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Over Pressure Layer Chromatography a Novel Technique

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ABSTRACT

OPLC is coming of age. Its rudimentary beginnings, some 25 years ago, have lead to innovative, high-performance products. The commercialization of modern, high efficiency columns has eliminated the need for analysts to prepare their own from TLC plates, making the technique even more accessible. Optimum performance laminar chromatography (OPLC) is a pumped flow chromatography technique that combines the user-friendly interface of HPLC with the capacity of flash chromatography and the multidimensionality of thin-layer chromatography. This review will describe the basis of modern OPLC instrumentation and the separation techniques, as well as give a brief account of some recent applications in OPLC.

Keywords: OPLC, Instrumentation, Application.

I. INTRODUCTION

Optimum performance laminar chromatography (OPLC) is a pumped flow chromatography technique that combines the user-friendly interface of HPLC with the capacity of flash chromatography and the multidimensionality of TLC. This review will describe the technique, as well as give a brief account of recent applications for which OPLC has found favour [1].

The acronym OPLC describes a technique that dates back to the late 1970s.[2] The instruments at that time used nuts and bolts to compress a modified TLC plate between metal or glass sheets. There was little control over the pressure applied to the layer, as this was a purely manual technique. The TLC plate was also modified by the user with the addition of a sealant to the edges of the plate. To distribute the solvent homogeneously across the layer, it also was necessary to scrape a horizontal line of silica from the plate directly below the solvent inlet. These aspects, and others not mentioned here, hindered the development of the technique in modern analytical laboratories. Three very important factors contribute to an optimized homogeneity of lateral and laminar flow inside the 200-mm thick sorbent bed. First, the introduction of electronic controls provides better control of the chromatographic conditions and the quality of the separation [3]. Second, factory-made flat columns have a high precision positioning and a more regular seal. Finally, the flowing eluent wall technology dramatically reduces disturbances at the layer edge and the inlet site (see text that follows and Figure 1). The technology described in recent articles demonstrates the jump in progress that justifies a new terminology introduced in 2000: optimum performance laminar chromatography [4] .This review will describe the basis of modern OPLC instrumentation and the separation techniques, as well as give a brief account of some recent applications in OPLC.

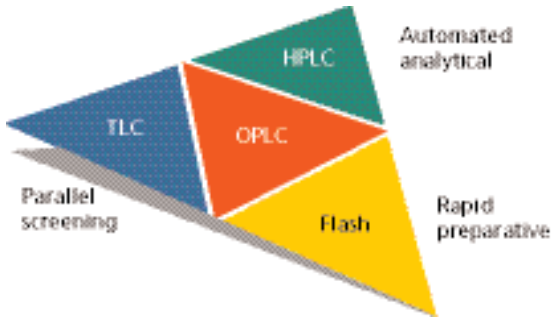

Figure 1: See text for details.

Instruments and Columns OPLC instruments: The basis of OPLC is similar to that of other chromatographic techniques in that a pump is used to force a liquid mobile phase through a stationary phase such as silica or a bonded-phase media (that is, C8, C18, amino or nitrile).

The uniqueness of OPLC lies in its column- housing structure, which allows flat planar columns to be used in the same way as cylindrical glass or stainless steel ones. An OPLC development chamber is composed of three basic structures: a planar column; a cassette constructed of a machined PTFE sheet and an aluminum support; and an electronically controlled hydraulic press. The planar column itself is a uniform sorbent bed layered on a glass or aluminum backing with an elastomeric polymer seal located at the periphery. The column is placed silica face-up in the cassette, in direct contact with the PTFE sheet. This in turn is placed inside the hydraulic press of the development chamber. Upon pressure, the PTFE sheet is pressed onto the sorbent bed and compresses the seal, creating a solvent- tight system ready for chromatographic separations. Mobile phase then is pumped through connections on the development chamber to the sorbent bed. The machined PTFE sheet is responsible for distributing solvent to particular zones on the column and recovering it from the sorbent bed as a chromatographic separation is performed. The first instruments, marketed some 25 years ago, applied relatively low pressures to the sorbent bed. Higher pressures, achieved in modern instruments, provide a better compaction of the sorbent bed and a more intimate contact of PTFE sheet and silica, which results in a noticeable increase in efficiency at a higher optimal flow rate (see Figure 2).

