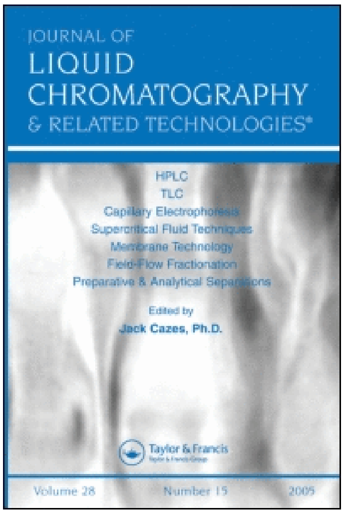

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APPLICABILITY OF EMPORE™ TLC SHEETS FOR FORCED-FLOW PLANAR CHROMATOGRAPHY. I. CHARACTERIZATION OF THE SILICA SHEETS

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ABSTRACT

Empore™ TLC silica sheets - a new separation medium for planar chromatography - are prepared from silica which is entrapped in an inert matrix of polytetrafluoroethylene (PTFE) microfibrils. It was established earlier that the separation power is only about 60 % of that of conventional TLC. This was attributed to the very slow solvent migration velocity resulting from capillary action.

In this paper the characteristics and applicability of Empore™ TLC silica sheets are reported for the various forced-flow planar chromatographic (FFPC) techniques, such as overpressured layer chromatography (OPLC) and rotation planar chromatography (RPC). The preparation of the sheets and the relationship between the solvent front migration distance and migration time are given. The influence of the linear flow rate on the theoretical plate height and the relationship between the resolution and the linear flow rate are determined. Also, the influence of the development mode and solvent system on the densitometric evaluation is discussed. The influence of external force (overpressure or centrifugal force) on the structure of Empore™ TLC silica sheets is demonstrated with the aid of scanning electron micrographs.

The experiments show that Empore™ TLC silica sheets allow extremely rapid separations (5-20 min) by FFPC methods in all development modes. Good resolution is obtained because FFPC techniques are able to ensure the suitable mobile phase migration.

INTRODUCTION

Empore™ TLC sheets were developed in the USA as a new separation medium for planar chromatography and were recently introduced as new sample extraction products [1]. The sheets consist of normal or chemically modified silica, suspended in a polytetrafluororethylene (PTFE) matrix. The average particle size of Empore™ TLC sheets is 8 µm, which suggests that the chromatographic properties should lie between those of TLC (11 µm) and those of HPTLC (6 µm) plates. The material of the sheets is approximately 90 % sorbent by weight, with the balance being largely PTFE. The increased loading capacity of the sheets was achieved by utilizing a mean 60 Å pore size with a surface area of approximately 500 m^2/g and the absence of a binder. After development, the spot or band of compound(s) of interest can be cut out easily from the sheets for analytical or micropreparative purposes.

Concerning the efficiency of this separation medium, Poole and Poole [2] established that the separation number is only about 60 % of that of conventional TLC plates. This was explained by the very slow solvent migration velocity on using capillary action for development. The development time for the mobile phase to migrate 5 cm is either similar to or shorter than observed for the HPTLC plate (e.g., in the case of acetonitrile this value was about 45 % shorter).

On using Empore™ TLC sheets, the migration distance is limited by the relatively slow flow rates, similar to those obtained with HPTLC plates. Therefore this sheet material cannot be used in conventional chamber systems over longer development distances (> 5cm) because of the diffusion caused by the rapidly decelerating migration of the solvent system. The flexibility of this separation medium requires that the sheets be hang on a rack. Therefore, these sheets are suitable for ascending and descending linear development, but circular and anticircular development modes would be rather complicated in practice.

The last decade has seen the introduction of forced-flow planar chromatographic (FFPC) methods [3] permitting optimization of the mobile phase velocity in order to achieve better resolution in a shorter development time. Overpressured layer chromatography (OPLC), developed by Tyihák et al. [4, 5], was the first FFPC technique, in which the vapor phase above the sorbent layer is completely eliminated. Using this method the mobile phase is transported through the cross section of the stationary phase assuming a constant flow similar to high performance liquid chromatography (HPLC). As such the separation power is assured over a longer separation distance without loss of resolution in a shorter development time. OPLC can be used for off-line and on-line analytical applications [6, 7], as well as for preparative separations [8].

A novel version of OPLC is overpressured multi-layer chromatography (OPMLC) [9], where two or more plates can be developed simultaneously. Therefore a great number of samples (100-500) can be separated during one development.

High performance planar liquid chromatography (HPPLC) developed by Kaiser and Rieder [10-11] is a special version of circular OPLC . Generally, HPPLC is used in the single sample mode, where one sample can be separated in the circular development mode within 1-2 min. The separation of the sample into individual compounds permits accurate quantification by an optical scanner in a circular mode of operation.

