITEC Corporation, Ohio, USA. Maisine® 35-1 (glyceryl monolinoleate; Maisine®) and Lauroglycol® 90 (propylene glycol monolaurate) were generously provided by Gattefossé, St-Priest, France. Cremophor® EL (polyoxyl 35 Castor oil; Cremophor®) was donated by BASF, Ludwigshafen, Germany. Tween 20® (polyoxyethylene sorbitan monolaurate) and polyethylene glycol 400 (PEG) were purchased from Oxford Laboratory Reagent, Maharashtra, India.

Absolute ethyl alcohol, sodium dihydrogen orthophosphate-1-hydrate and disodium hydrogen orthophosphate-1-hydrate were from El Nasr Pharmaceutical Chemicals, Abuzaabal, Egypt. Acetonitrile (HPLC grade), Formic acid (HPLC grade), Olmesartan (active metabolite; OL), Ornidazol (internal standard; ORN) and dialysis-tubing cellulose membrane (M. wt cut-off 12,000-14,000) were procured from Sigma Aldrich, St. Louis, Missouri, USA.

Methods:

Determination of the Saturated Solubility of OLM in Various Vehicles: The saturated solubility of OLM in various oils (Capmul®, Captex®, Maisine 35-1® and Lauroglycol 90®), surfactants (Cremophor® EL and Tween 20®) and a co-surfactant (Polyethylene glycol 400®) was determined, in triplicate, by adding an excess amount of drug to 3gm of each vehicle in 5mL stoppered vials, and mixed using a vortex mixer. The vials were kept at 25 ± 1.0 °C in an incubator shaker for 72 h to attain equilibrium. The

equilibrated samples were removed from the shaker and centrifuged at 3,000 rpm for 15 min. The supernatant was taken and filtered through a cellulose acetate membrane filter (0.45µm) [21]. The concentration of OLM was determined spectrophotometrically at 257nm after appropriate dilution [12, 22].

Construction of Ternary Phase Diagrams: On the basis of the saturated solubility studies of OLM, two ternary phase diagrams were plotted using varying concentrations of Capmul® as the oily phase, Cremophor® EL or Tween® 20 as the surfactant, and PEG 400 as the co-surfactant. The total of the three components always added to 100% for each system. An aliquot (1gm) of each system was introduced into 200mL of distilled water in a glass beaker at room temperature. The contents were mixed gently with a magnetic stirrer. Visual observations were made immediately as well as after storage for 48h at room temperature for clarity, phase separation and / or precipitation. All the studies were repeated twice with similar observations made between repeats [22 - 23]. Phase diagrams were plotted using Triplot software Ver. 4.1.2 (Graham and Midgley, Loughborough University, Leicestershire, England). The area under the curve (AUC) of the clear self-emulsifying region in each phase diagram was calculated according to the trapezoidal rule method. The statistical significance of the results was checked using one-way ANOVA test at $P < 0.05$ by Stat View® software Ver. 5.0.1 (SAS Institute Inc. San Francisco, CA, USA) [24].

Development of OLM - Loaded SNEDDS: A series of SNEDDS were prepared with varying weight ratios of oil (10% – 20% w/w), surfactant (60%, 70%, 80% and 90% w/w) and co-surfactant (0%, 10% and 20% w/w). In all systems, the concentration of OLM was kept constant at 1%, w/w. Briefly Capmul®, Cremophor® EL or Tween® 20, and PEG 400 were accurately weighed and vortex mixed in stoppered glass vials to ensure complete mixing. OLM - loaded SNEDDS were developed by dispersing an amount of OLM (10mg) with sonication into one gram of each system until a clear phase was obtained. The developed systems were equilibrated for 48h at room temperature for further studies. The composition of the investigated OLM-loaded SNEDDS is shown in **Table 1**.

TABLE 1: THE COMPOSITION (% w/w) OF OLM - LOADED SNEDDS

Systems	Oil	Surfactant		Co-Surfactant
	Capmul MCM®	Tween 20®	Cremophor EL®	PEG®
F1	10	90		0
F2	10	80		10
F3	10	70		20
F4	20	80		0
F5	20	70		10
F6	20	60		20
F7	10		90	0
F8	10		80	10
F9	10		70	20
F10	20		80	0
F11	20		70	10
F12	20		60	20

Characterization of OLM-Loaded SNEDDS:

Determination of the Emulsification Time: A predetermined weight (1gm) of each system was introduced into 200mL of distilled water maintained at 37 ± 0.5 °C in a glass beaker and the contents were mixed gently using a magnetic stirrer rotating at constant speed (100 rpm) [23]. The emulsification time - defined as the time required for the pre-concentrate (oil / surfactant / co-surfactant) to form a homogeneous clear phase upon dilution- was recorded visually [22].

