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FORMULATION, OPTIMIZATION AND EVALUATION OF FEBUXOSTAT LOADED SNEDDS FOR TREATING GOUT

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ABSTRACT: Febuxostat (FXT), used for treating gout, is poorly soluble and is susceptible to enzymatic degradation when administered as tablets. The current study aims to formulate, optimize and develop a stable Liquid self-nanoemulsifying drug delivery system (L-SNEDDS) and Solid SNEDDS to improve solubility, resulting in enhanced bioavailability and further to formulate Self-Nano emulsifying Osmotic Pump Tablets (SNEOPT) for controlled drug release. A ternary plot was constructed with Capmul MCM (oil phase), Tween 80 (surfactant), and PEG 400 (co-surfactant). Further optimization was done by D-Optimal design. Optimized L-SNEDDS (F8) was then converted to S-SNEDDS by the adsorption method. Aerosil 200 was used as an adsorbent. The L-SNEDDS and S-SNEDDS were characterized and evaluated. The particle size of the optimized batch was 97.25 nm (L-SNEDDS) and 155.2 nm (S-SNEDDS). *In-vitro* dissolution studies showed>75% release in pH 1.2 HCl buffer and >80% in phosphate buffer pH 7.4 in 1 hour. SEM (Scanning electron microscopy) studies indicated spherical particle morphology. SNEOPT was prepared by direct compression (with NaCl as the osmogent). *In-vitro* release from SNEOPT showed zero-order drug release kinetics. The developed formulation was found to be superior to pure FXT with enhanced solubility, which signifies that a lipidic system is an efficacious drug delivery system for treating gout.

INTRODUCTION: Gout is one of the commonest forms of rheumatological disorder and a type of inflammatory arthritis that can be diagnosed with certainty and treated properly. Imbalanced synthesis, as well as excretion of uric acid, increases the serum urate acid level [1]. Monosodium urate crystal (MSU) and Hyperuricemia (increase in urate level above 6.8mg/dl) are the most relevant feature of gout. The major co-morbidities related to gout are cardiovascular disorders as gout is allied with obesity, dyslipidemia, hypertension, and insulin resistance leading to diabetes [2, 3]. The relevance of gout is communal in men and less in women because of the uricosuric effect of estrogen. But in the postmenopausal period, the frequency of gout tends to rise [4]. Colchicine, NSAIDs, oral glucocorticoids, intraarticular glucocorticoids, interleukin inhibitors, *etc*., are widely used for the treatment, but rapid withdrawal lead to rebound gout attack [5].

Febuxostat (FXT) is one of the xanthine oxidoreductase inhibitors belonging to BCS class II with high lipophilicity but is considered more effective than allopurinol. It shows 99% of protein binding, 5-7 h of plasma half-life, and 45-50% of bioavailability. Its limited water solubility and degradation by enzymes limit bio-availability. Complexation with cyclodextrins, micronization, solid dispersions, solid mixtures, ODT and

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nanosuspensions are some techniques used to improve solubility, dissolution, and bioavailability Febuxostat [6-12]. Lipid-Based Drug Delivery Systems are an important class of drugs that have attracted the interest of researchers. The flexibility of the lipidic excipients, low-risk profile, amended solubility is some of the advantages favoring a lipid-based drug delivery system as an alternative technique to efficiently deliver poorly soluble drugs [13].

SNEDDS (Self nano emulsifying drug delivery system) is an isotropic, thermodynamically stable mixture of oils, surfactants, and co-surfactants, which, coming in contact with gastrointestinal fluids, leads to the formation of a fine o/w nanoemulsion on gentle agitation. The hydrophobic moiety is in the solution form, which maintains the drug in the dissolved phase. Here the dissolution stage is avoided due to which the release is faster. Novel approaches in SNEDDS have also emerged with improvement in solubility, reduction in the hepatic clearance of the drug, and bypassing the first-pass metabolism, no influence of lipid digestion process as compared to other lipid nanocarriers and ease of scale-up.

Absorption takes place by lymphatic transport *via* transcellular and paracellular mediated transport pathways, which obstruct cytochrome P-450 and P-glycoprotein efflux [14]. In spite of these advantages, there are certain shortcomings, including stability issues, possible interaction with the excipients and the capsule shell, and hence to overcome these limitations, L-SNEDDS (Liquid Self nano emulsifying drug delivery system) are converted to S-SNEDDS (Solid SNEDDS). High process control, better stability, and better patient compliance are some advantages as compared to the L-SNEDDS [15]. Intercurrent gout is one of the clinical presentations of acute gout, which starts at night and extends till morning [16]. Hence, the current work endeavors to formulate, optimize, and develop stable FXT SNEDDS to improve the solubility and stability, enhancing bioavailability with the objective of assessing *in vitro* release of optimized batches of L-SNEDDS and S-SNEDDS. Also, to develop a Self Nano-emulsifying Osmotic drug delivery (SNEOPT) system for improved bioavailability and controlled drug release to treat intercurrent gout.

MATERIALS AND METHODS:

Materials: FXT was obtained as the gift sample from Lupin Pharma, Vadodara, India. Capyrol 90, Labrafac, Labrasol, Masine were gifted by Gatteffose, Germany. Capmul MCM NP was a gift sample obtained from Abitec Corporation, USA. PEG 200, PEG 400, PEG 600 were bought from Dow Chemicals Pacific Limited, Europe. Aerosil 200 was acquired from Evonik India Pvt. Ltd. HPMC K4M and Opadry CA were obtained as the gift sample from Colorcon, Inc. Sodium Chloride, Sodium hydroxide, Hydrochloric acid, Disodium hydrogen phosphate, Potassium Chloride, and Methanol were supplied from Merck Specialties Pvt. Ltd, India. All the reagents and chemicals utilized were of analytical grade.

Methods:

Solubility Studies: Solubility measurement of FXT in oils, surfactants, and co-surfactants was done by dissolving an excess amount of drug in a fixed amount (2ml) of excipients followed by vortex mixing for 30 sec. Mixtures were stirred for 48 h on a magnetic stirrer and kept for 24 h. Mixtures were then centrifuged at 3000rpm for 10min, and the supernatant was filtered by 0.45 µm membrane filter to remove the undissolved drug. Samples were suitably diluted with methanol and drug concentration was obtained via UV validated method at 315 nm using methanol as a blank (UV Spectrophotometer Evolution 300- Thermo Fisher Scientific) [17, 18]. Further screening of surfactant and co-surfactant was done by transmittance study.

Pseudo Ternary Diagram:

a. Oil, surfactant and co-surfactant with highest FXT solubility were selected. A pseudo-ternary phase diagram was constructed by aqueous titration method, considering an oil phase, surfactant and cosurfactant (S mix), and aqueous phase. Firstly, optimized oil phase was dissolved in S_{mix} (1:1, 2:1, 1:2) in the ratio 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:1, and 1:9, in a glass test tube. Each ratio of surfactant and the oil phase was then titrated against the aqueous phase. Turbidity was referred to as an endpoint 19. The percentage composition of the component in each ternary system was determined following up with the plotting of observed results on triangular coordinates to

construct the phase diagram with the help of Prosim software, a ternary plot was put up to ensure the optimum self-emulsifying zone.

b. The ratios (S_{mix}) from (a) *i.e* water titration method which exhibited a wider region were further selected for transmittance study and emulsification time study.

c. The ratios which exhibited less emulsification time and more transmittance were selected for the ternary diagram. Phase diagrams were then constructed using Prosim software.

Optimization by QbD: D-optimal design was chosen since it minimizes the variance associated with the estimates of the coefficients in the model. Candidate points were selected by software as a base design (16 runs). These included various factorial points [20].

The Mixture Experimental Study was Designed Based on a 3 Component System: the oil phase X1 (Capmul MCM), the surfactant X2 (Tween 80) and the co-surfactant X3 (PEG 400).

The total concentration of the 3 components was rounded to 100%. The drug content was kept constant for the prepared SNEDDS.

Based on the previous results obtained from the phase diagram, the range of each component was selected as follows: X1 (30–50%), X2 (20–70%), and X3 (20–40%). The emulsification time of diluted SNEDDS (Y1), mean droplet size (Y2) and transmittance (Y3) were used as the responses (dependent variables).

The responses of all model formulations were treated by Design-Expert® software (version 12; Stat-Ease, Inc., Minneapolis, MN).

Cubic, quadratic and special cubic models were the suitable models for mixture designs consisting of three components.