Another variant of FFPC is rotation planar chromatography (RPC) [12, 13]. In RPC the mobile phase migrates mainly under the influence of centrifugal force, but also by capillary action, using different types of chromatographic chambers. The potential and efficiency of the various off-line analytical and on-line preparative RPC methods can be realized with the new ROTACHROM® model P rotation planar chromatograph.

In the present paper we report the implementation of FFPC techniques using this new separation medium. Furthermore, the changes of theoretical plate heights and resolution values for some lipophilic dyes at different flow rates and migration distances are given - as separation performance data - for the characterization of Empore™ TLC silica sheets.

EXPERIMENTAL

Empore™ silica gel TLC sheets with fluorescence indicator were obtained from Analytichem International (Harbor City, CA, USA). Sheets of the required size (20 x 20, 10 x 20, 10 x 10) were cut from 20 x 20 cm sheets of material supplied. All solvents were of HPLC quality, toluene of pro analysis grade and were obtained from ROMIL (Shepshed, Leics., UK).

The capillary flow-controlled developments were carried out in the ascending linear mode using twin-trough chromatographic tanks with chamber saturation (room temperature, 1h), and in the circular mode in a modified U-chamber from Camag (Muttenz, Switzerland).

The OPLC experiments were performed with a Chrompres 10 (linear and circular development modes) and a Chrompres 25 overpressured layer chromatograph (Laboratory Instruments Co. Ltd., Budapest, Hungary) at 10 and 25 bar overpressure. The use of Empore™ TLC sheets for linear OPLC separations demands special preparation. The stretched sheets placed on glass or teflon sheets must be impregnated on three sides with Impress II polymer suspension from Laboratory Instruments. Afterwards, an inlet channel must be cut out from the sheet. In case a prerun was necessary the plates were impregnated on all four sides and two channels (for the inlet and outlet) were prepared. For the OPMLC separations a hole was made in the center of the inlet channels on all plates. No impregnation was necessary for circular OPLC similar to the RPC developments.

A ROTACHROM® model P rotation planar chromatograph (Petazon Ltd., Zug, Switzerland) was used for RPC separations. The speed of rotation was varied between 700 and 2000 rpm. The RPC experiments were carried out also in the linear and circular development modes over a distance of 11 cm and 10 cm, respectively.

In all experiments, the solvents were delivered with a Waters M-45 chromatography pump (Milford, MA, USA). This pump was set to supply the mobile phase at a flow rate of 0.05 - 0.8 ml/min according to the exact setting.

Two dye mixtures (concentration of each compound between 0.05 and 0.1 %, dissolved in toluene) were applied. Mixture "A" contained violet 1, butter yellow, red, Sudan red G and indophenol blue, all obtained from Macherey-Nagel (Düren, FRG). Mixture "B" contained fat red and the components of test dye mixture III (HPTLC) from Camag (Muttenz, Switzerland). The samples were spotted either with a 200 nl (Pt-Ir) Nano Doser (Camag) and with 500 nl, 1, 2, or 5 µl minicaps or with a Camag Linomat III TLC spotter.

The densitometric evaluation was carried out with a Camag TLC Scanner II coupled to an HP 9000-216 computer. The absorbance was measured at 260 nm with a deuterium lamp. The monochromator band width was 10 nm and a slit dimension of 0.3 x 4 mm was used.

The cross sections for the electron micrographs were cut with a razor blade. The specimens were coated with Pt in an argon atmosphere. The micrographs were taken with a Hitachi S-700 scanning electron microscope (Tokyo, Japan) on both the surface and the cross section at 160 and 3600 times magnification.

RESULTS AND DISCUSSION

For all FFPC separations Empore™ TLC sheets had to be streched on glass or relatively soft teflon (PTFE) plates. Where as both supports are inert, the latter has the additional advantage on using the OPMLC technique that this inert material is always placed between the Empore™ TLC sheets where it holds and separates the individual chromatographic sheets. Moreover the soft teflon plate assures a uniform effect of overpressure on each individual chromatographic sheet (Figure 1). As shown in Figure 1a, the sheets were impregnated (the hatched zones symbolize the impregnation zone) on a minimum of three sides for linear OPLC and OPMLC separations. It was observed that the Empore™ TLC sheet is not suitable for micro-chamber RPC (M-RPC) [13] separations since the sheets become distorted due to the applied centrifugal force. On using ultramicro-chamber RPC (U-RPC) [12], the glass cover plate of the chamber is placed directly on the

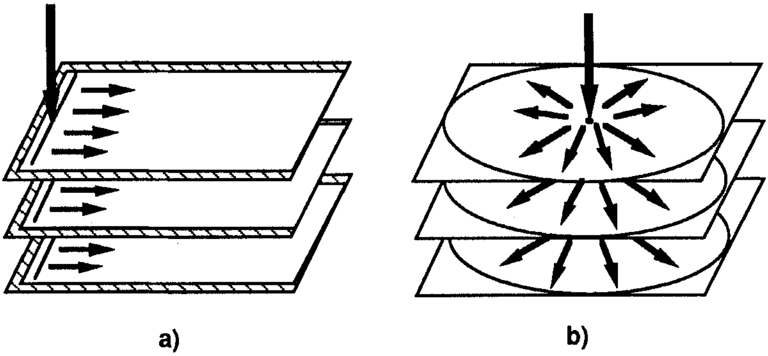

Figure 1 Preparation of the Empore™ TLC silica sheets for OPMLC separations; a) linear OPMLC, b) circular OPMLC.