Determination of Globule Size and Zeta Potential: The mean droplet size (z-ave) and the polydispersity index (PDI) were determined, in triplicate, by photon correlation spectroscopy (PCS) at 25 ± 0.5 °C. Each system was diluted (10 times) with de-ionized water to avoid the multi-

scattering phenomena. Measurements were performed at 90º to the incident beam using a Zetasizer Nano ZS [24]. PDI values ranging from 0.1 - 0.3 are considered optimum since they indicate uniform particle size distribution. In a parallel line, the zeta potential of the systems was determined, in triplicate, according to electrophoretic light scattering technology using a laser Doppler anemometer coupled with Zetasizer Nano ZS. Measurements were carried out at 25 ± 0.5 °C using the Helmholtz- Smoluchouski equation built into software [25].

Morphologic Examination: The morphologic characteristics of representative OLM-loaded SNEDDS (F6) were examined using transmission electron microscopy (TEM). SNEDDS were diluted 200 times by mixing with distilled water. One drop of each diluted system was placed on a copper grid for one minute and the excess was removed using a filter paper. For staining, one drop of an aqueous phosphotungstic acid solution (2%, w/v) was loaded and the excess was similarly removed. Finally, the grid was screened under a transmission electron microscope (Jeol, JXA-840A, Tokyo, Japan) under a magnification of 60,000X.

***In vitro* Drug Release Studies:** The *in vitro* release studies of OLM-loaded SNEDDS (1%, w/w), an aqueous drug suspension (10 mg/g) and Benicar® tablets (20mg) were assessed, in triplicate, using the bulk-equilibrium reverse dialysis technique in a USP Dissolution tester apparatus (type II) at 37 ± 0.5 °C (26-27). According to FDA specifications for the dissolution testing of OLM tablets [28], the release studies were conducted in 0.05M Sorensen's phosphate buffer (pH 6.8; 1000mL) and the paddles were adjusted to rotate at 50 rpm.

Five dialysis bags, each containing 5mL aliquots of the dissolution medium, were left to equilibrate in the dissolution medium within each dissolution flask for 12 h prior to the studies. On the study day, two g samples of OLM-loaded SNEDDS, the aqueous drug suspension or Benicar® tablets were directly placed into the dissolution flasks. At definite time intervals (5, 10, 15, 30 and 60 minutes), one dialysis bag was withdrawn from each dissolution flask. The withdrawn volume was equally replenished with fresh medium to maintain a constant volume.

The withdrawn samples were analyzed spectrophotometrically (Shimadzu spectrophotometer UV-1800, Kyoto, Japan) for the drug at 257nm. The mean (± S.D.) drug released percentages of each system were portrayed against time. For comparative studies, the drug released percentages after 5min (Q_{5min} %) and 1h (Q_{1h} %) as well as the dissolution efficiency percentages after 1h (DE_{1h} %) were estimated by calculating the area under the drug release profile curve (AUC) at 60 minutes using the trapezoidal rule. As proposed by Khan [29], DE% is expressed (equation 1) as a percentage of the area of the rectangle corresponding to 100% release, for the same total time (60 minutes);

$$DE\% = \frac{{}_0^t\int C \times dt \times 100}{C_{100} \times T}$$

Where, C represents the drug released percentage as a function of time t, C_{100} represents the complete drug release (100%) and T represents the total time of drug release.

***In vivo* Drug Absorption Studies:**

Study Design: The study was carried out to compare the pharmacokinetics of OL in rabbit plasma following oral administration of Benicar® tablets (Daiichi-Sankyo Co., Japan) and the best achieved OLM - loaded SNEDDS (F6) using a non - blind, two treatment, two-period, randomized, crossover design. The protocol of the studies (REC-FPSPI-5/38) was approved by the Research Ethics Committee for experimental and clinical studies at the Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University in Egypt. The use and the treatment of rabbits in this study were conducted in full compliance with the spirit of Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International's expectations for animal care and use/ethics committees.

Animals: Six healthy rabbits (weighing 2 – 2.5 kg) were housed in an air-conditioned room under controlled alternate day and night cycles; provided with artificial fluorescent light. The animals were fed a standard pellet diet, water *ad libitum*. These conditions were evaluated on a daily basis to ensure the safety and well-being of animal. A veterinarian checked the health of animals to ensure the lack of clinically observable abnormalities.