For the selection of the mathematical model, various statistical parameters, including the standard deviation (SD), the multiple correlation coefficient (R2), the adjusted multiple correlation coefficient (adjusted R2) and the predicted residual sum of square (PRESS)proved by Design-Expert software.

Preparation of FXT L-SNEDDS, S-SNEDDS, and SNEOPT:

L-SNEDDS: FXT (40 mg) was initially dispersed in a Tween 80 and PEG 400 (S mix) mixture and gently vortexed at room temperature for 10 min. Capmul MCM NF was accurately weighed and mixed with the S_{mix} and subjected to sonication for 20 min for obtaining a yellowish transparent emulsion. These were then kept for storage in air-tight containers at 25 °C for further experimentation.

S-SNEDDS: L-SNEDDS was converted into S-SNEDDS by adsorbing it to different porous solid carriers. Optimized batch (F8) of L-SNEDDS was taken, and increasing quantities of solid carriers were added till it formed a solid mass. Different solid carriers like Aerosil 200, Aerosil 300, Mannitol, Maltodextrin, spray-dried lactose were used to adsorption L-SNEDDS. Micromeritics parameters were noted for S-SNEDDS [21]. A solid carrier, which gave good flow properties, was selected.

SNEOPT [22, 23]: The formulation of S-SNEDDS which showed good powder characteristics was selected to prepare FXT osmotic pump tablets. The tablet core was prepared by the direct powder compression method.

The following procedure was prepared: Optimized L-SNEDDS (F8) batch was loaded on Aerosil 200 by simple mixing to form the S-SNEDDS. Avicel 102, HPMC-K4M, sodium chloride (osmotic agent), and Magnesium stearate were then mixed with the S-SNEDDS and punched by using a die of 12mm to form the core tablet. The concentration of the osmotic agent was varied in the range of100-200mg.

The coating solution was prepared with the help of a coating agent (Opadry®CA) and mannitol (pore-forming agent). Optimization of SNEOPT is shown in **Table 1.** The release behavior was investigated by carrying out the dissolution studies to check whether the formulated SNEOPT showed controlled release.

Effect of concentration of Coating agent (1%, 2%, 3%) pore-forming agent (2-5% w/w of Opadry®CA of NaCl and Mannitol) and osmotic agent (10% and 15 %) was studied.

TABLE 1: OPTIMIZATION CORE TABLET

Batch No	F1	F2	F3	F4	F5	F6	F7	F8
Qty (mg)								
Oil	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Surfactant	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Co-Surfactant	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106
Avicel 102	0.176	0.176	0.176	0.176	0.176	0.176	0.176	0.176
Aerosil 200	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium Chloride	0.110 (10%)	0.176 (15%)	0.110	0.176	0.110	0.176	0.110	0.176
HPMC K4M	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.017
Magnesium stearate	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011

COATING SOLUTION

Batch No	F1	F2	F3	F4	F5	F6	F7	F8
CA (%)	2	2	2	2	2	2	2	2
NaCl (% of CA)	2	2	5	5	---	---	---	----
Mannitol (% of CA)	---	----	----	----	2	2	5	5

Evaluation and Characterization of L-SNEDDS, S-SNEDDS and SNEOPT [24, 25]:

Drug Content: L-SNEDDS (1 ml) and S-SNEDDS (500 mg) were determined by dissolving in 10 ml methanol and sonicating for 5 min. The methanolic extract was suitably diluted and analyzed by UV at 315 nm, keeping methanol as the blank.

For SNEOPT, 6 tablets that contained 20 mg of the drug were weighed and finely powdered. This powder was completely dissolved in methanol, and the solution was filtered and evaluated by using a validated UV method.

Dilution Study: Dilution 50, 100, and 1000 times with distilled water of L-SNEDDS was done and evaluated for changes in transmittance. Also, the effect of pH, on optimized SNEDDS, was checked by diluting with 0.1 N HCl buffer (pH 1.2) and phosphate buffer (pH 6.8 and pH 7.4), and % transmittance was measured at 638.2nm [26].

Emulsification time: To assess the self-emulsification properties 50 mg of L-SNEDDS and S-SNEDDS was introduced into 50 ml of distilled water in a beaker at 25°C and gently stirred using a magnetic stirrer at 50 rpm. The tendency and time required to spontaneously form a transparent emulsion were recorded [27].

Cloud Point Measurement: Each formulation was diluted with water (1:100) and placed in a water bath with a gradual increase in temperature.

The temperature at which there was a sudden appearance of cloudiness was visually considered the cloud point.

Viscosity: The viscosity of FXT-SNEDDS was determined with Brookfield viscometer using spindle no 16 at 150 rpm at room temperature.

Emulsion Droplet size, PDI and Zeta Potential: The particle size and polydispersity index of L-SNEDDS and S-SNEDDS were analyzed using Malvern Zetasizer Nano ZS (by Malvern Instruments). This instrument worked on the principle of Dynamic Light Scattering (DLS) with 'Non-invasive backscatter optics (NIBS) optics. The dilutions were performed with double distilled water (1:100). The dispersion was filled into the cuvettes and was analyzed.

Thermodynamic Stability Studies: The stability of the optimized L-SNEDDS formulation was evaluated at different stress conditions such as heating-cooling cycles (4 °C and 40 °C) and freeze-thaw cycles (−21 °C and +25 °C) along with storage at the specified temperature for 48 hrs. To carry out the centrifugation stress study, 1 mL of the formulation was diluted to 100 ml with distilled water and centrifuged at 10000 rpm for 20 min, and visually observed for any phase separation.

***In-vitro* Drug Dissolution Studies:** SNEDDS was filled in size '00' hard gelatin capsules. *In vitro* dissolution profiles of L-SNEDDS and S-SNEDDS formulations were obtained and compared with the marketed (Febutaz 40) and pure drug, using USP apparatus II at 37±0.5 °C with a rotating speed of 50 rpm in dissolution media, namely, phosphate buffer 6.8 and 7.4 pH and pH 1.2 HCl buffer. During the study, 5 ml of the aliquot was removed at predetermined time intervals (0, 5, 10, 20, 30, 45

and 60 min) from the dissolution medium and replaced with a fresh medium to maintain the sink conditions. The amount of drug released in the dissolution medium was determined by UV spectrophotometrically at 315nm. For SNEOPT, the release studies were carried out in USP apparatus II rotating paddle at 50 rpm and 37±0.5°C, using a 900 mL suitable medium *i.e.* first 2hrs in pH 1.2 HCl buffer and further 6-8 h in phosphate buffer pH 7.4. Samples of 5 ml were withdrawn at predetermined time intervals (0.5, 1, 1.5, 2, 2.5, 3, 4, 6, and 8 h) and analyzed spectrophotometrically at 315 nm using a UV-Visible. Sink condition was maintained by adding equal volume maintained at the same temperature. A comparison was made between the release profile of FXT-SNEOPT and the marketed tablets.

X-ray Diffractometry (XRD): X-ray powder scattering measurements of FXT, adsorbent (Aerosil 200), and FXT-loaded S-SNEDDS were carried out with XRD (Bruker D8 Advance XRD) to look for any deviation in solid-state properties of the drug when L-SNEDDS is converted to S-SNEDDS.

Scanning Electron Microscopy (SEM): Scanning electron micrographs of the S-SNEDDS were taken using field emission gun-SEM (JSM-7600F, JEOL USA, Peabody, MA) to observe surface morphology as well as shape. The sample was dispersed in distilled water, and a drop was placed on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with platinum to a thickness of about 10 A ° under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The stub containing the coated samples was placed in the SEM chamber for analysis.

Physical Evaluation of SNEOPT: The thickness and diameter of tablets was measured using Vernier calipers, hardness by Monsanto Hardness Tester, and Friability by Roche Friability Tester. Mechanism of release (Release Kinetics) of SNEOPT An ideal osmotic pump system should exhibit zero-order kinetics (constant release rate).

To study the mechanism of drug release from osmotic tablets, release data were analyzed according to zero order, first order, and Higuchi kinetic equations. The model with the highest coefficient of determination (R^2) was considered to be the best fitting one.

RESULTS AND DISCUSSION:

Solubility Analysis: The components used in the system should have high solubilization capacity for the drug ensuring the solubilization of the drug in resultant dispersion.