chromatographic sheets, this permitting the separation process to be carried out without any distortion of the sheets. Preparation of the chromatographic sheet for the circular development mode is similar to the circular OPLC (see top sheet in Figure 1b). In spite of the centrifugal force, the mobile phase velocity can be linearized (linear development mode) for U-RPC separations. Here narrow strips have to be cut out and fixed to a glass holder plate. For the dependence of development time upon the separation distance this separation mode was tested using the U-RPC method, although the preparation is really complicated.

Relationship between the solvent front migration distance and time

The relationship between development distance (z_f) and time (t) illustrates the fundamental difference between the solvent velocity (κ) in capillary flow-controlled and that in forced-flow development techniques. To characterize the solvent velocity in different chamber types for linear and circular development modes, toluene was selected as solvent, which represents an average value ($0.071 cm^2/s$) on the scale of solvent velocity constants.

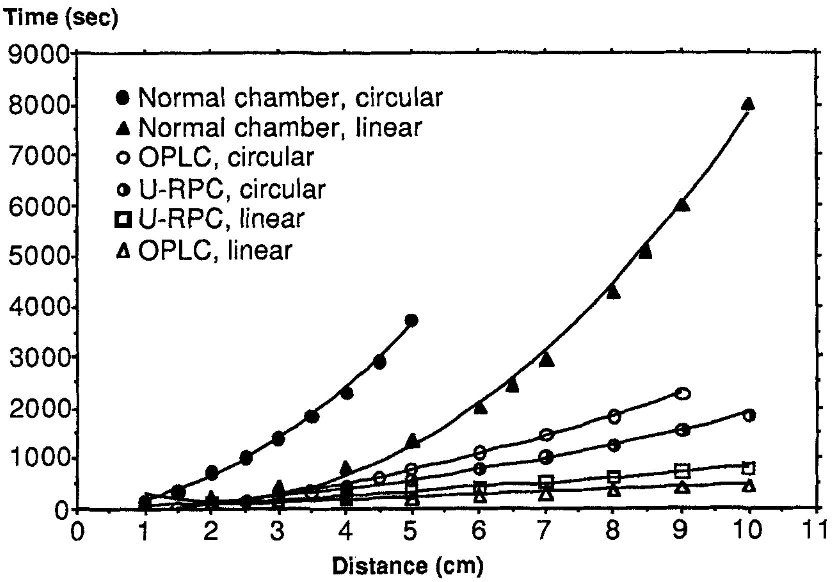

Figure 2 Relationship between the solvent front migration distance and time.

In a saturated chromatographic tank the movement of the eluent front during the separation time is governed by a quadratic function, *viz.*, $z^2 = \kappa t$, where κ is a coefficient dependent on the chromatographic system and the size of sorbent particles [14]. Figure 2 demonstrates the movement of the toluene front using linear and circular development modes in classical chromatographic tanks and applying the FFPC techniques. It can be seen that the relationship between the migration distance of the mobile phase and the development time using Empore™ TLC sheets is similar to that for coventional TLC plates.

The disadvantage of the new separation medium using capillary action for solvent migration is that the sheets exhibit very slow migration also over short development distances. In a saturated chromatographic tank the migration time was more than 3 hours over a 12 cm distance using the linear development mode, and applying the circular development mode the migration time was over 1 hour for a 5 cm distance.

It is clearly seen that, for longer development distances (> 5 cm), only the forced-flow techniques are able to ensure an appropriate mobile phase velocity. For circular FFPC developments, these are slightly curved functions (see Figure 2), because the volume of the stationary phase increases along the radius. Using linear development modes these relationships are linear. However, the slopes of these functions depend on the applied external forces.

Influence of the linear flow rate (u) on the plate height (H)

A major advantage of FFPC is that a linear flow rate (u) exists at which there is an optimum plate height (H) [15]. It was found that in linear OPLC the plate height passes through a minimum value over a certain range of linear flow rate. This fact was established using a Fixion chromatoplate, where 0.72 cm/min was found as optimum mobile phase velocity. Using a silica TLC plate for the same compounds, Kalász [16] determined that the minimum of the H/u value lies at 0.96 cm/sec. The results of Hauck and Jost [17] - working on HPTLC plates - indicated a velocity of about 1.2-1.4 cm/min for minimum plate height. Bearing in that, depending on the type of stationary phase, a relatively large range (between 0.72 and 1.4 cm/ min) was found for the optimum H/u, it seems to be important to determine this optimum value for the novel separation medium. The plate height (H) of the separated substances were calculated according to Guiochon and Siouffi [18] by the following formula:

$$H = \frac{(\sigma)^2}{(z_f - z_0)\, R_f}$$

where: z_f is the distance of the solvent front above the solvent in the reservoir (or inlet), z_0 is the start distance, σ is one-fourth of the width of the spot. Since the H value is strongly dependent on the Rf value [14], five different substances were investigated for the determination of the H/u optimum, using dichloromethane as solvent under controlled

