Analysis of TX-100 based microemulsion in the presence of TB drug isoniazid

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CERTIFICATE

This is to certify that the dissertation entitled "**Analysis of TX-100 based microemulsion in the presence of TB drug isoniazid**" being submitted by Shailesh **Kumar** to the Department Of Chemistry, National Instuitute Of Technology, Rourkela-769008, for the award of the degree of Master Of Science in Chemistry, is a record of bonafide research carried out by him under my supervision and guidance. The dissertation report has reached the standard fulfilling the requirements of the regulations relating to the nature of the degree.

I further certify that to the best of my knowledge Mr. Shailesh Kumar bears a good moral character.

NIT-Rourkela

Date: (**Sabita Patel**)

DECLARATION

I Mr. Shailesh Kumar, National Institute of Technology, Rourkela declare that all my research works are original and no part of this thesis has been submitted for any other degree or diploma. All the given information and works done are true to my sense and knowledge.

(Shailesh Kumar)

Date:

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(Shailesh Kumar)

CONTENTS

ABSTRACT:

The present study delineate the formulation of microemulsion composed of TX-100:AcOH(1:1), Chloroform, Water and to investigate its potential as drug delivery system for an antitubercular drug isoniazid. The pseudo-ternary phase diagram (Gibbs Triangle) were constructed for these systems with and without isoniazid. Microemulsion system showed the occurrence of structural changes from water-in-oil to oil-in-water microemulsion. It has been observed that the microemulsion remained stable after the incorporation of isoniazid. After dilution a controlled release of drug is expected from o/w emulsion droplet. Further to study the incorporation of drug in TX100 based microemulsion in presence of cetyl trimethyl ammonium dichromate (CTADC), a lipophylic oxidant, phase diagrams of systems containing TX-100:AcOH(1:1), Chloroform, CTADC, Water has been constructed in the presence and absence of isoniazid. The results suggested that isoniazid binds to the CTADC in o/w and w/o microemulsion. Thus drug release is possible where there is demand for the drug. The present TX 100 based microemulsion in presence of CTADC appears beneficial for the delivery of the isoniazid.

1. Introduction

Now a days delivery of drugs to the target cell is a challenging problem to the chemists and biologists. Various drug delivery systems are in use and more and more are proliferate to reduce the loss of drug due to absorption, metabolism, distribution and elimination in the body and to enhance the bioavailability of the drug. Drug delivery systems such as liposomes, polymeric micelles, polymeric vesicles and niosomes, microemulsions, lipoproteins, solid lipid nanoparticles, lipid coated submicron sized particles, nanocapsules, dendrimers, cochleates, aerosols, magnetic Nanoparticles, DQAsomes etc. are extensively reviewd [1].

1.1 Microemulsion as drug delivery system

The concept of microemulsion was first introduced by Hoar and Schulman in 1943 [2]. Microemulsion is a system of water, oil, and amphiphilic compounds (surfactant and co-surfactant) which is a transparent, optically isotropic, and thermodynamically stable liquid [3]. Microemulsions [4,5] has seeked a lot of attention for the design and development of new drug delivery systems because of their high solubilization capacity, thermodynamic stability, ease of preparation, and high diffusion and absorption rates. They help in the improvement of drug bioavailability [6], protection against enzymatic hydrolysis, and decrease toxicity [7].

Fig.1 [8] shows three types of microemulsions which are formed depending on composition. It can be seen while the three structures shown are quite different, in each there is an interfacial surfactant monolayer separating the oil and water domains. Note that while the oil-in-water (o/w) and water-in-oil (w/o) droplets are represented in Fig. 1 as spheres they may be asymmetric in shape, frequent adopting the shape of a prolate ellipsoid.