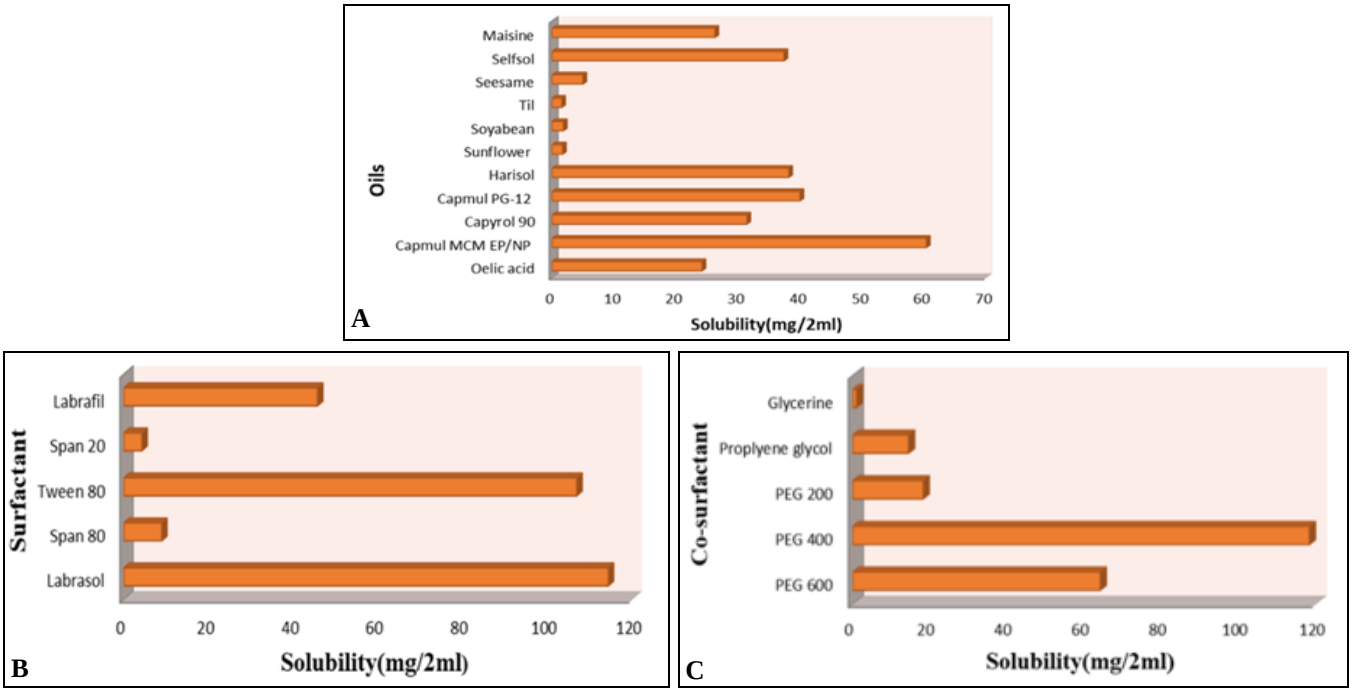


FIG. 1: (A) SOLUBILITY OF FXT IN VARIOUS OILS, (B) SOLUBILITY OF FXT IN VARIOUS SURFACTANTS, (C) SOLUBILITY OF FXT IN VARIOUS CO-SURFACTANTS

The solubility of FXT was tested in different oils, surfactants, and co-surfactants, which are commonly utilized in SNEDDS formulation.

The graphical representation of results of solubility studies of FXT is shown in **Fig. 1A**, **Fig. 1B,** and **Fig. 1C,** which revealed that the drug shows the highest solubility in Capmul MCM (60.37mg/ 2ml) and Sefsol (37.42mg/ 2ml).

In the case of surfactants, the drug exhibited good solubility in Labrafil (45.7mg/2ml), Tween 80 (106.85 mg/2ml), and Labrasol (114.29mg/ 2 ml), which may be due to their medium carbon chain (C8-C16) while among the co-surfactants higher solubility was found in PEG-400 (118.21 mg/2ml) and PEG-600 (64.07 mg/ 2ml) which may be due to the fact that PEGs are miscible with both aqueous and organic solvents.

A right blend of low and high HLB surfactants is necessary for the formation of a stable nanoemulsion in the development of a self-emulsified formulation. In this study, Labrafil, Tween 80, and Labrasol were selected further based on the solubility study. Also, it is reported that they possess bioactive effects like lymphotropic character (Tween 80) and an effect on the tight junction (labrasol). It has been reported that well-formulated SNEDDS is dispersed within seconds under gentle stirring conditions.

Surfactants were screened collectively on the basis of the % transmittance study **Table 2.** Based on solubilization potential, emulsification ability, and % transmittance, Tween 80 and Labrasol were considered for further study.

Among all co-surfactants, PEG 400 showed maximum transmittance Table 3 with Tween 80 and then PEG 600. Therefore, PEG 400 was selected as co-surfactants with tween 80 and labrasol as surfactants for further studies.

TABLE 2: EMULSIFICATION EFFICACY WITH SELECTED OIL AND DIFFERENT SURFACTANTS

Oil	Surfactant	Transmittance (%)
Capmul MCM	Tween 80	93.924
Capmul MCM	Labrafil	58.462
Capmul MCM	Labrasol	68.217
Sefsol	Tween 80	86.963
Sefsol	Labrasol	83.642
Sefsol	Labrafil	50.741

TABLE 3: EMULSIFICATION EFFICACY WITH SELECTED OIL, SURFACTANT AND DIFFERENT CO-SURFACTANTS

Oil	Surfactant	Co-surfactant	Transmittance (%)
Capmul MCM	Tween 80	PEG 400	87.548
Capmul MCM	Labrasol	PEG 400	83.452
Capmul MCM	Tween 80	PEG 600	79.401
Capmul MCM	Labrasol	PEG 600	74.892
Sefsol	Tween 80	PEG 400	81.182
Sefsol	Labrasol	PEG 400	79.420
Sefsol	Tween 80	PEG 600	75.854
Sefsol	Labrasol	PEG 600	71.452

Pseudo Ternary Diagram: Identification of self-nano emulsifying region and determining the concentration of the excipients required was done by plotting pseudo ternary plot [29]. The yellow color in **Fig. 2A–2C** indicates the region of self-emulsification. From the phase diagram, it is clearly observed that Smix 1:1 **Fig. Fig, 2A** and **2:1 Fig 2B** had a good region of self nano emulsification compared to 1:2 **Fig. 2C,** which might be due to the synergistic effect of surfactant and co-surfactant. Increasing the ratio of Smix would further lead to the generation of the liquid crystalline phase. Hence, there is no need to increase the concentration because the SNEDDS zone started to decrease (data not shown) [28]. **Fig. 2D** shows the actual zone with nanosized droplets.

Tween 80 decreases the interfacial tension, and PEG acts as the moiety, which enhances the wetting and improves the emulsification Capmul MCM C8® penetrates through surfactant film, creating void spaces hereby increasing the interfacial fluidity.

The surfactants with an HLB value greater than 10 are significantly superior in providing small and uniform microemulsion droplets with less particle size [30].