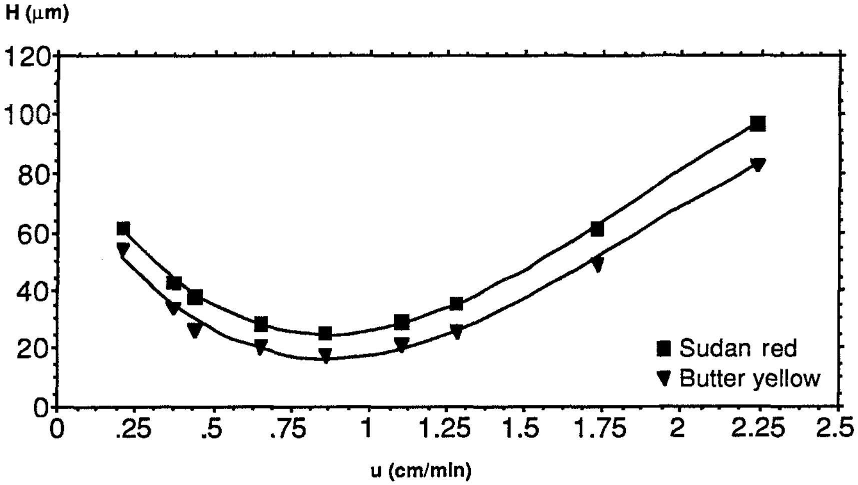

Figure 3 Dependence of the plate height (H) on the linear flow rate using Sudan red and butter yellow dyes from Macherey-Nagel.

development parameters. Since a constant linear flow rate of the mobile phase can be produced by a constant flow pump, this relationship was measured in linear OPLC. The compounds used to measure these values were chosen in the Rf range between 0.3 and 0.7.

Using dye mixture "A" the H values of four compounds were measured. In the very low Rf range, the H values for indophenol (Rf=0.08) varied between 270 µm and 610 µm, therefore this compound was not considered further in the study. Because the properties of butter yellow (Rf=0.7) and the red (Rf=0.5) compound were practically the same, in Figure 3 only the plate heights of Sudan red (Rf=0.3) and butter yellow are plotted against the linear flow rate, using a constant migration distance of 18 cm.

Using the dye mixture "B", the H/u values were determined for at least two compounds. The compounds located below Rf=0.2 were not suitable for these measurements. Because the blue (Rf= 0.35) and red (Rf=0.4) components of the mixture "B" could not be separated at all, the mobile phase velocities of these values were also neglected. Figure 4

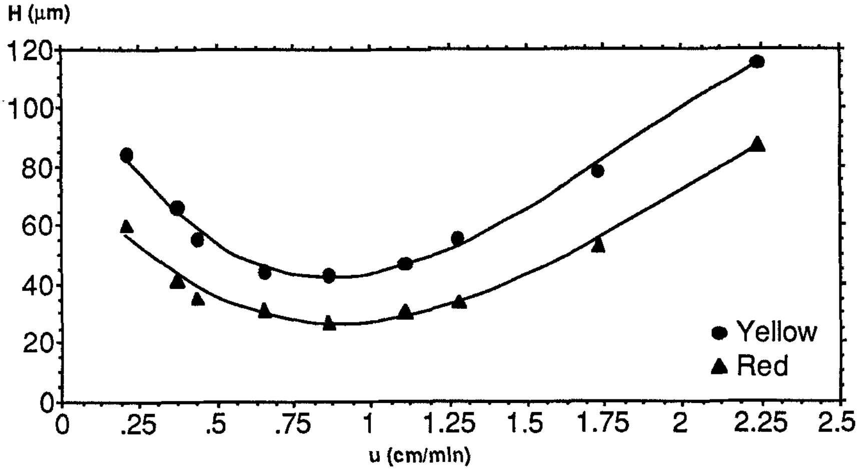

Figure 4 Dependence of the plate height (H) on the linear flow rate using yellow and red dyes from Camag.

shows the plate heights, using a constant migration distance of 18 cm of the red (Rf=0.6) and yellow (Rf=0.8) compounds of mixture "B" at different flow rates in the range of 0.25-2.25 cm/min.

For all five components investigated, the optimum mobile phase velocity range was found to be 0.83-0.89 cm/min, this value being independent of the Rf values. The optimum theoretical plate height varied between 17.2 and 43 µm, depending on the concentration and Rf values of the tested compounds.

