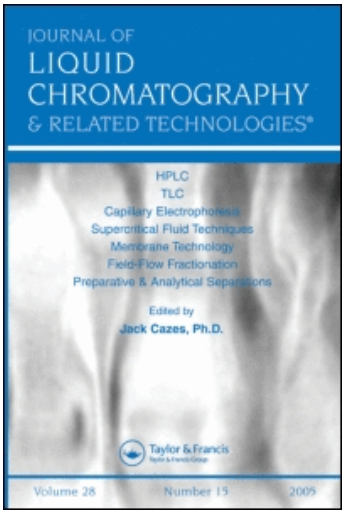

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Issaq, Haleem J. , Seburn, Kendra E. and Hightower, Jeffrey R.(1991) 'Thin Layer Chromatographic Separations by Conventional Glass Backed Plates and Empore Sheets: A Comparative Study', Journal of Liquid Chromatography & Related Technologies, 14: 8, 1511 — 1517
To link to this Article: DOI: 10.1080/01483919108049632
URL: http://dx.doi.org/10.1080/01483919108049632

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(8), 1511-1517 (1991)

THIN LAYER CHROMATOGRAPHIC SEPARATIONS BY CONVENTIONAL GLASS BACKED PLATES AND EMPORE SHEETS: A COMPARATIVE STUDY

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ABSTRACT

A comparison of separations using conventional TLC plates and Empore sheets is presented. The results show that in certain cases the Empore sheets failed to reproduce the separations obtained using silica gel plates, however, the retention times were comparable. The reversed phase C-18 Empore sheets were found to: (a) require much longer development times than C-18 TLC plates; (b) small increases in water content in the mobile phase (2%) contributed to considerable increase in development time; and (c) the maximum recommended percent water (v/v) in the mobile phase for meaningful and acceptable experimental time should not exceed 20%. It was observed that the Empore sheets were easier to spot than the TLC plates but harder to write on. Day-to-day and experiment to experiment R_f reproducibility of Empore sheets was better than 5%.

INTRODUCTION

Thin layer chromatography (TLC), a separation technique which can be used at the micro or macro level, was first introduced in 1938 by Izmailov and

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Shraiber (1) as spot chromatography. Eleven years later (2), surface chromatography was used to fractionate inorganic salts on adsorbent-coated glass plates. In 1951 Kirchner et al (3) used glass strips coated with adsorbents for the separation of terpenes. TLC became popular after Shahl in 1956 (4) introduced a spreader which could prepare uniform and reproducible layers of adsorbents. The adsorbent's slurry was spread on uniform sheets of glass, plastic or aluminum. This procedure of preparing TLC plates is the dominant one today. In 1964 Gelman Instrument Company (Ann Arbor, MI), introduced instant TLC. The ITLC sheet of glass micro fiber is impregnated with the adsorbent which saturates and surrounds the glass microfiber cloth. Mobile phase and reagents penetrate from both sides. The ITLC silica gel sheets are prepared by dipping ultrapure micro fibers of glass into a freshly prepared supersaturated solution of potassium silicate in ammonium chloride. The finely precipitated silicic acid forms lustrous granules, i.e., contains water in an amorphous form of hydrated silica. The solution gels rapidly and the ammonia is removed by distilled water chromatography and extended heating of over 300°C. The resultant medium is slightly acidic (pH 5), possesses weak stationary phase and has a porous matrix which makes it extremely sensitive (5). Recently, 3M Company (St. Paul, MN) introduced the Empore TLC sheets whereby the silica gel or silica gel bonded phases are entrapped into a poly(tetrafluoroethylene) micro fibrils. No chemical binder or a support sheet is required (6). Both Empore and ITLC sheets are easy to handle and can be cut by scissors to any size or shape. Poole and Poole (7) and Poole et al (8) evaluated the Empore sheets. In this evaluation we wish to compare different aspects than those done earlier (7-8). This study compares the separation characteristics and reproducibility of Empore sheets (silica gel and bonded silica gel) with those obtained using conventional TLC plates. Also, diffusion of the spots (ratio of diameters of developed spot to spotted) will be evaluated along with the separation factor (α), time of development and volume of water content of the mobile phase (for reversed-phate sheets) that will allow the completion of an experiment in a reasonable time. Other parameters will also be studied and commented on.