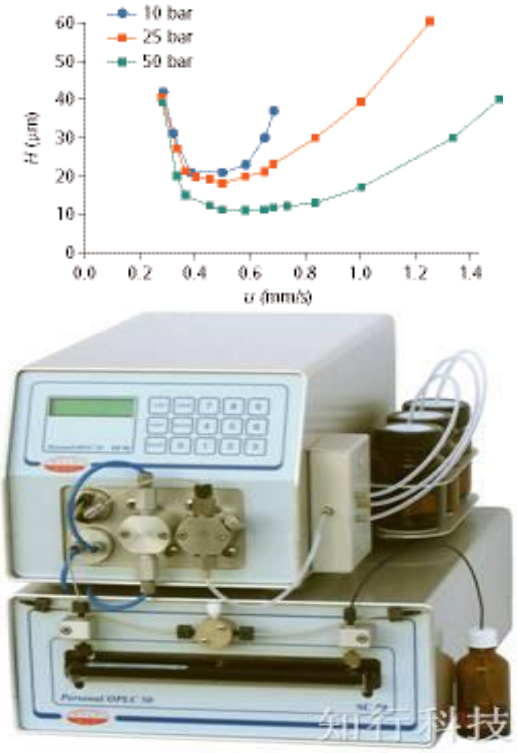

Figure 2: Plate height versus linear flow velocity as a function of applied pressure in OPLC for HPTLC layers (5-mm particle size) b) Instrument of OPLC

II. METHODS AND MATERIAL

A. Cassettes

The cassette has two main functions: creating solvent-tightness under compression and determining solvent flow within the sorbent bed. A cassette comprises an aluminum base plate and a PTFE cover. The PTFE sheet has 1-mm holes that allow flow to the sorbent bed. Microchannels (250-mm wide, 350-mm deep) on the underside of the PTFE sheet direct and recover solvent from predetermined zones on the stationary phase. Different cassette designs provide different separation modes. With two standard cassettes, it is possible toperform basic 1-D separations, as well as bidirectional, 2-D, and simultaneous four- sample 2-D development. Figure 3 shows schematics of both mono- and bidirectional elution cassettes. Cassette (a) is used for one-sample on-line separations or for the off-line separations of 1–50 samples in a single run (on- and off-line separation techniques are described later). This cassette format delivers mobile phase evenly over the entire width at one end of the column with recovery of eluting solvent at the other end. Samples are placed directly on the column 1 cm from the inlet. Twice as many samples can be separated on the same column using a bidirectional elution cassette (Figure 3b), which introduces solvent over the midline of the column. This cassette type also serves as a rinsing cassette when changing solvent systems. With this cassette, separation occurs out- wards in two directions toward the extremities of the column. Here, samples are deposited 1 cm to either side of the inlet trough, and the separation distance is only half the length of the column. 2-D separations can be achieved with mono directional cassette formats. The sample is placed in one corner of the column, 1 cm from the inlet trough. After development in the first direction, the column is removed, turned 90° such that the developed line is parallel to the inlet trough, reinserted and a second development is performed. Similarly, parallel four-sample, 2-D analyses are run using a bidirectional cassette format, starting from the central corner of each quadrant of a 20 3 20 cm flat column. Again, each separation is performed over half the column length.