Relationship between the resolution (R) and the linear flow rate (u)

Using OPLC technique with HPTLC plates, Hauck and Jost [17] first demonstrated that the best resolution can be achieved at the optimum flow rate. Therefore, using the Empore™ TLC sheets the resolution was calculated for the three compounds of test dye mixture "A". The resolution (R) between two neighbouring substances was calculated

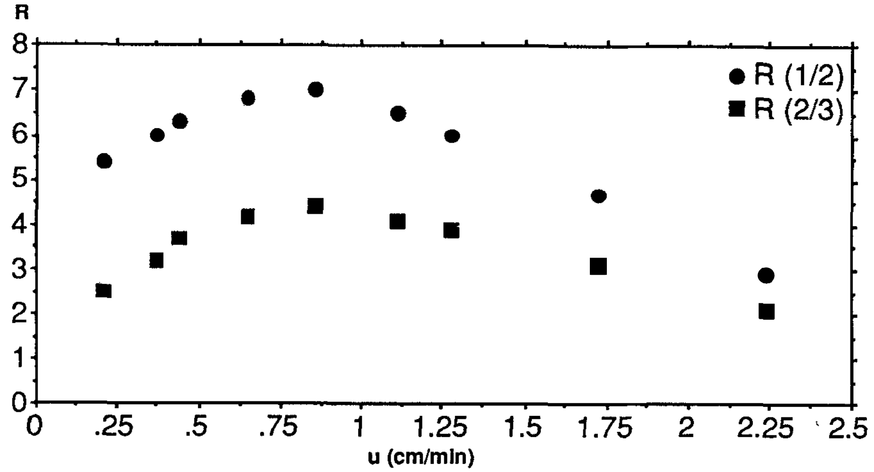

Figure 5 Relationship between the resolution (R) and the linear flow rate (u) using 3 compounds of test dye mixture "A".

according to the equation [14]:

$$R = \frac{(z_x)_2 - (z_x)_1}{2\,(\sigma_2 + \sigma_1)}$$

where: $z_x = (z_f - z_o)\,R_f$

Figure 5 shows that the resolution is maximal at the optimum mobile phase velocity (0.86 cm/min). R (1/2) represents the resolution between butter yellow and the red compounds; R (2/3) indicates the separation efficiency between the red and Sudan red components of the applied dye mixture "A".

Correlation between the H values at the optimal linear flow rate and the migration distance (z)

In capillary flow-controlled planar chromatography using Empore™ TLC silica sheets, the optimum migration distance for the

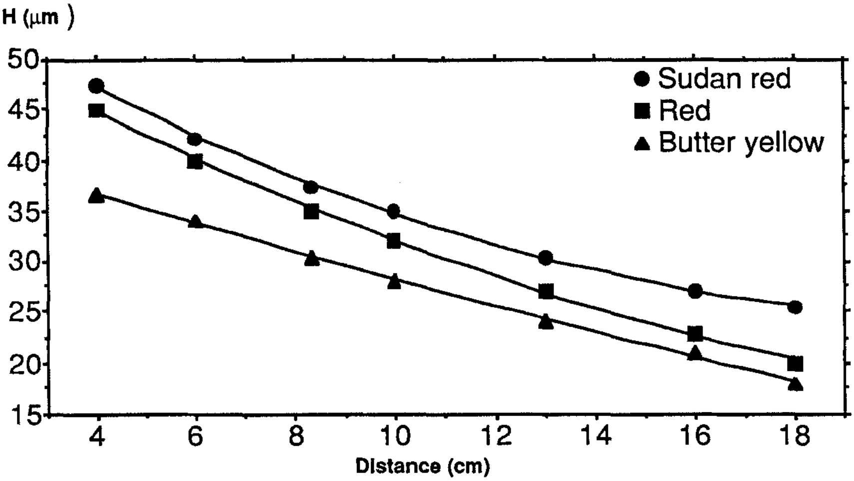

Figure 6 Correlation between the theoretical plate height (H) at the optimal linear flow rate and the migration distance (z)

smallest theoretical plate heights was only 5 cm. Figure 6 shows the correlation between the optimum plate heights and the migration distance using linear OPLC. The curves demonstrate one of the main advantages of the FFPC techniques, since the plate heights decrease under optimum condition with increasing migration distance.

The increasing diffusion of the spots, which is the main source of trouble in development over longer distances under capillary flow-controlled conditions, can be reduced because of the optimal flow rate and the short development time using FFPC methods.

This advantage is especially obvious for long distance OPLC [19]. On using this technique the development distance can be easily increased to more than 50 cm. Therefore, it open up new vistas in FFPC, particularly in the separation of complex samples.

Influence of development on the densitometric baseline

The stationary phases of TLC plates are generally contaminated by impurities from the materials used in the manufacture of the plates (binder, holding, backing material), by impurities adsorbed from packing materials and from the atmosphere, and by additives and impurities in the mobile phase [20]. All of these impurities can disturb a reliable baseline for quantitative densitometric analysis or they can cause incorrect absorbance values in the case of quantitative determination by spot elution. It was established [2] that the disturbing feature of the Empore™ TLC sheet is due to the high level of layer contamination compared to HPTLC and TLC plates. After cleaning the sheets by continuous development for 24 hours the baseline problem was exaggerated by the appearance of a wavy and rising baseline when only capillary action was used for development.

