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# Direct use of Empore sheets in overpressured thin-layer chromatography

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## ABSTRACT

The impregnation of the edges of plates before carrying out forced-flow thin-layer chromatography (TLC) as overpressured TLC is time consuming. This paper reports the optimisation of analytical parameters and the direct use of a new separation medium, Empore TLC sheets, for forced-flow TLC. The pressure of the water pump ($P$) and the flow-rate of the eluent ($F$) were studied and optimum values of $P$ = 2–4 bar and $F$ = 0.4–0.6 ml/min were determined. The separation of a mixture of dyes and a bergamot oil was investigated and the results demonstrated that the direct utilisation of Empore TLC sheets in forced-flow TLC gave performances similar to pre-impregnated high-performance TLC plates.

## INTRODUCTION

One of the major advantages of the use of forced-flow thin-layer chromatography (TLC) as overpressured TLC (OPLC) over other planar chromatography techniques is the decrease in migration time resulting from the absence of a vapour phase and no capillary flow. The distance of the front, $Z$, is a linear function of development time, $t$: $Z = kt$ [1–4]. However, the overpressured chromatographic separation technique requires that the edges of the chromatographic plate are sealed by a polymer solution, which prevents the eluent from flowing off the chromatographic plate in an unwanted direction. This procedure, called impregnation, is time consuming and needs careful attention and skill.

Empore TLC sheets were recently introduced in the USA as a new separation medium [5]; they are prepared from 8 μm diameter and 60 Å pore size particles entrapped in a matrix of poly(tetrafluoroethylene) (PTFE) microfibrils. Their flexibility suggests an interesting means of eliminating the impregnation step.

Poole *et al.* [6] have recently demonstrated the successful use of forced-flow development with an Empore sheet, but pretreatment of the edges of the plate was still needed. To improve the analytical conditions, preliminary tests without pretreatment have been performed to check the performance of these plates under OPLC conditions.

To assess their performances in OPLC, these plates were compared, after pa-

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rameter optimisation, to those most commonly used for the separation of dyes and bergamot oils.

## EXPERIMENTAL

*Characteristics of the Empore plates*

The Empore plates (also called sheets) were from 3M Labs. (Analytichem International Harbor City, CA, USA). They consist of 90 ± 2% silica entrapped in a matrix of PTFE, which gives great flexibility and allows the sheet to be cut easily in the laboratory (using, for instance a pair of scissors). The sheets consist of a 0.5 mm thick layer with 60 Å diameter silica pores and 8 μm diameter silica particles. The absorption capacity of the particles is greater than that of similar plates. The tests were performed with untreated Empore sheets.

*Preliminary tests*

Seven streaks of 1 μl of dye solution containing violet 1, butter yellow, red, sudan red G and indophenol blue (Labor MIM, Budapest, Hungary), mixed with heptane, were placed in a 4-mm band on an Empore 10 × 20 cm silica plate without fluorescence indicator (Analytichem International) using an automatic applicator (Linomat IV, Camag, Muttenz, Switzerland).

The dyes were eluted by toluene using a Chrompres 25 (Labor MIM) over a migration distance of about 8 cm. The conditions were as follows: water pump pressure, 25 bar; eluent pump pressure, 6 bar; initial volume used to fill the trough and form a uniform solvent front, 1.2 ml; and flow-rate, 0.3 ml/min.

*Influence of the water cushion pressure*

The tests were carried out on an Empore 10 × 10 cm silica plate (without fluorescence indicator). The selected solvent was ethanol, which allows the observation of potential leaks on the press base, the study of the linearity of the solvent front and the calculation of migration distance. The initial conditions were as follows: eluent pump pressure, 6 bar; solvent volume, 0.7 ml; flow-rate, 1 ml/min; and migration distance, 7 cm.

The cushion pressure was then varied from 0 to 20 bar in steps of 1 bar; the appearance of potential leaks, and the time and migration distance of the $\alpha$ and $\beta$ fronts were observed. Each test was carried out in duplicate.

*Influence of the eluent flow-rate*

The operating conditions were as described for the influence of water cushion pressure. The cushion pressure was 4 bar and the eluent pump flow-rate varied from 0.1 to 2 ml/min.