Administration of Drug Treatment to Rabbits: After overnight fasting, the rabbits randomly divided into two equal groups. The rabbits of the first group were administered two gm samples of OLM-loaded SNEDDS (F6) (Test, treatment A). Meanwhile, the rabbits of the other group received Benicar® tablets (Reference, treatment B). Before withdrawal of blood samples, the marginal ear vein was dilated, using warm water and/or swapping with cotton, and then punctured (24 gauge needle) to allow withdrawal of blood samples (2mL) at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24, 48 hours (post - dose). The samples were collected in EDTA tubes.

Sample Processing: The plasma was derived from the blood samples by centrifugation (3000 ×g) for 10 min, pipetted into glass tubes and then frozen (− 20 °C) until analyzed by LC – MS/MS. The tubes were labelled with the animal code, the study period, the run date, the treatment type; test or reference. The plasma samples (250µl) were spiked with ORN solution in acetonitrile (500µl; 0.5µg/mL). The spiked samples were vortexed (2 min) and centrifuged (4000 x g) for 5 min to allow for the separation of plasma proteins and injection of samples of the supernatant, *via* the auto-sampler, for analysis.

Determination of OLM in Rabbit Plasma by LC – MS/MS: The chromatographic separation of OL and ORN in rabbit plasma was performed on Shimadzu Prominence LC system (Kyoto, Japan) equipped with an auto-sampler (SIL-20A/HT), a solvent delivery pump (LC-20AT) and a degasser (DGU-20A3). A previously reported selective and sensitive LC-MS/MS method for the determination of OL in plasma was adopted [11]. The separation was performed on a SunFire C_{18} column (4.6 x 50 mm; 5µm particle size) (Waters Corp., Milford, MA, USA), at 25 °C, using an isocratic mobile phase composed of acetonitrile: 0.1% formic acid (80: 20, v/v) running at a flow rate of 1mL/min into the electrospray ionization (ESI) chamber. The API-3200™ triple quadrupole LC/MS/MS mass spectrometer (AB Sciex Instruments, Foster City, CA, USA) was equipped with an electrospray ionization source (ESI) operating in the positive ion mode with multiple-reaction monitoring (MRM) mode to detect the transitions for OL and the *m/z* 446.909 precursor ion to the *m/z* 207.100 product ion and for ORN; the *m/z* 219.949 precursor ion to the *m/z* 128.100 product ion. The peak area ratio of (OL/ORN) was plotted against OL concentration of 1.7 – 1002.9 (ng/mL) in blank rabbit plasma. The Analyst® Software 1.6 (AB Sciex Instruments, Concord, Ontario, L4K, 4V8, Canada) was used for the processing of the LC-MS/MS data.

Pharmacokinetic and Statistical Analyses: The pharmacokinetic parameters following oral administration of both treatments for each animal were estimated based on the non - compartmental analysis using Win Nonlin® software Ver.1.5 (Scientific consulting, Inc., Cary, NC, USA). The estimated pharmacokinetics parameters included; C_{max} (the maximum drug concentration; ng/mL), t_{max} (the time to reach C_{max}; h), $MRT_{0-\infty}$ (the mean residence time, h), $AUC_{(0-48h)}$ (the area under the plasma concentration - time curve from zero to 48 h; ng h/mL) and $AUC_{(0-\infty)}$ (the area under the curve from zero to infinity; ng h/mL) [30]. The relative bioavailability was calculated by dividing $AUC_{(0-48h)}$ of F6 over $AUC_{(0-48h)}$ of Benicar® tablets. The results are expressed as mean values of six rabbits ± S.D. The statistical significance of the results was checked using one - way ANOVA at a P-value of 0.05.

RESULTS AND DESCUSSION:

Development of OLM-Loaded SNEDDS: OLM suffers from a limited oral bioavailability in humans (26%), possibly due to its limited aqueous solubility, uncontrolled enzymatic conversion to the active metabolite OL as well as the efflux by P-gp in GIT. These factors restrict the overall permeability of the drug. In a parallel line, the concomitant use of OLM has been associated with the incidence of diarrhoea and certain intestinal problems. In fact, FDA had approved label changes to include intestinal problems (sprue-like enteropathy) linked to OLM [31]. The physical contact of OL with intestinal villi might be a trigger to local cell mediated immune response [13].

In view of the aforementioned, the design of OLM - loaded SNEDDS - where OLM was incorporated within the oily core of the resulting Nanoemulsion - was explored in an attempt to surmount such limitations and improve the oral drug bioavailability. It should be noted that the proper selection of the oil, surfactant, and co-surfactant

would allow complete solubility and optimum drug loading in SNEDDS. So that, it should be promptly dissolved as clear monophasic liquid, without physical accumulation, when introduced to gastrointestinal fluids.