Table 1

Physical Properties of Empore TLC Sheets

Formulation	90% ± 2% adsorbent particle 10% + 2% PTFE
Particle Size	8 micron silica based
Pore Size	60 Angstron
Particle Shape	Irregular
Sheet Thickness	500 micron

EXPERIMENTAL

Silica gel plates were purchased from EM Science (Gibbstown, NJ) while bonded C-18 silica gel plates (KC-18F) were purchased from Whatman (Hillsboro, OR). Empore silica gel and C-18 reversed-phase sheets were purchased from Analytichem International (Harbor City, CA). Test dye mixture was obtained from Camag (Wrightsville, NC) while the aflatoxins B_1, B_2, G_1, and G_2 were purchased from Aldrich Chemical Co. (Milwaukee, WI). Plates were spotted using a microsyringe and developed in a rectangular glass developing tank. They were viewed, after development, when needed, in a viewing box equipped with short and long wave UV lamps. Plates and sheets were developed to the same distance (6-7 cm) in a saturated tank (normal phase) at room temperature and under the same experimental conditions. All experiments were run in triplicates.

RESULTS AND DISCUSSION

Table 1 lists the physical properties of the Empore TLC sheets.

Spot Diffusion Ratio:

Spot diffusion ratio is a parameter which would indicate the diffusion after development, of the spots on the stationary phase. This parameter, which is the ratio of the diameter of the spot after development divided by the diameter of the applied spot, would indicate: (a) how well the adsorbent layer (particle size and distribution, density and compactness) is made; and

Table 2

Diffusion ratios, R_f and α values for a dye mixture using silica gel TLC plates and Empore sheets

	TLC Plates			Empore Sheets		
Spot Color	Ratio	R_f	α	Ratio	R_f	α
Purple 2	1.00±.01	0.10±.01	-	1.15±.21	0.10±.01	-
Light Purple	1.88±.18	0.18±.01	1.80	-	-	1.00
Light Pink	1.10±.14	0.26±.01	1.44	1.15±.21	0.28±.01	2.80
Purple 1	1.90±.14	0.32±.01	1.23	1.90±.57	0.33±.01	1.18
Dark Grey	1.20±.01	0.38±.01	1.19	-	-	1.00
Dark Pink	2.35±.21	0.44±.01	1.16	2.75±.35	0.44±.01	1.33
Dark Blue	3.00±.01	0.51±.01	1.16	3.65±.92	0.51±.01	1.16
Pink	2.80±.01	0.59±.01	1.16	2.90±.57	0.57±.01	1.12
Yellow	3.75±.35	0.66±.01	1.12	4.5±.71	0.64±.01	1.12
Blue	2.90±.14	0.70±.01	1.06	3.40±.85	0.69±.01	1.08
Grey	1.35±.21	0.79±.01	1.13	1.35±.21	0.80±.01	1.16

(b) degree of resolution. The larger the ratio the worse the layer and vice versa. Table 2 shows the diffusion ratios, R_f and α values of a dye mixture (5 μl) spotted on silica gel TLC plates and Empore sheets, and developed for 7 cm in toluene:hexane:ethyl acetate (50:35:15). These results are the mean and standard deviation of three different developments (Tables 2, 3 and 5). The results show that the Empore sheets failed to resolve the light purple and dark grey spots, while the conventional silica gel plate resolved all the components in the mixture. The R_f and α values of resolved spots on both systems are comparable, and reproducible. In most cases, the spot diffusion ratio is better on the TLC plates than on the Empore sheets. Also, the standard deviation is larger using the Empore sheets. Similar results were obtained when 1 μl and 10 μl of the dye mixture solution were spotted. Run time was 12 minutes for both TLC and Empore sheets.

