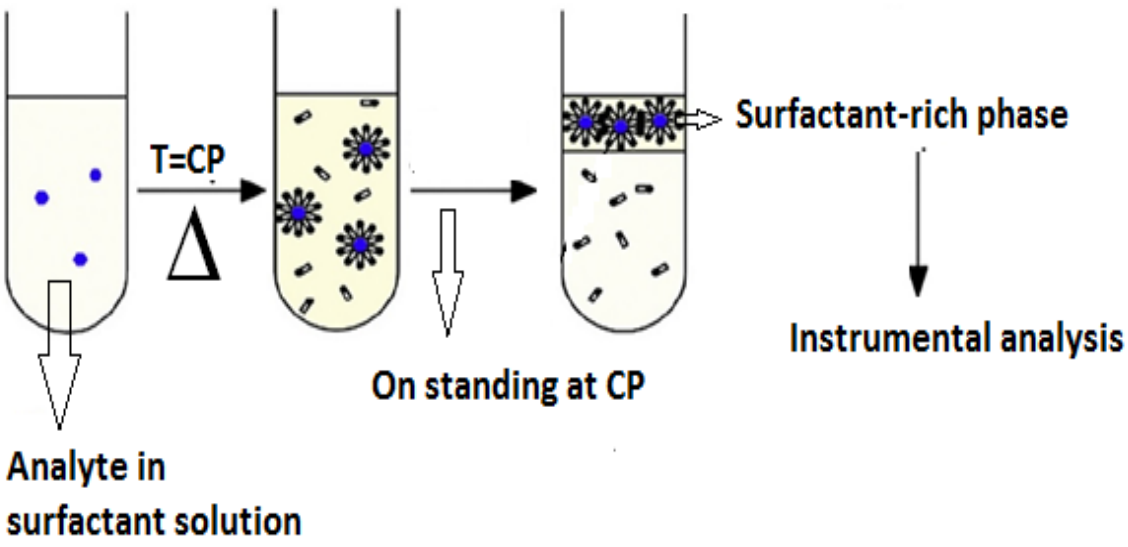
## 6.1. Introduction

In pharmaceutical field, interaction of various amphiphilic molecules with drugs has extensively been studied. A most common challenge is to formulate drug and drug carriers with good aqueous solubility together with no effect on potency. Surfactant solutions are reported to be used advantageously as a drug carrier over other alternatives [184, 185] because higher order aggregates can act as drug containing reservoirs. Specific modification to this composition can adjust the sustained and targeted release depending upon the external stimulus [186]. It has been reported that surfactants form mixed micelles with drug molecules. Thus, it is always desirable to know the solution behavior of drug-surfactant combinations under various conditions (temperature, additives, pH or concentration). As reported in previous chapters, both ionic and non-ionic surfactants can undergo clouding under variety of conditions mentioned above. The phase transfer surfactant-based extraction methods like the CPE or coacervative extraction (CAE) provides distinct advantages for pre-concentration and sample preparation as they are simple, efficient and greener having wide spectrum of applications. However, in surfactant-based extraction methods, solutions of surfactant aggregates are phase separated, with one phase rich in surfactant and the other phase rich in water [40].

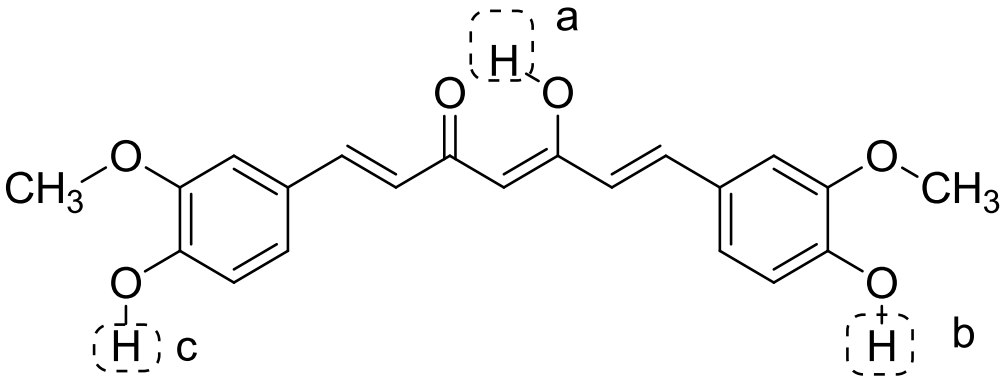
The analysis of industrial samples, their preparation and purification, extraction, isolation and/ or preconcentration of one's present in trace amount have also been the major challenge for analytical chemists. To solve this problem, many conventional methods like liquid-liquid extraction (LLE) and liquid-solid extraction (LSE) coupled with mass spectrometry, gas chromatography and many other chromatographic techniques, are reported. These methods have the ability to separate the target analytes from the sample solution, reduce, control and even eliminate the interferences originally present. Most of these methods are expensive when using large amount of chemicals, time consuming and use the complicated instrumentation. This also poses the adverse effects to the environment as large amount of solvents are required for the analysis. Therefore, a continuous demand of an analytical technique offering the best environment friendly advantages is persisting from long time. In many applications CPE has served as a primary isolation step in purification and extraction of metal ions as well as the bio active compounds. [42, 187]