The saturated solubility of OLM in various vehicles was shown in **Fig. 1**. For the investigated oils, the highest solubility for OLM was achieved with Capmul MCM® (7.26mg/g). The solubility of OLM in Tween 20® (40.2mg/g) was greater than that in Cremophor® EL (10.7mg/g). Yet, both non-ionic surfactants were selected for incorporation in SNEDDS for many reasons, including; (i) high safety, low toxicity,high stability, and biodegradability [32], (ii) minimal irritation potential when compared to other ampholytic, anionic or cationic surfactants [11], (iii) their highly hydrophilic nature (HLB values > 12) would allow lowering of the surface tension and formation of fine, uniform Nanoemulsion droplets upon contact with gastrointestinal fluids [22], and (iv) their transporter inhibition activity on P-glycoprotein in Caco-2 cell monolayers of the intestinal mucosa *via* increasing apical-to-basolateral permeability and decreasing basolateral - to - apical permeability of P-gp substrates [33]. On the other hand, PFG 400 was the co-surfactant of choice where the solubility of OLM was 44.8mg/g. Recent reports showed that polyethoxylated excipients, like PEG, can inhibit P-gp activity in Caco-2 cell monolayers.

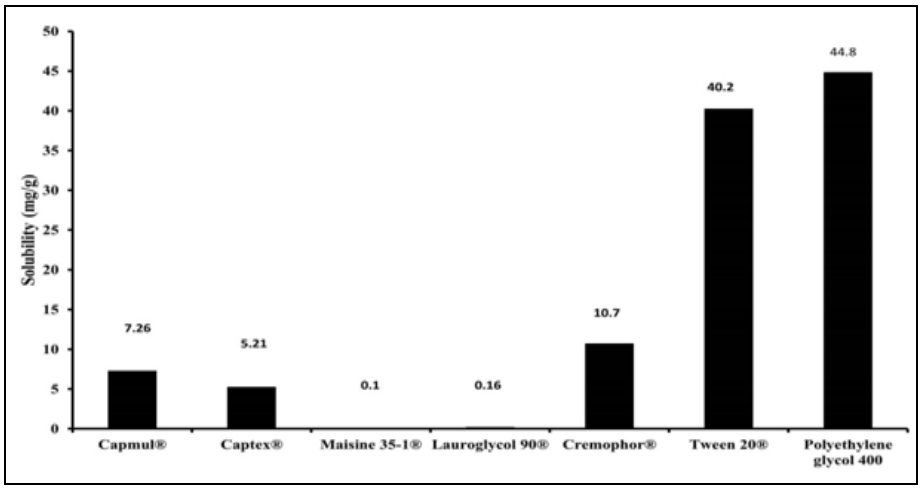

FIG. 1: SATURATED SOLUBILITY BAR CHART OF OLM IN VARIOUS VEHICLES

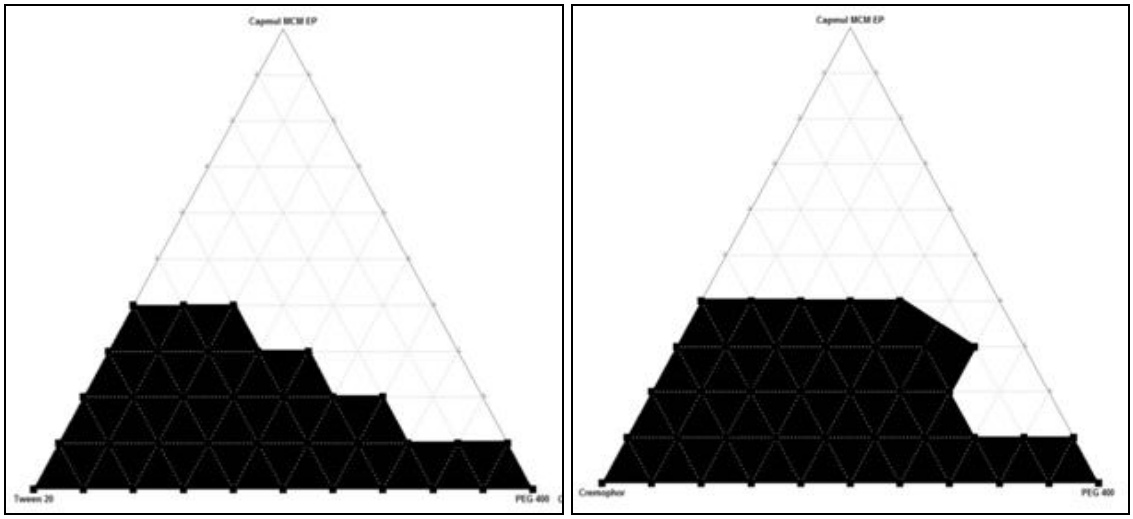

FIG. 2: TERNARY PHASE DIAGRAMS CONSISTING OF CAPMUL, TWEEN 20 AND PEG 400 (A) AND CAPMUL, CREMOPHORE EL AND PEG 400 (B) [BLACK DOMAINS INDICATE THE SELF-EMULSIFICATION REGION]

