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Original Research Paper

ANALYSIS OF THREE COMPONENT SYSTEM FOR NANDROLONE DECANOATE TO PREPARE NANOEMULSION FORMULATION

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ABSTRACT

Microemulsion based gel formulations are successfully used to improve the aqueous solubility and bioavailability of drugs with less aqueous phase solubility. Phase diagram of drug Nandrolone Decanoate (ND) is prepared and studied as three component system. The pseudo-ternary system Ethyl oleate + Kolliphor HS + ethanol (3:1v/v)/water witnessed a small clear zone of 30.25%, which was reduced to 25.30% when ratio reduced to 2:1v/v. And, for another combination of Ethyl oleate + Kolliphor HS + IPA (3:1v/v)/water witnessed clear zone of 28.63%, which was reduced to 19.33% with reduction in ration to 2:1v/v. The purpose of this study is to provide information about the significance of pseudo-ternary phase diagram construction, as an important tool for the development of microemulsion formulation. The performed experimental work results are presented in detail and discussed with regard to prepare a microemulsion based formulation for transdermal drug delivery of the drug.

Keywords: Pseudoternary phase diagram, Lipophilic drug, Non-ionic surfactants, Chronic kidney diseases.

INTRODUCTION

Route of drug administration primarily depends on bioavailability of the active form of the drug. Most of the drugs exhibit lipophilic characters with poor water solubility.[1,2] Most popular approach to impart enhanced water solubility is by preparing a dispersed system (emulsion, microemulsion, nanoemulsion etc.). To prepare a dispersed system (emulsion, microemulsion, or nano-emulsion) based formulation, study of phases obtained from various combinations of oil, surfactant and co-surfactant is required.[3] Selection of surfactant and co-surfactant plays an important role to prepare a stable productive formulation. Non-ionic surfactants are preferred over anionic and cationic surfactants because of their lower irritation potential and better cutaneous tolerance and toxicity. Cationic surfactants are generally more expensive, because of the high hydrogenation reaction to be carried out during the synthesis.[4-6]

To study the phase behavior of different formulation components, most critical step is construction of pseudoternary phase diagram.[7] A pseudo-ternary phase diagram of oil, surfactant, co-surfactant, and water can be helpful in preparing a stable microemulsion formulation. This is done by plotting the three components surfactant: co-surfactant (S/CoS), oil and water each of them representing an apex of triangle with apexes that represent 100% of each pseudo component and the opposite side of apex represents 0% of that pseudo component. For any ternary mixture formed the total of surfactants, co-surfactants and oil concentrations always added to 100%.[8] Usually there are three types of phases encountered in a pseudo-ternary phase diagram: microemulsion (ME), liquid crystal (LC) and coarse emulsion (CE). A large microemulsion region provides more flexibility to find optimal dosage composition.

Microemulsions are identified with their clear and transparent appearance. Liquid crystal (LC) possess oil streaks under stirring condition. They also exhibit birefringence under crossed polarized microscope. Coarse emulsion (EM) is thermodynamically unstable and it appears as milky white during the preparation and storage.[9] Nandrolone Decanoate (ND) is an androgenic anabolic steroid (AAS) used for the treatment of anemia associated with chronic kidney diseases (CKD). ND is a relatively lipophilic drug in nature. Its molecular weight is 428.64 g/mol with logP value of 7.32.[10,11] In marketed formulation of ND is an intramuscular injection (IM), with strengths varying from 50 to 250 mg once a week dosing regimen. The use of Decanoate ester results in a depot effect, so that an effective plasma Nandrolone level is not obtained rapidly after an intramuscular injection, also this Nandrolone level may persist for several weeks.[12] Studies indicates that various intramuscular oily injections could lead to necrosis, skin eruption and even physical deformity.[13] Also, it cannot be administered to the patients who are allergic to peanut oil.[14,15] When ND is given orally, it is scarcely active, or in some cases much less active. The only advantage of administrating via parenteral route is that a good effect can be achieved with relatively low dosage. Parental form of administration always come with certain limitations. A doctor or a trained person is required to administer injection.[12] To overcome all the limitations and bypass the gastric environment, a transdermal drug delivery system (TDDS) form would therefore be far more preferable route than a parenteral. Objective of this study is to analyzing the most critical step in microemulsion preparation of ND which is pseudo-ternary phase diagrams.

MATERIAL AND METHOD

Nandrolone Decanoate used in this work was received as gift sample from ASG Biochem. Pvt. Ltd. and its mass purity was 98.98%. Kolliphor HS 15 and Kolliphor HS 40 used were received from BASF, Mumbai. Ethyl oleate, Oleic acid, PEG400, Tween 20, 60, 80 were received from Apeejay Stya University, Gurgaon. Edible sunflower oil and castor oil was purchased from local market. The solvents used were anhydrous methanol from Merck, with a mass purity of 99.9%. Other chemicals were of AR grade and used directly without further purification.

Solubility Study of Nandrolone Decanoate in Various Oils, Surfactants and Co-Surfactants

Drug powder of ND was added in excess to each of the oils, surfactant (S) and co-surfactant (CoS). Each glass vial was then vortexed for a period of 10 min using a vortex mixer. The vortexed vials were then kept at 37±0.5°C in an isothermal shaker for 72 hours to achieve equilibrium. The equilibrated samples were then removed from the shaker and centrifuged at 8000 rpm for a period of 15 min to remove undissolved drug.[16] The aliquots of supernatant were filtered through a 0.45 µm membrane filter and the solubility of ND was determined by UV spectrophotometer by scanning through a range of wavelength from 200-400nm.

Preliminary Screening of Surfactant and Co-Surfactant

For screening of oil, surfactant and co-surfactant with good solubilizing capacity for Nandrolone Decanoate, the solubility of Nandrolone Decanoate was investigated in oils like Ethyl Oleate, Oleic acid, sunflower oil and surfactants and co-surfactants like Tween 20, Kolliphor RH 40, Kolliphor HS 15, IPA, ethanol etc. Selected oily phase and surfactant were further used for screening of different co-surfactants for their emulsification study.

