Faculty of Chemical Engineering

Ph.D. Thesis

Overpressured layer chromatography (OPLC) with special emphasis on the separations in the sorbent layer segmented by flowing eluent wall

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INTRODUCTION

The principle of the present scientific and technological revolution is based on the increased importance of sophisticated instruments, which become more and more an indispensable means of the progress.

The development of separation methods in the field of analysis and isolation deserves a particular attention. The boom started in the fifties surpass of the evolution of all other instrumental techniques. The intensive development of instruments means above all the advance of chromatographic, electrophoretic as well as electrochromatographic techniques. In the last decade the high performance column chromatographic techniques became especially dominant involving the advantages of automation and data processing based on computer technology. By today the high performance column liquid chromatography (HPLC) has became a leading technique in different laboratories. In the last decade the miniaturization, the additional increase of efficiency and the reduction of analysis time can be monitored. The progress is valuable in the field of hyphenation of chromatographic and spectroscopic techniques.

Both column and layer liquid chromatographic techniques - as supplementary techniques due to their arrangements - have been characteristically developed in constant mutual interaction. It was especially typical in the seventies, when a renewing of TLC, the efficient fine particle sorbent layers (HPTLC) and the more sophisticated instruments (sample applicators, densitometers) were introduced. In contrast with that, the separation remained conventional using capillary action. In order to increase the efficiency the forced eluent flow using a pump system is introduced. This can ensure the constant, optimum linear velocity yielding the maximum accuracy by the sorbent layer given.

THESIS

1. Based on the ultramicro chamber a new forced flow planar layer liquid chromatographic technique, the overpressed layer chromatography (OPLC) has been developed integrating the advantages of TLC and the HPLC (e.g. optimal linear velocity generated by a pump, high number of parallel separations, applicability of specific reagents).

2. Basic conditions of the linear separations, such as the layer sealing, eluent introduction onto the layer close to the edge, eluent distribution for the linear front formation and a rapid eluent admission at the starting period of separation have been developed. The developed sealed layers and also the cover sheet having linear troughs ensure the easy use of the system. Based on the scientific knowledge’s cumulated different OPLC systems have been developed.

3. The relationships of the movement of the mobile phase have been revealed in the case of OPLC. In the linear transfusion OPLC (when the end of the layer is in open stage) the movement of alpha front, the front of total wetness generated by the pore filling of particles as well as the secondary fronts caused
by eluent demixing produce straight relationship and their movements are proportional regarding the alpha front.

4. Based on the original off-line transfusion OPLC, a new version, the on-line OPLC has been developed by building into the chamber an outlet connection. If the system is equipped with injector at the inlet and a flow cell detector at the outlet it is well suited for on-line and/or off-line combinations of chromatographic operating steps, such as sample application, separation, detection and isolation. The fully on-line OPLC operation corresponds to the HPLC configuration. A special combination of on-line and off-line detection in OPLC (combined on-line/off-line OPLC) yields two times better spot/peak capacity comparing the values with off-line or on-line separation-detection.

5. In order to reduce the efficiency loss of the conventional transfusion OPLC caused by the front of total wetness, infusion as well as infusion-transfusion operations have been developed. In infusion OPLC the outlet of the chamber is closed and the eluent is introduced into the totally closed sorbent layer. It is suitable only for off-line separation without overrun. The infusion-transfusion operation allows continuous and on-line developments, and serves better conditions for on-line detection than transfusion OPLC, flushing out the compressed air (no bubbles).

6. The efficiency parameters of OPLC—theoretical plate height (H), theoretical plate number (N), spot and/or peak capacity (n), resolution (Rₙ)—have been studied using different separation conditions and different versions of OPLC. The effect of development distance on H shows the principle of OPLC, because of its value is roughly constant along the layer contrary to the TLC, where an efficiency loss exists over a distance. The differences between TLC and OPLC are higher in the case of use of fine particle sorbent layer. The elevation of external pressure on the surface of sorbent layer results in more efficient separation in off-line OPLC and also broader optimum range of liner velocity. Such effect was not found in the case of on-line OPLC. Comparing the effect of different operating modes on efficiency it was found that fully off-line OPLC yields better efficiency, than fully on-line process.

7. The combination of OPLC with HPLC is especially effective for cleanup and separation. The cleanup step can be carried out in OPLC prior to the HPLC separation. Another solution of combination is, when the pre-separation is fulfilled in a normal tank and OPLC works as an interface transferring separated components from the localized area of the layer to the HPLC system.

8. A new OPLC separation procedure has been developed for single- and multi-channel separation using a non-segmented sorbent bed and flowing eluent wall (FEW) for operating segmentation. The FEW detaches the sorbent bed into active and non-active parts regarding separation during the process. Only mobile phase is introduced into the non-active part, while, for the active part, eluent and also the sample can be admitted, thus the non-homogeneous part of the sorbent bed is excluded from the separation process. The FEW helps the elimination of the edge effect of overpressured layer chromatography (OPLC)
in case of single sample injection and abolition of the sample mixing effect of neighboring lanes in the case of a multi-channel separation process. In the case of dirty samples, the one-channel FEW-OPLC system is well suited for quick isolation in different preparative ranges using preparative chromatoplates. The multi-channel solution will be a tool for high throughput analysis using efficient fine, superfine, or monolithic layers. The four-channel version can be applied for high throughput multi (parallel) analysis as well as micro- and semi-preparative parallel isolation using efficient analytical or preparative layers. The FEW provides the possibility for real multi-channel liquid chromatographic separation on a non-segmented sorbent bed.

**PRACTICAL MOMENTS OF THE RESULTS**

Based on the patent system and know-how’s led to the fundamental renewal of the planar layer liquid chromatography, TLC. The technical solutions of analytical and preparative off-line and on-line OPLC (developed simple and automated systems, sealed layers and layer systems) demonstrate the significant improvement of the technology. The present instrument versions of OPLC (especially the automated OPLC 50) and the ready to use sealed layers having different phases offer the possibilities of the efficient best ever analysis and isolation of synthetic and natural compounds in the field of planar layer liquid chromatography. Owing to the high flexibility of OPLC, contrary to other layer liquid chromatographic techniques, it gives a special exploitation in the field of chemical-biochemical analysis and isolation.

The practical exploitation of the results means also the fabricated and the sold instruments in Hungary as well as abroad.

The validated methods help on the spreading of the OPLC technology. Over ten validated methods are only in Richter Gideon Ltd (Budapest, Hungary) in the daily routine work.

The newly developed one-, four- and eight-channel flowing eluent wall systems (FEW) give further possibilities for the parallel on-line analysis and micro- and semi-preparative works, which will be especially attractive in the field of combinatorial chemistry and also in the field of the natural compound research.

The theory and practice of OPLC technique is already an educational material of universities.

**REFERENCES**