It was revealed that PEG-induced changes in P-gp activity are probably related to changes in the fluidity of the polar head group regions of cell membranes [20]. PEG 400 is a short-chain alcohol that can reduce the critical packing parameter of the investigated surfactants and would facilitate the

formation of stable o/w microemulsions [34]. Furthermore, it has a positive influence on the fluidity of the surfactant film and provides sufficient flexibility to the interfacial film to be able to attain different curvatures required to form promising microemulsions [35]. In view of the aforementioned, two ternary phase diagram plots were constructed to identify the clear self-nanoemulsifying areas suitable for loading OLM (1% w/w); **Fig. 2**. The AUC of Cremophor® EL-based system (2338.26 square units) was wider than that of Tween® 20-based one (19.9185 square units); indicating better self-nanoemulsifying ability of the former system. This difference was proved to be statistically significant ($P > 0.05$). These results were in line with those reported by Yin *et al*., [36] and Tayel *et al*., [24] who found that Cremophor® EL - based systems showed wider AUCs than the corresponding Tween® 80 based systems; suggesting the better emulsification ability of the former surfactant with respect to reduction in the interfacial energy as well as the provision of a mechanical barrier to coalescence [14].

Characterization of OLM-Loaded SNEDDS:

Determination of self-Emulsification Time: Previous reports showed that no significant differences were observed in the dispersibilty of non-ionic surfactant - based Nanoemulsions upon dispersion in water or simulated gastric or intestinal fluids. Therefore, water was used as a dispersion medium to simulate the emulsification efficiency of SNEDDS upon infinite dilution in GI fluids [12].

The self-emulsification times for OLM - loaded SNEDDS were represented in **Table 2**. It was clear that all systems exhibited rapid rates of emulsification with very short self-emulsification times upon contact with water; ranging from 12 s (F12) to 20 s (F1 and F8). These findings could be attributed to the proper selection of the type and concentration of SNEDDS ingredients.

Determination of Droplet Size, PDI and Zeta Potential: The droplet size is a crucial factor influencing the *in vivo* performance of SNEDDS. An inverse correlation could be established between the droplet size and the interfacial surface area. Larger surface areas have been associated with rapid drug absorption rates and higher drug bioavailability. The droplet size, PDI and zeta potential of the investigated OLM-loaded SNEDDS (F1 – F12) were shown in **Table 2**.

TABLE 2: CHARACTERIZATION OF OLM - LOADED SNEDDS, SUSPENSION AND BENICAR® TABLETS

System	Particle size (nm)	PDI	Zeta Potential (mV)	Self-emulsification time (s)	$Q_{5\ min}$ (%)	$Q_{1\ h}$ (%)	DE (%)
Suspension	-	-	-	-	11.20±1.98	34.73±1.02	24.55±1.26
Benicar®	-	-	-	-	6.66±3.29	65.57±1.04	33.71±1.67
F1	119.70±39.68	0.19	-15.23	20	22.23±2.46	60.02±3.65	39.27±2.00
F2	89.63±9.99	0.15	-11.6	14	24.45±1.11	57.79±1.45	40.19±2.01
F3	66.68±46.00	0.16	-11.26	15	24.45±2.76	55.57±1.11	41.49±2.04
F4	43.34±21.06	0.12	-7.53	15	33.34±1.01	51.12±1.87	40.10±2.50
F5	12.19±0.55	0.29	-9.64	14	26.67±2.21	62.24±1.10	37.60±1.86
F6	60.00±14.20	0.25	-14.4	13	29.78±1.00	66.69±0.91	47.96±1.09
F7	20.60±0.14	0.25	-10.12	15	26.67±3.28	46.68±1.33	34.54±1.65
F8	18.54±0.45	0.23	-11.2	20	29.78±0.21	57.79±3.29	40.77±2.07
F9	22.10±1.06	0.23	-7.92	14	24.45±2.21	51.12±1.86	35.84±1.23
F10	21.13±0.98	0.19	-9.93	13	26.67±2.09	44.46±1.75	33.99±2.03
F11	19.52±0.09	0.20	-6.65	13	31.12±2.09	57.79±2.22	42.77±2.21
F12	21.06±7.31	0.17	-9.5	12	28.89±3.02	51.12±1.75	38.80±1.20

Cremophor® EL-based systems ranged in size from 18.54 nm (F8) to 22.10nm (F9) while Tween® 20-based systems extended from 12.19nm (F5) to 119.70nm (F1). It could be inferred that the former systems had smaller droplet sizes than the corresponding ones of the latter system; confirming the better emulsification ability of Cremophor® EL; as noted earlier [24, 36].

