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Review

Microemulsion in Chemical Analyses by Thin-Layer Chromatography

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Key Words:

Microemulsion
Surfactants
Thin-layer chromatography
Sodium dodecyl sulfate

Summary

This review encapsulates the work which appeared in the literature during 1995–2015 on the use of microemulsion as the mobile phase in the analysis of inorganic anions and organic compounds by thin-layer chromatography (TLC). Among anionic, cationic, and non-ionic surfactants used as one of the components of oil-in-water (O/W) and water-in-oil (W/O) microemulsions, sodium dodecyl sulfate (SDS) (anionic surfactant) has been found the most effective for the analysis and separation of different compounds. Compared to the work performed with the use of W/O microemulsion, little work has been reported on use of O/W microemulsion in the TLC–high-performance thin-layer chromatography (HPTLC) analysis of organic compounds. In contrary to TLC, more work has been done on use of O/W microemulsion in high-performance liquid chromatography (HPLC). Out of the sorbent phases, silica gel in combination with microemulsion eluent has been favorable for realizing analytically useful separations. Classification of microemulsions and surfactants is also discussed in the present review.

1 Introduction

Emulsions, sometimes called macroemulsions, are heterogeneous systems where one immiscible liquid is dispersed as droplets in another liquid. Generally, water is used as one of the liquids. Emulsions have comparatively higher interfacial tension and are kinetically stable. The droplet size of the emulsions may be greater than 10^5 nm. Systems with sizes ranging between 10^2 and 10^5 nm are termed "miniemulsions" which are thermodynamically unstable.

The term "microemulsion" was first proposed by *J.H. Schulman and coworkers* in 1959 [1]: they described the methodology for the preparation of microemulsion systems. Microemulsions are optically transparent, thermodynamically stable, and apparently homogenous single-phase solutions containing oil (a non-polar solvent, *e.g.,* heptanes or octane), surfactant, and a cosurfactant which is generally a medium chain length alcohol,

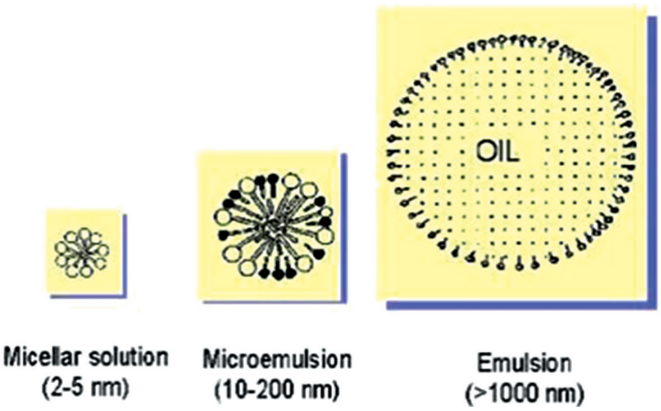

Figure 1

The structural representations of micellar solution, microemulsion, and emulsion.

Table 1

Difference between microemulsion and macroemulsion.

S. No.	Property	Microemulsion	Macroemulsion
1	Appearance and optical isotropy	Transparent and isotropic	Cloudy and anisotropic
2	Interfacial tension	Ultra low	High
3	Microstructure and droplet size	Dynamic and 10–200 nm	Static and >1000 nm
4	Stability	Thermodynamically stable	Kinetically stable, but thermodynamically unstable
5	Viscosity	Monophasic with low viscosity	Biphasic with high viscosity

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amine, or a similar polar organic molecule [2]. Compared to emulsions, microemulsions provide better contact between the oil and water phases. **Figure 1** illustrates the microstructures of micellar solutions, microemulsions, and emulsions. The parameters differentiating macroemulsions and microemulsions are listed in **Table 1.**

1.1 Characteristics of Microemulsions

Some interesting characteristics of microemulsions are listed below:

1. relatively low viscosity,
2. inhomogeneous nature at microscopic level,
3. differential polarity of oily microdomain and aqueous microdomain,
4. optical clarity,
5. dynamic nature (microdomains are in continuous formation–destruction processes),
6. small drop size which provides intimate contact between the water and oil phases,
7. enhanced solubilization and protection of solubilized components from undesired degradation reactions,
8. capability of incorporating solutes within the dispersed droplets,
9. utility as novel reaction and/or extraction media.