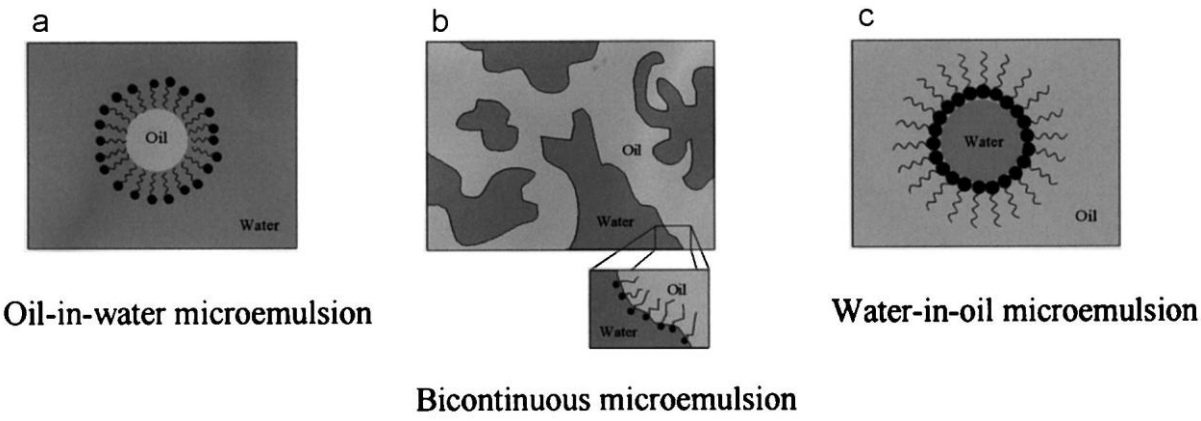


Fig. 1. Schematic representation of the three most commonly encountered microemulsion microstructures: (a) oil-in-water, (b) bicontinuous, and (c) water-in-oil microemulsion [8].

In addition to the use of microemulsions based system as drug delivery vehicles, these are also for various applications such as (i) Microemulsion in enhanced oil recovery (ii) Microemulsions as fuels (iii) Microemulsions as lubricants, cutting oils and corrosion inhibitors (iv) Microemulsions as coatings and textile finishing (v) Microemulsions in detergency (v) Microemulsions in cosmetics (vi) Microemulsions in agrochemicals (vii) Microemulsions in food (viii) Microemulsions in environmental remediation and detoxification (ix) Microporous media synthesis (microemulsion gel technique) (x) Microemulsions in analytical applications (xi) Microemulsions as liquid membranes (xii) Novel crystalline colloidal arrays as chemical sensor materials etc.

The use of microemulsion as drug carriers are extensively reviewed by different workers [8, 9, 10]. Mehta et al. [11] have studied the use of tween based microemulsion system for potential application as drug carrier for TB drug rifampicin. They have formulated microemulsion composed of oleic acid + phosphate buffer (PB) + Tween 80 + ethanol and investigated its potential as drug delivery system for an antitubercular drug rifampicin. From conductivity and viscosity studies with variation in weight fraction of aqueous phase and molar concentration ratio, they proposed structural changes from water-in-oil (w/o) microemulsion to oil-in-water (o/w). Along with the solubility and partition studies of

rifampicin in microemulsion components, the changes in the microstructure of the microemulsion after incorporation of drug have been evaluated to show that the microemulsion remained stable after the incorporation of rifampicin (in terms of optical texture and phase separation). From the dissolution studies they inferred a controlled release of rifampicin from o/w emulsion droplet. The experimental and theoretical study of drug release from microemulsions structured as a dispersion of oil droplets in water or vice versa have been focused by Sirotti et. al. [12]. Nimesulide (anti-inflammatory action) is chosen as model drug for its industrial relevance while isopropyl myristate (oil-phase), benzyl alcohol (co-surfactant), Tween 80 (surfactant), are taken to form the microemulsion (45.7% (w/w) water, 30.8% (w/w) surfactant, 11.75% (w/w) oil-phase and 11.75% (w/w) co-surfactant).