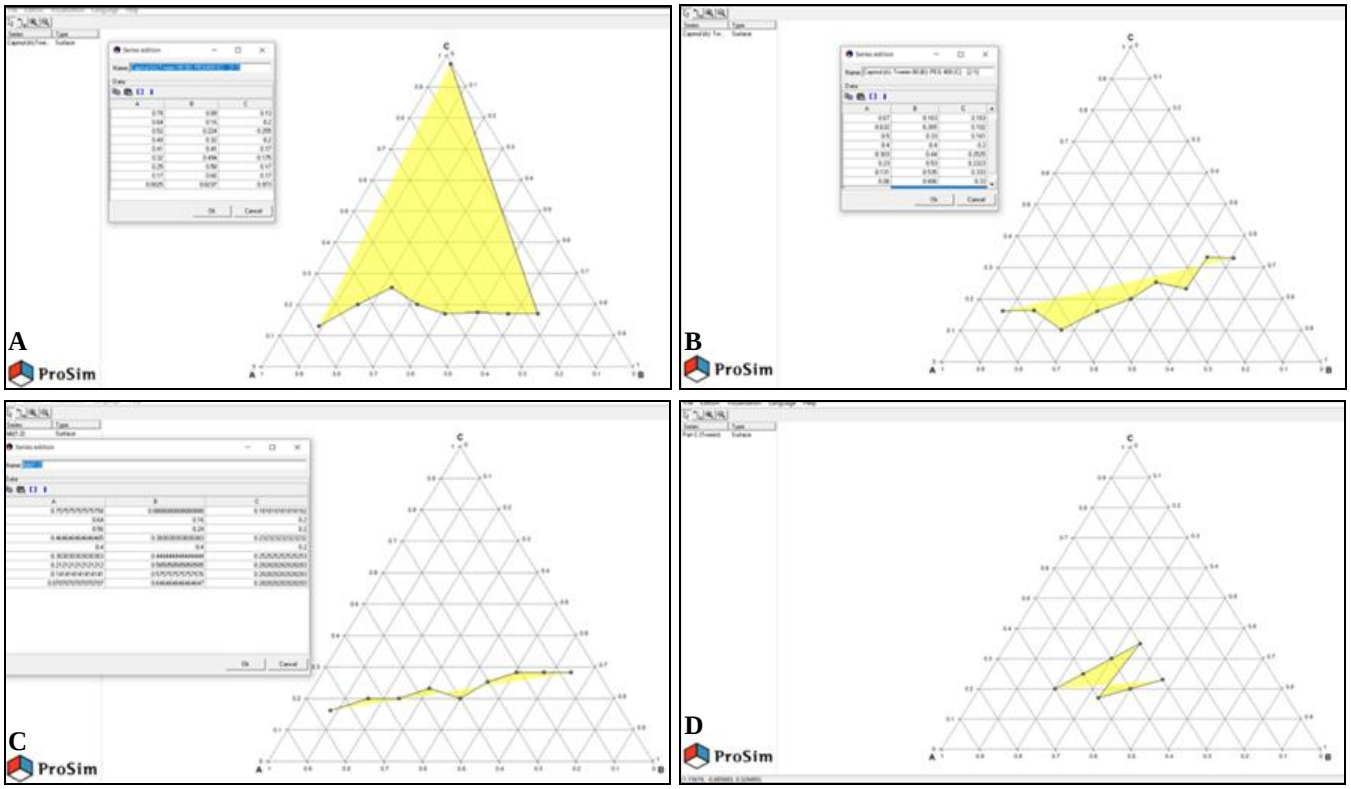


FIG. 2: (A) PSEUDO TERNARY PLOT OF CAPMUL MCM EP/NF: TWEEN 80: PEG400[S_{MIX}=1:1], (B) PSEUDO TERNARY PLOT OF CAPMUL MCM EP/NF: TWEEN 80: PEG400[S_{MIX}=2:1], (C) PSEUDO TERNARY PLOT OF CAPMUL MCM EP/NF: TWEEN 80: PEG400[S_{MIX}=1:2], (D) TERNARY PLOT OF CAPMUL MCM EP/NF (OIL): TWEEN 80(SURFACTANT): PEG400 (CO-SURFACTANT)

Optimization Using Design of Experiments: As per the ternary phase diagram, oil phase (30–50%), surfactant phase (20–50%), and co-surfactant phase (20-40%) concentrations were chosen for the D-optimal design. A sum of 16 runs was carried, and the responses are recorded in **Table 4.** The independent and response variables were related using a polynomial equation with statistical analysis through Design-Expert® software.

TABLE 4: ACTUAL DESIGN

Batch No	A (%Oil)	B (% Surf)	C (% Co Surf)	Y1 (% Transmittance)	Y2 (Emulsification Time in sec)	Y3 (PS in nm)
F1	0.3	0.3	0.4	94.17	40	189.44
F2	0.3	0.3	0.4	94.16	42	185.25
F3	0.5	0.2	0.3	82.43	49	193.65
F4	0.4	0.2	0.4	79.19	70	285.45
F5	0.44	0.27	0.27	87.41	33	146.32
F6	0.3	0.5	0.2	99.35	18	115.65
F7	0.5	0.3	0.2	73.57	36	152.26
F8	0.3	0.38	0.31	100.44	10	97.25
F9	0.4	0.2	0.4	85.116	58	280.63
F10	0.3	0.5	0.2	99.35	18	120.31
F11	0.38	0.33	0.28	94.055	24	136.21
F12	0.35	0.42	0.22	100.44	8	115.2
F13	0.37	0.27	0.34	90.385	38	165.21
F14	0.42	0.37	0.2	96.858	20	141.35
F15	0.5	0.2	0.3	52.419	50	278.45
F16	0.5	0.3	0.2	73.67	38	152.26

% Transmittance (Y1): Response (Y1) of nanoemulsion showed a Transmittance in the range of 73.57 to 100.44%. The model found appropriate by design was cubic. The F-value was 48.05 with a *p value* < 0.05, indicating that model terms are significant.

The p values of the model terms AB, BC, AC, ABC, AB (A – B), AC (–C) and BC (B–C) were found to be < 0.05, indicating their significant role on the response. These terms are considered as significant as described in **Fig. 3A** and **3B;** the contour and surface plots show the transmittance which is impacted by each and every factor. The relationship can be seen by the equation below:

Transmittance = 10.79*A + 99.36 *B + 47.50* C+147.10*AB+239.18*AC+106.74*BC424.4*AB C+8.29* AB (A-B) + 84.48*AC (A-C)-76.38*BC (B-C).

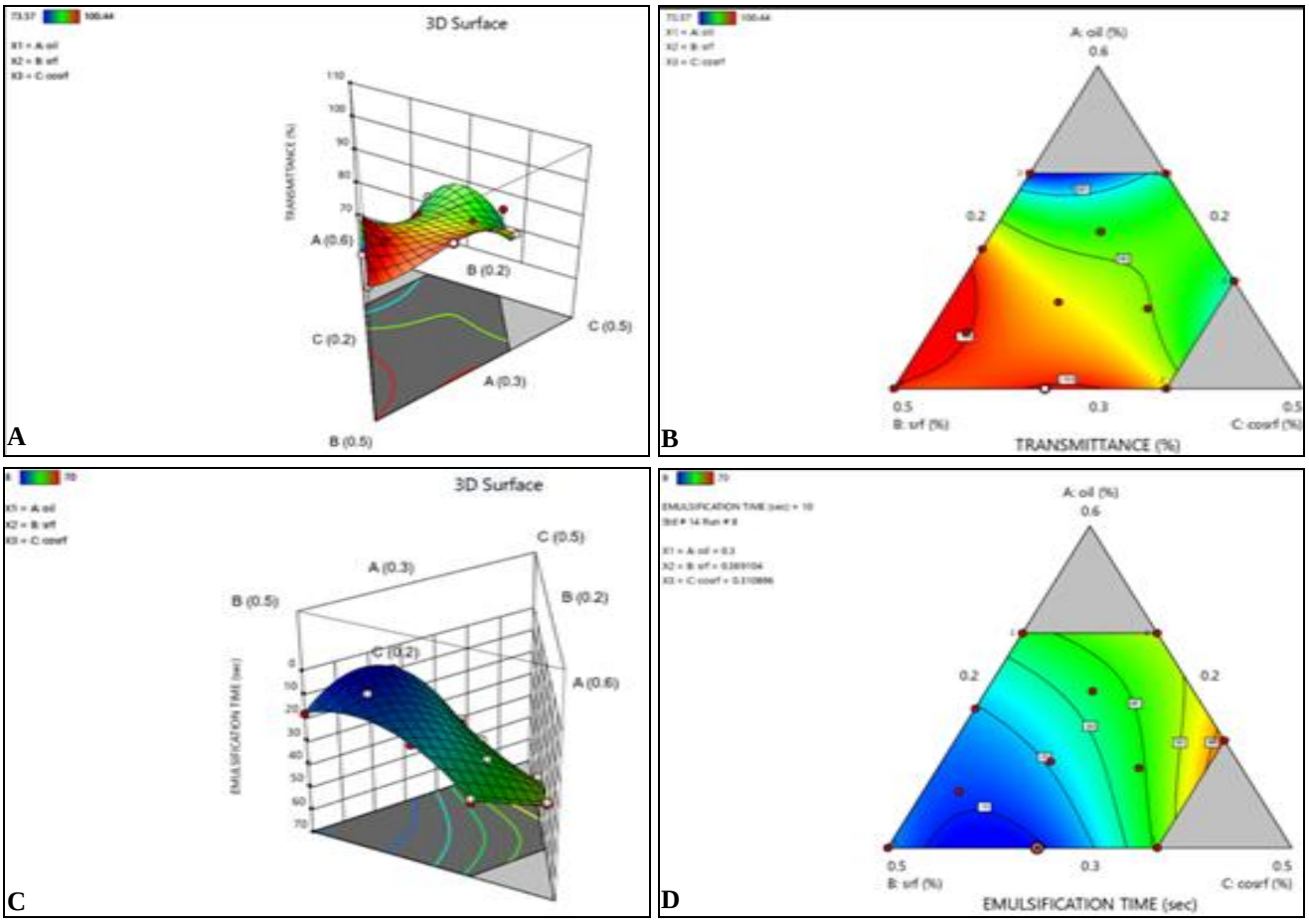
Emulsification Time (Y2): Similarly, Response (Y2) of nanoemulsion showed emulsification time in the range of 8 – 70 sec. The design suggested a special cubic model where F-value was 61.38 with a *p-value* < 0.05, indicating that model terms are significant. The p values of the model term AB, BC, AC, ABC were found to be < 0.05, indicating their significant role on the response. This is depicted in **Fig. 3C,** and **3D** by contour as well as surface plots show the emulsification time, impacted by every factor, and the relationship can be seen by the equation below:

Emulsification Time = 80.2141 * A + 17.5303 * B + 121.021 * C + -102.433 * AB + -197.778 * AC + -201.955 * BC + 324.094 * ABC.