Unfortunately, the possible formation of microemulsions within specific ranges of temperature, pressure, and composition limits their wide spectrum applications. However, the attractive features of microemulsion thin-layer chromatography (TLC) include:

(a) the unique differential distribution of solute molecules among the external aqueous or oil phase, the stationary phase, and the droplets of microemulsion,

(b) enhanced separation selectivity,

(c) high peak capacity,

(d) increased fluorescent detection capability.

1.2 Classification

L1 (oil-in-water, O/W), L2 (water-in-oil, W/O), and bicontinuous classification of microemulsion has been convenient and popular. The O/W microemulsions (also called swollen micelles) are formed when oil microdroplets, enclosed in a surfactant–cosurfactant layer, are dispersed in an aqueous continuous phase. However, W/O microemulsions (also known as reversed micelles) are formed when droplets, surrounded by a surfactant, are dispersed within an oil continuous phase. In a bicontinuous microemulsion, both water and oil microdomains overlap in continuous phase which has a sponge-like structure. Therefore, in the case of bicontinuous microemulsion systems, it is not possible to differentiate between the dispersed phase and the dispersion phase. In general, surfactants with bulky hydrophilic head groups associated with small hydrophobic tail favor the formation of O/W microemulsion as it is easy for the short tail to fit properly within a relatively confined core. In contrast, the surfactants having small head group and large tail favor the formation of W/O microemulsion.

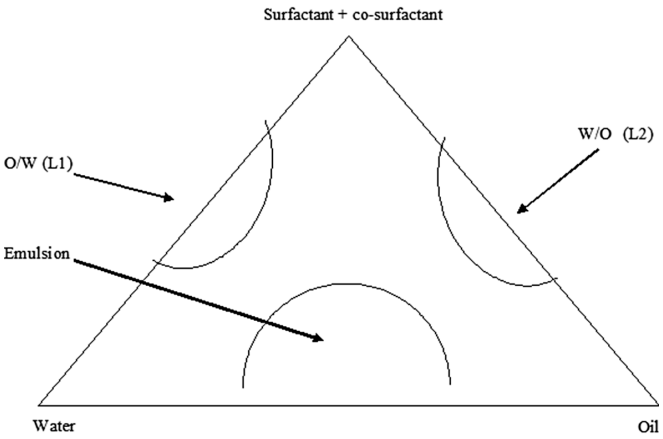


Figure 2

Pseudoternary phase diagram illustrating the phase behavior of microemulsions.

From **Figure 2,** it is evident that each corner of the diagram represents 100% of the particular component. The region can be separated into L1 (oil-in-water, O/W) and L2 (water-in-oil, W/O) microemulsion, depending upon the composition whether it is a water-rich or oil-rich region.

1.3 Components of Microemulsion

The main components of microemulsions include surfactant, cosurfactants, oil, and water.

1.3.1 Surfactants

Surfactants are amphiphilic compounds containing both hydrophilic (water-loving) and hydrophobic (water-repellent) parts in the same molecule **(Figure 3).** Surfactants have the ability to adsorb on the surface or interface (solid–solid, solid–liquid, solid–gas, liquid–liquid, and liquid–gas) and, hence, they modify the surface or interfacial properties. Surfactants lower the surface tension between two immiscible interfaces and also act as emulsifying, foaming, and wetting agents. Due to these properties, surfactants are widely used in the chemical industry, pharmaceuticals, petroleum industry (during the drilling of mud), ore-processing (as floating agents), and household-cleaning products. Recently, the applications of the surfactants have extended to biotechnology, viral research, magnetic recording, electronic printing, and separation science [3].

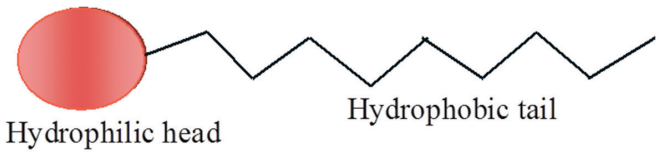


Figure 3

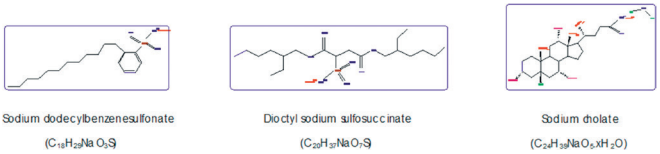
The representative structure of surfactants.