Screening of Surfactants

For this study, 150 mg of surfactants were added to 150 mg of oily phase and then this mixture was heated at 50 ºC for homogenization of the components. Then from each mixture prepared 100 mg was withdrawn and diluted to 100 ml in a volumetric flask. The ease of emulsification was judged by the number of flask inversions required to yield homogeneous emulsion. The emulsions were allowed to stand for 24 hours and then % transmittance was evaluated at 650 nm by using UV spectrophotometer. They were also observed visually for turbidity or phase separation.[17-20]

Screening of Co-surfactants

For screening of selected co-surfactants, the oil: surfactant: co-surfactant was taken as 300 mg: 200 mg: 100 mg i.e. in the ratios of 3:2:1. Out of the total 600 mg of the mixture 100 mg was withdrawn and then added dropwise into 100 ml volumetric flask containing distilled water dropwise; then it was inverted 50-60 times and kept overnight. After which the % transmittance was determined by scanning in the range from 800-200 nm (wavelength 650 nm) using UV-visible spectrophotometer. After the completion of screening the next step was to optimize the combination showing good % transmittance.[18-20]

Construction of Pseudo-ternary Phase Diagrams

To find out the concentration range of components for the existing range of ME, pseudo-ternary diagrams were constructed using water titration method. Two different combinations of surfactant to co-surfactant were tried. Combination I was Ethyl oleate + Kolliphor HS 15 + ethanol and combination II was Ethyl oleate + Kolliphor HS 15+Isopropyl alcohol. The ratio of surfactant to co-surfactant were varied from 1:1, 2:1, 1:2, 3:1, 1:3 on weight basis for both the combinations. Oil was mixed with S/CoS mixture at ratio of 0.5:9.5, 0.5:9, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 3:7, 4:6 and 5:5 (w/w). The mixture was then gently heated at 45–50ºC and vortexed to form homogenous mixture. At certain weight ratios, the mixture of oil, S/CoS were diluted with distilled water dropwise, under moderate magnetic stirring. The samples were then sonicated for 3 min and kept at room temperature for 24 h. After 24 h, the mixtures were inspected visually and determined as emulsion, ME or ME gels.[19] The stable ME formulation were also observed under polarizing light to confirm their isotropic nature. The different ratios of oil and Smix were taken as Ternary mixtures were formed in these ratios and then quantity of water forming transparent solution was plotted with other components in the pseudo-ternary phase diagram. No attempts were made to distinguish between oil in water, water in oil or bicontinuous type ME.

RESULT AND DISCUSSION

Screening of Formulation Ingredients

Solubility of oil, surfactants and co-surfactant

The solubility of ND in different oils, surfactants and co-surfactants is listed in Table 1. Figure1,2 and 3 is indicating solubility graphs of ND in different oils, surfactants and co-surfactants, respectively. From solubility study of ND in different oils, maximum solubility was found in ethyl oleate and oleic acid with the solubility of 214.50 ± 4.796 and 3330.73 ± 41.68 mg/ml, respectively. The solubility of drug in Tween 20 and Kolliphor HS 15 was found to be 275.63 ± 1.07 and 98.833 ± 1.41 mg/ml, respectively. The solubilisation capacity of other three surfactants was quite low than 60 mg/ml. Addition of co-surfactants provides reduction in the interfacial tension and enhances the fluidity of interfacial surfactant film, which can take up different curvatures, thus expanding the area of existence of microemulsion system. Consequently, isopropyl alcohol, ethanol and propylene glycol 400 (PEG 400) with solubility of 3264.458 ± 20.905, 120.394 ±3.891 and 39.289 ± 2.371, respectively were selected as co-surfactants.

Screening of Surfactants and Co-Surfactants

The results of emulsification study involving the surfactants and co-surfactants are presented in Table 2 and 3, respectively. The surfactants for developing ME of ND were selected based on number of flask inversions required to yield homogeneous emulsion of oil, surfactant and co-surfactants. The ease of emulsification was judged by the number of flask inversions required to yield homogeneous emulsion. The emulsification capacity of surfactants for co-surfactants was determined for all the combinations. Combination of ethyl oleate, Kolliphor HS 15 and ethanol showed 9± 3 number of flask inversion with 93.6 ± 0.36 % transmittance. Another combination selected was Ethyl oleate, Kolliphor HS 15 and IPA with 10± 2 number of flask inversions and 96.9 ± 0.11% transmittance. Figure4 and 5 is indicating the screening graphs of surfactants and co-surfactants, respectively.

Construction of Pseudo-Ternary Phase Diagram

The construction of pseudo-ternary phase diagrams was used to determine the appropriate concentration ranges of components (aqueous phase, oil phase, surfactant and co-surfactant) in the regions of forming microemulsion. Figure 6 presents the pseudo-ternary phase diagrams of Ethyl oleate, Kolliphor HS 15 and water systems in the presence of co-surfactant (ethanol) with various weight ratios of Kolliphor HS 15/ethanol. Percentage area for all the pseudo-ternary phase diagrams was calculated on weight basis method and tabulated in table no 4. The pseudo-ternary system shown in Figure 6(d), which was Ethyl oleate + Kolliphor HS + ethanol (3:1v/v)/water witnessed a small clear zone of 30.25%, which was reduced to 25.30% when ratio reduced to 2:1v/v shown in Figure6(b). Figure7 presents the pseudo-ternary phase diagrams of Ethyl oleate, Kolliphor HS 15, IPA and water systems with various weight ratios of Kolliphor HS 15/IPA. For second combination shown in Figure 7(d), which was Ethyl oleate + Kolliphor HS + IPA (3:1v/v)/water witnessed clear zone of 28.63%, which was reduced to 19.33% in fig 7(b) with reduction in ration to 2:1v/v. The phase behavior of Ethyl oleate +Kolliphor HS + IPA mixture did not show any improvement of clear zone. The percent areas of clear zones of microemulsion systems herein studied are presented in Table 4. All these systems were stable for more than six months and over variations of 4-40ºC.

CONCLUSION

This is the first report, where the phase behavior of number surfactants is presented and compared. All Pseudo-ternary combinations were found to form microemulsion with varied area under microemulsion region. Combination Ethyl oleate + Kolliphor HS + ethanol (3:1v/v)/water witnessed best results with maximum area of microemulsion of 30.25%. The studied systems were much simple and showed very good stability towards time and temperature range of 4-40 ºC.