Each of the four samples migrates outwards from the center over a 10 3 10 cm area of the flat column (Figure 4). While other designs exist (such as radial, stacked parallel, or serial elution), these are less common in routine use. Specific cassettes are available for different column widths. More often, method development is performed on the smallest column, 5 cm wide, while screening and semi- preparative work is often done on intermediate and full-width columns (from 10 to 20 cm). Recently, a new technology has been introduced, known as flowing eluent wall (FEW) for on-line and semipreparative purposes[5]. Conceptually, FEW is a stream of sample-free eluent that is pumped to the lateral wall of the column, near the elastomer seal. This confines the sample to a central portion of the column and eliminates direct contact of the sample stream and the wall, improving band (peak) shape. The FEW does not require any additional equipment. The solvent stream simply is divided upstream of the injector: one stream goes to the injector and onto the portion of the column where the sample is to be separated, while the FEW stream is taken directly to the lateral walls of the column (Figure 5). Figure 6 shows a comparison of the separation of dyes injected on both a modern OPLC system with FEW and an older non- FEW version of the same instrument. We can see that flow is affected at the edges in the non-FEW instrument. We also can see that the sample band is deformed at its centre in the non-FEW version. This has been corrected in the FEW system by a modification of the inlet structure of the cassette.

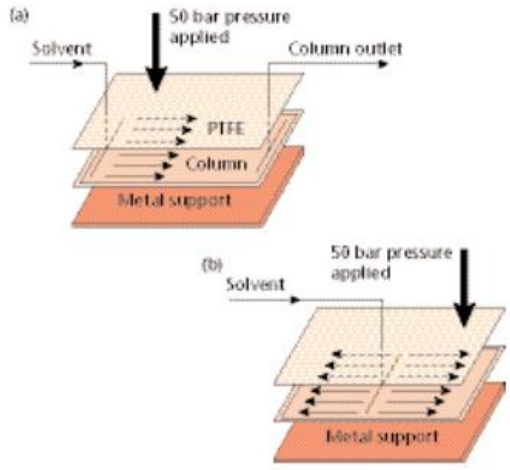

Figure 3: OPLC cassette design for (a) linear one-dimensional and (b) bidirectional chromatography separations steps.

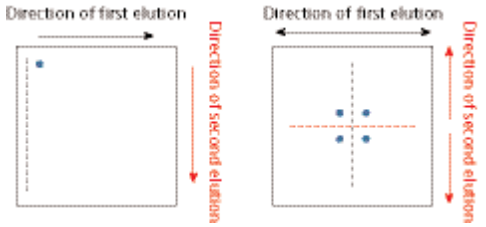

Figure 4: 2-D development on a planar OPLC column. Dotted lines correspond to solvent inlet microchannel. Blue corresponds to sample, and white, black, and red are the sequential development

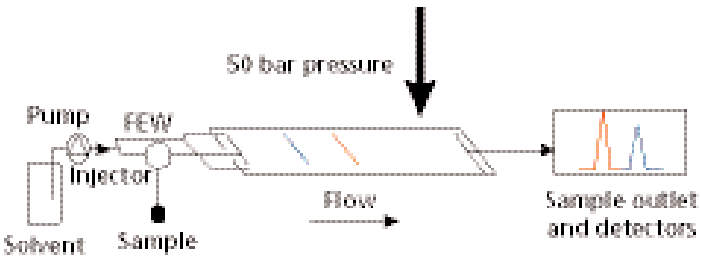

Figure 5: Concept of FEW with the distribution of solvent at sample channel and flowing walls.

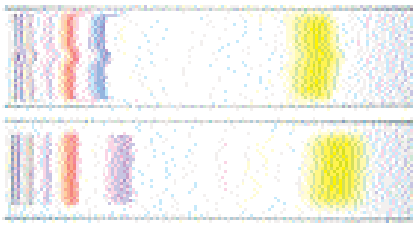

Figure 6: Close-up of dye molecules at column wall during a chromatographic separation with (a) non-FEW and (b) FEW instruments.