1.3.2 Classification of Surfactants

1.3.2.1 Anionic Surfactants

These surfactants are negatively charged. These are sulfonic acid salts, alcohol sulfates, alkyl benzene sulfonates, phosphoric acid esters, and carboxylic acid salts and are roughly

considered non-toxic. These surfactants are sensitive to the hardness of water and are used as detergents in petroleum oil recovery operation. They are also used as contaminants in hydrogeology remediation.



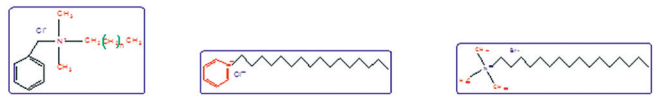
Sodium dodecylbenzenesulfonate ($C_{18}H_{29}NaO_3S$)

Dioctyl sodium sulfosuccinate ($C_{20}H_{37}NaO_7S$)

Sodium cholate ($C_{24}H_{39}NaO_5.xH_2O$)

1.3.2.2 Cationic Surfactants

These surfactants are positively charged. They include long-chain amines which are soluble in pH < 7 and quaternary ammonium salts which are stable in the whole pH range and are used as antiseptic agents, textile softener, corrosion inhibitors, flotation chemicals, and cosmetic formulation ingredients. These surfactants are also widely used in pharmaceutical products as preservatives (in eye and ear drops or sprays) and disinfectants (in cleaning products).



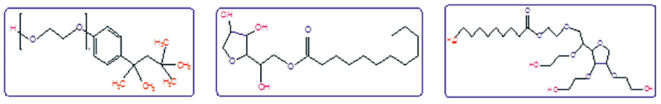
Benzalkonium chloride ($C_6H_5CH_2N(CH_3)_2RCl$) R= C_8H_{17} to $C_{18}H_{37}$

Cetylpyridinium chloride ($C_{21}H_{38}ClN$)

Cetyltrimethylammonium bromide ($C_{19}H_{42}BrN$)

1.3.2.3 Non-Ionic Surfactants

These surfactants consist of a hydrophilic head group without bearing any charge. Alcohol ethoxylates, alkylphenol ethoxylates, alkanolamides, and sugar surfactants belong to this group. These surfactants are widely used in petroleum and environmental applications. Being non-toxic, these are also used as emulsifying agents in pharmaceutical products, foods, and cosmetics.



Triton X -100 ($C_{14}H_{22}O(C_2H_4O)_n$(n= 9-10)

Span 20 ($C_{18}H_{34}O_6$)

Tween 20 ($C_{58}H_{114}O_{26}$)

1.3.2.4 Zwitterionic Surfactants

Zwitterionic surfactants contain both positively and negatively charged groups within the same molecule. The positive charge is invariably due to the presence of primary, secondary, or ternary amines or quaternary ammonium compounds, while the negative charge may be due to the presence of sulfate, carboxylate, and sulfonate moieties. These surfactants are widely used in the pharmaceutical, paint, and textile industries as well as in animal feed. Lecithin, cocamido-2-hydroxypropyl sulfobetaine ($C_{19}H_{39}N_2O_3$), dodecyl betaine, and lauramidopropyl betaine are important surfactants of this group.

1.3.2.5 Gemini Surfactants

These surfactants consist of two hydrophobic chains and hydrophilic groups attached through a spacer unit. They are subclassified as anionic, cationic, non-ionic, zwitterionic, and heterogemini surfactants. These are used to modify different types of chemical reactions and also as antibacterial agents. *N*,*N*-Bis(dimethyldodecyl)-1,4-butanediammonium dibromide belongs to this group.

1.3.2.6 Hybrid Surfactants

These surfactants consist of hydrocarbon and fluorocarbon chains. These are used as tooth surface modifiers and as novel emulsifiers for water-in-supercritical CO_2 microemulsion system. $(C_7H_{15})(C_7F_{15})CHSO_4^-Na^+$ is the most useful surfactant of this group.

1.3.3 Cosurfactants

Cosurfactants are polar molecules such as medium-chain alcohols, acids, and amines which have the capability to reside between the tails of the surfactants. The functions of cosurfactants include the following:

(a) lowering of interfacial tension sometime near to zero value,

(b) increasing fluidity,

(c) adjusting the hydrophilic–hydrophobic balance (HLB),

(d) destroying the liquid crystalline and/or gel structures,

(e) expanding the microemulsion region.