Particle size (Y3): Response (Y3) *i.e.*, particle size, was found in the range of 97.25 – 285.45nm where The software suggested a quadratic model. The F-value was found to be 22.15, with a p-value < 0.05 indicating that model terms are significant. The p values of the model terms AB, BC, AC were were found to be < 0.05, indicating their significant role on the response.

The contour and surface plots show the Particle size, which is impacted by each and every factor in **Fig. 3E –3F**. The equation below gives the relationship between the response and the factors.

Particle Size = 219.984 * A + 121.155 * B + 383.751 * C + -134.484 * AB + -204.357 * AC + -505.393 * BC.



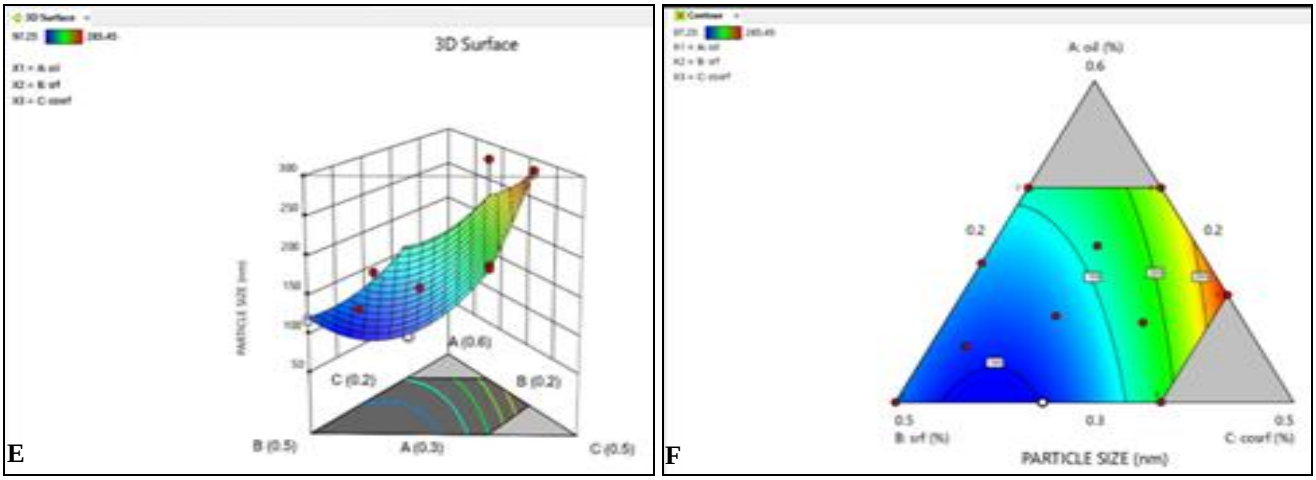


FIG. 3: (A) 3D RESPONSE OF TRANSMITTANCE, (B) CONTOUR PLOT OF TRANSMITTANCE, (C) 3D RESPONSE SURFACE OF EMULSIFICATION TIME, (D) CONTOUR PLOT OF EMULSIFICATION TIME, (E) 3D RESPONSE SURFACE OF PARTICLE SIZE, (F) CONTOUR PLOT OF PARTICLE SIZE

Characterization of L-SNEDDS and S-SNEDDS:

Drug Content: The Drug content of the optimized L-SNEDDS (F8) was 101.25 ± 0.42%.

Dilution Study: To mimic *in-vivo* conditions, SNEDDS formulations were exposed to various folds of dilution.

Effect of dilution medium and robustness to dilution was observed by diluting the SNEDDS 50, 100, and 1000 times with distilled water, 0.1 N HCl buffer (pH 1.2), and phosphate buffer (pH 6.8) [31, 32].

The results of this study indicated that resultant nanoemulsion was stable and transparent at all pHs having % transmittance of more than 95%.

No drug precipitation was observed, indicating the stability of the reconstituted emulsion. Optimized L-SNEDDS showed % transmittance of 100.44% on dilution with distilled water.

Cloud Point: The cloud point is a significant factor in the SNEDDS consisting of non-ionic surfactants, and it is responsible for the efficacious formation of a stable nanoemulsion. At a temperature higher than cloud point, irreversible phase separation occurs due to dehydration of ingredients, which may affect drug absorption [33].

Phase separation can occur due to dehydration. Due to this, the drug release from the formulation may get affected. To avoid this, the cloud point of the formulation should be over 37 °C.

The cloud point of the optimized SNEDDS was found to be 82 - 84°C.

Viscosity: The viscosity of the optimized L-SNEDDS (F8) was 106.4cP at RT.

Particle size: Particle size after nano emulsification is the most important property of SNEDDS as the droplet size of the emulsion determines the rate and extent of drug release as well as absorption.

The smaller the droplet size, the larger the interfacial surface area provided for drug absorption. It is reported that larger droplets are less neutralized by mucin than smaller droplets [32].

Mechanisms of the particle size effect on drug absorption may include improved release and facilitated lymphatic transport.

The average droplet size of optimized L-SNEDDS (F8) was found to be 97.25 nm **Fig. 4A** and PDI value 0.12, indicating uniform droplets.

The measurement of zeta potential, the electric potential at the plane of shear, is a useful method to predict the physical stability of nanoparticles during storage.

The surface charge is consistently negative, which indicates that the Nano Carriers are stable.

Optimized SNEDDS had zeta potential value -21.5 mV **Fig. 4B**, indicating stability.

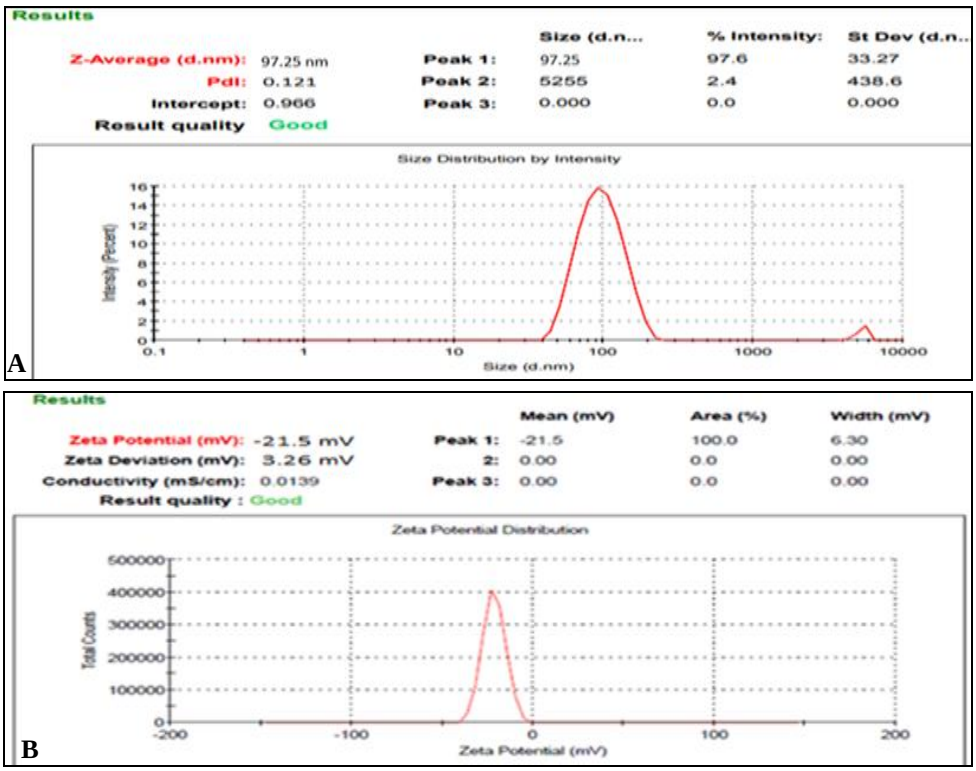


FIG. 4: (A) PARTICLE SIZE OF BATCH 8, (B) ZETA POTENTIAL OF BATCH 8

Emulsification time: Self-emulsification time of the L-SNEDDS was found to be 10 sec which indicated spontaneous emulsification,

Thermodynamic Stability: On centrifugation, neither phase separation nor any precipitations were observed, indicating that the batches were stable. The stability of the batches was assessed at various stress conditions such as heating-cooling cycles (4 °C and 40 °C) and freeze-thaw cycles (−21 °C and +25 °C) along with RT at specified temperature for 48 h, and all the batches were found to be stable in all conditions.