Generally it is reported the preparation of nanoemulsions stabilized using ionic surfactants is not possible as temperature does not change the behavior of ionic surfactants [188]. But as discussed in previous chapter, the quaternary surfactants respond to the temperature and also, the solution separates into surfactant rich and surfactant lean phase these surfactants can be of great potential if used in the area of interest. Whenever a hydrophobic molecule is present in surfactant solution and the system phase separates at CP, the hydrophobic molecule is expected to concentrate in surfactant rich phase [32]. As the surfactant rich phase is small in volume and if hydrophobic molecule is a drug, its concentration may increase many fold in a small volume (Scheme 1). These systems resemble the biphasic aqueous-organic solvents even at the macroscopic level, but the surfactant-rich phase provides a much safer, more economical alternative to hazardous, expensive organic solvents used in LLE. Such changes may affect drug's activity as well as bioavailability. Hence, influence of drugs on the solution behavior of drug carrier (e.g., surfactant solution), is a problem of genuine interest.

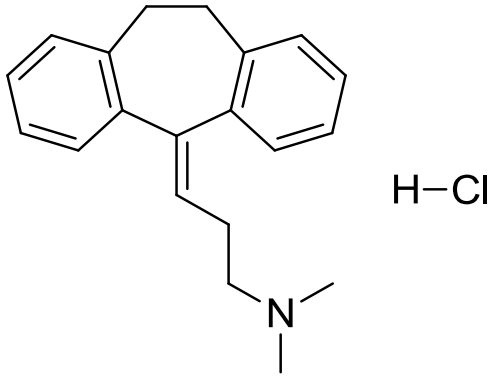
In the light of above view point, a few drugs have been added to TBADS solution and clouding behavior has been studied. The drug selected are AMT and curcumin. AMT belongs to the family of tricyclic antidepressants and possesses a rigid, almost planar tricyclic ring system and a short hydrocarbon chain carrying a terminal nitrogen atom (Scheme 2). A major challenge in using curcumin for treatment of diseases is the poor aqueous solubility, which significantly limits its availability in biological systems. An attractive alternative approach to addressing the poor aqueous solubility issue is to encapsulate curcumin in surfactant micelles; several studies have shown that curcumin has a significantly higher solubility in micellar solutions [189, 190].




**Scheme 1**: Solution behavior of surfactant on heating




**Keto –enol form of Curcumin**




**AMT**

**Scheme 2**: Chemical structures of drugs

## 6.2. Results and Discussion

### *6.2.1. Clouding Behaviour of TBADS in presence of Drugs*

From the previous studies it has been found that TBADS shows clouding on heating. The effect of [TBADS] variation on CP shows that 0.03M TBADS has CP (43 °C) which provides enough temperature below and above it to see the influence of various additives. CP data for 0.03M TBADS in presence of AMT and curcumin are compiled in Table 1. The tricyclic portion of the AMT molecule is hydrophobic, and the tertiary amine portion is hydrophilic. The latter portion becomes protonated (cationic) at low pHs, and deprotonated (neutral) at high pHs. The ionization constant, pKa, of AMT in the free molecular state is 9.4 [191]. Therefore, AMT is expected to behave as a neutral molecule in the present experimental conditions. AMT addition decreases the CP of 0.03M TBADS. Due to hydrophobic nature of the AMT, it is expected to interact with TBADS micelle and may get solubilised in the interior of the micelle. It has been reported that hydrophobic alcohols (medium to large chain alcohols) solubilise in the micellar interior and causes micellar growth and decrease the CP of TBADS solution [99]. The similar situation can be expected in TBADS-AMT system and CP decrease can be interpreted in terms of AMT solubilisation and its effect on increased hydrophobicity of the mixed micelle. Above interrelated factors are responsible for CP decrease as indeed observed (Table 1). NMR data of TBADS-AMT system (Figure 1) show a clear cut downfield shift wit increasing temperature. A few peaks either split or get broadened with the increase of temperature. This peak broadening and splitting indicate the formation grown micelles followed by the presence of two populations near the CP (see Chapter 4 for more discussion). The deeper interaction of AMT with micellar interior was also confirmed by the appearance of cross peaks in 2D NOESY NMR spectra (Figure 2b) absent in 2D COSY spectra (Figure 2a). This discussion finds support from the POM data (Figure 3). Due to increased solubilisation near CP, the grown aggregates are observed in optical micrographs.

**Table 1: CP data for 0.03M TBADS in presence of different concentrations of AMT/ Curcumin**

| Concentration (M) | CP °C | |
|---|---|---|
| | AMT | Curcumin |
| 0.000 | 43.0 | 43.0 |
| 0.001 | 42.2 | 42.1 |
| 0.002 | 41.2 | 41.0 |
| 0.003 | 40.2 | 32.0 |
| 0.004 | 38.8 | 29.0 |
| 0.005 | 37.5 | 24.3 |
| 0.010 | 27.5 | - |