B. Columns:

Presently, flat columns are made of irregularly shaped, preparative-grade silica (5 and 11 mm; 500 m2/g)[6] placed on an aluminum or glass backing (that is, HTSorbTM columns [Bionisis, Le Plessis Robinson, France]). They are 20 cm in length and come in three different widths: 5, 10, and 20 cm depending upon the capacity desired. Standard thicknesses are 200 mm and 500 mm. An elastomeric seal is placed at the periphery of each flat bed column. Normal silica and standard bonded phases are available, including C8, C18 nitrile, chiral, and amino phases. Plate height is independent of the sol- vent-front migration distance and achieves a minimum value for high-performance silica layers of about 10 mm at a mobile phase velocity of 0.5 mm/s. [7] This is sufficient for some analytical methods and is very acceptable for micro preparative isolation (20–200

mg). In addition, the possibility to observe residual non migrating products on the column provides a higher degree of confidence in the final analytical result.

C. Basic Operation

A basic OPLC unit can be configured into an HPLC system and can either be used on- line, in the same way as a standard cylindrical HPLC column, or off-line, as with a high performance TLC development. In general, one uses the term "mode" to describe the way in which samples are applied and detected: off-line means that the column is handled outside the development chamber while on-line indicates that the column is left inside the chamber and is connected to the pumping and/or detection modules. A good technical description of the relationships between retention time and migration distance in OPLC has been developed by Siouffi and Mincsovics [8].

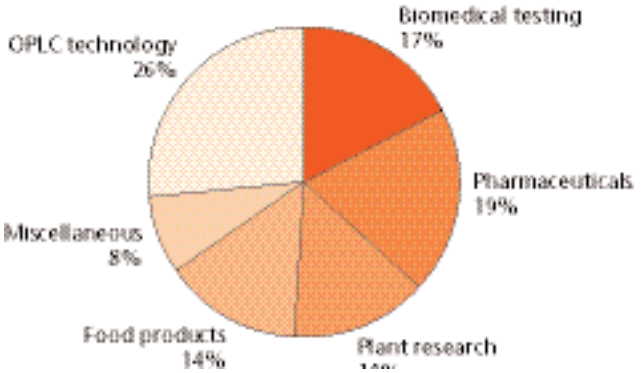

Figure 7: Published articles on OPLC technology and applications in various domains of research.

D. Fully on-line mode:

The fully on-line mode has very few examples to date, mainly because a dedicated unit for this type of work only recently has become available. In brief, the OPLC unit is installed in an HPLC system much like an HPLC column. The programmable workstation of the HPLC system ensures column equilibration, sample injection, detection, and monitoring of the separation; that is, UV, mass spectrometry (MS), nuclear magnetic resonance (NMR), refractive index (RI), and evaporative light scattering (ELS). On-line mode can be used as an analytical or semi- preparative method [9]. FEW technology gives better peak shape in on-line separations.

E. Fully off-line mode:

This mode corresponds to application of the sample and the subsequent analysis of the separation directly on the stationary phase. The different steps of sample application, separation, and detection are effectively decoupled in off-line mode. Formulated products or crude extracts can be applied directly on the stationary phase without prior sample preparation steps. In many instances, the active ingredients can be determined among the formulating agents [10]. The possibility of depositing a sample on the column is an advantage, particularly when the sample is in a solvent that is not miscible with the eluting solvents, or when increased sample loading is desirable. The quantity of sample applied is not limited by a development [11].

Otherwise, it is possible to flush the air from the column with an a polar solvent that does not cause migration of the products, then change solvents to provide the desired separation. A second phenomenon, known as solvent demixing, occurs when using a solvent mixture as the mobile phase and silica as the stationary phase. Demixing describes depletion of the more polar components of the mobile phase as a result of their strong interaction with a non-equilibrated (dry) stationary phase. [12] Resaturation with the solvent will reduce this effect dramatically and could represent a solution to the problem. [13] However, demixing can be useful. For a two-solvent system, two migrating fronts are observed: the total wetness front, as discussed previously, and a b-front resulting from demixing. Above this b-front, the mobile phase is almost devoid of the polar component of the mixture, while below it, the mobile phase is complete. In general, a small amount of a highly polar solvent will give a b-front at low Rf, while a larger fraction of a less polar solvent will give a b-front at a higher Rf value. By properly adjusting the amount and nature of the polar modifier, it is possible to create zones of "defined" polarity at predetermined areas on the column. Properly done, this can provide higher peak capacity for complex sample mixtures. Maintaining the sample components behind all secondary fronts is necessary when OPLC serves as a precursor to HPLC method development. Elution should be continued beyond one column volume of solvent. Samples either can be maintained injection loop as in HPLC. Sample components are not eluted from the sorbent bed, as the results of the separation are observed directly on the

