Water-in-Oil Microemulsion as Mobile Phase in Thin-Layer Chromatographic Retention Studies of Anions

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ABSTRACT: A water-in-oil (W/O) microemulsion, consisting of sodium dodecyl sulphate (SDS)/1-pentanol/water/heptane, has been used as mobile phase for the separation of inorganic anions from their binary mixtures, for example, IO_4^--NO_2^-, IO_4^--BrO_3^-, IO_4^--I^-, MnO_4^--BrO_3^-, MnO_4^--NO_2^-, and MnO_4^--Br^-. The weight ratio, SDS/*n*-pentanol was kept constant at 1/2.46 for all compositions. The retention efficiency of anions on layers of silica gel G, alumina, microcrystalline cellulose, kieselguhr G, and mixtures of kieselguhr and cellulose in 4:1 and 3:2 ratios has been examined with the W/O microemulsion system as a mobile phase. Thin layers of kieselguhr were most useful for differential migration of anions. Quantitative separation of IO_4^- from accompanying ions, limits of identification, and dilution of few anions are reported. The effects of amines, phenols, and heavy metals on the separation efficacy of IO_4^- also have been investigated.

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Microemulsions are isotropic, clear, or translucent thermodynamically stable dispersions of oil, water, surfactant, and often a small amphiphilic molecule, called a cosurfactant (1). The droplet diameters in microemulsions range from 100 to 1000 Å. The microemulsion systems are usually transparent because of their much smaller droplet size compared to macroemulsions. One can distinguish three different types of microemulsions: Oil microdroplets enclosed in a surfactant–cosurfactant film are dispersed in an aqueous continuous phase (oil-in-water, O/W type); water droplets are dispersed in the oil-continuous phase (water-in-oil, W/O type); and if oil and water droplets overlap in each other, the system is called bicontinuous (2). W/O microemulsions are complex fluids that have wide-ranging applications, for example, as novel lubricants, reaction media for new synthetic chemistry, and mobile phases in separation science (3,4). Since the first report by Armstrong and Henry (5), micellar chromatography has been a subject of interest to many analytical chemists (6–8). It recently has been shown that W/O microemulsions could be used as normal liquid chromatographic mobile phases (9). Interesting selectivities, along with poor efficiencies, were obtained. It must be noted that the W/O microemulsions used did not contain any alcohol cosurfactants. It was reported earlier that the addition of 1-pentanol to the mobile phase greatly improves efficiencies by reducing the adsorbed amount of emulsifier (surfactant). In this work, a W/O microemulsion with 1-pentanol as cosurfactant was used as eluant because use of such phases in thin-layer chromatography (TLC) is an unexplored area of research. In these systems, water microdroplets, enclosed in surfactant/cosurfactant film, are dispersed in an oil-continuous phase (10). One characteristic feature of microemulsions is their wide domain of existence in the pseudoternary phase diagram (9,11).

In continuation of our earlier studies on TLC of anions (12–14), this report describes simple methodology for identification and separation of anions under optimal experimental conditions with a W/O microemulsion as novel eluant. The use of such phases may offer unique solutions for the change in retention data, due to the influence of water on the stationary phase (15) because water is present in the core of W/O microemulsion droplets.

EXPERIMENTAL PROCEDURES

Chemicals. Specially pure sodium dodecyl sulphate (SDS) and *n*-heptane (99%) were obtained from BDH (Poole, United Kingdom), and *n*-pentanol was a Riedel product (99%). Demineralized water, redistilled from alkaline potassium permanganate, was used. Alumina, microcrystalline cellulose, and kieselguhr were obtained from CDH Laboratories (India), while silica gel G was obtained from Glaxo (India). All other chemicals (Analar grade) were used as supplied.

Test solutions. The test solutions (1%) were either Na or K salts of all anions studied, except SCN^-, which was used as ammonium thiocyanate. Solutions of the nitrates of lead, silver, and bismuth, and the chloride of mercury were prepared in demineralized water, which contained small quantities of the corresponding acid to limit the extent of hydrolysis. The

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solutions (1%) of various amines and phenols used were prepared in methanol.