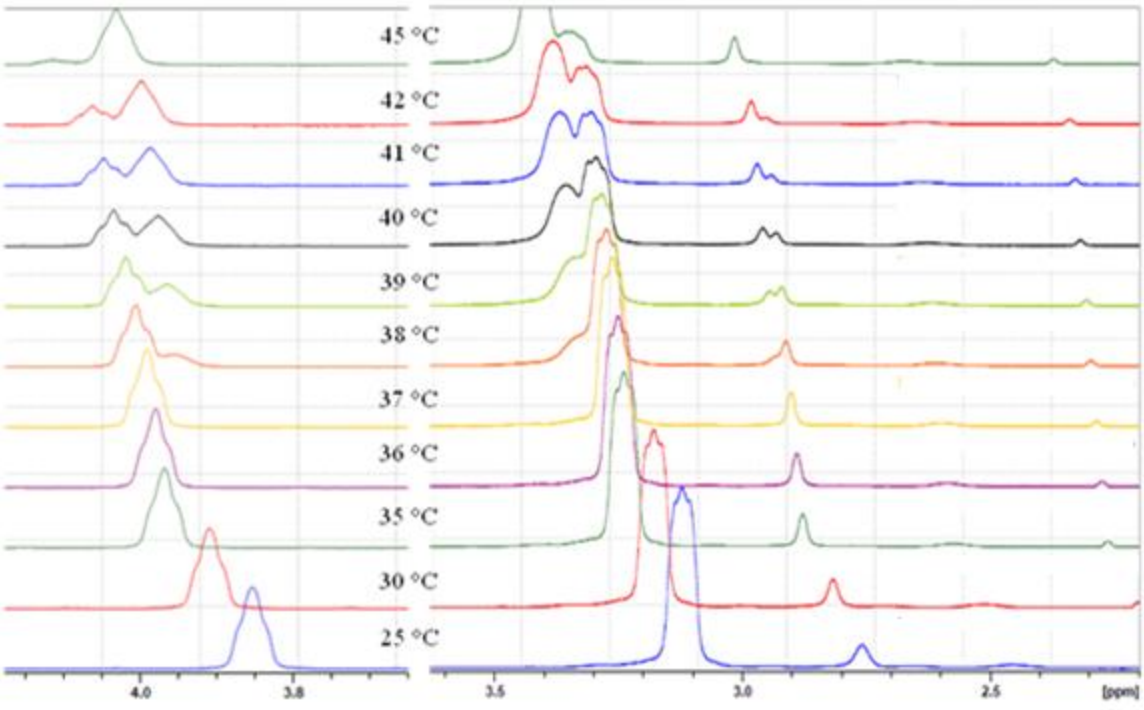



**Figure:** 1 $^{1}$H NMR spectra for 0.03M TBADS solution in presence of 0.003 M AMT at different temperatures (below and above CP) showing the broadening and splitting of the peaks.