Table 1: Solubility of Nandrolone Decanoate in various formulation components

Component	Solubility (mg/ml) ± SD
Oils	
Ethyl Oleate	214.50 ± 4.796
Oleic acid	3330.73 ± 41.68
Castor oil	109.59 ±2.631
Myristol	96.529±1.989
Sunflower oil	154.82 ± 1.407
Surfactant	
Tween20	275.63 ± 1.07
Tween60	1.627 ±0.3328
Tween80	74.359 ±2.07
Kolliphor RH 40	56.133 ± 2.80
Kolliphor HS 15	98.833 ± 1.41
Co-surfactant	
IPA	3264.458 ± 20.905
Ethanol	120.394 ±3.891
Glycerol	2.276 ± 0.597
Propylene Glycol	36.350 ± 1.908
PEG200	12.938 ± 1.426
PEG400	39.289 ± 2.371

Table 2: Screening of surfactants

Formulation Code	Oils (in mg)	Surfactants (in mg)	Oil+ Surfactant (in mg)	No. of Inversion	% Transparency
O1	Oleic acid (150mg)	Kolliphor HS15 (150mg)	100mg	27± 1	11.2± 1.15
O2	Oleic acid (150mg)	Tween20 (150mg)	100mg	23± 2	55.5± 1.32
M1	Ethyl Oleate (150mg)	Tween20 (150mg)	100mg	22± 2	26.5± 0.76
M2	Ethyl Oleate (150mg)	Kolliphor HS15 (150mg)	100mg	18± 3	75.6± 1.22

Table 3: Screening of co-surfactants

Form. code	Oil (mg)	Surfactant (mg)	Co-surfactant (mg)	Mixture (mg)	No. of Inversion	% transmittance
OT1	Oleic acid (300)	Kolliphor HS15 (200)	Ethanol (100)	100	18± 2	88± 1.5
OT2	Oleic acid (300)	Kolliphor HS15 (200)	IPA (100)	100	19± 2	82.5± 0.26
OT3	Oleic acid (300)	Kolliphor HS15 (200)	PEG400 (100)	100	23± 3	75.4± 0.32
OT4	Oleic acid (300)	Tween20 (200)	Ethanol (100)	100	17± 2	91.7± 0.64
OT5	Oleic acid (300)	Tween20 (200)	IPA (100)	100	18± 3	89.5± 0.30
OT6	Oleic acid (300)	Tween20 (200)	PEG400 (100)	100	15± 4	85.8 ± 1.13
MT1	EthylOleate (300)	Kolliphor HS15 (200)	Ethanol (100)	100	9± 3	93.6 ± 0.36
MT2	EthylOleate (300)	Kolliphor HS15 (200)	IPA (100)	100	10± 2	96.9 ± 0.11
MT3	EthylOleate (300)	Kolliphor HS15 (200)	PEG400 (100)	100	11± 2	89.6 ± 0.51
MT4	EthylOleate (300)	Tween20 (200)	Ethanol (100)	100	8± 2	56 ± 0.32
MT5	EthylOleate (300)	Tween20 (200)	IPA (100)	100	6± 1	78± 0.17
MT6	EthylOleate (300)	Tween20 (200)	PEG400 (100)	100	12± 2	80± 0.40

Table 4: Percent areas of clear micro-emulsion forming zones in the phase diagrams for the studied systems

System	% Area[a]
Ethyl oleate + Kolliphor HS 15 + Ethanol (1:1w/w)/Water	19.86
Ethyl oleate + Kolliphor HS 15 + Ethanol (2:1w/w)/Water	25.30
Ethyl oleate + Kolliphor HS 15 + Ethanol (1:2w/w)/Water	13.11
Ethyl oleate + Kolliphor HS 15 + Ethanol (3:1w/w)/Water	30.25
Ethyl oleate + Kolliphor HS 15 + Ethanol (1:3w/w)/Water	9.54
Ethyl oleate + Kolliphor HS 15 + IPA (1:1w/w)/Water	15.99
Ethyl oleate + Kolliphor HS 15 + IPA (2:1w/w)/Water	19.33
Ethyl oleate + Kolliphor HS 15 + IPA (1:2w/w)/Water	11.34
Ethyl oleate + Kolliphor HS 15 + IPA (3:1w/w)/Water	28.63
Ethyl oleate + Kolliphor HS 15 + IPA (1:3w/w)/Water	8.10