column. Much longer time can be spent in the detection mode when it effectively is decoupled from the separation technique, because detection is not limited by the rate of elution. In some instances, this can lead to significant gains in the limits of detection, particularly with radio labeled metabolites [14] A fluorescent indicators (254 nm) can aid detection, or one could choose to derivative with a spray-on reagent solution to visualize the sample components on the column [15]. Semi quantitative screening and sample-comparison assays typically are per- formed with multiple samples on the same column, including calibration standards. Analytical determinations of UV-active sub- stances are obtained with a full-spectrum scanning densitometric instrument, or more simply with a single-wavelength desktop scanner [16].Other types of detection that have been used in conjunction with OPLC include radio-detection, Raman spectroscopy [17] and bioautography. Finally, the column is removed, stored with experimental records, and if necessary, retrieved for analysis later or at another location. With off-line OPLC, there is less waste (from 10 to 100 times less solvent consumption and fewer disposables used) as sample preparation is reduced to a bare minimum and column equilibration and regeneration are not necessary. In some instances, applying crude sample mixtures to the column can lead to improved reproducibility as the number of sample pretreatment steps is limited, and all of the sample components actually reach the stationary phase where the separation is performed. From a practical standpoint, fully off-line separations are more complex than on-line separations, and this can be disconcerting for uninitiated users. First, it is necessary to program a rapid solvent injection at the start of an off-line experiment, particularly for large column widths. This flash volume establishes a homogeneous flow over the width of the column, a requirement for linear bands and good peak shape. As the sol- vent passes into the sorbent bed, air is dis- placed. If the column outlet is open to allow gas to escape from the column, two solvent fronts can be observed: a faster eluting partial wetness front and a slower total wetness front. By simply closing the outlet on the OPLC, a pressure build-up occurs within the separation chamber, preventing vaporization of the eluting solvent and causing the air in the column to dissolve in the sol- vent. The upper pressure limit of the pump is set automatically by the instrument so that the pump stops when the development is complete. In this manner, the partial wet- ness front is not observed in full column on the stationary phase and observed directly or they can be eluted to an appropriate detector.

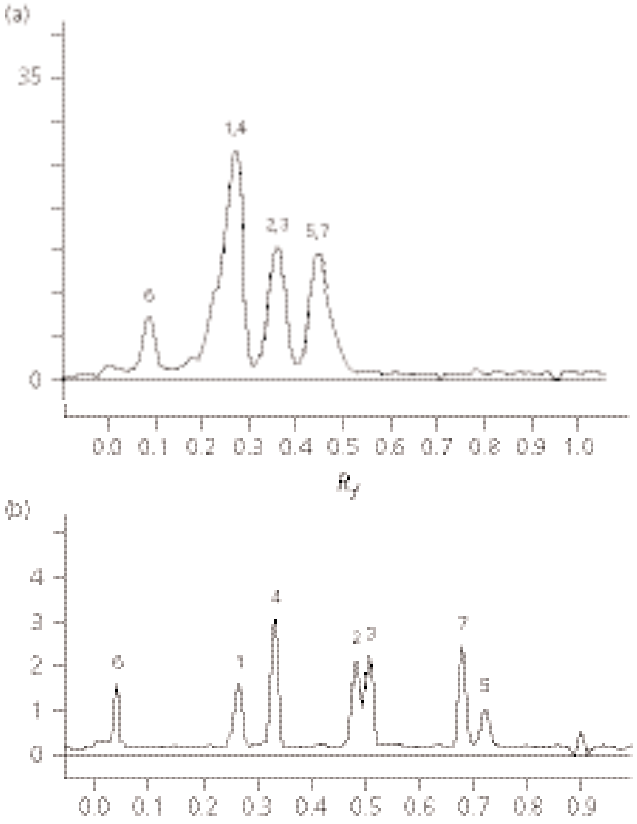

Figure 8: Densitometric analysis of nandralone, analyzed by (a) TLC and (b) MD-OPLC. Reproduced from reference 13 with permission