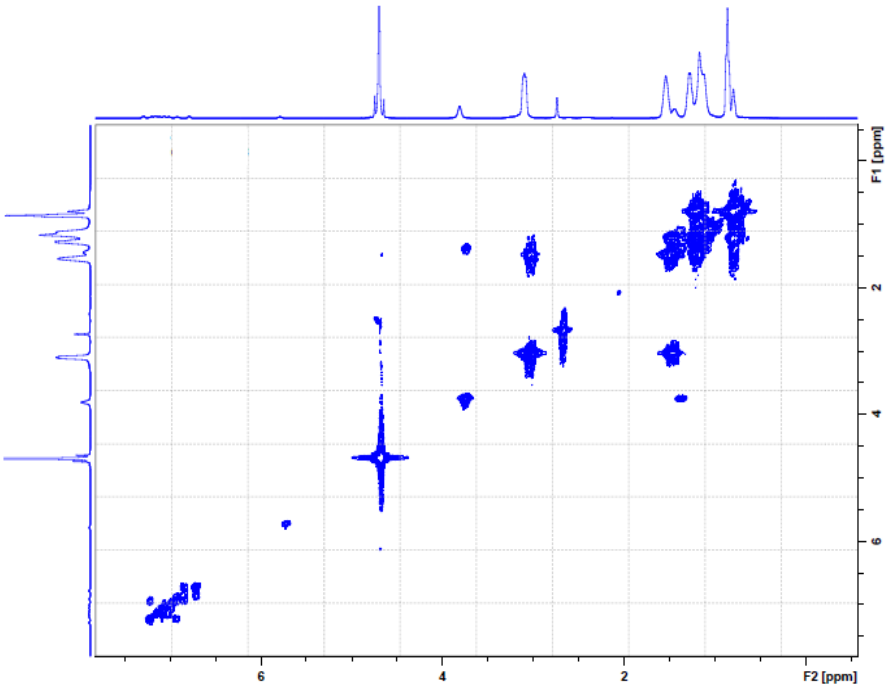



**Figure 2a**: 2D COSY NMR spectra of 0.03M TBADS+0.003M AMT at 18 °C

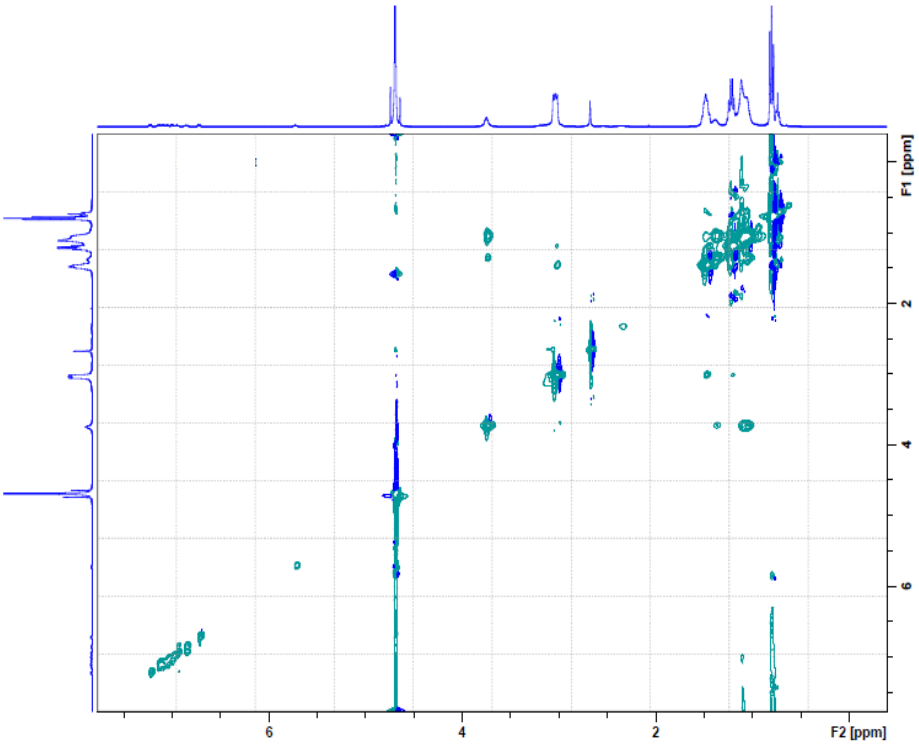
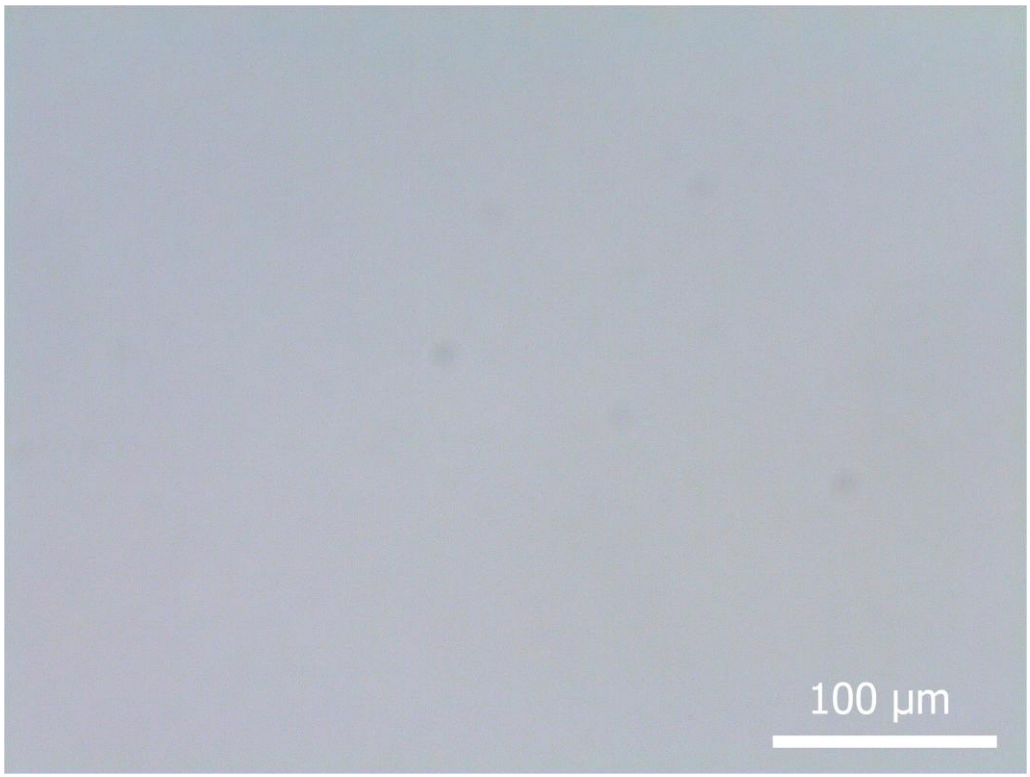
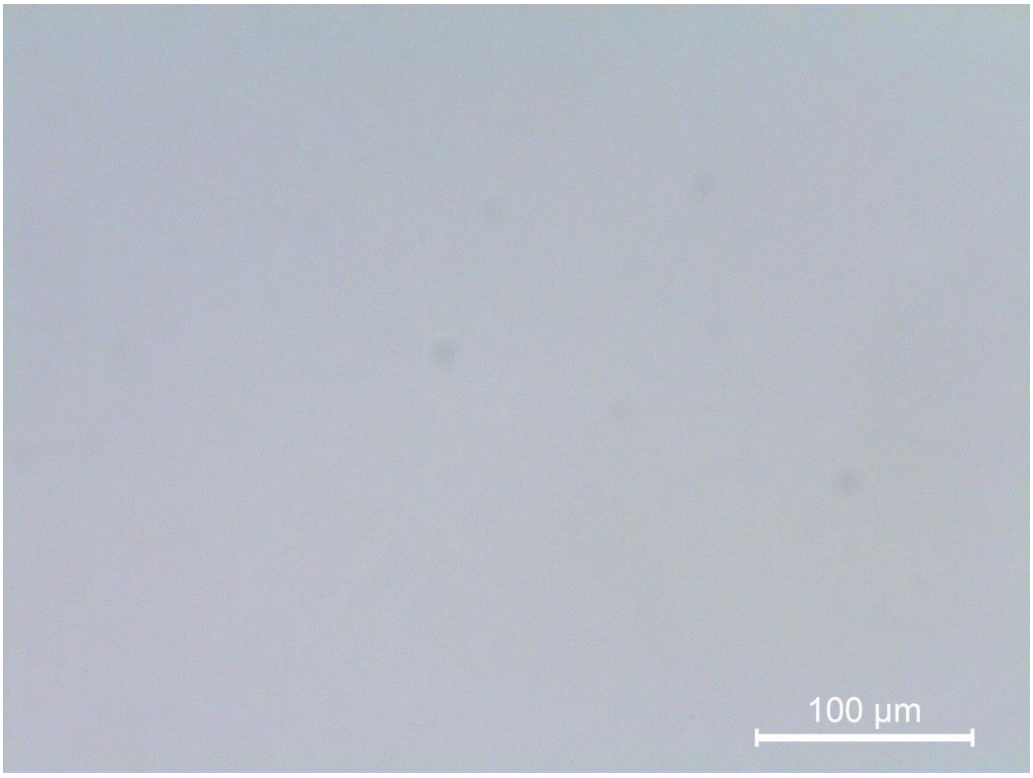