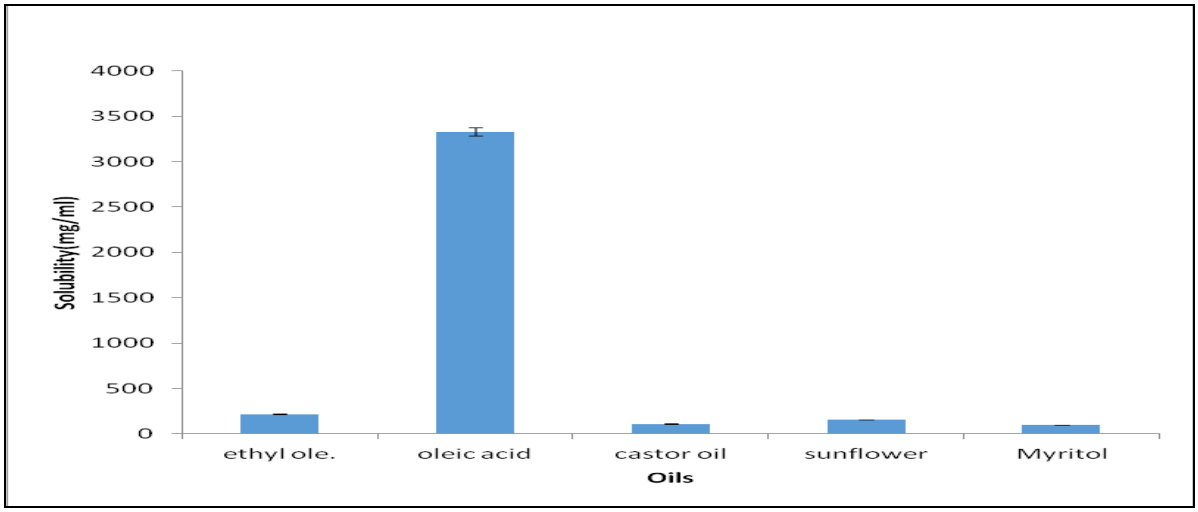


Figure 1: Solubility of Nandrolone Decanoate (ND) in different oils

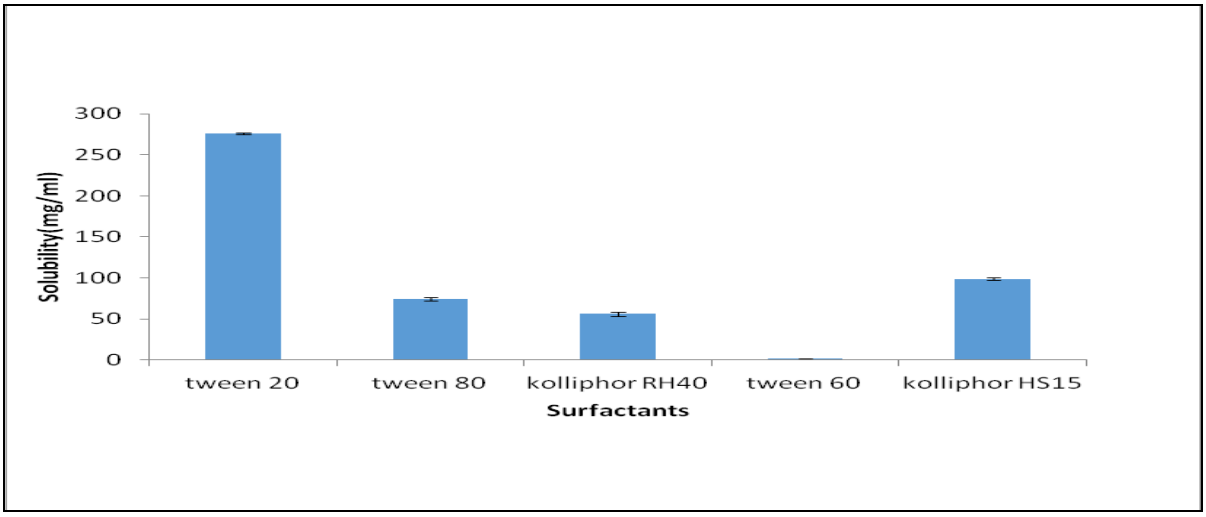


Figure 2: Solubility of Nandrolone Decanoate (ND) in different surfactants

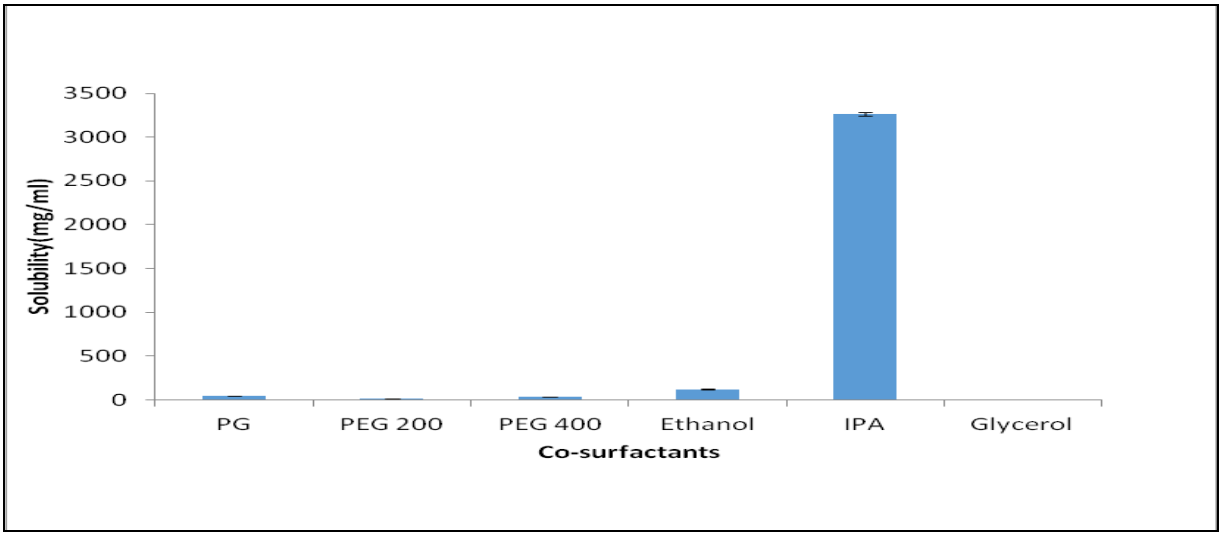


Figure 3: Solubility of Nandrolone Decanoate (ND) in different co-surfactants