Chromatography. A TLC apparatus (Toshniwal, India) was used for the preparation of 20 × 3.5 cm^2 glass plates. The chromatography was performed in 24 × 6 cm^2 glass jars.

Detectors. The following spray reagents were used for the detection of various anions. (i) Saturated $AgNO_3$ solution in methanol for I^-, Br^-, Cl^-, F^-, CrO_4^{2-}, $Cr_2O_7^{2-}$, SO_3^{2-}, and PO_4^{3-}; (ii) diphenylamine (0.2—0.5%) in 4M H_2SO_4 for NO_2^-, NO_3^-, IO_4^-, BrO_3^-, MnO_4^-, and WO_4^{2-}; (iii) ferric chloride (10%) in 2M HCl for SCN^-, $Fe(CN)_6^{3-}$, and $Fe(CN)_6^{4-}$; (iv) alcoholic pyrogallol (0.5%) solution for MoO_4^{2-} and $Mo_7O_{24}^{6-}$.

Chromatographic systems. The following sorbent layers were used as stationary phases: S_1 = silica gel G; S_2 = alumina; S_3 = microcrystalline cellulose; S_4 = kieselguhr G; S_5 = kieselguhr + cellulose (4:1), (3:2).

Mobile phase. The W/O microemulsion, used as mobile phase, was prepared by titrating a coarse emulsion of *n*-heptane (160 mL), water (8 mL), and SDS (8 g) with *n*-pentanol (24 mL). Heptane was chosen for the oil phase becaue of low toxicity of the odd-carbon number alkanes. The microemulsion was produced at 30°C.

Preparation of TLC plates. The TLC plates were prepared by mixing adsorbents with demineralized water in a 1:3 ratio by weight (1:4 ratio for S_3). The resultant slurry was mechanically shaken for 10 min, after which it was applied on well-cleaned glass plates to give a uniform layer of 0.25 mm thickness. The plates were air dried at room temperature and then heated at 100 ± 5°C for 1 h. After activation, the plates were stored in an air-tight chamber. No binder was added during the preparation of plates.

Procedure. A sample volume (1.0–10 μL) with a sufficient amount of analyte (0.1–10 μg) was applied with the aid of micropipette about 2.0 cm above the lower edge of the TLC plate. The spots were dried, and the plates were developed by the one-dimensional ascending technique, in glass jars (24 × 6 cm^2) that contained the mobile phase. Before the development of plates, the glass jars, containing mobile phase, were covered with a lid for about 20 min, so that the glass jars may get presaturated with the mobile phase vapors. The solvent was allowed to migrate up to 10 cm from the starting line in all experiments. After development, plates were dried at room temperature, and the spots were visualized with the appropriate spraying reagent.

The identification limits of various anions were determined by spotting different amounts of anionic solutions on the chromatoplates. The plates were developed, and the spots were detected as described above. The method was repeated with successive lowering of the amount of anion until spots could no longer be detected. The minimum amount of anion just detectable was taken as the limit of detection.

RESULTS AND DISCUSSION

We have tried identification and separation of anions on various adsorbents (S_1–S_5). Kieselguhr thin layers were highly effective for differential migration of anions. Table 1 summarizes some binary separations of IO_4^- and MnO_4^- from accompanying anions by using the W/O microemulsion as mobile phase and kieselguhr as the stationary phase. Separations achieved are well resolved and compact in this chromatographic system. It is an important aspect of this study because our past experience (14) has shown that kieselguhr, being only slightly active, fails to provide any fruitful data when used as stationary phase for normal TLC of anions with organic or mixed aqueous-organic solvent systems. This indicates that the W/O microemulsion plays a crucial role in enhancing the differential migration of anions on kieselguhr. This is possibly due to the presence of a water core in W/O microemulsion droplets which provides a restricted volume (16) and are responsible for specific selectivities. The hR_F values of separated anions are given in Table 1. The R_F value of each individual anion changes slightly when it is chromatographed in mixture with other anions.