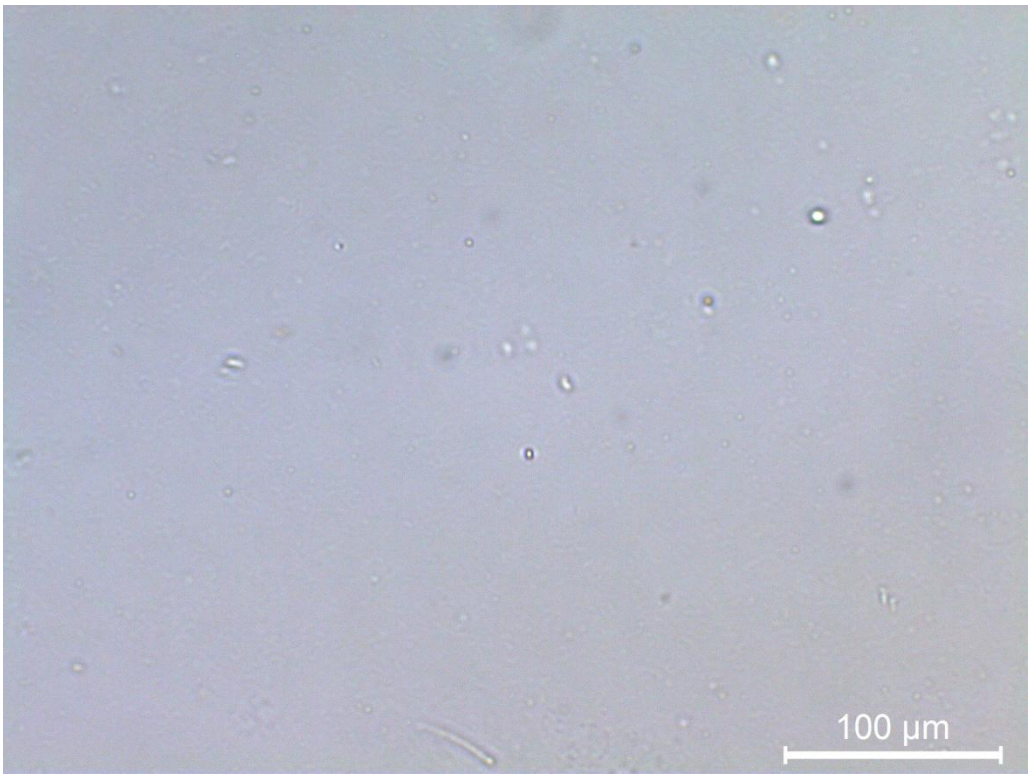

**Figure 2b**: 2D NOESY NMR spectra of 0.03M TBADS+0.003M AMT near CP.