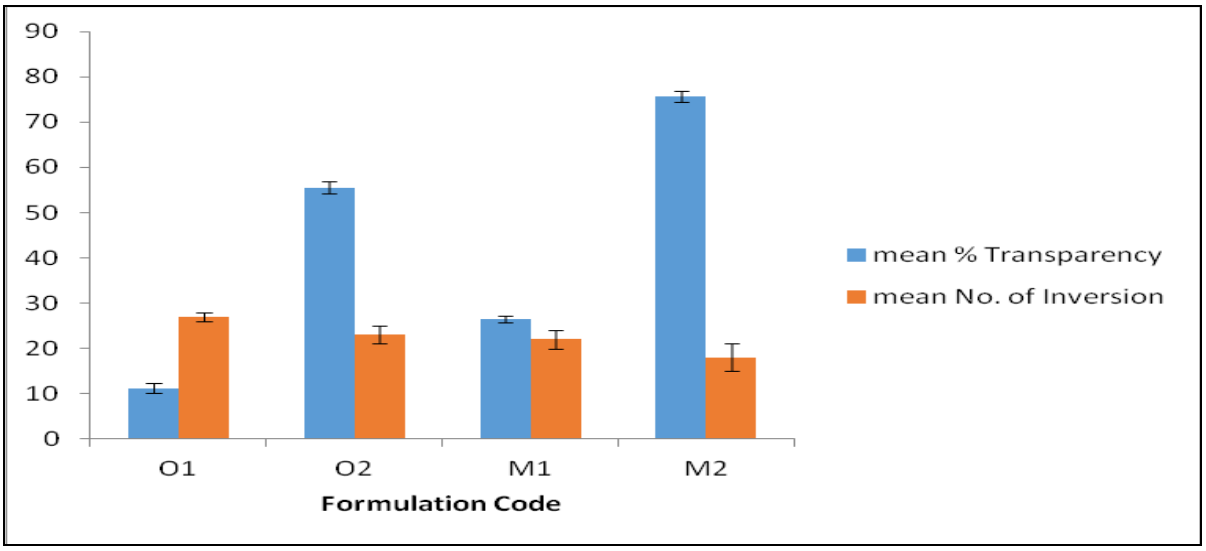


Figure 4: Screening of surfactants

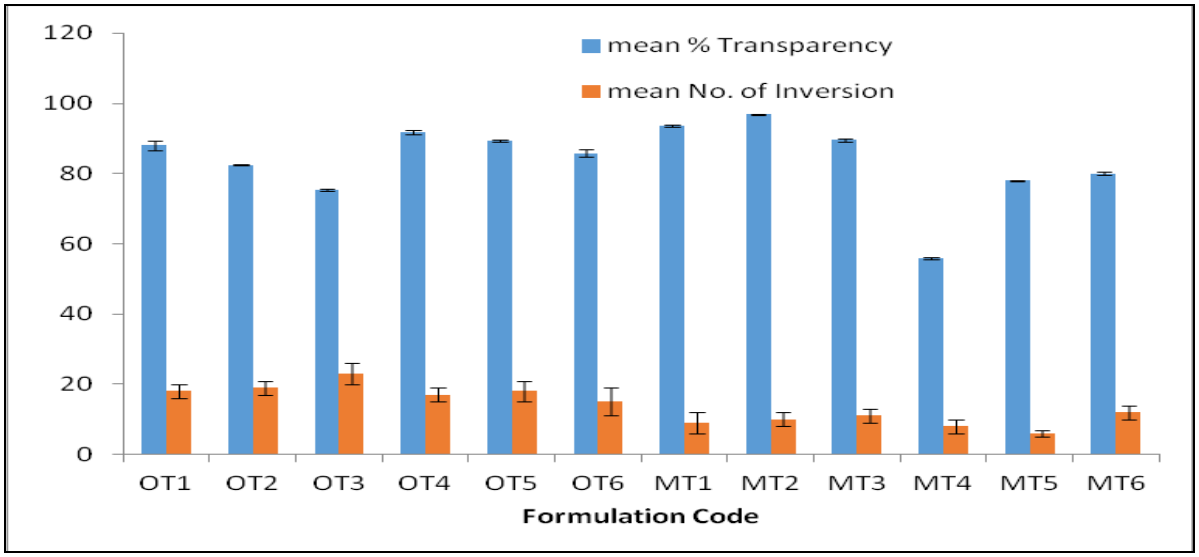


Figure 5: Screening of co-surfactants

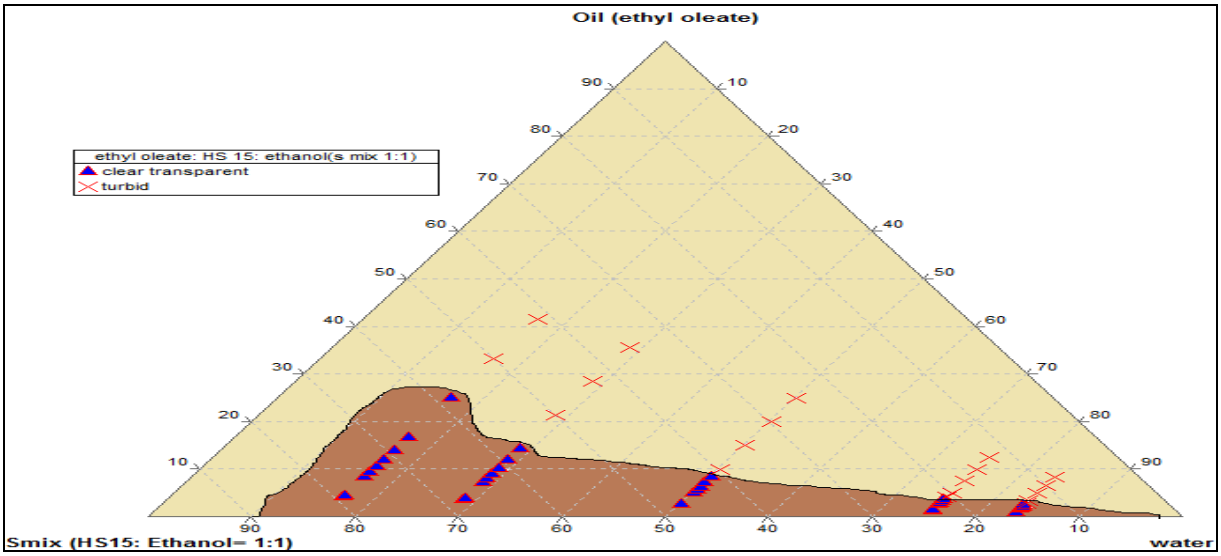
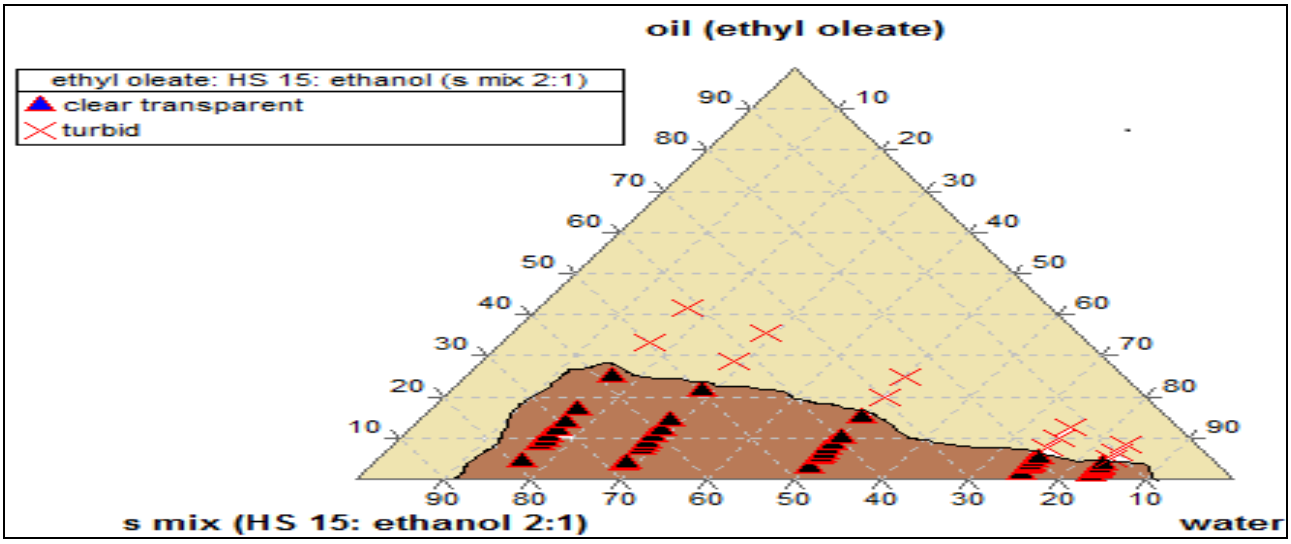
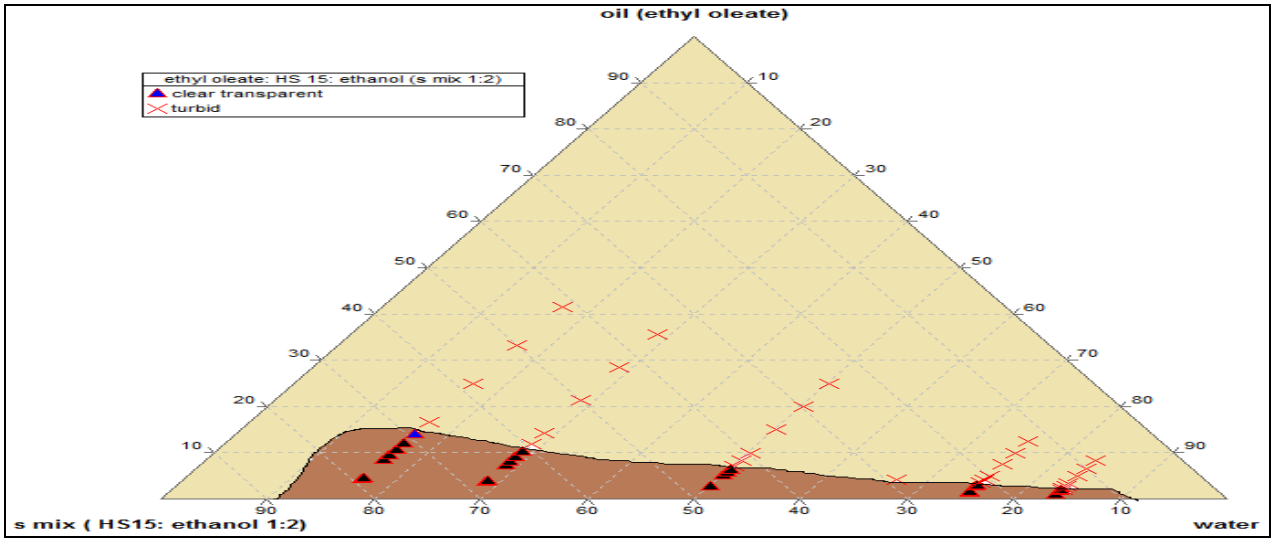