The dye mixture was also used to test the results obtained on reversed phase C-18 TLC plates and Empore sheets when developed in a mobile phase of

Table 3

Diffusion ratios, R_f and α values for a dye mixture using reversed phase C-18 TLC plates and Empore sheets

	TLC Plates			Empore Sheets		
Spot Color	Ratio	R_f	α	Ratio	R_f	α
Light Blue	1.02±.12	0.07±.01	-	1.00±.01	0.04±.01	-
Blue	1.35±.07	0.20±.01	2.86	1.50±.14	0.10±.01	2.5
Dark Pink	1.25±.07	0.30±.02	1.50	1.35±.07	0.18±.02	1.80
Dark Blue	1.70±.42	0.36±.02	1.20	1.40±.01	0.23±.01	1.28
Pink	1.15±.21	0.43±.03	1.19	1.20±.28	0.29±.01	1.26
Yellow	2.30±.01	0.46±.04	1.07	2.45±.21	0.30±.01	1.03
Grey	1.00±.01	0.52±.02	1.13	1.05±.07	0.39±.01	1.30
Light Yellow	1.62±.07	0.58±.04	1.12	1.64±.10	0.44±.02	1.13
Purple	1.54±.05	0.68±.04	1.17	1.64±.10	0.57±.04	1.30

methanol:acetonitrile:water (63:28:9). Note that the normal phase (silica gel) system resulted in 11 spots while the RP system gave only 9 spots for the same mixture. No effort was made to optimize the mobile phase because we were interested in comparing the TLC plates with the Empore sheets and not both systems (normal and reversed phase). Table 3 lists the results obtained in this mobile phase.

The table shows that overall the separation is better using the Empore sheets judging from the α values for both systems. While the spot diffusion ratios are comparable, they slightly favor the TLC plates. The R_f values using TLC plates are higher than those using the Empore sheets, although the development time was shorter for conventional plates (10.5 minutes) than for Empore sheets (47 minutes) although the mobile phase contained only 9% water. Table 4 shows the effect of water content in the mobile phase on time of development. The table shows that a small increase in the water content results in a big increase in the development time. The RP-Empore sheets, in our opinion are unpractical to use in mobile phases that contained more than 20% water by volume.

Table 4

Effect of water content in the mobile phase on development time of reversed phase Empore sheets

Mobile Phase Composition	Distance (cm)	Time (min)
20% H_2O:80% Organic*	2.10	70
22% H_2O:78% Organic	1.65	42
24% H_2O:76% Organic	0.80	33
26% H_2O:74% Organic	0.35	56

*Organic phase is made of 3:2 Acetonitrile:methanol.

Table 5

Separation of aflatoxins B_1, B_2, G_1, and G_2 on silica gel TLC plates and Empore sheets using a mobile phase of chloroform: tetrahydrofuran (9:1) in a saturated tank. Development distance 65 cm

	TLC Plates			Empore		
Aflatoxin	R_f	Ratio	α	R_f	Ratio	α
B_1	0.30±.01	1.33±.14	-	0.31±.02	1.25±.25	-
B_2	0.25±.01	1.08±.14	1.20	0.26±.03	1.08±.14	1.24
G_1	0.21±.01	1.08±.14	1.19	0.23±.02	1.08±.14	1.14
G_2	0.18±.01	1.06±.01	1.17	0.19±.02	1.06±.16	1.16

Separation of Aflatoxins B_1, B_2, G_1, and G_2:

Aflatoxins B_1, B_2, G_1, and G_2 are an important class of compounds. Their separations by TLC is a classic one (9). A comparison of the separation of these aflatoxins on silica gel conventional plates and Empore sheets is given in Table 5 (normal phase-silica gel). The results show that both plates and sheets resolved the four aflatoxins. The time required for a development distance of 6.5 cm was the same (12 minutes) using both systems. Also, spot diffusion ratios were comparable.

CONCLUSION

The results of the study show that the silica gel Empore sheets performed almost as well as the glass backed conventional TLC plates. The

diference in performance was most prominent when the RP-18 Empore sheets were used with mobile phases that contained more than 20% water, where the development time became excessively long and experimentally unpractical. The advantages of the Empore sheets is in its higher capacity, and spot elution after development. Since the Empore sheets are very flexible, the recommended bracket, for development, should be used, otherwise the sheet might stick to the sides of the tank, which is not a problem using conventional glass backed TLC plates. The Empore sheets were easier to spot than the conventional plates, but harder to write on. These results agree with those observed earlier (7,8). Also, the reproducibility of R_f values using the Empore sheets from experiment to experiment and from day to day was better than 5%.

ACKNOWLEDGEMENTS

By acceptance of this article, the publisher or recipient acknowledges the right of the U.S. Government to retain a nonexclusive, royalty-free license and to any copyright covering the article.

This project has been funded at least in part with Federal funds from the Department of Health and Human Services under contract number NO1-CO-74102. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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