The potential protective effect, regarding the dermal application of DNAzyme, of multiple (W/O/W) emulsions, W/O emulsions, submicron emulsion and microemulsions were investigated using a HPLC method. It was found that the degradation of an aqueous solution of DNAzyme is depending on the DNase I activity as well as on the incubation time. Investigation of the protective character of different delivery systems revealed that formulations containing DNAzyme in the outer water phase (submicron emulsion and microemulsion) did not exhibit any form of protective effect, whereas formulations containing DNAzyme in the inner water phase (multiple emulsion and W/O emulsion) were able to prevent the DNAzyme degradation to a considerable degree. Consequently, these formulations are promising candidates for the dermal drug delivery of oligonucleotides [13].

Li et. al. [14] have studied the effects of combined use of two nonionic surfactants on the characteristics (i.e., appearance, emulsification time, and particle size) of oil-in-water microemulsions generated from flurbiprofen-loaded drug. From different experimental observation they found that, preconcentrates using single surfactant, either O 20T80 or O20C80, the dilution generated emulsions with visible cloudiness. The particle size increased

as the drug loading increased and for preconcentrates using two surfactants O20T40C40, the dilution generated clear microemulsions with small particle sizes (10-11 nm), and the increased drug loading seemed to have little effect on the particle size. The microemulsions from preconcentrate O20T40C40 was also found to be stable at ambient temperature over 20 days without significant change in particle size at differentdrug loadings. Dilution with different aqueous medium, either water, or simulated gastric fluid or simulated intestinal fluid, also did not change the particle sizes of the microemulsions. The combined use of surfactants in preconcentrate showed the promise in generating desired self-emulsifying microemulsions with small particle size, increased drug loading, and improved physical stability. This will have significant implications in future dosage development for poorly water-soluble drugs in using self-emulsifying microemulsions drug delivery system (SMEDDS).

Topical microemulsions of nimesulide, a poorly water-soluble nonsteroidal anti inflammatory drug, using olive oil as oil phase and Tween 80/iso-octanol as surfactant/co-surfactant were designed [15]. Various concentrations of surfactant: co-surfactant (2:1, 3:1, 4:1) were used in constructing the pseudoternary phase diagram. Oil-in-water microemulsions with ratio 15/35/50 (o/w/s) was chosen for the study. Using 4:1 ratio of surfactant : co-surfactant, a microemulsion-based gel of nimesulide was prepared with 1% Carbopol 934. In vitro permeation study of the gel was carried out through excised hairless rat skin and compared with a marketed preparation. The drug release after 24 h from the prepared microemulsion gel and marketed formulation was found to be 72% and 81% respectively. No significant difference was observed between the release rates of nimesulide from both formulations despite their differences in alcohol content. Microemulsion-based gel of poorly water-soluble nimesulide was successfully developed with in vitro release rates comparable to that of the marketed gel formulation.

2. Experimental

2.1 Preparation of microemulsion

The microemulsion was prepared by using oil (chloroform), surfactant (TX-100) (fig. 2) and acetic acid as co-surfactant with constant surfactant: co-surfactant mass ratio (1:1). TX-100 and acetic acid were first mixed with chloroform, water was then added to obtain the desired microemulsion compositions. The samples were observed visually.

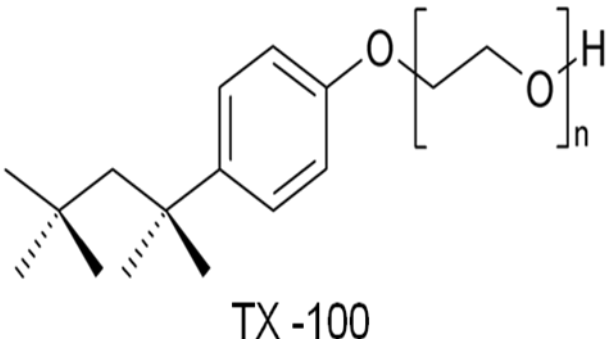


Fig. 2 Structure of TX-100

2.2 Drug incorporation in microemulsion

A solution of desired concentration of isoniazid (fig. 3) in acetic acid was first prepared followed by the addition of remaining components.