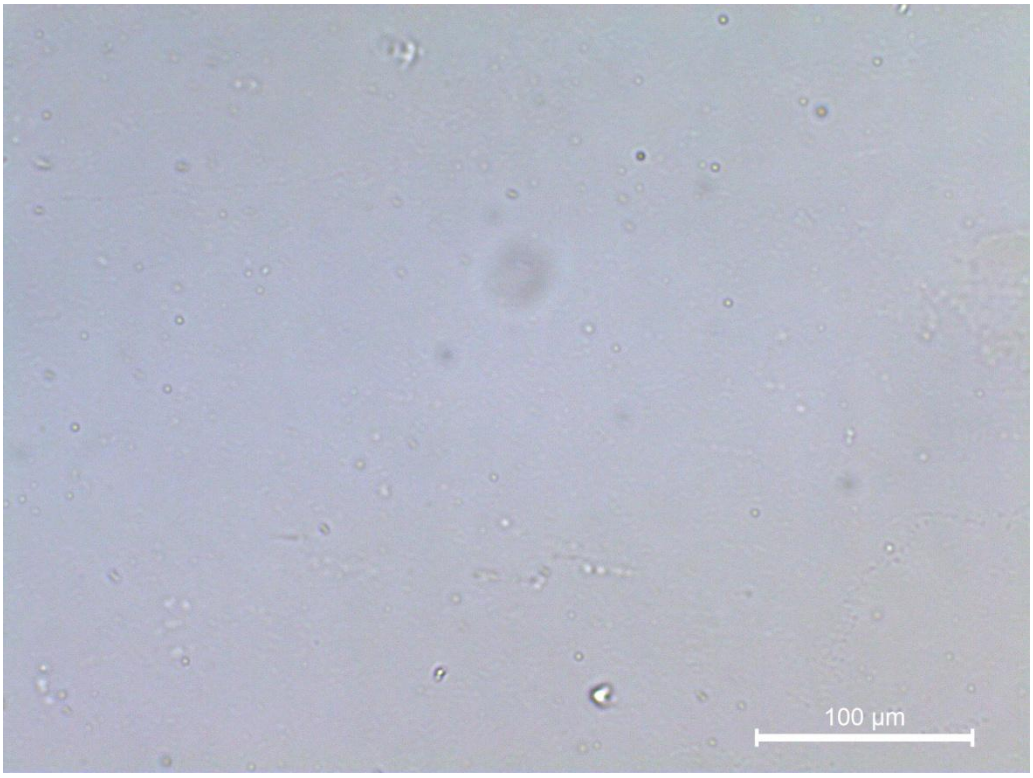

(a)



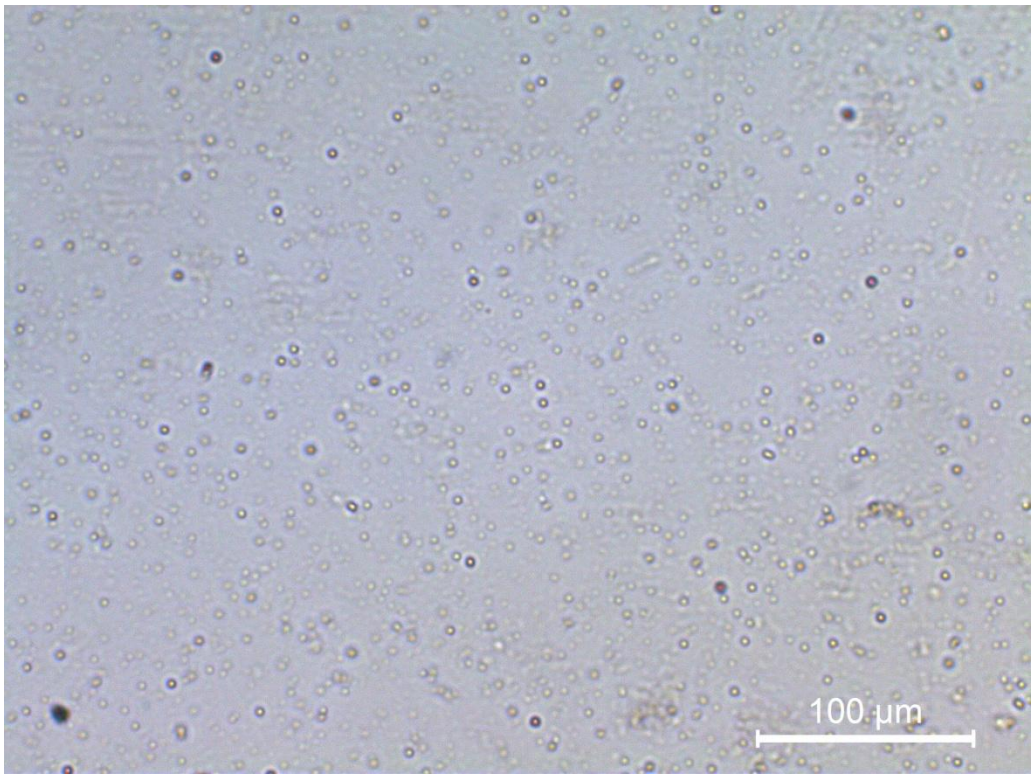

(b)



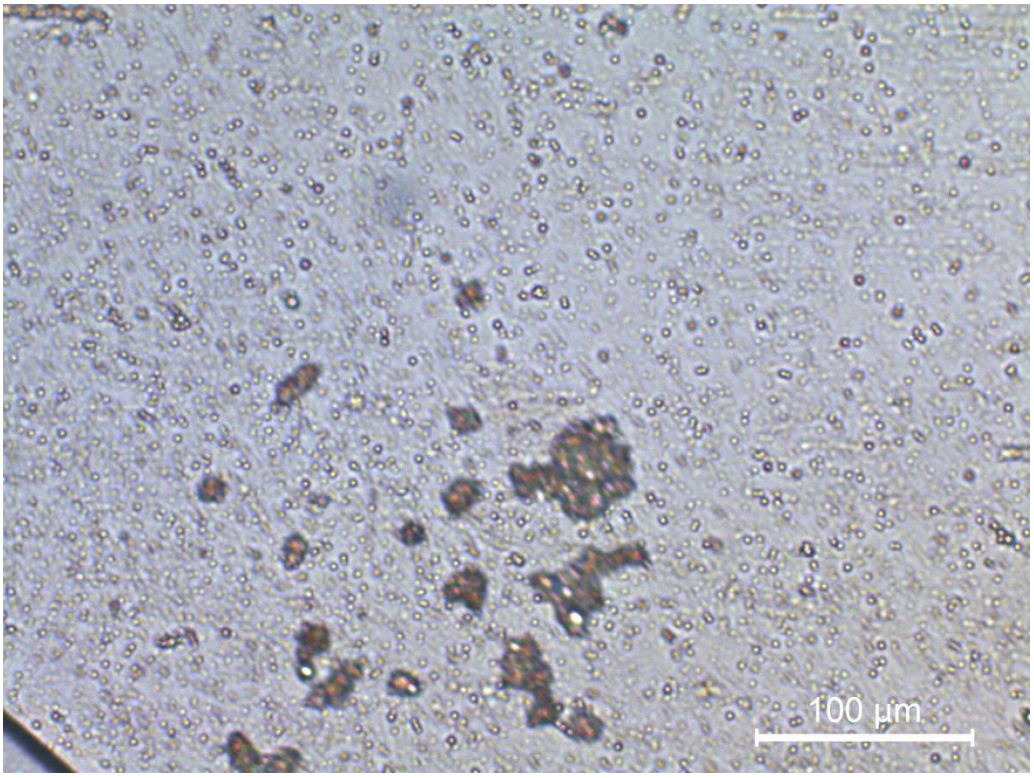

(c)