1.4 Applications of Microemulsions

Microemulsions have a wide range of applications in different fields [4] such as pharmaceuticals, biotechnology, food industry, detoxification processes, agrochemicals, cosmetics, enhanced recovery of oil, and analytical operations. In addition, microemulsions have also been used as liquid membranes, reaction media, lubricants, corrosion inhibitors, and fuels.

2 Chromatography

The word “chromatography” has been derived from the Greek words ‘chromatus’ (means “color”) and ‘graphein’ (means “to write”). Chromatography originated from the fundamental separation of plant pigments by passing the extract of green leaves through a column, resulting in the formation of a series of coloured bands. This was published by *Michael Tswett* in the form of two research papers in the leading scientific journal *Berichte der Deutschen Botanischen Gesellschaft* in 1906.

The original work of Tswett was initially ignored but it gained attention 10 years after his death. The English version of Tswett’s original article is now available [5]. Between 1906 and 1952, planar chromatography had been developed and thin layers of silica gel were introduced as alternatives to paper in the late 1950’s.

The pioneering work of *Martin et al.* [6, 7] who introduced gas–liquid partition chromatography as a novel analytical technique for the resolution and estimation of volatile fatty acids ranging from formic acid to dodecanoic acid activated the development in the field of column chromatography. The major advantages of gas–liquid partition column chromatography over liquid–liquid partition chromatography are the following: (a) the low viscosity of the mobile phase which allows relatively longer columns

Table 2

Development of chromatography since Tswett's discovery.

Year of origin	Discoverer	Chromatographic technique
1906	Tswett	Adsorption chromatography
1937	Konig	Electrophoresis chromatography
1938	Izmailov and Schraiber	Thin-layer chromatography (adsorption)
1941	Martin and Synge[a)]	Gas–liquid partition chromatography
1944	Martin, Consden, and Gordon	Paper chromatography
1944	Craig	Counter-current chromatography
1946	Claesson	Gas–solid chromatography
1947	Mayer and Tompkins	Ion-exchange chromatography
1951	Kirchner, Miller, and Keller	Thin-layer chromatography (partition)
1952	James and Martin	Gas–liquid chromatography
1959	Porath and Per Flodin	Gel-filtration chromatography or size-exclusion chromatography
1960	Kirkland, Horvath, and Huber	High-performance liquid chromatography (HPLC)
1962	Klesper, Corwin, and Turner	Supercritical fluid chromatography (SFC)
1964	Moore	Gel-permeation chromatography
1975	Small, Stevens, and Bauman	Ion chromatography
1976	Zlatkis and Kaiser	High-performance thin-layer chromatography (HPTLC)
1979	Tyihák, Mincsovics, and Kalász	Over-pressurized layer chromatography
1981	Jorgensen and Lukacs	Capillary electrochromatography
1990	Manz	Open tubular liquid chromatography
1995	Mohammad et al.	Microemulsion thin-layer chromatography
2004	Waters Corporation	Ultra performance liquid chromatography

[a)]A.J.P. Martin and R.L.M. Synge got the Nobel Prize in 1952, for gas–liquid partition chromatography

to be used with a corresponding gain in efficiency and (b) it is easier to detect changes in the composition of a gas than of a liquid stream.

The development stages of chromatography after Tswett's discovery are encapsulated in **Table 2.**

2.1 Thin-Layer Chromatography

Thin-layer chromatography, a subdivision of liquid chromatography, is carried out on a flat surface, and hence, it is sometimes referred to as a planar chromatographic separation technique. In TLC, the mobile phase (a liquid) migrates through the stationary phase (thin layer of porous sorbent on a flat surface) by capillary action. A Dutch biologist, *Beyerink* [8] was the first to use TLC for the separation of hydrochloric and sulfuric acids in the form of fine rings on thin layer of gelatine.

The first application of TLC for the separation of inorganic ions was reported in 1949 by two American chemists, *Meinhard* and *Hall,* who realized the separation of Fe^{2+} from Zn^{2+} on microscope slides coated with alumina (an adsorbent) and celite (a binder) [9].

The applicability of the thin-layer chromatographic method received impetus with the entry of high-performance thin-layer chromatography (HPTLC) plates in 1975. Furthermore, ultra thin-layer chromatography (UTLC) which emerged in 2001 [10] seems to add a new dimension in advancing the applicability of thin-layer chromatography in the future.

HPTLC–TLC methods with minor variations in the experimental conditions can be used for qualitative analysis (to identify the presence or absence of a particular substance in a mixture), quantitative analysis (to determine the amount of a particular substance in a mixture precisely and accurately), and preparative analysis (to purify and isolate a particular substance for subsequent use).