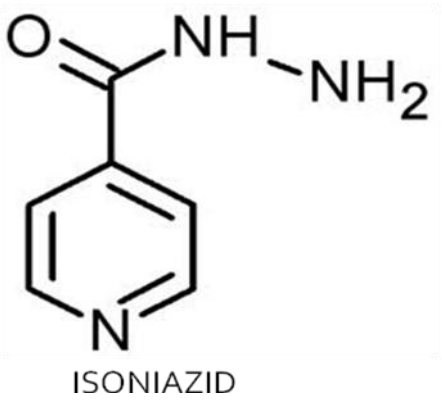


Fig. 3 Structure of Isoniazid (INH)

In the present work we have analysed the phase behaviour for the following system (table 1) for analysing the incorporation of drug isoniazid in the microemulsion. We have also added a lipophylic oxidant CTADC (cetyltrimethylammonium dichromate) to the microemulsion to study the solubilisation of isoniazid in that microemulsion.

Table 1: Components used to study the phase behaviour

Sl.No	**Components**
1	TX-100:AcOH(1:1), Chloroform, Water
2	TX-100:AcOH(1:1), Chloroform, Isoniazid, Water
3	TX-100:AcOH(1:1), Chloroform, CTADC, Water
4	TX-100:AcOH(1:1), Chloroform, Isoniazid, CTADC, Water

2.3 Construction of phase diagrams

Pseudo-ternary phase diagrams were constructed to examine the formation of different organized assemblies using 4 components: oil, surfactant, co-surfactant, and aqueous phase system. Pseudo-ternary phase diagrams were constructed keeping the ratio of surfactant (TX-100) and co-surfactant (acetic acid) constant and varying the remaining two components. The phase diagrams were constructed by drawing "water dilution lines" representing increasing water content and decreasing surfactant:co-surfactant levels [16]. The mixture of surfactant: co-surfactant and oil was titrated with water along dilution lines drawn from the emulsifyer-oil line to the opposite water apex (water 100%) of the triangle. If turbidity appeared followed by a phase separation, the compositions were considered to be biphasic. If clear and transparent mixtures were visualized after stirring, the samples were considered monophasic. The samples were marked as points in the phase diagram as per their weight percentage. The area covered by these points was considered to be the microemulsion region of existence.

3. Results and Discussion

3.1 Phase Behaviour

The phase behaviour [17] of microemulsion system composed of oil, water, surfactant and co-surfactant was studied with the aid of pseudo ternary phase diagram. In this diagram, the three apex E, O and W represents emulsifier (surfactant+cosurfactant), oil and water respectively.

The pseudo-ternary phase diagram and area of existence of microemulsion for TX-100/acetic acid/chloroform/water is presented in Fig.4. Microemulsion in the present study formed spontaneously when their components were brought into contact. The phase behaviour initially exhibits w/o microemulsion which transformed through bicontinuous phase to o/w microemulsion.