TABLE 1
Binary Separations Achieved Experimentally with Water-in-Oil Microemulsion[a]

Stationary phase	Separations (hR_F)
Kieselguhr G	IO_4^- (5.75)-NO_2^- (89.5)
	IO_4^- (6.75)-BrO_3^- (89.25)
	IO_4^- (7.25)-I^- (88.25)
	IO_4^- (7.5)-Br^- (94.0)
	MnO_4^- (5.0)-BrO_3^- (91.0)
	MnO_4^- (5.0)-NO_2^- (90.0)
	MnO_4^- (2.5)-Br^- (93.25)

[a] Note: $hR_F = R_F \times 100$, $R_F = (R_L + R_T)/2$. R_L = R_F of leading front of spot. R_T = R_F of trailing front of spot.

Table 2 presents the detection and dilution limits of anions. The proposed method is highly sensitive for most ions, except Br^-, I^-, and IO_4^-. The most interesting behavior is exhibited by MnO_4^-, which can be detected easily, even if it is present at 1 μg. Similarly, CrO_4^{2-} and BrO_3^- can be detected easily. This may well be attributed to the fact that, in W/O microemulsion, these solutes are localized in the hydrophilic core, which is responsible for some sort of preconcentration

TABLE 2
Detection and Dilution Limits of Anions as Their Salts on Kieselguhr Layers Developed with Water-in-Oil Microemulsion

Anion	Lower detection limit (μg)	Dilution limit[a]
IO_4^-	20	1:1000
IO_3^-	10	1:2000
BrO_3^-	2	1:10,000
I^-	20	1:1000
Br^-	20	1:1000
MnO_4^-	1	1:20,000
CrO_4^{2-}	2	1:10,000

[a] Dilution limit = 1:(volume of test solution × 10^6)/[limit of detection (μg)].

TABLE 3
Quantitative Separation of IO_4^- (20–100 μg) from Milligram Amounts of NO_2^-, Br^-, and I^- with Chromatographic System as in Table 1

Amount of accompanying anion salt (mg)		Separations (R_L-R_T)
$NaNO_2$	1.25	IO_4^- (0.15–0.00)-NO_2^- (1.0–0.46)
KBr^a	1.25	IO_4^- (0.11–0.00)-Br^- (1.0–0.76)
	2.5	IO_4^- (0.10–0.00)-Br^- (1.0–0.68)
	5.0	IO_4^- (0.10–0.00)-Br^- (1.0–0.48)
KI	1.0	IO_4^- (ND^b)-I^- (1.0–0.70)
	2.0	IO_4^- (ND-I^- (1.0–0.65)
	4.0	IO_4^- (ND)-I^- (1.0–0.20)

[a]Spots of Br^- appear after 20 min. [b]ND = not detected.

of anions (17). Further, the anionic charge on the microemulsion droplet is responsible for fast transfer of anionic species out of the microemulsion droplet and easily available to interact with the detection reagents.

Table 3 presents some quantitative separations of anions. KBr, up to 5 mg, and $NaNO_2$, up to 1.25 mg, can be easily separated from KIO_4 (100 μg). Separation of KIO_4 from milligram quantities of KI does not take place, KIO_4 remains undetected while KI produces a tailed spot. Thus, increasing the KI amount produces a deleterious effect and causes poor or no detection of KIO_4. This possibly may be due to reduction of KIO_4 to iodine in solutions of moderate acidity. Because of this fact, we carried out the separation of IO_4^- from NO_2^-, Br^-, and I^-.

TABLE 4
Effect of Additives on Some Binary Separations of IO_4^- from NO_2^- and Br^{-a}

Additives	Separations (R_F)
1. Amines	
(a) β-Napthylamine	IO_4^- (0.05)-NO_2^- (0.85)
	IO_4^- (0.06)-Br^- (ND^b)
(b) Diphenylamine	IO_4^- (0.21)-NO_2^- (0.88)
	IO_4^- (0.21)-Br^- (0.74)
(c) 2-Nitroaniline	IO_4^- (0.20)-NO_2^- (0.65)
	IO_4^- (0.17)-Br^- (0.78)
2. Phenols	
(a) Phenol	IO_4^- (0.03)-NO_2^- (0.82)
	IO_4^- (0.10)-Br^- (0.85)
(b) Resorcinol	IO_4^- (0.07)-NO_2^- (0.90)
	IO_4^- (0.07)-Br^- (0.81)
(c) Pyrogallol	IO_4^- (ND)-NO_2^- (0.80)
	IO_4^- (ND)-Br^- (0.81)
3. Heavy metals	
(a) Hg^{2+}	IO_4^- (0.05)-NO_2^- (0.92)
	IO_4^- (0.08)-Br^- (0.83)
(b) Pb^{2+}	IO_4^- (0.03)-NO_2^- (0.90)
	IO_4^- (0.01)-Br^- (ND)
(c) Ag^+	IO_4^- (0.05)-NO_2^- (0.94)
	IO_4^- (0.05)-Br^- (ND)
(d) Bi^{3+}	IO_4^- (0.04)-NO_2^- (0.94)
	IO_4^- (0.06)-Br^- (ND)