All the three cases require the common procedural steps but different experimental conditions. For example, analytical TLC differs from preparative TLC as the sample solution/or amount is applied on thinner layers in the former case, whereas thicker TLC plates are used for preparative TLC.

2.2 Chromatographic Systems

The stationary and mobile phases together comprise the chromatographic system. The proper selection of stationary and mobile phase conditions decides the degree to which effective separation of components in a mixture can be achieved.

2.2.1 Mobile Phase (Solvent System)

In TLC, the separation of components from a mixture mainly depends on the competitive interactions of the analyte with the stationary and mobile phases. In TLC, the selection of the proper mobile phase composition is a most difficult and time-consuming process. Solubility, affinity, and resolution are the main factors which should be considered before selecting a solvent system.

The solvents used as mobile phases in TLC for the development of plates include (a) organic solvents, (b) inorganic solvents, (c) mixed aqueous–organic solvents, (d) surfactant-mediated solvents, and (e) green solvents.

The green solvents are currently replacing volatile organic solvents successfully and minimizing the human and environmental hazards as these are basically derived from the processing of agricultural crops. On the other hand, surfactant-mediated mobile phase systems are receiving the attention of chromatographers because of their favorable properties and unique separation potentiality.

According to the literature, *Armstrong et al.* [11] were the first to use normal and reversed micellar sodium dodecyl sulfate (SDS) solutions as mobile phase systems in TLC. They separated pesticides, decachlorobiphenyl, and nucleosides using polyamide and alumina thin-layer sheets. However, microemulsions or reversed micellar solutions were first used in high-performance liquid chromatography (HPLC) in 1986 by *Hernandez et al.* [12]. These experts successfully used reversed micelles of dioctyl sodium sulfosuccinate under the name of aerosol OT formed in hexane for the normal-phase chromatography of phenol, naphthol, and dinitrotoluene. Microemulsion was first applied in TLC for the analysis of inorganic anions in 1995 by *Mohammad et al.* [13]. In contrary to TLC and HPLC, microemulsions have been extensively used in electrokinetic chromatography (EKC) as mobile phases. So far, more than 150 research papers have been reported on the use of microemulsion in EKC. The publication trend using different chromatographic techniques has been found in the following order: microemulsion EKC > HPLC > TLC/HPTLC **(Figures 4–6).** The literature survey also revealed that SDS (anionic surfactant) as one of the components in microemulsions has been the most widely used component by many of the researchers in all the chromatographic techniques. Since the first use of microemulsion, only a few papers have been published on its use in TLC–HPTLC; the works reported during 1995–2015 therein is briefly summarized in **Table 3** [13–39]. From the contents of Table 3, it is clear that SDS (anionic surfactant) has been frequently used as one of the components of microemulsion for the analysis and separation of organic compounds such as amino acids, alkaloids, flavonoids, *etc.* by thin-layer chromatography using polyamide film as the stationary phase, whereas silica gel, alumina, and other adsorbents have been used for the separation of both organic and inorganic compounds. It is also interesting to note that O/W microemulsion was first used as an eluent in the thin-layer chromatography of aliphatic and aromatic amino acids using plain as well as monovalent cation impregnated alumina stationary phase. In some studies, the combination of W/O–O/W microemulsions was found useful for the analysis of amino acids using silica gel as the stationary phase. Silica gel 60 F_{254} plates were also used for the analysis of vitamins and drugs.

In addition, among the several reviews written on different aspects of microemulsions [40–81], none has dealt with TLC technique. However, certain interesting reviews dealing with the use of microemulsions in HPLC [50, 51, 57, 74] and microemulsion electrokinetic chromatography (MEEKC) [42, 43, 45, 46, 56, 69] have appeared in the literature. Recently, the opportunities of contemporary planar chromatography in pattern recognition and fingerprint analysis have been nicely described by *Milojkovic-Opsenica et al.* [82]. As far as we are aware, the present communication is the first review summarizing the literature reports on the fascinating effectiveness of microemulsions in the analysis of inorganic ions and organics. It is suggested that more work is done in this area if we wish to increase the application of this novel eluent in HPTLC–TLC.