Figure 7 demonstrates the densitometric baseline after development with different solvents in various chamber types, such as normal (N) and OPLC chambers. The densitograms show that the contaminants of Empore™ TLC sheets migrate with the solvent or solvent system and give a rise to very noisy and rising baselines. It should be noted that the different solvents result in different cleaning effects. On using dichloromethane for washing the sheets (Figure 7a, b) in an N-chamber, an increasing baseline could be recorded, while in the OPLC chamber two sharp peaks were observed. Figure 7c shows the effect of precleaning with methanol-water 1:1 (the maximum allowed water concentration using capillary action). With the OPLC chamber, thanks to the FFPC technique even water could be used as solvent, the contaminations could not be located in sharp bands.

On using methanol (Figure 7e,f) for precleaning the contaminants in the sheets migrate close to the α-front. The efficiency of the cleaning procedure is better with FFPC development than in the N-chamber because the migration of the impurities can also be achieved over a longer development distance with a higher mobile phase velocity. The impurities were located behind the α-front in a broad band, this cleaning method yields the best baseline.

The off-line UV spectra were recorded for the observed peaks. In all cases, a similar character was obtained (range from 200 to 350 nm), which shows the difficulties for quantitative determination with scanning densitometry, and also the presence of these impurities disturb the quantitation in the whole UV range.

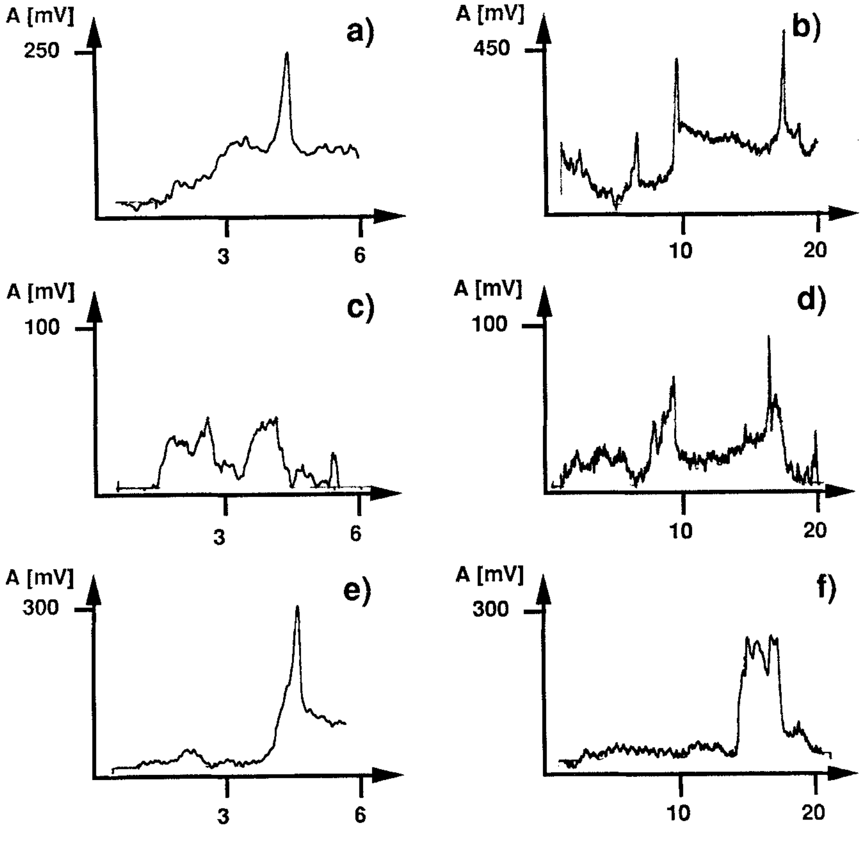

Figure 7 Influence of development on the densitometric baseline a, b) development with dichloromethane in N-chamber and OPLC chamber, respectively; c) development with methanol-water 1:1 in N-chamber; d) development with methanol-water 1:1 in OPLC-chamber; e,f) development with methanol in N-chamber and OPLC chamber, respectively.

In contrast to the N-chamber, the band of contaminations can be easily removed from the sheet by the forced flow, because the eluent may carry them out of the planar medium. Prior to U-RPC or OPLC cleaning, it may be impossible to obtain a stable densitometric baseline, particularly with UV detection.