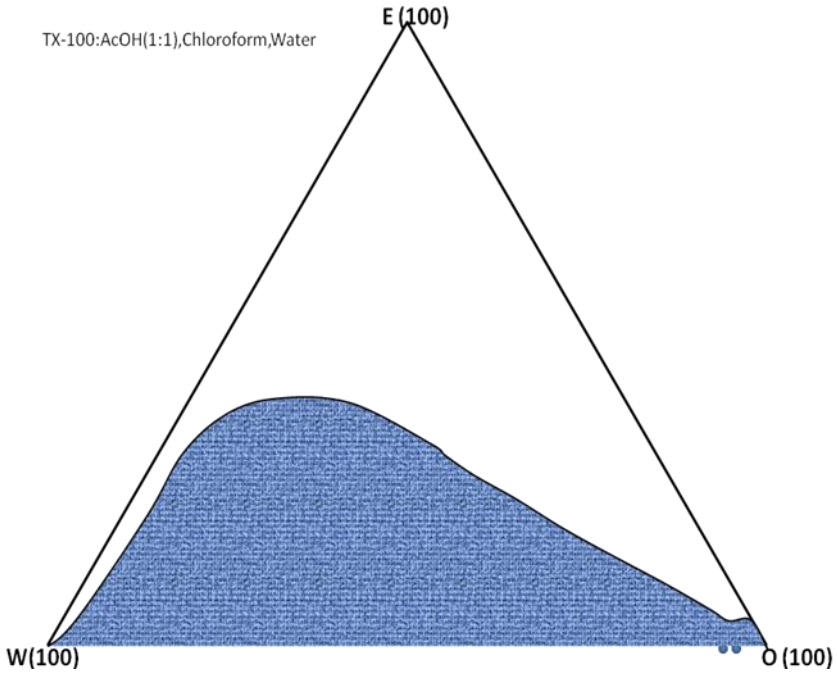


Fig. 4 Pseudo-ternary phase diagram of TX-100/AcOH/chloroform/water. Shaded zone represents turbid biphasic region and the rest is clear monophasic zone.

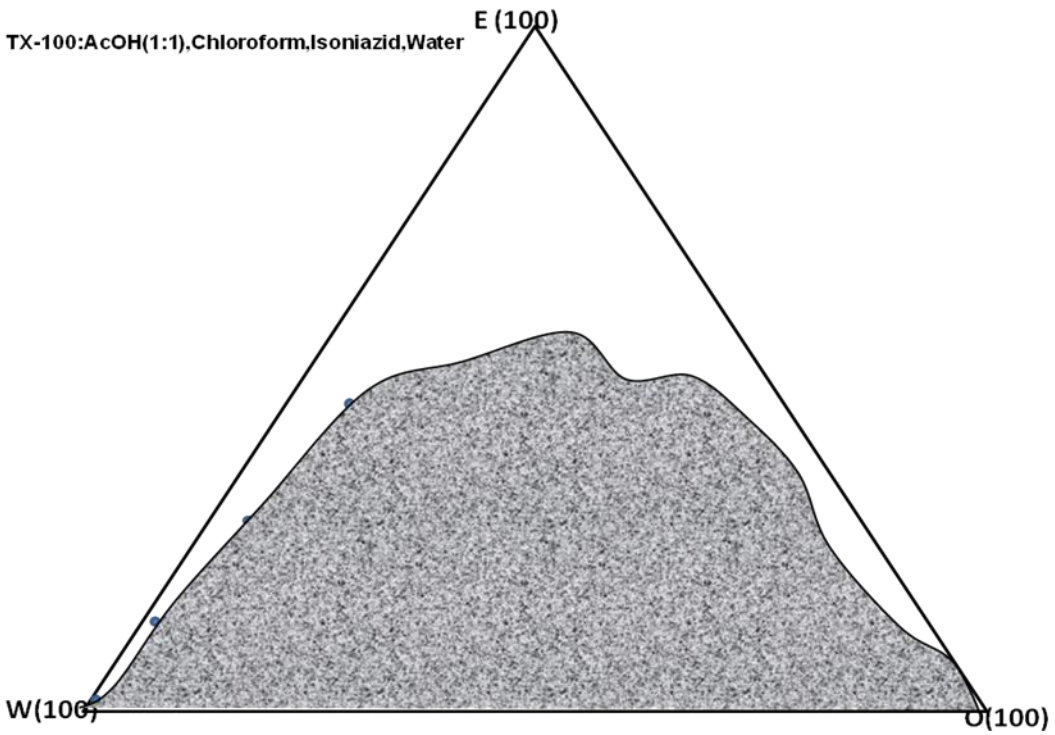


Fig. 5 Pseudo-ternary phase diagram of TX-100/AcOH/chloroform/water inpresence of isoniazid Shaded zone represents turbid biphasic region and the rest is transparent monophasic zone.