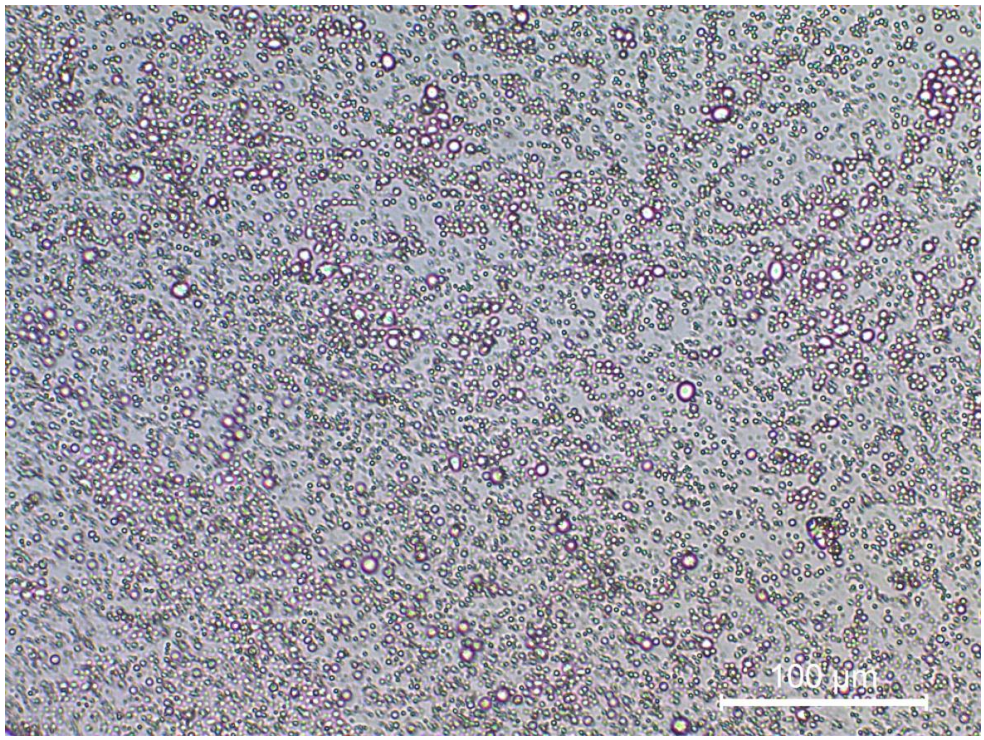

(d)



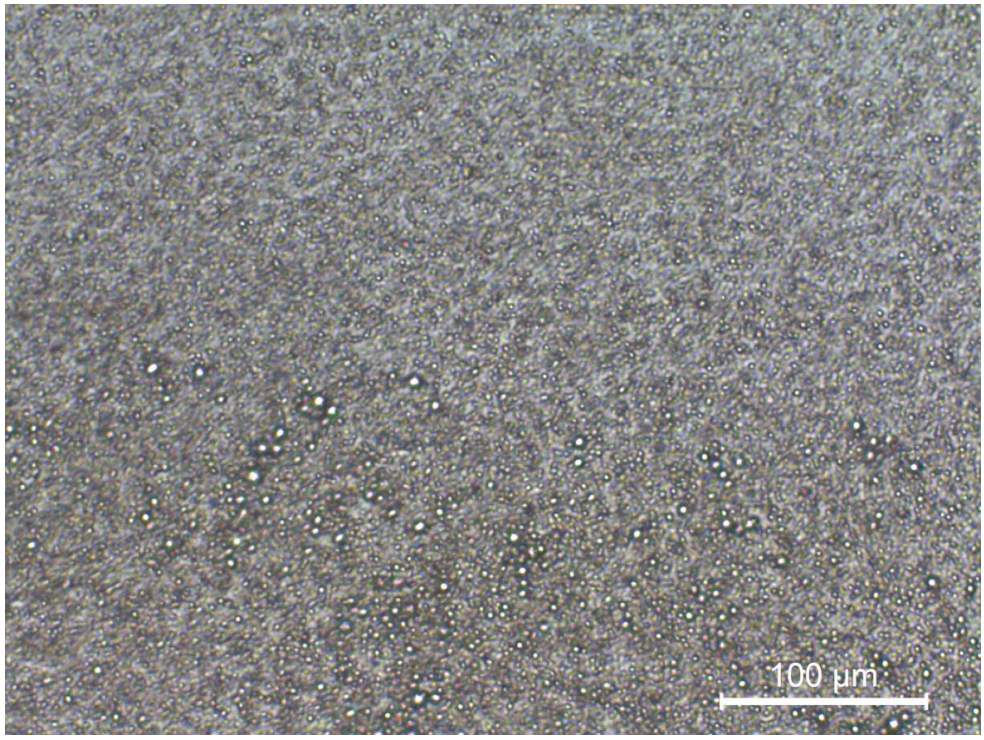

(e)