Influence of external force on the structure of Empore™ TLC sheet

Roeraade and Flodberg [20] studied the influence of external pressure (up to 350 bar) on silica coated aluminum. They observed that silica, being a refractory material, has a very small range of elasticity and will suddenly break at a particular point when sufficient force is applied. They assumed that at approximately 250 bar the largest particles have been broken into medium sized particles. In fact better H values are obtained, probably due to the formation of a more uniform particle layer, both in respect to packing density and particle size. These results suggested that the external pressure, which is applied on using the FFPC techniques, could also be lead to a deformation of the structure of the Empore™ TLC sheets. Therefore the separation media were subjected to electron microscopy. Views of cross sections at two different magnifications are shown in Figure 8a and b. It is clearly seen that the structure of the sheet is loose, the silica particles are enmeshed in a matrix of PTFE microfibrils. Between the silica particles there is unfilled volume present. This could lead to inefficient capillary forces, which seems to be the reason for the rapidly decreasing solvent velocity and therefore the extra band broadening.

Figure 9a and b show the scanning electron micrographs of Empore™ TLC sheets after subjecting them to an external pressure of 25 bar over 24 hours. Comparing the cross sections of unmanipulated (see Figure 8) and pressurized structures, the permanent change of form of sheet thickness, more than 15% using 25 bar overpressure (Figure 9a). Also it is clearly visible that the structure is much more compact (Figure 9b), the dead volume is decreased and a better particle size distribution could be achieved.

Therefore, the text mixture "A" was spotted on two Empore™ TLC sheets, the first for an unmanipulated, the second one for a manipulated

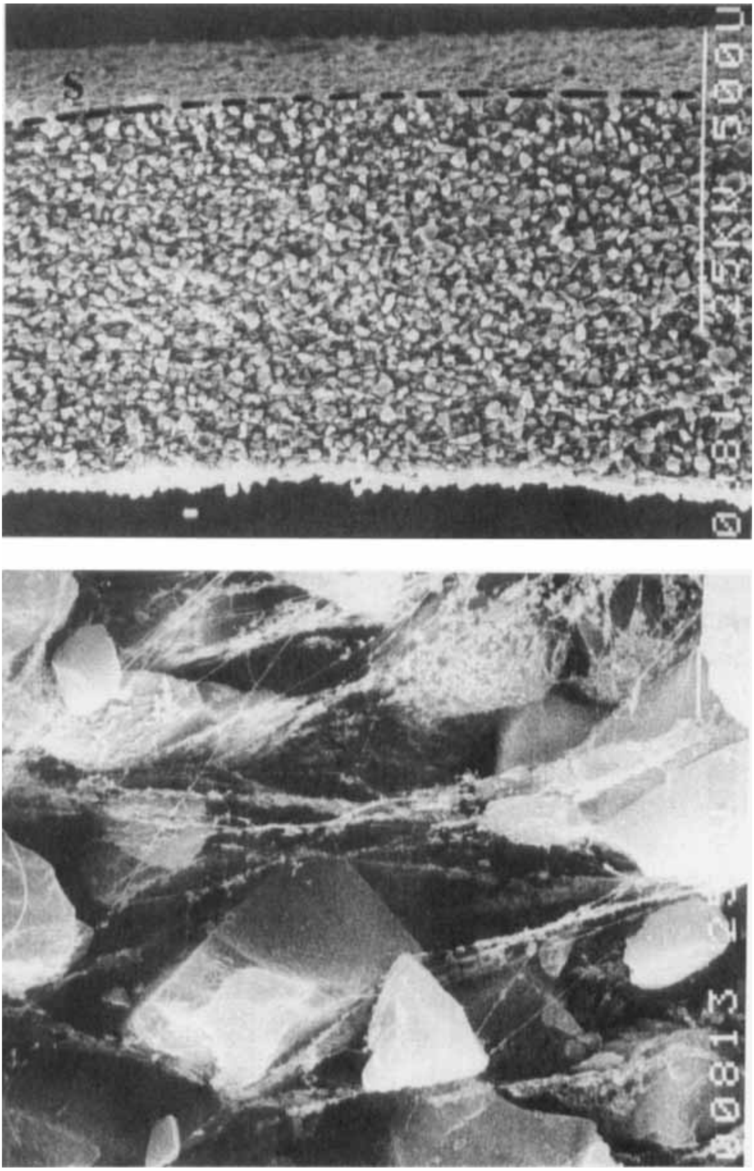

Figure 8 Scanning electron micrographs of the cross section of original Empore™ TLC silica sheet.
a) magnification x 160 (S: surface of the sheet)
b) magnification x 3600.