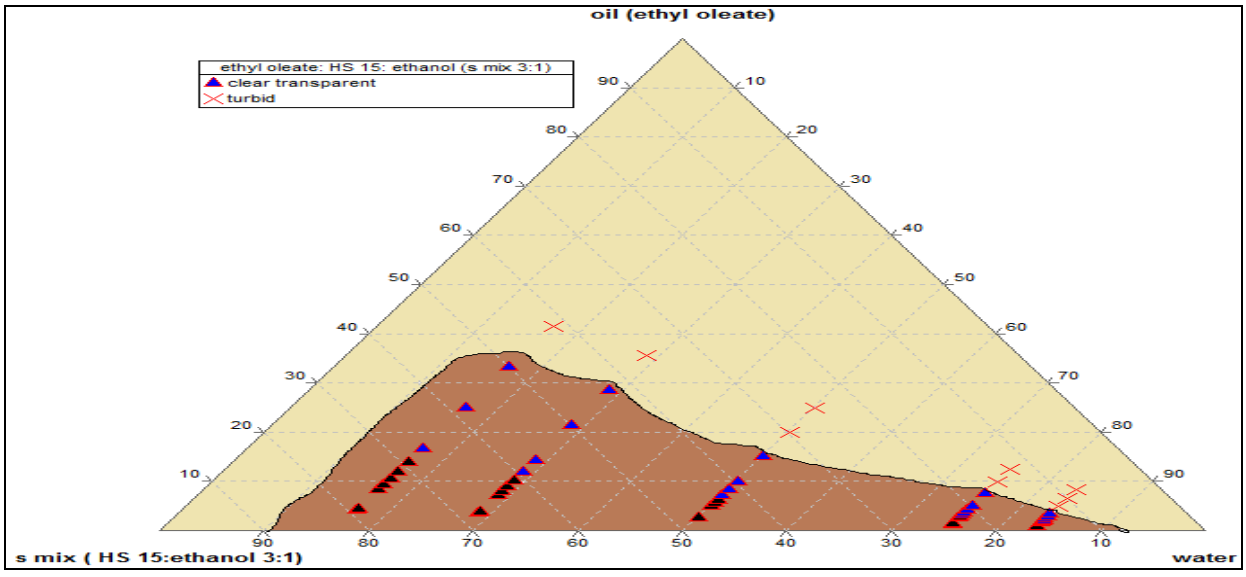
Figure 6 (a): Ternary phase diagrams for combination I


oil (ethyl oleate)
ethyl oleate: HS 15: ethanol (s mix 2:1)
clear transparent
turbid
s mix (HS 15: ethanol 2:1)
water

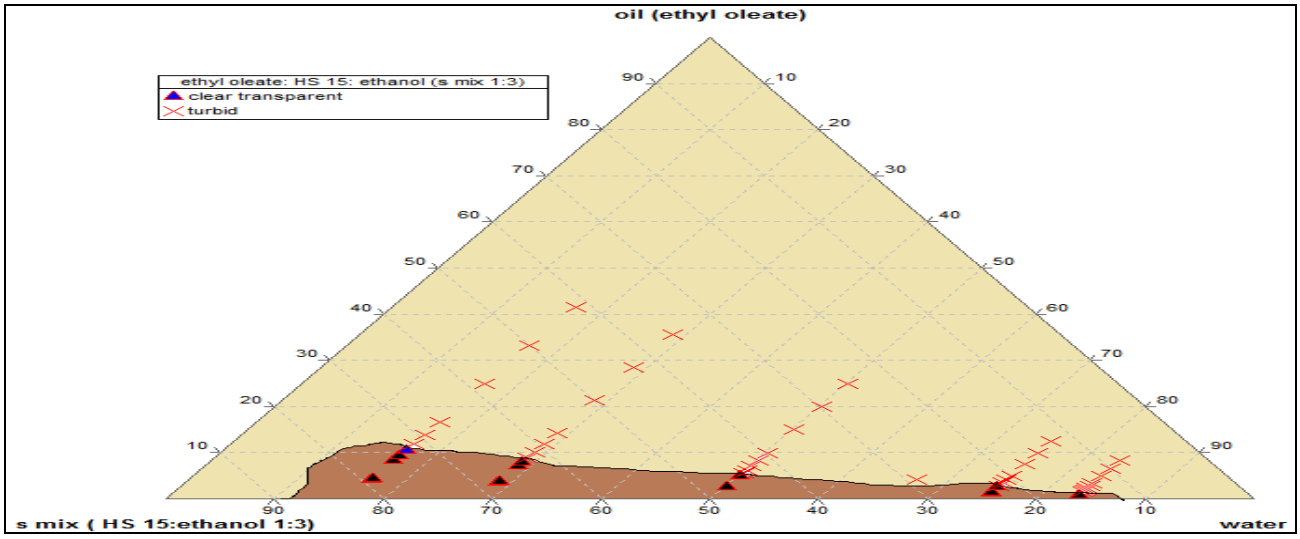
6(b)


oil (ethyl oleate)
ethyl oleate: HS 15: ethanol (s mix 1:2)
clear transparent
turbid
s mix (HS15: ethanol 1:2)
water