*Separation of a dye mixture after optimisation*

The dye mixture was re-used to test the optimisation of the utilisation parameters of the Empore plates in OPLC.

Volumes of 1 μl of dye mixture were manually applied to an Empore 10 × 10 cm silica plate (without fluorescence indicator). The conditions were as follows: water cushion pressure, 2 bar; flow-rate, 0.6 ml/min; initial volume, 0.7 ml; and eluent pump pressure, 6 bar. The elution was performed with toluene.

*Separation of bergamot oil*

A comparative study of bergamot oil (Man C[ie], Bar sur loup France) separation in chamber and by OPLC was carried out using the Empore silica $F_{254}$ 10 × 10 cm plates and HPTLC silica $F_{254}$ 10 × 10 cm plates (Merck, Darmstadt, Germany) on a glass support (chamber) and an aluminium support (OPLC).

Streaks of 6 μl of bergamot oil were added to the plates in a 6-mm band using Linomat IV. The eluting phase was chloroform–methanol–1 *M* sodium hydroxide solution (95:5:0.1, v/v/v).

The operating conditions in the OPLC were the same as those used for optimising the parameters for the Empore plates. For standard plates, the water cushion pressure was 25 bar, the eluent pump pressure 6 bar, the initial volume 0.7 ml and the flow-rate 0.25 ml/min.

Readings were taken using a Desaga CD 60 densitometer (Desaga, Heidelberg, Germany) at 254 nm in the reflectance mode.

## RESULTS AND DISCUSSION

*Optimisation of parameters*

During the preliminary tests, the elution was not linear, but followed a radial path (Fig. 1). This phenomenon is a result of applying too high a pressure to the Empore plate. The distortion is imprinted on the inlet as well as on the cushion membrane groove, which is obstructed, and therefore the solvent no longer has free access to the whole length of the plate. The eluent moves centrifugally away from the inlet. An optimisation of the utilisation parameters of the Empore plate in OPLC [water cushion pressure (*P*) and solvent flow-rate (*F*)] was therefore necessary.

The results of pressure optimisation study (Fig. 2a) show that (1) the leaks are proportional to pressure; (2) the migration time remains very similar; and (3) the α and β fronts are altered. The migration time is unacceptable for high pressures ($> 4$ bar) and for low pressures ($< 2$ bar) as there are numerous leaks and the solvent fronts are non-linear. However, these phenomena are considerably reduced and the solvent front is almost linear for pressure from 2 to 4 bar.

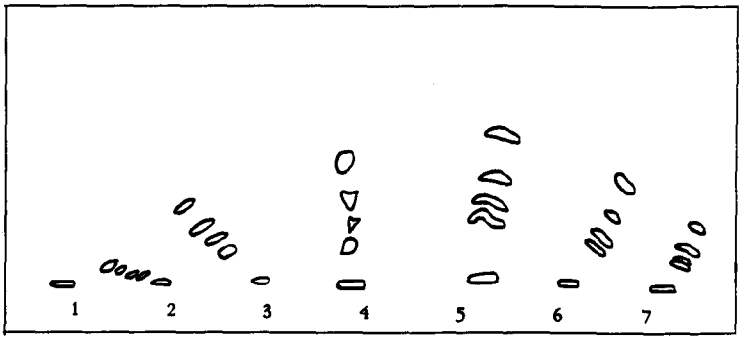


Fig. 1. Overpressured thin-layer chromatography of dyes. Elution order: violet 1, sudan red G, indophenol blue, red, butter yellow. Seven streaks of the same solution were applied. Conditions: eluent, toluene; water pump pressure, 25 bar; eluent pump pressure, 6 bar; initial volume, 1.2 ml; flow-rate, 0.3 ml/min.