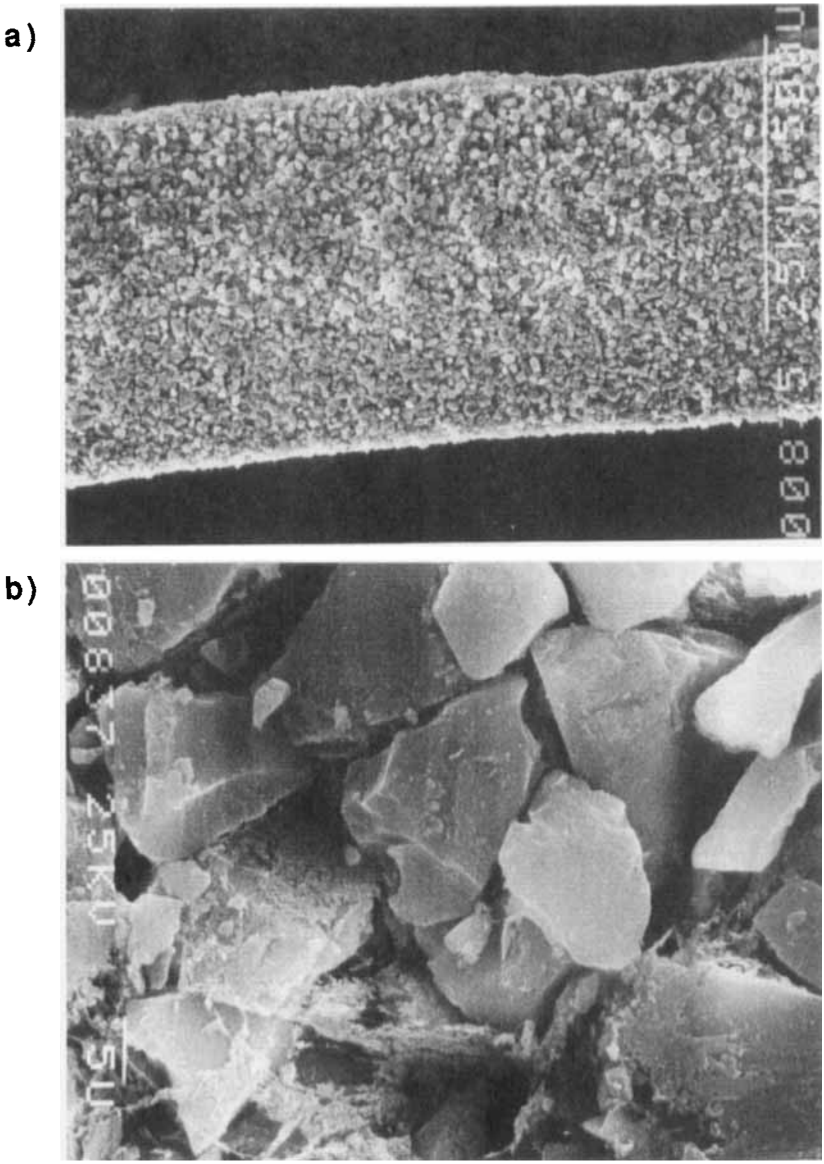

Figure 9 Scanning electron micrographs of the cross section of pressurized Empore™ TLC silica sheet.
a) magnification x 160, b) magnification x 3600.

sheet. The latter was earlier pressurized at 25 bar for two hours, and precleaned with methanol over 30 min. Using the pressurized separation medium in a saturated chromatographic tank the TLC separations were compared. No significant migration properties could be obtained under capillary flow-controlled technique.

These differences in the results visibly demonstrate the influence of external force on the structure of the sheet. The overpressure causes a higher density of the layer, which could be one of the reason for the demonstrated higher separation efficiency.

CONCLUSION

The use of FFPC techniques with Empore™ TLC sheets leads to the following conclusions:

- the applications of Empore™ TLC sheets for linear OPLC separations demands special preparations. The sheets can easily be impregnated along the edges with a special polymer suspension, then inlet and outlet channels must be cut out of the lower and upper part of the layer. For circular OPLC and U-RPC no impregnation is necessary.
- the relationship between development distance and separation time clearly illustrates the fundamental difference between the solvent velocity in capillary flow-controlled and forced-flow development techniques. The curve of linear OPLC and U-RPC shows a linear relationship between the distance of the solvent front and time. In circular FFPC this relationship becomes a quadratic function.
- the dependence of the H value on the linear flow rate clearly illustrates that an optimum range of flow rate (0.83-0.89 cm/min) exists, at which the plate height is optimal. At the optimum flow rate the resolution is also at a maximum.
- the correlation between the optimum plate heights and the migration distance demonstrates that the plate heights decrease with the migration distance. Based on this relationship the long distance OPLC technique opens up a new field of application for Empore™ TLC sheets.
- the densitometric baselines obtained after development with various solvents show that Empore™ TLC sheets are generally contaminated

with unknown substances that usually migrate with the solvent front and give rise to very noisy and rising baselines. The impurities can cause incorrect absorbance values in cases of quantitative determination by spot elution. Precleaning can be easily accomplished using FFPC methods.

- the scanning electron micrographs demonstrate that application of overpressure causes a higher density of the layer, which could be one of the reason for the demonstrated higher separation efficiency.

The results show that Empore™ TLC silica sheets allow extremely rapid separations (5 - 20 min) with FFPC methods in all development modes and give good resolutions. The sheets are especially useful for micropreparative separations [21] using the different FFPC techniques.

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