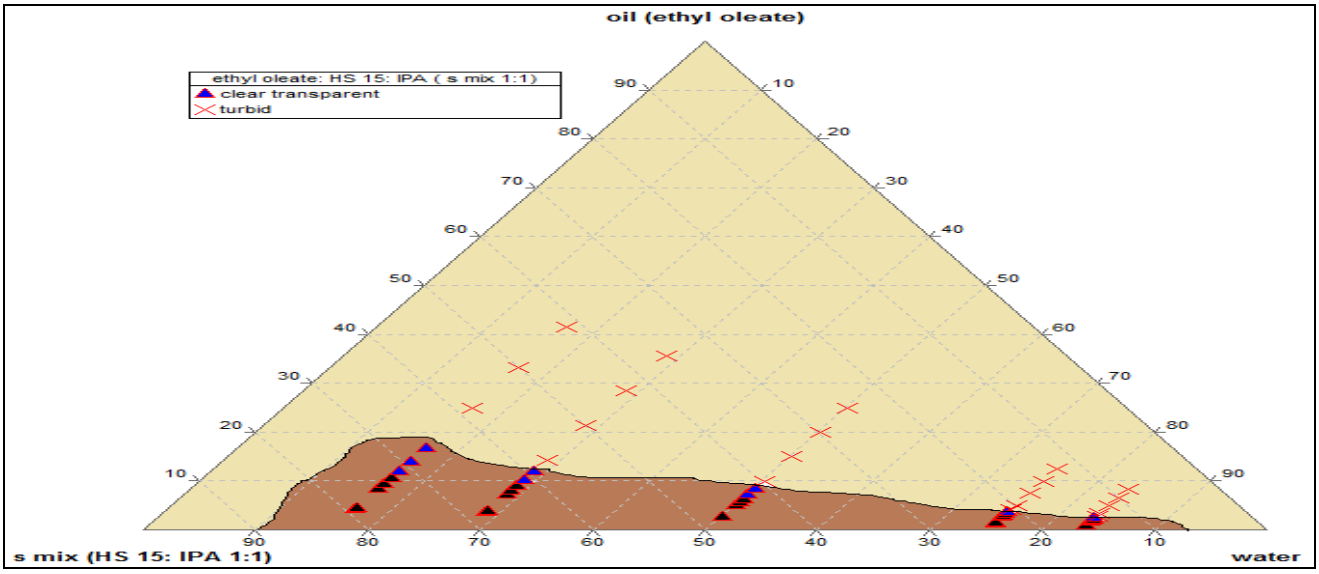
6(c)


oil (ethyl oleate)
ethyl oleate: HS 15: ethanol (s mix 3:1)
clear transparent
turbid
s mix (HS 15:ethanol 3:1)
water

6(d)

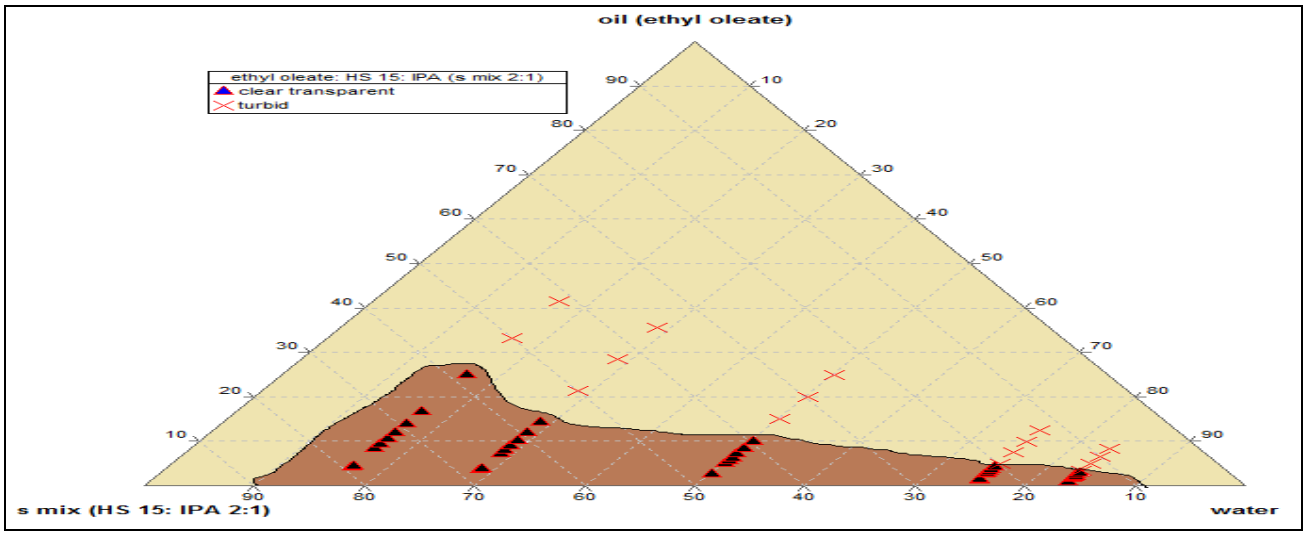


6(e)