3 Conclusion

After the discovery of microemulsions in 1959, their application in chemical analyses by TLC–HPTLC has been limited till date. Although promising applications of microemulsions in analytical chemistry are found in the literature, only a few have been reported on TLC. Hence, more efforts are to be initiated on the use of microemulsions in chemical analyses by TLC in both academic institutes and industrial sectors. It is hoped that TLC has more to offer for the study of microemulsions than microemulsion mobile phases to chemical analyses performed by TLC.

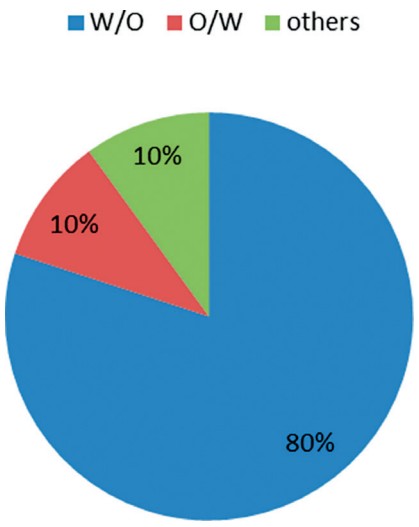


Figure 4

The magnitude of publications (%) on TLC using different types of microemulsions as mobile phases.

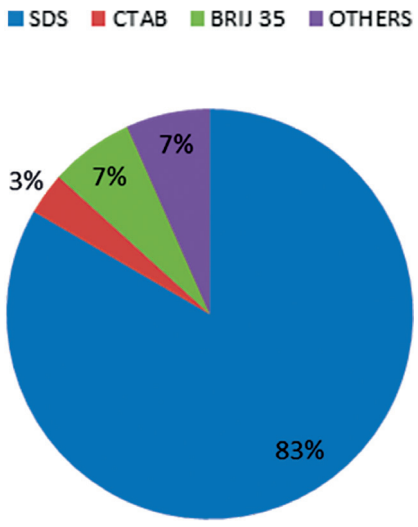


Figure 5

The magnitude of publications (%) on HPLC using microemulsions as mobile phases containing different surfactants.

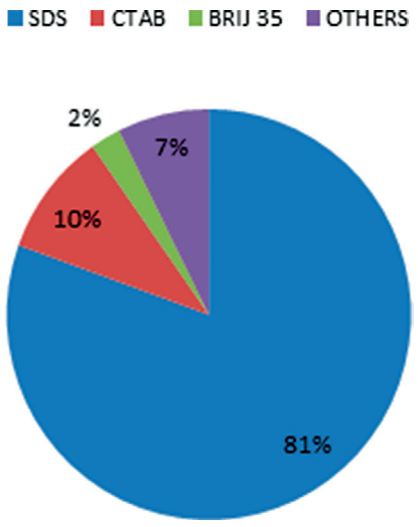


Figure 6

The magnitude of combined publications (%) on HPLC and TLC using microemulsions as mobile phases containing different surfactants.

Table 3

List of studies performed on the use of microemulsion as mobile phase in thin-layer chromatography of organic compounds and inorganics.