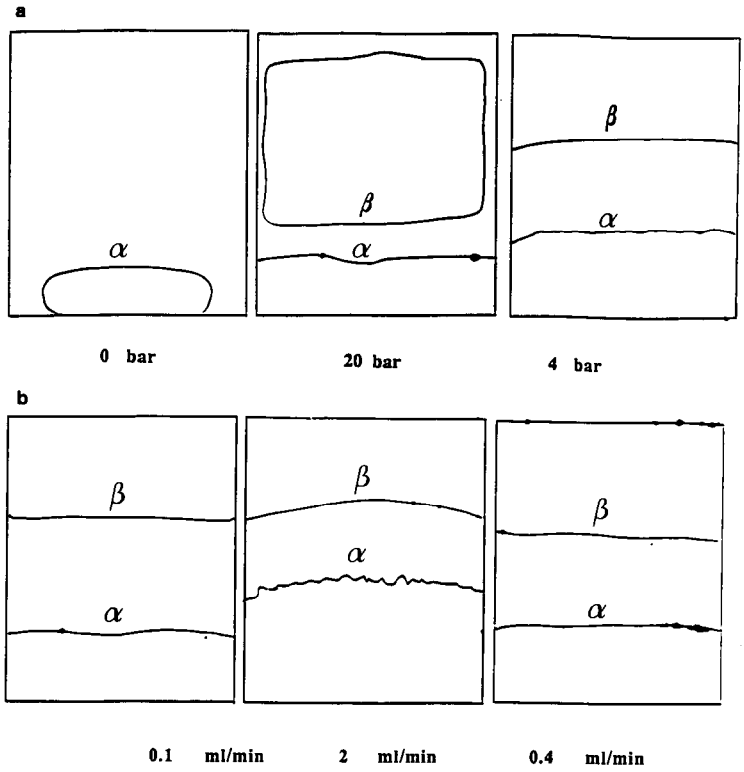


Fig. 2. (a) Optimisation of pressure. Conditions: eluent pump pressure, 6 bar; initial volume, 0.7 ml; flow-rate, 1 ml/min. $\alpha$ and $\beta$ fronts can be observed for a pressure of 4 bar. (b) Flow optimisation. Conditions: eluent pump pressure, 6 bar; initial volume, 0.7 ml; water pump pressure, 4 bar. $\alpha$ and $\beta$ fronts can be observed.

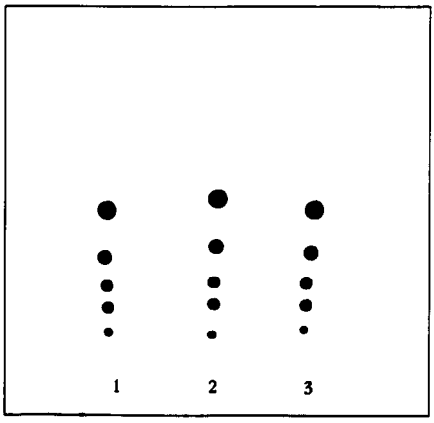


Fig. 3. Overpressured thin-layer chromatography of dyes. Three streaks of the same solution were applied. Conditions: eluent pump pressure, 6 bar; water pump pressure, 2 bar; initial volume, 0.7 ml; flow-rate, 0.6 ml/min. For elution order of dyes, see Fig. 1.