All OLM-loaded SNEDDS had PDI ≤ 0.3; indicating a homogenous droplet size population and narrow globule size distribution. Negative zeta potential values were observed with OLM - loaded SNEDDS ranging from of - 6.65 (F17) to - 15.23 (F4) mV. The results might be attributed to the presence of negatively charged carboxylic acid groups of the free fatty acids in Capmul®[12].

Topographic Examination of SNEDDS: Representative photomicrographs of OLM - loaded SNEDDS (F6) were illustrated in **Fig. 3**. It was clear that the developed SNEDDS were fairly dispersed in aqueous media and formed homogeneous small-sized discrete spherical Nano emulsion droplets. The estimated droplet sizes from TEM images were in good agreement with those measured by the photon correlation spectroscopy; as noted earlier.

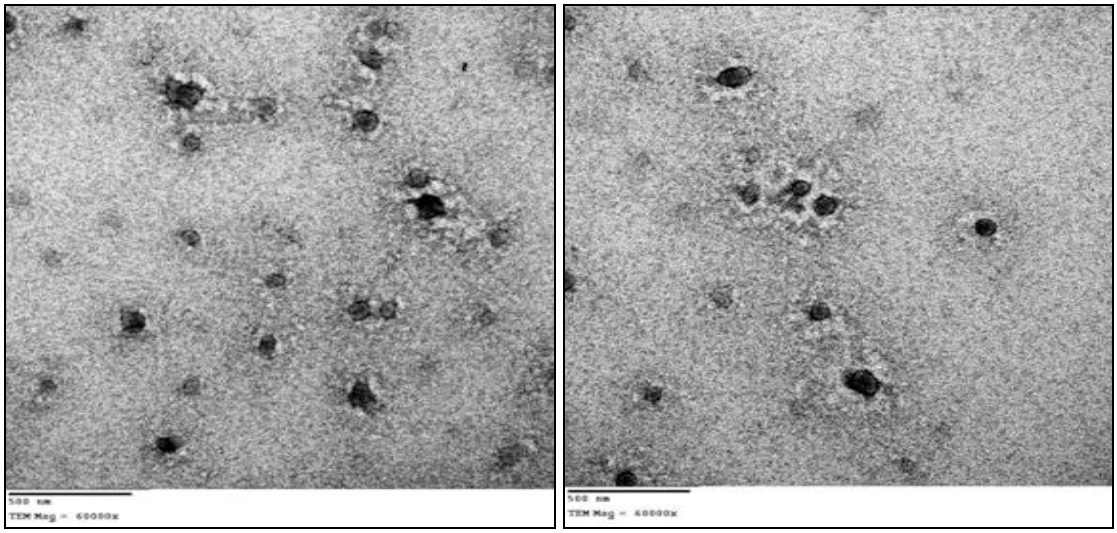

FIG. 3: REPRESENTATIVE TEM PHOTOMICROGRAPHSOF OLM LOADED SNEDDS

***In vitro* Drug Release Studies:** The *in vitro* release profiles of OLM from an aqueous suspension, Benicar® tablets in comparison to Tween® 20-based SNEDDS and Cremophor® EL-based SNEDDS in Sorensen's phosphate buffer (pH 6.8) at 37 ± 0.5 °C were illustrated in **Fig. 4** and **Fig. 5**. The drug released percentages after 5 min (Q_{5min} %) and 1 hour (Q_{1h} %) and the dissolution efficiency percentages after 1h (DE_{1h} %) were reported in **Table 2**.