Stationary phase	Mobile phase	Remarks	Reference
Alumina, silica gel G, microcrystalline cellulose, kieselguhr G and kieselguhr plus cellulose (4:1 and 3:2 weight ratio)	Water-in-oil (W/O) microemulsion containing sodium dodecyl sulfate (SDS)–1-pentanol–water–heptane	This is the first report on the use of W/O microemulsion as eluent in the analysis of inorganic anions by thin-layer chromatography using seven different adsorbents. Out of the seven stationary phases used, kieselguhr was the most useful for the quantitative separation of IO_4^- from other oxo-anions. Selective separation of IO_4^- was not influenced by the presence of phenols, amines, and heavy metal cations.	[13]
Alumina, microcrystalline cellulose, silica gel G, silica gel H, and kieselguhr	W/O microemulsions consisting of surfactant (SDS or *N*-cetyl-*N*,*N*,*N*-trimethyl ammonium bromide), water, heptane, or hexane, and a cosurfactant (1-pentanol or butanol)	Fourteen aromatic amines were chromatographed, and separations from their ternary and binary mixtures were achieved on alumina layers. The separation efficacy of various adsorbents used as layer materials was in the order: alumina > silica gel > kieselguhr > cellulose. In the case of aniline, m-isomer has a lower R_F value compared to o- and p-isomers. The effects of inorganic cationic and anionic species on separation pattern of certain amines were examined.	[14]
Plain silica gel as well as silica gel layers impregnated with cationic and anionic aqueous solutions	W/O microemulsion	The impregnated plates were stable and gave well-formed spots. Silica gel impregnated with 2% $CuSO_4$ solution was found the most suitable layer material for the selective separation of DL-phenylalanine from other amino acids.	[15]
Polyamide layer	SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O	The developed microemulsion system containing variable amounts of water was found very useful for the separation of forty-one alkaloids.	[16]
Polyamide film	Oil-in-water (O/W) microemulsion (SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O)	This microemulsion system containing 70% water in the presence of 5% formic acid was very efficient for the separation and identification of flavonoid drugs.	[17]
Polyamide film	(SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O)	Microemulsion (containing 75% water) was used for the separation and identification of Rhizoma Coptidis drugs.	[18]
Polyamide film	(SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O)	The mobile phase containing 70% water as one of the components of the microemulsion was found the best for the accurate separation and identification of Eucommia flavonoids and its products.	[19]
Silica gel	SDS–*n*-butyl alcohol–*n*-hexane–water	Both W/O microemulsion and the bicontinuous microemulsion system composed of SDS–*n*-butyl alcohol–*n*-hexane–water were found suitable as developers for the TLC analysis of twenty-three amino acids.	[20]
Plain (or non-impregnated) alumina and alumina layers impregnated with 2–8% aqueous solutions of lithium, sodium, and ammonium chlorides	Five O/W microemulsion systems comprising of SDS–pentanol (1:2 *w/v*) + heptanes–water in different volume ratios	The first report on the use of O/W microemulsion as eluent in thin-layer chromatography. Twenty-three aliphatic and aromatic amino acids were chromatographed on plain as well as monovalent cation impregnated alumina stationary phases. Several important separations including the separation of non-polar (proline and valine) from polar (glycine, histidine, serine, and threonine), of non-polar (iso-leucine) from polar (leucine), and of polar (glutamic acid) from polar (aspartic acid) were achieved. The salting-out effect of lithium impregnant on alumina layers was observed for the first time in the case of neutral amino acids. The highly compact spots on alumina layer led to an enhanced sensitivity for the detection of amino acids in the range of 2–3.9 µg per spot.	[21]

Table 3 (continued)

Stationary phase	Mobile phase	Remarks	Reference
Silica gel	SDS–butyl alcohol–*n*-hexane–water	Both W/O microemulsion and the W/O–O/W microemulsion were found suitable for the analysis of twenty-three amino acids on using silica gel static phase.	[22]
Silica gel impregnated with a 1:1 mixture of 2% aqueous $CuSO_4$ and 3% Brij-35	W/O microemulsion	Under optimized experimental conditions, selective separation of DL-phenylalanine from other amino acids was achieved.	[23]
Silica gel	W/O microemulsion (SDS–water–heptanes–*n*-pentanol, 8.0 g:8.0 mL:160.0 mL:25.0 mL) as the mobile phase	The proposed thin-layer chromatography system was successful for the separation of o-, m-, and p-nitroaniline isomers from their mixtures. The effects of the basicity of the amines on their R_F values were examined.	[24]
Polyamide film	Unique microemulsion system containing 2.8 mL of *n*-heptane, 19.0 mL of 1-butanol, 75 mL water and 7.7 g of SDS with added 14.6 mL formic acid	Microemulsion thin-layer chromatography (ME-TLC) has been developed for the fingerprinting of the aqueous extract of licorice (*Glycyrrhiza* spp.). The separation conditions and operational processes were optimized, and the chromatographic characteristics were compared by conventional TLC. The ME-TLC fingerprinting images of different species appeared as well-resolved bands with strong intensities to reveal distinctively different compositional features of the samples.	[25]
Polyamide film	SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O	Twelve gingko flavonoids of different natures were separated by the developed TLC method. The polarity of the microemulsion was regulated through adding or reducing the water content. Compared with the general mobile phase, the sensitivity of the detection was improved markedly for the gingko flavonoids under study.	[26]
Silica gel	W/O microemulsion system used cetyltrimethylammonium bromide (CTAB)–water–heptane–*n*-pentanol (8 g:8 mL:160 mL:25 mL), AOT–water–heptanes (8 g:8 mL;160 mL), SDS–water–heptane–*n*-pentanol (8 g:8 mL:160 mL:25 mL)	The proposed method was applicable for the identification of L-tryptophan in drug samples such as Astymin-m (forte), Astymin (liquid), and Alamine (forte). The separation up to 10 μg of tryptophan from milligram quantities (up to 0.77 mg) of other amino acids has been realized. The quantitative estimation of tryptophan by spectrophotometer (λ_{max} 570 nm) after separation from other amino acids has been done. The recovery of tryptophan was around 98%, and its limit of detection on silica gel layer was 0.13 μg.	[27]
Silica gel, alumina, cellulose, and kieselguhr TLC plates	W/O SDS and CTAB surfactants containing W/O microemulsion systems	Useful separations of phenolic compounds from their multicomponent mixtures on silica gel layers were achieved preferably by SDS containing microemulsion system.	[28]
Polyamide film	SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O–HCOOH (0.27:0.63:0.10:3:0.2)	This TLC system has been used for the simultaneous determination of rutin and quercetin in flos sophorae where the recoveries of rutin and quercetin were found 99.8 and 97.1%, respectively.	[29]
Silica gel	W/O and bicontinuous microemulsion; CTAB–*n*-butyl alcohol–*n*-octane–water as W/O microemulsion	The chromatographic behavior of 23 amino acids on the silica gel thin layers was investigated using W/O microemulsion and the bicontinuous (BC) microemulsion systems. Several amino acids from their multicomponent mixtures were successfully separated using microemulsion with 40% hydrous contents.	[30]
Polyamide film	SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O	Microemulsion TLC has been used for the separation of flavonoids extracted from mulberry leaves in the form of twelve spots on polyamide layer. The SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O microemulsion containing 70% water was used as the eluant, and the pH adjustment was done by 10% formic acid.	[31]