***In-vitro* Release Study from L-SNEDDS:** Drug release studies were performed in phosphate buffer pH 6.8 and 7.4 and pH 1.2 HCl buffer. Percent drug release at 30 and 60 mins revealed that FXT release was maximum from Optimized L-SNEDDS F8 (about 75.95% in 0.1 N HCl while 91.44% in phosphate buffer pH 7.4 within 30 min)as compared to other formulations (marketed Febutaz 40) and plain drug shown in **Fig. 5**.

The higher release of drug from Optimized batch may be attributed to the higher surfactant/ co-surfactant mixture content per gram of the formulation, which may facilitate the drug to remain in solubilized form in lipid solution during the course of its residence in the gastrointestinal (GI) tract. There was also a good correlation between the release rate of FXT and the particle size distribution on most of the formulations.

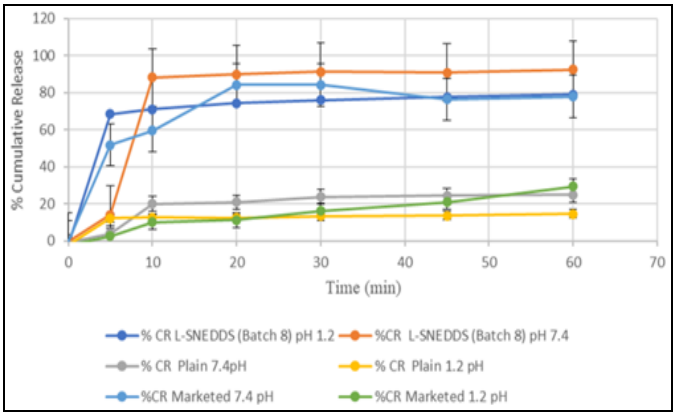


FIG. 5: COMPARATIVE RELEASE PROFILE OF OPTIMIZED BATCH (8) WITH PLAIN DRUG AND MARKETED (FEBUTAZ 40)

Selection of carrier for adsorption of L-SNEDDS: Optimized L-SNEDDS (F8) was converted into S-SNEDDS by adsorbing it to different porous solid carriers (Aerosil 200, Aerosil 300, Mannitol, Maltodextrin, Calcium Di Phosphate, MCC, and spray-dried lactose) by using porcelain dish and pestle. Aerosil 200 showed good adsorption capacity and had good flow property (angle of repose= 20.70 ± 0.03); hence was selected

further. The S-SNEDDS was characterized for flow property by measuring the angle of repose, Carr's index, and Hausner's ratio; values for these tests are 20.70 ± 0.03, 14.54 ± 0.13, and 1.17 ± 0.14, respectively, which indicated that prepared S-SNEDDS have good flowability. The drug content of optimized S-SNEDDS was found to be 98.52 ± 0.14%. S-SNEDDS, when evaluated for self emulsification time and droplet size, had a value of 40 ± 2 s, 155.2 nm, and 0.397 (PDI), respectively, signifying that when it comes in contact with physiological fluid, nanoemulsion is formed rapidly, and droplets are nano size, and so it should show fast absorption.

***In-vitro* Drug Release Study of S-SNEDDS:** The dissolution from both L-SNEDDS and S-SNEDDS formulations took place immediately.

The percentage dissolution of FXT at 30 min was 75.95% and 73.97% from L-SNEDDS and S-SNEDDS in pH 1.2, respectively shown in **Fig. 6A**. Also, the percentage dissolution of FXT at 30 min was 91.44% and 84.32% from L-SNEDDS and S-SNEDDS in pH 7.4, respectively shown in **Fig. 6B**. The solid carrier used (Aerosil 200) in the present study did not interfere with the dissolution of FXT from the S-SNEDDS. At the end of the study, the dissolution was more than 75%for for both formulations.

No precipitation or aggregation was observed. The release profile results suggested that the S-SNEDDS preserved the enhancement of *in-vitro* dissolution of SNEDDS and would eventually enhance the dissolution of the drug *in-vivo.*

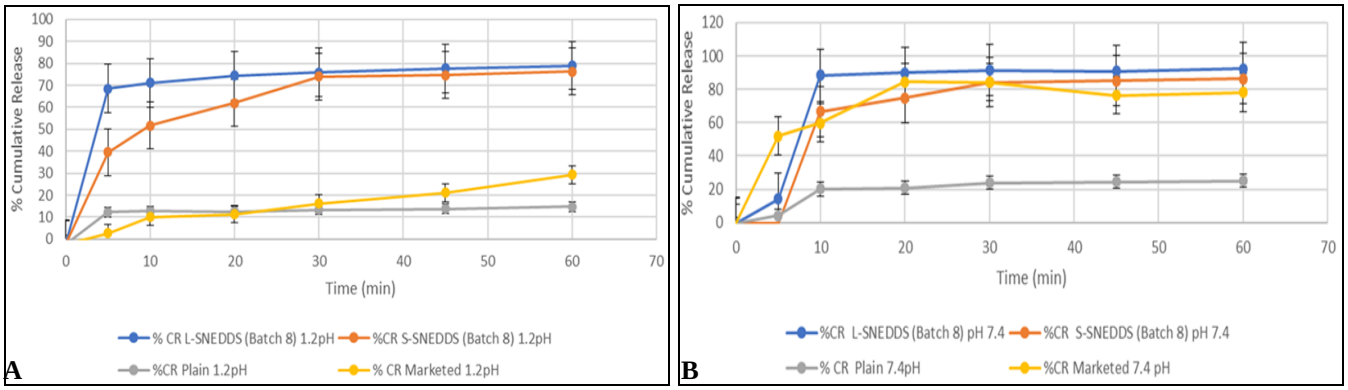


FIG. 6: (A) COMPARATIVE RELEASE PROFILE OF L-SNEDDS, S-SNEDDS, PLAIN AND MARKETED (FEBUTAZ 40) IN HCL BUFFER (PH 1.2), (B) COMPARATIVE RELEASE PROFILE OF L-SNEDDS, S-SNEDDS, PLAIN AND MARKETED (FEBUTAZ 40) IN PHOSPHATE BUFFER (pH 7.4)

XRD: FXT showed sharp, characteristic peaks at different diffraction angles (2θ), indicating its crystalline form. The X-ray spectrum of S-SNEDDS had a halo type of pattern describing the amorphous nature of the drug. The XRD pattern of S-SNEDDS showed an absence of drug instability in its polymorphic transitions when the transformation of L-SNEDDS to S-SNEDDS was occurring. While low-intensity characteristic peaks of the drug were visible in XRD patterns of S-SNEDDS could be due to the added excipient interferences at specific angular responses and even the non-appearance of a few peaks indicates the transformation of FXT from crystalline to unstructured form in S-SNEDDS [34].

SEM: The surface topology of the S-SNEDDS was checked using SEM, and images of the same are depicted in **Fig. 7A** and **Fig. 7B**. SEM studies revealed irregular surface morphology of S-SNEDDS with discrete, spherical structure.