(f)




(g)




(h)

**Figure 10 :** Optical micrographs of 0.03M TBADS+0.003M AMT below (a) 30 °C, (b) 35.0 °C, (c) 38.5 °C, (d) 39.0 °C, near (e) 40 °C, at (f) 40.5 °C , on standing (g ) 40.5 °C and above CP (h) 42.0 °C

### *6.2.2. Preconcentration studies with Curcumin/ $HgCl_2$*

Solubility of curcumin in TBADS solutions has been studied at 25 °C and compared with other conventional ionic surfactants (SDS and CTAB). It has been found that solubility (absorbance at $\lambda_{max}$ = 419.7nm) increases upto certain concentration followed by a level off (Table 2). Therefore, 0.03M TBADS has been selected for the comparison with conventional ionic surfactants (Table 2.). At equal concentration, TBADS was found more effective in solubilizing curcumin. This may be due to less hydrated micelle formed by TBADS (Chapter 4) which can provide enough volume for hydrophobic moiety (e.g., curcumin) to get solubilised with a concomitant increase in the absorbance values. Though it has already been mentioned, that micellar solutions increase the curcumin solubility. The present study is an addition as it shows further increase in aqueous solubility of curcumin with TBADS and also can be used to increase the bioavailability of curcumin (due to pre-concentrating effect) under physiological conditions. To establish this point, a calibration data was collected for curcumin by dissolving it into ethanol at ($\lambda_{max}$ = 419.7nm) (Table 3). To get the CP near ambient temperature and to get an increased volume of rich phase, [TBADS] was taken 0.1M. The curcumin was solubilised followed by standing the system (0.1M+curcumin) at CP. The surfactant rich phase shows high solubility of curcumin (out of range of the instrument). Therefore, surfactant rich phase was diluted with ethanol and absorbance of this diluted system and surfactant lean phase was noted. Even after dilution (25 time), absorbance value was distinctly high indicating the potential of quaternary surfactants to work in pre-concentrating step of the CPE.

**Table 2**: Effect of different surfactants on solubility of curcumin

| Surfactant | Concentration (M) | Absorbance |
|---|---|---|
| TBADS | 0.015 | 2.40 |
| | 0.03 | 3.40 |
| | 0.05 | 3.42 |
| SDS | 0.03 | 1.9 |
| CTAB | 0.03 | 2.3 |

**Table 2**: Absorbance of curcumin in ethanol and the separated phases

| [Curcumin] ppm | Abs at ($\lambda_{max}$ = 419.7) |
|---|---|
| 2 | 0.31 |
| 10 | 1.39 |
| 25 | 1.94 |
| 50 | 2.10 |
| 100 | 2.18 |
| 125 | 2.20 |
| 250 | 2.27 |
| 500 | 2.33 |
| 1000 | 2.38 |
| Lean phase | 0.65 |
| Rich phase | 2.23 |

The effect of $HgCl_2$ addition on the CP behaviour of 0.03M TBADS has been studied. It was found that CP decreases as the [$HgCl_2$] increases. To establish the pre-concentrating role of quaternary surfactants, 0.006M $HgCl_2$ (1630ppm) solution was prepared in 0.1M TBADS aqueous solution (CP=31°C). This solution was heated till 40°C (as it is known that the % fraction of rich phase increases at higher temperature and also depends on the initial concentration of surfactant) and allowed to stand at the same temperature for 48hrs). The system was separated into two phases and was allowed to cool at RT. The surfactant rich phase was separated and diluted with water. The final solution was introduced to the flame by conventional aspiration. Similarly, AAS was also used to analyse the surfactant lean phase. It has been found that even at double dilution of surfactant rich phase (Table 4), the absorbance (0.103) was nearly 2.5 times than that of the lean phase (0.04). This indicates that the present surfactant system is able to concentrate the mercury without any chelating or hydrophobicity providing agent [192].
It was found out that preconcentration of $HgCl_2$ has taken place.

**Table 4**: The AAS data for $HgCl_2$ in TBADS phases.

| [$HgCl_2$] ppm | Absorbance | |
|---|---|---|
| 0.1 | 0.004 | |
| 0.2 | 0.008 | |
| 0.3 | 0.011 | |
| 0.4 | 0.016 | |
| 0.5 | 0.21 | |
| Separated phase | | Dilution |
| Lean phase | 0.040 | 25 times |
| Rich Phase | 0.103 | 50 times |

## 6.3. Conclusions

AMT and curcumin have been found to decrease the CP of TBADS solution. The rate of CP decrease was found to be dependent on hydrophobic interactions and interior penetration of micellar core. $^{1}$H NMR, 2D NMR, DLS and POM show the growth of the TBADS-AMT mixed micelle. Hence a potential of the above higher order aggregates can be envisioned because of the response of the above systems at physiological temperature. Few exploratory studies show that the present quaternary surfactants (e.g., TBADS) have a potential to use them as solubility booster (for hydrophobic molecules like curcumin) as well as for the pre-concentration of hazardous metal ion (e.g., $Hg^{+2}$) too. Study shows that metal ion can be pre-concentrated with lesser steps involved in CPE.