Table 3 (continued)

Stationary phase	Mobile phase	Remarks	Reference
Polyamide film	SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O	W/O microemulsion containing 30% water was found the best mobile phase for the separation and identification of alkaloids of Sophara and its patent medicine, Shiwei compound capsule.	[32]
Polyamide film	Sodium lauryl sulfate–*n*-butanol–skellysolve C–H_2O microemulsion system	This TLC system being simple, environmental-friendly, and sensitive with ideal separation efficacy was found very effective for the separation and identification of various compositions in Chinese patent medicines. Rhizoma Coptids was detected using microemulsion (75% water content) as the developing agent and *Corydalis yanhusuo,* saposhnikovia, divaricata, chlorogenic acid and radix et Rhizoma Glycyrrhizae in Changiian prescription were detected using microemulsion (75% water content)–formic acid (9.0:1.0) as the developing agent.	[33]
Silica gel 60 F_{254} and PEI cellulose F_{254} HPTLC plates	W/O microemulsion (heptane [160 mL], water [8 mL], CTAB [8 g], and *n*-pentanol [25 mL])	The proposed method was used for the selective separation of bromocresole green from other anionic as well as cationic dyes on cellulose HPTLC plates for the resolution of vitamins (thiamine, pyridoxine, and riboflavin in marketed formulations).	[34]
Polyamide film	SDS–*n*-butane–*n*-heptanes–aqueous microemulsion as developer in combination with aminic acid and methanol as the modifier	The microemulsion containing 4% SDS and 80% water–methyl acid–methanol (1.0:2.0:1.0) was found suitable for separating and identifying (Z)- and (E)- diastereomers of resveratrol and piceid in polygonum cuspidatum.	[35]
Silica HPTLC plates	W/O microemulsion (SDS–water–*n*-hexane–1-butanol, 8 g:8 mL:160 mL:25 mL)	The developed method was used for the identification, separation, and quantification of caffeine and paracetamol in formulated drug and in spiked urine sample. After separation of caffeine from paracetamol on silica HPTLC plates with W/O microemulsion system, HPLC was used for quantification after their extraction from HPTLC plates.	[36]
Polyamide thin layer	SDS–*n*-butylacohol–*n*-heptane–H_2O	This TLC system was found useful for the separation of flavonoids in pyrrosia leaf using formic acid–acetone (0.8 mL:0.8 mL) as the modifier.	[37]
Polyacetamide	SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O	Microemulsion containing 70% water in the presence of formic acid in 9.0:1.0 ratio was optimum for the identification of flavonoids in *Taraxacum* herb.	[38]
Polyamide film	SDS–*n*-C_4H_9OH–*n*-C_7H_{16}–H_2O	O/W microemulsion containing 75% water as developer was used for the identification and separation of flavones in *Filipendula ulmari.*	[39]

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