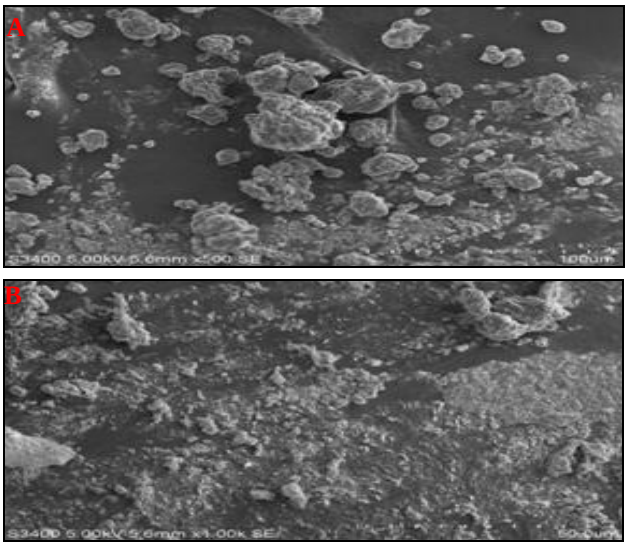


FIG. 7A, B: SEM IMAGE OF FXT S-SNEDDS

Investigations of Release Behavior of SNEOPT: Release of the drug from F1-F8 is shown in **Fig. 8.**

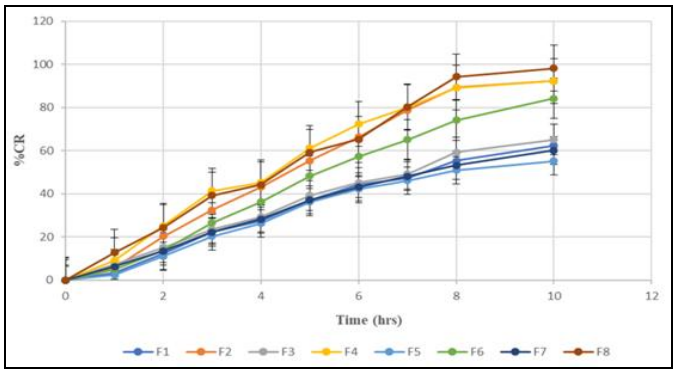


FIG. 8: *IN-VITRO* RELEASE BEHAVIOUR OF SNEOPT

Effect of Concentration of Coating Agent: *In-vitro* release studies indicated that as the concentration of the coating agent increased, the drug release retarded. When the polymer concentration was 2% drug release was appropriate 3% showed the lag time. Hence, 2% of polymer concentration was used further. (Data not shown)

Effect of Concentration of Ratio of Drug to Osmogent: It is clear from **Fig. 8** that osmogent enhances the release of the drug. This finding is evidenced by a formulation devoid of any osmogent in the core and showed around 45% drug release at 24 h. However, the use of osmogent enhanced the release beyond 75% at 10 hours, depending on the amount of osmogent present in the core formulation. A greater amount of osmogent would create a higher osmotic pressure difference, hence a greater driving force to push the drug solution from the system. It was observed that the release rate from the formulation F2, F4, F6, and F8 was more as compared to the other.

Effect of Pore Forming Level: It is evident that the level of pore-forming agent (NaCl and mannitol) directly affected drug release. As the level of pore former increases, the membrane becomes more porous after coming into contact with the aqueous environment, resulting in faster drug release.

It was observed that when the concentration of mannitol increased upto 5%, there was burst release as compared to 2%, which showed controlled release of 75% till 10 hrs and was better than that of NaCl.

Mechanism of Release: Release date of the prepared formulations were fitted to various mathematical models (zero-order, first-order, and Higuchi models) to describe drug release kinetics. The coefficient of determination (R^2) was taken as a criterion for selecting the most appropriate model. All the Opadry CA-coated tablets followed zero-order kinetics. The produced porous membrane upon content with the dissolution media make the drug diffusion through the created pores is the predominant pathway. The drug release from the porous osmotic systems is influenced by osmotic pressure. The osmotic agents' dissolution forms this pressure in the water permeating through the membrane and the diffusion through the pores produced by the pore formers. 4 out of the 8 formulae showed zero-order release kinetics. This suggests that the rate of water imbibition across the coating membrane was perfectly controlled so that a saturated solution of sodium chloride in the tablet core was maintained. Although R^2 value was high for F2, F4, and F8, those tablets released the whole FXT contents (more than 80%) in 8 h. On the other hand, F6 had R^2 equal to 0.9866, and FXT release was continued until 24 h, which matched the aim of formulating once daily controlled release formulation.

Characterization of FXT-SNEOPT: All the QC tests were performed. The diameter and thickness of the prepared FXT SNEOPT ranged between 11.02 - 11.05 mm and 6.5–6.7 mm, respectively, for uncoated tablets. The weight gain per tablet reached after coating was found to be between 0.01 - 0.02 g, and the weight of the tablet was in the range of 1045-1155 mg. The friability and hardness was found to be 0.65 ± 0.015% and 2-2.5kg/cm^2 for uncoated tablet and 0.79 ± 0.015% and 2-3kg/cm^2 for coated tablets. Drug content was found to be 95.64 ± 0.25%.

***In-vitro* Release Studies of SNEOPT:** Release of FXT from the FXT-SNEOPT and the marketed is shown in **Fig. 9** in pH 1.2 HCl buffer and phosphate buffer pH 7.4.

It was found that the marketed formulation showed rapid release in 2 hrs. The dissolution profile of the optimized formulation (F6) coated by 2% Opadry® CA clearly indicated a controlled release pattern over 8 h of the experiment, where 75.35% FXTwas

released. Along with optimized formulation parameters, the concentration of coat and pore-forming agent, the presence of HPMC K4M form gel-like layer on the surfaces leading to tablet swelling upon contact with the dissolution media. This gel delayed water penetration into the tablet and thus reduced the drug release rate. Still, the extent of drug release was nearly complete at the end of the dissolution experiment (after 24 h). The incorporation of FXTinto the S-SNEOPT helped in achieving a constant drug release in both media (pH 1.2 and 7.4) and dissolution rate. Also, the disadvantage faced with osmotic pump tablets when lipophilic drugs are formulated and are difficult to be released completely was resolved.

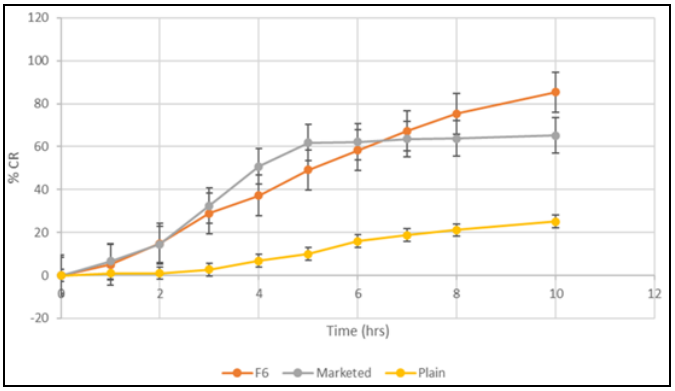


FIG. 9: *IN-VITRO* RELEASE STUDIES OF SELECTED BATCH (F6)

CONCLUSION: The FXT-SNEDDS were successfully formulated, optimized, and evaluated. Release of L-SNEDDS was found to be 75.95% in HCl buffer pH 1.2 and 91.44% in pH 7.4 (PBS), S-SNEDDS was 73.97% in HCl buffer pH 1.2, and 84.32 in pH 7.4 (PBS) compared to market, which was 16.27% in HCl buffer pH 1.2 and 75.24% in pH 7.4 (PBS) whereas plain drug showed release of 13.41% in HCl buffer pH 1.2 and 23.47% in pH 7.4 (PBS) in 30 min. It could be concluded that the release was independent of pH. Also, the SNEOPT showed more than 75% release in 8 h with the zero-order release. Incorporation of FXT into lipids, surfactants & co-surfactants enhanced solubility, which formed S-SNEDDS and tailored as tablets to attain Controlled release characteristics using coating agents to develop SNEOPT. The major advantage included precise control of zero-order with a consistent release which can be achieved irrespective of the environmental factors at the delivery site. Also, the challenges faced with osmotic pump tablets when lipophilic drugs are formulated and are difficult to be released completely was resolved. All the formulations were stable for three months.