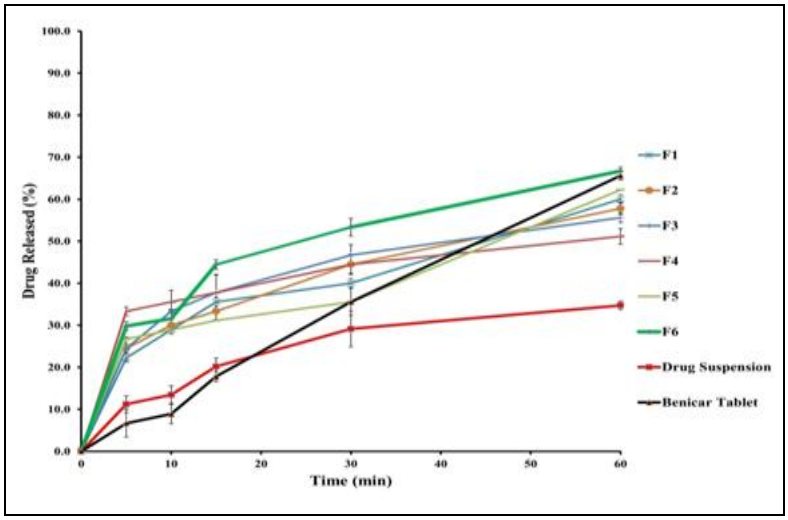

FIG. 4: *IN VITRO* RELEASE PROFILES OF AN AQUEOUS OLM SUSPENSION, BENICAR TABLETS AND OLM - LOADED SNEDDS COMPOSED OF CAPMUL, TWEEN 20 AND PEG 400 IN SORENSEN'S PHOSPHATE BUFFER (pH 6.8) AT 37 ± 0.5 °C (MEAN ± S.D., n = 3)

All SNEDDS showed higher Q_{5min} %, Q_{1h} % and DE_{1h} % than the aqueous drug suspension. This finding can be attributed to the small size of the developed Nanoemulsion droplets; thereby providing larger surface areas and faster drug release percentages. In a parallel line, all SNEDDS showed higher Q_{5min} % and DE_{1h} % than Benicar® tablets. Only one SNEDDS (F6) succeeded to achieve greater Q_{5min} %, Q_{1h} % and DE_{1h} % (29.78%, 66.69% and 47.96 %, respectively) than the corresponding values of Benicar® tablets (6.66%, 65.57% and 33.71%, respectively).

Yet, no-significant difference ($P > 0.05$) was observed between Q_{1h} % of both treatments. Based on the previous findings, SNEDDS (F6) was selected for further *in-vivo* studies.

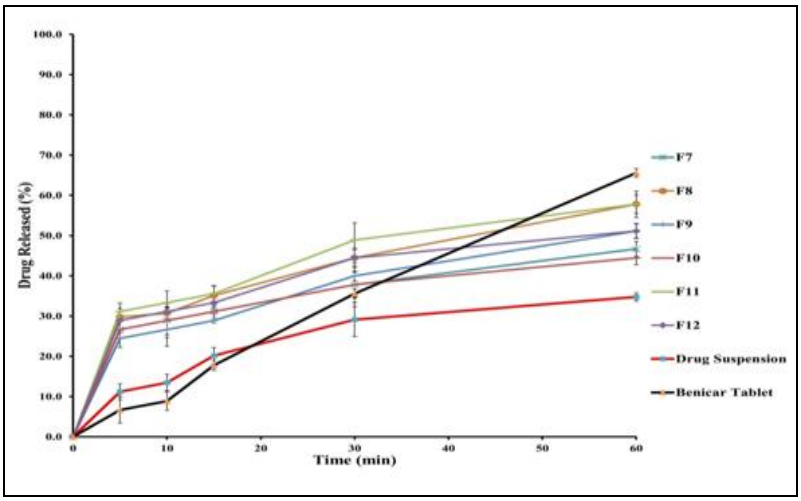

FIG. 5: *IN VITRO* RELEASE PROFILES OF AN AQUEOUS OLM SUSPENSION, BENICAR TABLETS AND OLM-LOADED SNEDDS COMPOSED OF CAPMUL, CREMOPHORE EL AND PEG 400 IN SORENSEN'S PHOSPHATE BUFFER (pH 6.8) AT 37 ± 0.5 °C (MEAN ± S.D., n = 3)

***In vivo* Drug Absorption Studies:** A previously reported selective and sensitive LC-MS/MS method for the determination of OL in plasma was adopted [11]. For the reported chromatographic conditions, the detection of OL and ORN was achieved at 0.48 and 0.53 min, respectively. A linear (r^2 = 0.999) calibration curve was constructed by plotting the peak area ratio of (OL / ORN) against OL concentration of 1.7 – 1002.9 ng/mL in rabbit plasma. The LLOQ (lower limit of quantification), representing 10: 1 signal / noise ratio was 1.7ng/mL. The Plasma concentration-time curves of OL following oral administration of OLM - loaded SNEDDS (F6) and Benicar® tablets in rabbits (mean ± S.D., n = 6) were portrayed in **Fig. 6**. The estimated pharmacokinetic parameters of both treatments, derived by non-compartmental fitting of data, were summarized in **Table 3**.