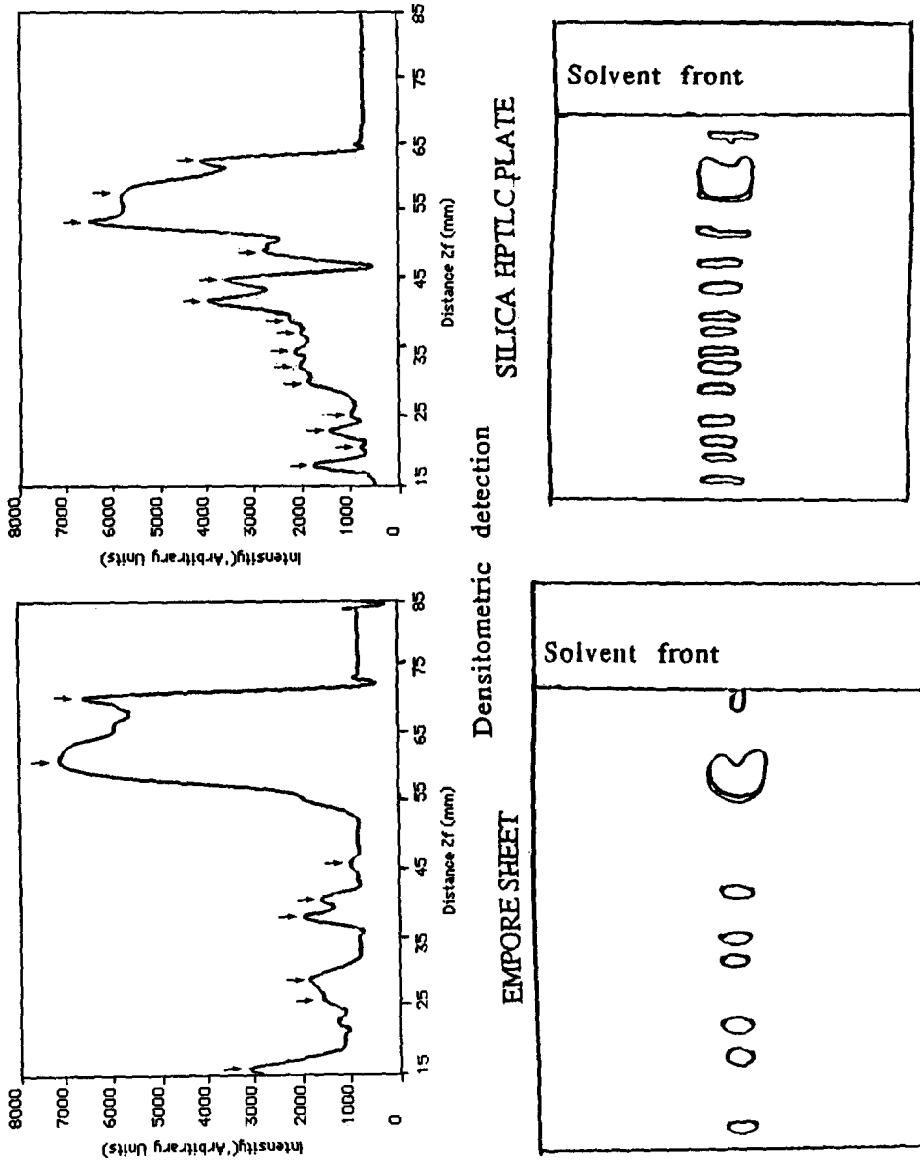


Fig. 4. Planar chromatography of bergamot oil in a tank using a silica high-performance TLC (HPTLC) plate and an Empore sheet. Arrows indicate that the integrated compounds are visually detectable. $Z_f$ is the elution distance.