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REFERENCES:

1. Rangaraj N, Shah S, Maruthi AJ, Pailla SR, Cheruvu HS, Sujatha D and Sampathi S: Quality by design approach for the development of self-emulsifying systems for oral delivery of febuxostat: pharmacokinetic and pharmacodynamic evaluation. AAPS Pharm Sci Tech 2019; 20(7): 1-6.
2. R, Vedder D, Baniaamam M, Tausche AK, Gerritsen M and Nurmohamed MT: Cardiovascular risk in inflammatory arthritis: rheumatoid arthritis and gout. The Lancet Rheumatology 2021; 3(1): 58-70.
3. Borghi C, Agabiti-Rosei E, Johnson RJ, Kielstein JT, Lurbe E, Mancia G, Redon J, Stack AG and Tsioufis KP: Hyperuricaemia and gout in cardiovascular, metabolic and kidney disease. European Journal of Internal Medicine 2020; 80: 1-11.
4. Dehlin M, Jacobsson L and Roddy E: Global epidemiology of gout: prevalence, incidence, treatment patterns and risk factors. Nature Reviews Rheumatology 2020; 16(7): 380-90.
5. Pillinger MH and Mandell BF: Therapeutic approaches in the treatment of gout. In Seminars in Arthritis and Rheumatism WB Saunders 2020; 50(3): 24-30.
6. Ahmed TA, Felimban RI, Tayeb HH, Rizg WY, Alnadwi FH, Alotaibi HA, Alhakamy NA, Abd-Allah FI, Mohamed GA, Zidan AS and El-Say KM: Development of Multi-Compartment 3D-Printed Hansildaar Tablets Loaded with Self-Nanoemulsified Formulations of Various Drugs: A New Strategy for Personalized Medicine. Pharmaceutics 2021; 13(10): 1733.
7. Kanojia N: Development and characterization of solid dispersions and microspheres for solubility enhancement of bcs class ii drug: comparative study (Doctoral dissertation, Chitkara University Publications) 2021: 1-140.
8. Savjani KE, Gajjar AN and Savjani JI: Modified formulation of Febuxostat: improved efficacy and safety. Int J Pharm Pharm Sci 2016; 8(1): 359-66.
9. Ahuja BK, Jena SK, Paidi SK, Bagri S and Suresh S: Formulation, optimization and *in-vitro* and *in-vivo*

evaluation of febuxostat nanosuspension. Int J Pharm 2015; 478(2): 540-52.
10. Amin OM, Ammar A and Eladawy SA: Febuxostat loaded β-cyclodextrin based nanosponge tablet: An *in-vitro* and *in-vivo* evaluation. J of Pharma Inve 2020; 50(4): 399-411.
11. Sharma A, Garg V, Gupta M and Sharma V: Design, Formulation and Evaluation of Fast Disintegrating Tablets of Febuxostat. The Pharmaceutical and Chemical Journal 2018; 5(1): 163-73.
12. Rani S, Rana R, Saraogi GK, Kumar V and Gupta U: Self-emulsifying oral lipid drug delivery systems: advances and challenges. AAPS Pharm Sci Tech 2019; 20(3): 1-2.
13. Zubair AH, Sheshe SM, Bashir MR, Sade SM. Lipid Based Drug Delivery System: A Review Journal of Applied Life Sciences International 2021; 24(3): 33-46.
14. Buya AB, Beloqui A, Memvanga PB and Préat V: Self-nano-emulsifying drug-delivery systems: From the development to the current applications and challenges in oral drug delivery. Pharmaceutics 2020; 12(12): 1194.
15. Singh D: Self-nanoemulsifying Drug Delivery System: A Versatile Carrier for Lipophilic Drugs. Pharmaceutical Nanotechnology 2021; 9(3): 166-76.
16. C. van Durme R: Landewé. Gout. The Heart in Rheumatic. Autoimmune and Inflammatory Diseases 2017; 349-64.
17. Park JH, Kim DS and Mustapha O: Comparison of a revaprazan-loaded solid dispersion, solid SNEDDS and inclusion compound: physicochemical characterisation and pharmacokinetics. Colloids Surfaces B Biointerfaces 2019; 162: 420–26.
18. Okawa S, Sumimoto Y, Masuda K, Ogawara KI, Maruyama M and Higaki K: Improvement of lipid solubility and oral bioavailability of a poorly water-and poorly lipid-soluble drug, rebamipide, by utilizing its counter ion and SNEDDS preparation. European Journal of Pharmaceutical Sciences 2021; 159: 105721.
19. Van Staden D, Du Plessis J and Viljoen J: Development of a self-emulsifying drug delivery system for optimized topical delivery of clofazimine. Pharmaceutics 2020; 12(6): 523.
20. Syukri Y, Oktari RN, Munawiroh SZ and Nugroho BH: Optimization and preparation of Self-Nanoemulsifying Drug Delivery System (SNEDDS) loaded glimepiride. In AIP Conference Proceedings 2021; 2370(1): 060008.
21. Pandey NK, Singh SK, Gulati M, Kumar B, Kapoor B, Ghosh D, Kumar R, Khursheed R, Awasthi A, Kuppusamy G and Wadhwa S: Overcoming the dissolution rate, gastrointestinal permeability and oral bioavailability of glimepiride and simvastatin co-delivered in the form of nanosuspension and solid self-nanoemulsifying drug delivery system: a comparative study. Journal of Drug Delivery Science and Technology 2020; 60: 102083.
22. El-Zahaby SA, AbouGhaly MH, Abdelbary GA and El-Gazayerly ON: Zero-order release and bioavailability enhancement of poorly water soluble Vinpocetine from self-nanoemulsifying osmotic pump tablet. Pharmaceutical Development and Technology 2018; 23(9): 900-10.
23. Yanfei M, Guoguang C, Lili R and Pingkai O: Controlled release of glaucocalyxin–a self-nanoemulsifying system from osmotic pump tablets with enhanced bioavailability. Pharm dev Technol 2017; 22(2): 148-55.
24. Patel G, Shelat P and Lalwani A: Statistical modeling, optimization and characterization of solid self-nanoemulsifying drug delivery system of lopinavir using design of Experiment. Drug Delivery 2016; 23(8): 3027-42.
25. Garg V, Kaur P, Singh SK, Kumar B, Bawa P, Gulati M and Yadav AK: Solid self-nanoemulsifying drug delivery systems for oral delivery of polypeptide-k: formulation, optimization *in-vitro* and *in-vivo* antidiabetic evaluation. European J of Pharmaceu Sciences 2017; 109: 297-315.
26. Alghananim A, Özalp Y, Mesut B, Serakinci N, Özsoy Y, Güngör S. A Solid Ultra Fine Self-Nanoemulsifying Drug Delivery System (S-SNEDDS) of Deferasirox for Improved Solubility: Optimization, Characterization and *In-vitro* Cytotoxicity Studies. Pharmaceuti 202; 13(8): 162.
27. Kazi M, Al-Swairi M, Ahmad A, Raish M, Alanazi FK, Badran MM, Khan AA, Alanazi AM and Hussain MD: Evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for poorly water-soluble talinolol: preparation, *in-vitro* and *in-vivo* assessment. Frontiers in Pharmacology 2019; 10:459.
28. Dhoot AS, Naha A, Priya J and Xalxo N: Phase Diagrams for Three Component Mixtures in Pharmaceuticals and its Applications. Journal of Young Pharmacists 2018; 10(2): 132-7.
29. Razzaq FA, Asif M, Asghar S, Iqbal MS, Khan IU, Khan SU, Irfan M, Syed HK, Khames A, Mahmood H and Ibrahim AY: Glimepiride-Loaded Nanoemulgel; Development, *In-vitro* Characterization, Ex Vivo Permeation and *In-vivo* Antidiabetic Evaluation. Cells 2021; 10(9): 2404.
30. Azmi NA, Elgharbawy AA, Motlagh SR, Samsudin N and Salleh HM: Nanoemulsions: factory for food, pharmaceutical and cosmetics. Processes 2019; 7(9): 617.
31. Baral KC, Song JG, Lee SH, Bajracharya R, Sreenivasulu G, Kim M, Lee K and Han HK: Enhanced Bioavailability of AC1497, a Novel Anticancer Drug Candidate, *via* a Self-Nanoemulsifying Drug Delivery System. Pharmaceutics 2021; 13(8): 1142.
32. Nasr A, Gardouh A and Ghorab M: Novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of olmesartanmedoxomil: design, formulation, pharmacokinetic and bioavailability evaluation. Pharmaceutics 2016; 8(3): 20.
33. Patel G, Shelat P and Lalwani A: Statistical modeling, optimization and characterization of solid self-nanoemulsifying drug delivery system of lopinavir using design of experiment. Drug Delivery 2016; 23(8): 3027-4.
34. Patel VP, Patel AP and Shah A: Optimization of amorphous solid dispersion techniques to enhance solubility of febuxostat. Folia Medica 2021; 63(4): 557-68.

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