The pseudo-ternary phase diagram and area of existence of microemulsion for TX-100/acetic acid/chloroform/water inpresence of drug isoniazid is presented in Fig. 5. We can observe that the turbid region increases after addition of drug. This may be attributed to the higher solubility of the water soluble drug leading to desolubilization of other components in the phase, which increases the turbid region in the phase diagram. In the isotropic domain, the structure of the surfactant assemblies changes from water in oil microemulsion (w/o) at low water content to oil in water microemulsion (o/w) at higher water content through bicontinuous zone. Mehta et.al [18] have studied the delivery of isoniazid through tween based microemulsion systems and explained the release of the drug through the structural change from w/o to o/w microemulsion. In w/o microemulsion the drug remains in the water pool. But on dilution this reverse micellar system transformed to micellar system through bicontinuous phase and the drug releases to the bulk aqueous phase from where it can be

transported to targeted site (Fig. 6). Thus the microemulsion system can be used as drug delivery system for isonniazid. Before dilution it remains in the water pool separated by the outside environment through a layer of emulsifier and thus it can be protected against the loss due to adsorption, metabolism, distribution or elimination from the body. This increases the bioavailability of the drug at the target site.

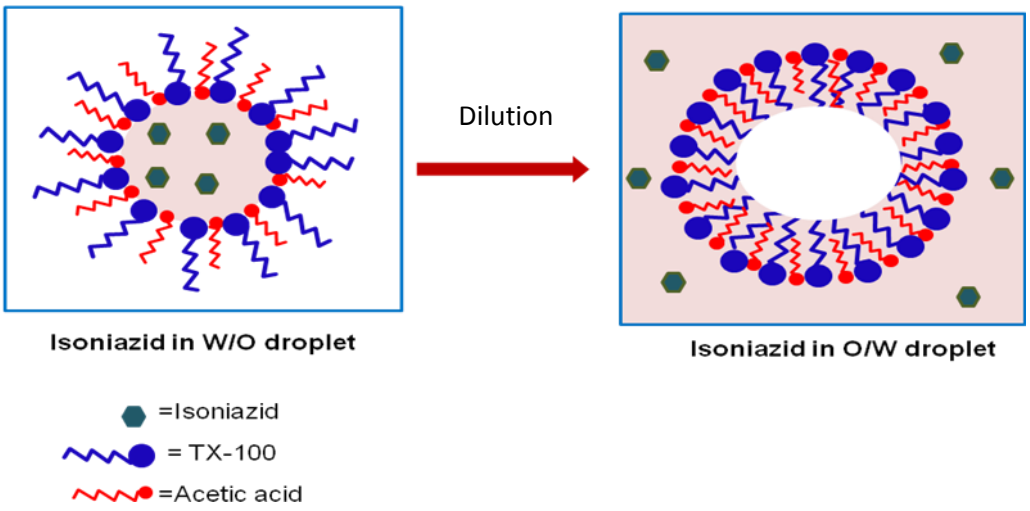
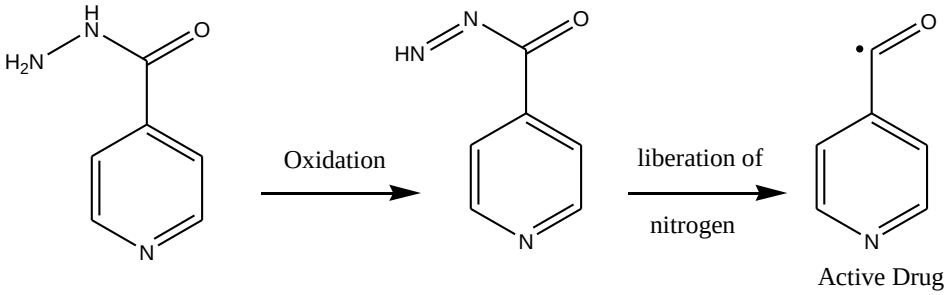