[a]Standard R_F values of individual ions are: IO_4^- (0.00), NO_2^- (0.92), and Br^- (0.90).
[b]ND = not detected.

Finally, Table 4 summarizes the effect of various additives on the separation efficacy of IO_4^- from Br^- and NO_2^-. Amines by and large do not influence the separations, but there are drastic changes in the R_F values of ions, compared with their standard R_F values. The separation mixture precipitates by the addition of amines. In case of β-napthylamine, Br^- remains undetected because the entire spot (R_F range of Br^-) is superimposed by the solvent uptake of β-napthylamine (deep purple color spot). The situation for NO_2^- is similar, but the spot of NO_2^- is clear. Spots of Br^-, when chromatographed with diphenylamine and 2-nitroaniline, appeared as streaks after one-half hour. Phenols neither hamper separations nor produce precipitation. However, addition of pyrogallol produces slight turbidity, and 10_4^- remains undetected when separated from NO_2^- or Br^-. In this case, the R_F range of IO_4^- gets superimposed by pyrogallol. Heavy metals also do not hinder the separations except silver, which causes dense precipitation as a result of which Br^- remains undetected. Addition of lead to the separation mixture of IO_4^- and Br^- leads to poor detection of Br^-. Mercury shows no effect on the separation of IO_4^- from Br^- or NO_2^-.

An attempt has been made for the semiquantitative determination of IO_4^- by employing a peak height measurement procedure. For this purpose, 0.1 mL standard aqueous solu-

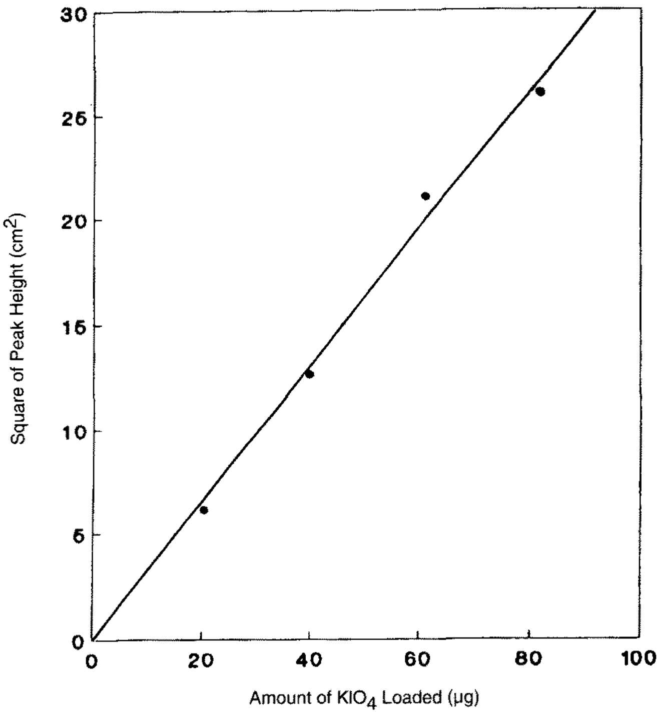

FIG. 1. Standard calibration curve for semiquantitative determination of IO_4^- by peak height measurement method.

tions of KIO_4 (0.02–0.1%) were spotted on kieselguhr thin layers. The chromatograms were developed and detected. The spots obtained were copied directly onto tracing paper from the chromatoplates. A linear relationship was obtained when the square of peak heights of the spots was plotted against microgram quantities of KIO_4 (Fig. 1).

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