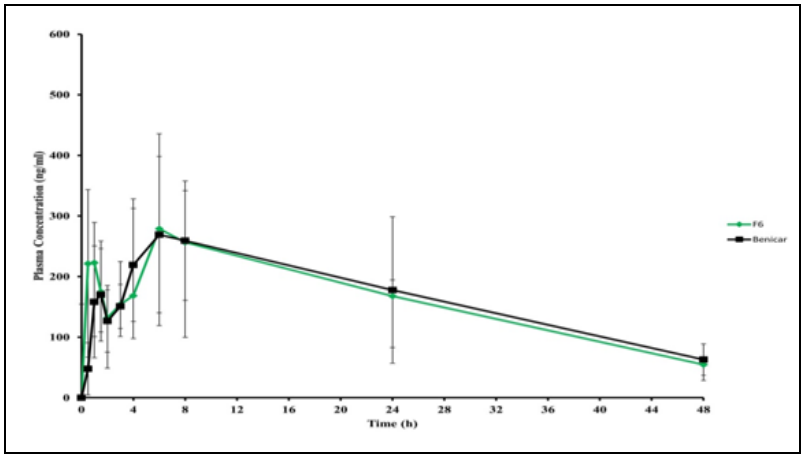

FIG. 6: PLASMA CONCENTRATION – TIME CURVE OF OLM FOLLOWING ORAL ADMINSTRATION OF OLM LOADED - SNEDDS (F6) AND BENICAR TABLETSINRABBITS (MEAN ± S.D., n = 6)

TABLE 3: PHARMACOKINETIC PARAMETERS OF OLM FOLLOWING ORAL ADMINISTRATION OF OLM - LOADED SNEDDS (F6) AND BENICAR® TABLETS IN RABBITS (MEAN ± S.D., n = 6)

Pharmacokinetic parameters	Benicar® tablets	OLM-loaded SNEDDS (F6)
C_{max} (ng/mL)	335.41 ± 98.12	387.55 ± 141.17
T_{max} (h)	5	3.5
$^{a}MRT_{(0-\infty)}$ (h)	31.93 ± 13.90	31.32 ± 16.54
$AUC_{(0-48\ h)}$ (ng.h.mL^{-1})	7040.89 ± 1793.92	7696.67 ± 3478.74
$AUC_{(0-\infty)}$ (ng.h.mL^{-1})	9205.62 ± 2438.83	9603.73 ± 3980.31

Clearly, the immediate release pattern of Benicar® tablets and SNEDDS (F6) were revealed. Benicar® tablets showed a maximum drug concentration (C_{max}) of 335.41ng/mL at a median T_{max} of 5 h. On the other hand, a higher C_{max} value of 387.55ng/mL was estimated for SNEDDS (F6) at a shorter median T_{max} of 3.5 h. In fact, the $MRT_{(0-\infty)}$ of SNEDDS (F6) was almost similar (31.32 h) to that estimated for the reference Benicar® tablet (31.93 h). The $AUC_{(0–48h)}$ and $AUC_{(0–\infty)}$ values of SNEDDS (F6) (7696.67 and 9603.73ng.h/mL, respectively) were larger than the corresponding values of Benicar® tablets (7040.89 and 9205.62ng.h/mL, respectively). Based on the calculated $AUC_{(0–48h)}$ values, the relative bioavailability % was found to be 109.31%. The higher drug bioavailability of SNEDDS could be related to a number of factors, including; (i) the generation of fine o/w Nanoemulsions upon contact with gastrointestinal fluids.

The fine oil droplets provide a large interfacial area for pancreatic lipases to hydrolyze the oily core and hence promote immediate drug release and/or the formation of drug-loaded mixed micelles of bile salts [21, 37]. (ii) the ability of Tween® 20 and / or PEG 400 to lower the interfacial tension and hence promote faster drug dissolution in gastrointestinal fluids, to increase intestinal epithelial permeability, to increase tight junction permeability and to inhibit P-gp efflux transporter activity [21, 33, 38, 39].

CONCLUSION: OLM - loaded SNEDDS were successfully developed using Capmul MCM®, Cremophor® EL or Tween20® and PEG400®. At an optimum Capmul MCM®: Tween20®: PEG400® ratio of 20: 60: 20, the best achieved SNEDDS (F6) showed short emulsification time, fine droplet size, low PDI, negative zeta potential, promising *in vitro* dissolution parameters with respect to Q_{5min} %, Q_{1h} % and DE_{1h} %. When compared to Benicar® tablets, the later system showed shorter T_{max}, higher C_{max} and larger $AUC_{(0–48h)}$ in rabbit plasma. Further *in-vivo* absorption studies on a larger number of animals are needed to minimize the variations in the estimated pharmacokinetic parameters.

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