SUMMARY

The work summarized in this thesis is related to the use of Thin-Layer Chromatography (TLC) as an analytical method for identification and separation of amphipathic compounds (amino acids, surfactants and dyes). In quest of developing inexpensive methodologies for the identification and separation of amphipathic compounds, several chromatographic systems involving cost effective layer materials and novel eluents have been identified. The results presented in the thesis contribute substantially to the advancement of normal-phase TLC procedures. As an extension to the existing knowledge, several new TLC systems have been searched out for quick analysis of amino acids (chapters 2-5), surfactants (chapter 6) and dyes (chapter 7). The interesting features of the present study include:

- Use of different adsorbents in combination of organic solvents for analysis of surfactants
- Examination of effect of impurities on the separation of coexisting analytes
- Estimation of limit of detection of studied analytes with proposed TLC systems.
- Use of commercially available silica gel, alumina, kieselguhr and cellulose sorbents as layer material
- Use of surfactant (in the form of electrolyte and micelles) as the component of mobile phases to investigate its role in modifying the retention pattern of analytes on TLC plates.
Use of surfactant modified silica layers for separation and identification of amino acids in TLC.

CHAPTER-1 An introductory part, summarizes brief history of chromatography, a comprehensive description on the use of thin layer chromatography as an analytical technique, usefulness of surfactants as an eluent in chromatography. Besides, it also provides a general idea and structural formulae of amino acids surfactants and dyes, their classification alongwith complete literature survey of last fifteen years on the application of TLC to the analysis of amino acids, surfactants and dyes.

CHAPTER-2 Thin layer chromatography (TLC) has been used as an analytical technique for the identification and monitoring the adsorption behavior of 27 amino acids through soil static flat bed in contact of aqueous solutions of an inorganic electrolyte (ammonium sulphate) and an organic non-electrolyte (urea) at different concentration levels. Certain amino acids show salting-out and/or salting-in effects under limited concentration range of ammonium sulphate. As a result of differential adsorption, it was possible to separate closely related amino acids from their mixtures on soil bed using 0.1M solutions of ammonium sulphate and urea. In addition to the simultaneous separation of glycine from arginine and glutamic acid, mutual separations of amino acids having non-polar side chains from amino acids having charged or ionic polar side chains (basic and acidic) were worth mentioning. Effects of particle size, activation temperature, irradiation of soil by γ-rays and pH of the soil bed, on the mobility sequence of amino acids were also examined. The most fascinating aspect of present study is the investigation of adsorption behavior of amino acids in the presence of cationic, anionic and non-ionic surfactants at their different concentration levels.
CHAPTER-3 A new thin layer chromatographic system comprising silica gel layer impregnated with micellar solution of cetrimide (5.0 mM) as stationary phase and 40% (w/v) aqueous solution of dextrose as mobile phase has been proposed for the analysis of fifteen amino acids. The impregnation of silica gel with micellar solution of cetrimide brings about a substantial change in the mobility of lysine. Separation of lysine (ketogenic) from arginine (glucogenic) is important from physiological point of view. Surface modification of silica gel on impregnation, as indicated by FTIR and SEM studies, was responsible for improved chromatographic performance. Effect of presence of heavy metal cations as impurities in the sample on the separation was examined. The limit of detection for lysine and arginine was found to be 0.17 μg and 0.12 μg respectively. For validation, stability of the mixture, reproducibility, chromatographic parameters like ΔRf, separation factor (k) and resolution (Rs) were calculated. The proposed method is simple, rapid and free from the use of volatile organic solvents.

CHAPTER-4 A new thin layer chromatographic system comprising of silica gel impregnated with 0.1% aqueous solution (below CMC) of nonionic surfactant Brij-35 as stationary phase and 0.1% aqueous solution (below CMC) of cationic surfactant (cetrimide) as eluent has been found most suitable for the resolution of closely related amino acids (phenylalanine and tyrosine) from their mixture. Effect of substitution of Brij-35 by cetrimide and sodium cholate has been examined to assess the impact of charge of impregnant on the chromatographic behavior of analytes. The changes brought about by the impregnation in the structure and homogeneity of silica gel have been studied by scanning electron microscopy (SEM) and fourier transform infra-red spectroscopy (FTIR) techniques. Optimizations of concentration of Brij-35 and cetrimide
in the stationary and mobile phases respectively and the effect of bed height on the separation have also been examined. Chromatographic parameters like $\Delta R_f$, separation factor ($k$) and resolution ($R_s$) were calculated for the separation of tyrosine from phenylalanine and separation mechanism have been proposed. The limits of detection for phenylalanine and tyrosine were found to be 0.25 and 0.23 $\mu g$ respectively. The proposed method studied is simple, rapid and free from the use of volatile organic solvents.

CHAPTER-5  Thin layer chromatographic studies of amino acids were performed on three differentially charged surfaces of silica gel, alumina and cellulose with 40% aqueous solution of five carbohydrates namely dextrose, fructose, maltose, lactose and sucrose. 40% dextrose-alumina and 40% dextrose-cellulose TLC systems were identified as most favorable for selective separation of glutamic acid and tryptophan from the mixture of other amino acids. In addition to this, several combinations of amino acids have been resolved on silica gel and alumina layers with 40% dextrose as eluent. The lowest detectable limit of glutamic acid and tryptophan, stability of mixtures of amino acids and reproducibility of $R_f$ values were determined. The proposed method is environmentally acceptable because of the use of non-toxic nature of eluents used.

CHAPTER-6  Thin layer chromatography (TLC) cationic and nonionic surfactants has been performed on soil, silica gel, alumina and keiselguhr layers using aqueous solutions of ammonium sulfate and urea as mobile phases. Four stationary phases and fifteen mobile phases were used to examine the mobility of the surfactants and to discover the best TLC system for selective separation of dodecytrimethyl ammonium bromide (DTAB) from multicomponent mixtures of other surfactants. The mobility of all the surfactants was insignificant on soil, alumina, and silica gel, irrespective of the nature of
the mobile phase. Some of the surfactants were mobile on keiselguhr. Among the TLC systems studied, keiselguhr-0.1M ammonium sulfate was best for achieving selective separation of DTAB from other surfactants. A salting-out effect on DTAB is reported. The stability of the mixture of surfactants on the plate and in solution was also examined. The limit of detection of DTAB was 0.3 µg per zone.

CHAPTER-7 A new green thin layer chromatographic system comprising of biphasic alumina-keiselguhr (1:1) as stationary phase and equal volume mixture of 1% (w/v) aqueous solution of cationic surfactant (cetylpyridinium bromide) and nonionic surfactant (Brij-35) in 1:1 ratio as mobile phase has been proposed for the analysis of ionic (anionic and cationic) dyes. From the point of view of resolution of dyes, mixed alumina-keiselguhr has been found more effective than its individual components. In the same way, synergism between cationic and nonionic surfactant proves to be more efficient for the separation of dyes from their quaternary mixture than the individual surfactants. Effect of presence of heavy metal cations as impurities in the sample on the separation was examined. The limits of detection of bromocresol green, malachite green, rhodamine B and congo red were 0.27, 0.19, 0.21 and 0.23 µg respectively. Chromatographic parameters like ΔR₁, separation factor (α) and resolution (Rₛ) were calculated for the separation of bromocresol green- malachite green- rhodamine B-congo red. The proposed method is simple, rapid and free from the use of volatile organic solvents.