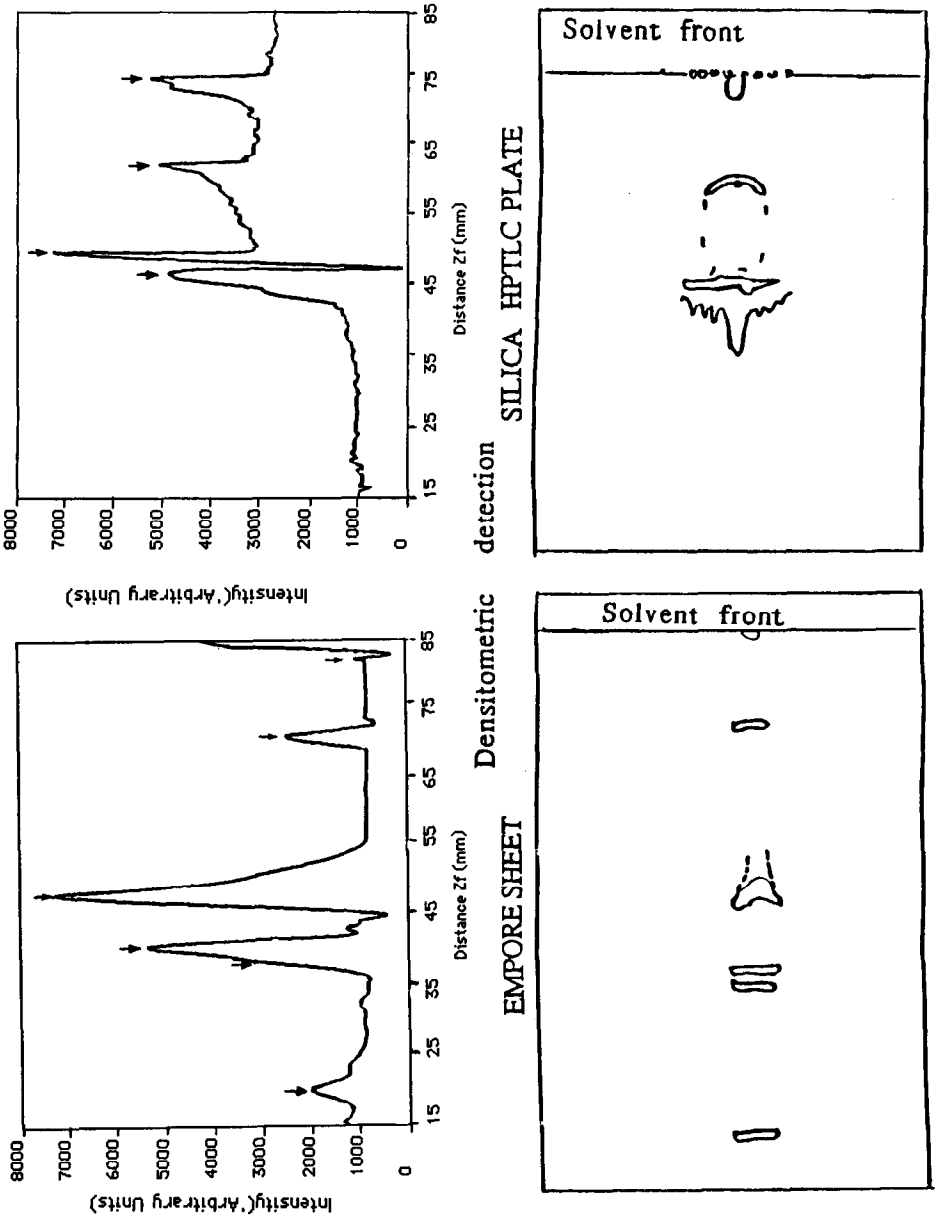


Fig. 5. Overpressured thin-layer chromatography of bergamot oil using a silica HPTLC plate and an Empore sheer. Arrows as in Fig. 4. $Z_f$ is the elution distance.

The results of the flow-rate optimisation (Fig. 2b) show that the migration time is inversely proportional to the flow-rate. In contrast, the number of leaks is proportional to the flow-rate and a modification of the linearity of the $\alpha$ and $\beta$ fronts is observed for flow-rates $>0.75$ ml/min. The maximum flow-rate therefore lies between 0.4 and 0.6 ml/min for ethanol.

*Application to specific cases*

*Separation of a dye mixture.*The migration of the dyes is similar to that observed with conventional plates and leaks are almost non-existent (Fig. 3). The lower pressure of the water cushion no longer distorts the plate, allowing the solvent to migrate freely.

*Separation of bergamot oil.*The results observed do not show any correlation between separation in tank and by OPLC. This is because in a tank the solvents migrate together, whereas in OPLC each solvent migrates independently as a result of their different physicochemical properties [7].

In a first analysis, the results in the chamber with Empore sheets are therefore more favourable than a separation on conventional plates; it enables the display of fifteen spots compared to the eight on the Empore plates, and these have a higher peak intensity (Fig. 4). In addition, these results confirm the work of Poole *et al.* [8], which showed the inferior performance of these new plates. However, these results are reversed in OPLC; the number of compounds detected densitometrically is greater on the Empore plate and the intensity of the common peaks is higher with a better resolution. The baseline is perfectly linear (Fig. 5).

## CONCLUSION

The direct utilisation of the new Empore plates in OPLC is possible. Parameter optimisation was necessary, although the results are encouraging and suggest that further development may allow this product to become of much more use in the future.

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