Fig. 6 Probable drug site before and after dilution

We have also studied the incorporation of isoniazid in presence of lipophylic oxidant CTADC. CTADC is a Cr(VI) containing oxidant with amphilic cetyltrimethylammonium counter ion[19-27]. This reagent is water insoluble and stable at room temperature for more than a year when kept in sealed bottle. Exists as a tight ionpair and forms monolayer on water surface with area/molecule 51 Å2 [28]. Although Cr(VI) is undisputedly carcinogenic, the insolubility in water reduces contamination of Cr(VI) in aqueous medium, and the compound thus can be used as a green reagent. Further, CTADC is devoid of an acidic proton and thus is relatively milder than other Cr(VI) oxidants. In the absence of acid, CTADC exhibits some bizarre reactions with nonconventional products. Aromatic amines are found to yield corresponding diazo compounds,[19] and arylaldoximes yielded corresponding nitriles. [20] In an oxidation reaction of cholesterol with CTADC, Patel et al. [22] have observed that 7-

dehydrocholesterol is obtained instead of usual product cholestenone. This dehydrogenation is a rare event in Cr(VI) oxidation studies, and it is explained through remote functionalization mechanism where the cetyltrimethylammonium ion provides a favourable environment for proper orientation of the dichromate group so that the removal of hydrogen from 7^{th} and 8^{th} position becomes easier. In most of the cases CTADC acts as a dehydrogenating agent.

In the process of activation of isoniazid to form the true drug the first step is a dehydrative oxidation (Scheme 1) [29]. CTADC in the microemulsion may enhance the drug activity through dehydrative oxidation. Thus we have studied the phase behaviour in presence of CTADC.



(Scheme 1)

Fig. 7 and 8 represents the phase behaviour of TX100/acetic acid/ water/oil and CTADC in the absence and in precence of isonizid. From the figure it is clear that, there is no considerable change in the area of different phases when compared with fig. 7. From this observation we can infer that, although isoniazid is water soluble but in the presence of CTADC it binds with CTADC in interfacial region (Fig. 9), thus solubility of other components remains same. Thus in the o/w microemulsion also isonizid may be bounded to CTADC and it may release where the demand for the drug will be more. Thus the presence of

CTADC may enhance the possibility of this microemulsion as a better drug carrier and releases the drug wherever necessary reducing the loss due to elimination

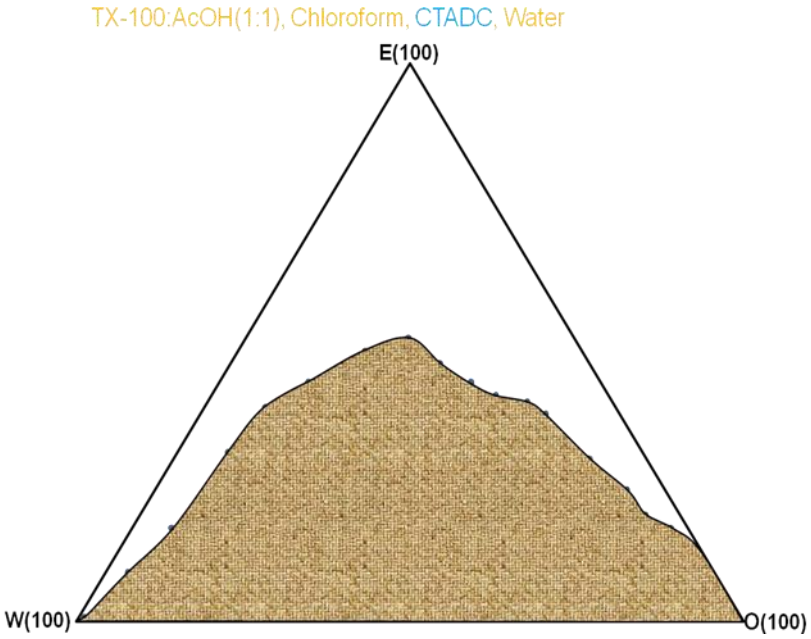


Fig. 7 Pseudo-ternary phase diagram of TX-100/AcOH/chloroform/water/CTADC. Shaded zone represents turbid biphasic region and the rest is transparent monophasic zone.