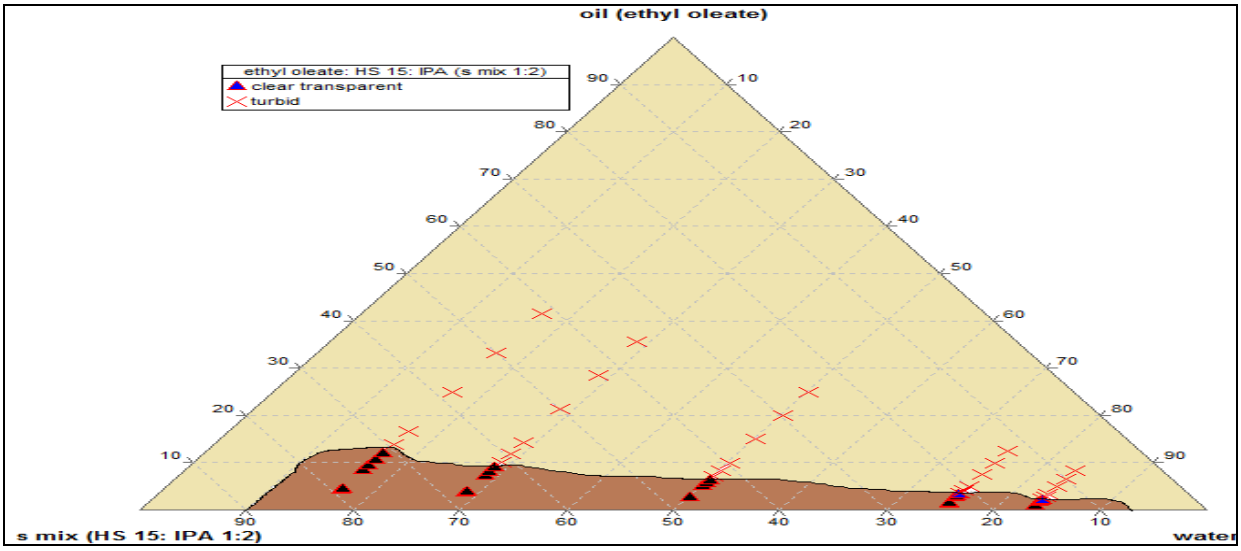


7 (a)

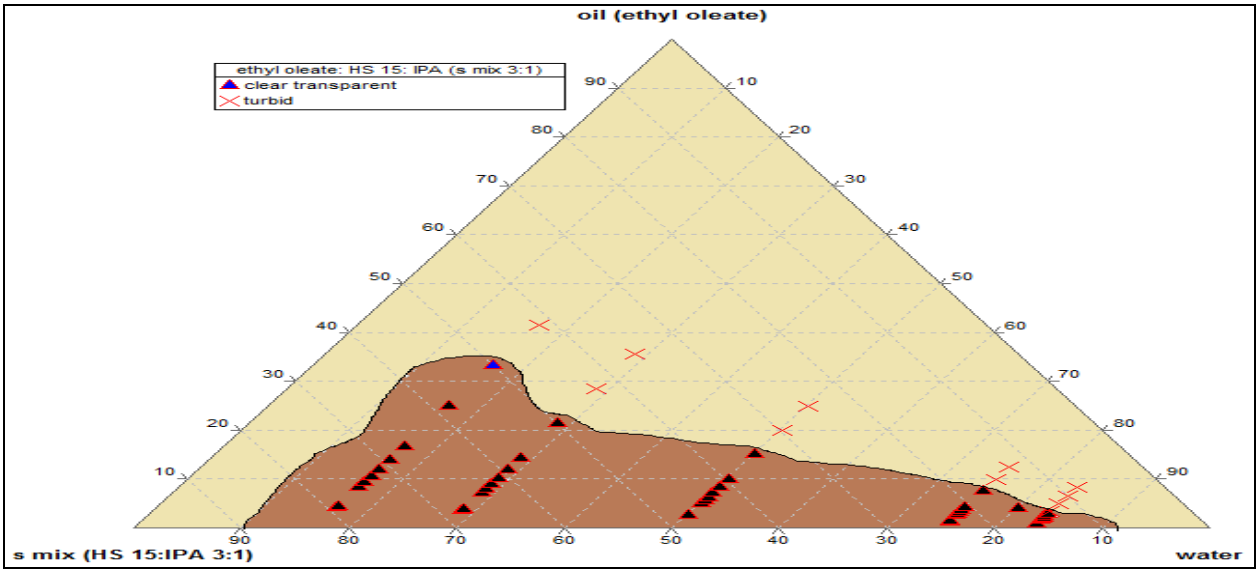
Figure 7: Ternary phase for combination II



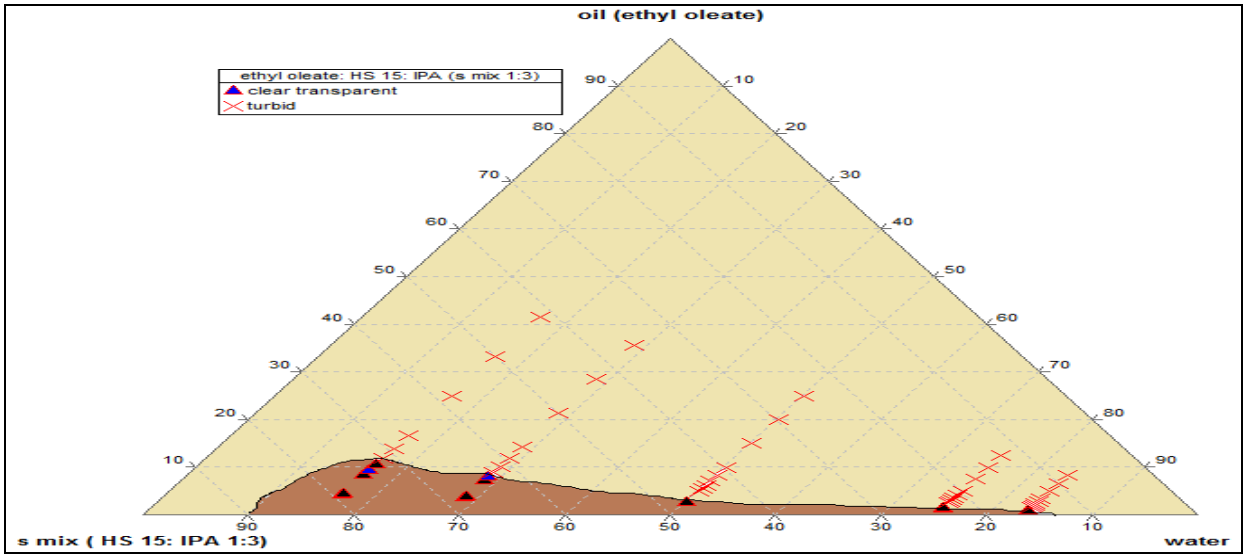
7(b)


oil (ethyl oleate)
ethyl oleate: HS 15: IPA (s mix 1:2)
clear transparent
turbid
s mix (HS 15: IPA 1:2)
water

7(c)


oil (ethyl oleate)
ethyl oleate: HS 15: IPA (s mix 3:1)
clear transparent
turbid
s mix (HS 15:IPA 3:1)
water

7(d)


oil (ethyl oleate)
ethyl oleate: HS 15: IPA (s mix 1:3)
clear transparent
turbid
s mix (HS 15: IPA 1:3)
water

7(e)

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