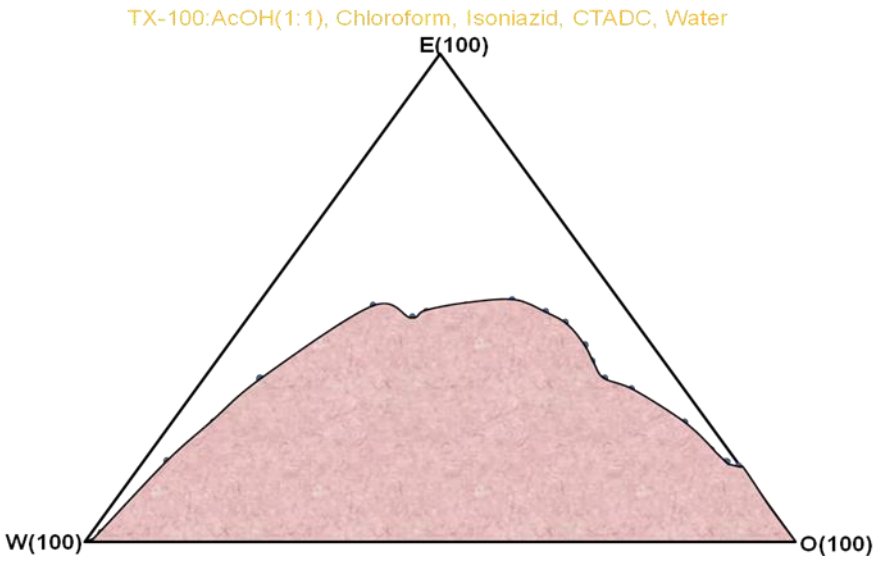


Fig. 8 Pseudo-ternary phase diagram of TX-100/AcOH/chloroform/water and CTADC in presence of isoniazid. Shaded zone represents turbid biphasic and the rest is transparent monophasic zone.

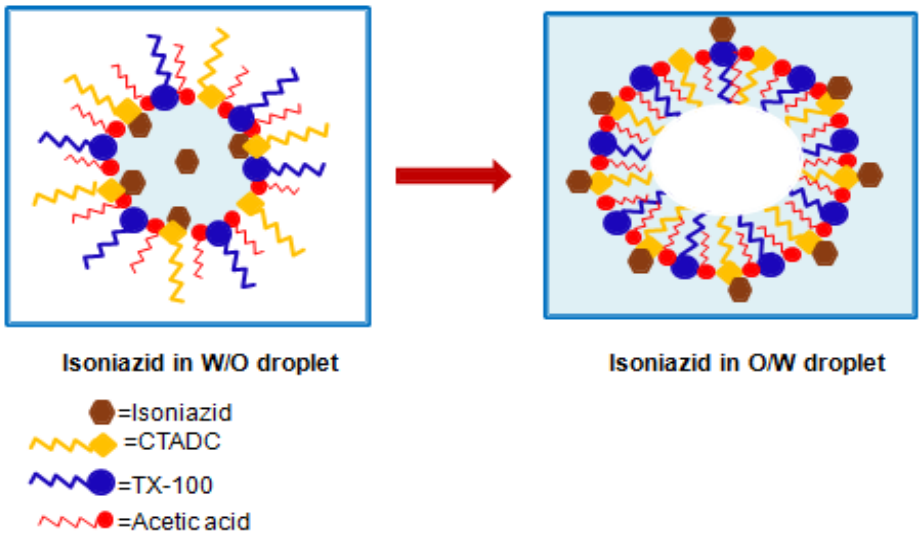


Fig. 9 location of isoniazid in presence of CTADC in w/o and o/w microemulsion.

4. Conclusions

Incorporation of the drug in the microemulsion, it remains stable and optically clear with no phase separation. The presence of drug in the microemulsion is not influencing the measured physical parameters to a larger extent and therefore is not affecting the microstructure of the drug delivery vehicle. At infinite dilution the W/O microemulsion droplet changes in to O/W emulsion type and there was a sustained release of isoniazid from this microemulsion drug delivery vehicle. Microemulsion protect labile drug, control drug release, increase drug solubility and increase bioavailability.

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