F. Other on-line and off-line techniques:

When OPLC is used as a tool for HPLC method development, it is possible to examine the column as development occurs to see how a particular method performs. If the conditions are inadequate for a satisfactory elution of the products, it is possible to put the column back into the OPLC, change the solvent conditions, and continue with the new elution method. Even when a method has been optimized for a particular product, the analyst always can recover the column to verify that the entire sample has been eliminated from the column. Mass balance analysis in drug-stability testing is just one application for which this mixed-mode technique finds an added advantage relative to HPLC. If non migrating products are observed on the column, it is possible to recover them by extraction and identify them using another method. Recent developments in the field of TLC–MS are pertinent for structural determination directly from the OPLC stationary phase [18]. Further improvement in resolution

can be obtained by using multiple development techniques. [19] Multiple developments is a series of development and drying cycles that cause solvent to flow over the sample several times during the course of a separation. Multiple developments takes advantage of the peak compression that occurs as the mobile phases pass over products on a dry sorbent bed and can provide very high resolution. Multiple development-TLC, which has been known for years, is an under- exploited high-performance TLC technique, mainly because it requires a long analysis time. With the pumped-flow of an OPLC, multiple developments become more accessible and has been applied successfully in pharmaceutical analysis for difficult separations [20].

III. RESULT AND DISCUSSION

A. Features and Benefits

- High chromatographic efficiency.
- Flexibility in monitoring the progress of the separation by either on-line or off-line detection.
- Simple scale-up from analytical to semi-preparative purification of up to 200 mg.
- Visualization of compounds retained on the stationary phase prevents any loss of information.
- The Personal OPLC-50 is a standalone unit that comes with its own pump.
- Inexpensive, disposable sorbent beds, low solvent consumption (up to 1000x less than other LC techniques) and fast separations (5-20 minutes) make this technique particularly economical.

B. Typical Applications

i OPLC is a general separation technique that has been successfully applied to problems such as:
ii QC of pharmaceutical products.
iii Determination of impurities in drugs and reaction mixtures and cleaning validation of manufacturing vessels.
iv Natural products: Extraction of compounds of pharmacological interest from natural products.
v Drug metabolism: Isolation of metabolites in biological fluids.
vi Sample preparation: Purification of reaction mixtures to extract the compounds of interest for additional studies (e.g. for NMR or MS).
6) Optimization of HPLC methods: determination of optimal solvent system for HPLC to eluate all compounds out of the column thanks to the possibility to inspect the flat column for retained compounds.
vii Oligomers and synthetic polymers: Separation of oligomers of natural (e.g. peptides) and synthetic polymers
(e.g. polystyrene).
viii Toxicology: Determination of toxins in foodstuffs (e.g. aflatoxins in wheat).

C. Applications

OPLC technology has integrated several disciplines including pharmacognosy (plant research), pharmaceutical development, drug metabolism, drug abuse assays and others (see Figure 7). At this stage, in the development of OPLC, journal articles related to OPLC technology outnumber specific applications. However, many specific applications have been explored over the past 20 years. These include the detection of drugs and metabolites in animal tissues (homogenates, urine), potentially active ingredients in plant extracts,[21] formulating agents in cosmetics (lipsticks, shampoos and creams),[22] toxins in food stuffs, as well as chemical substances in crude reaction mixtures. Detection limits can reach 0.1–1000 ng depending on the product and the detection techniques employed. The aspects that make OPLC a preferred technique for many of these assays include limited sample preparation, the semi- disposable nature of the column, high capacity suitable for semi-quantitative analysis and micro-preparative scale-up,multiple parallel samples in a single run, and the possibility of direct on-column detection. The following paragraphs highlight four application types to exemplify a few aspects of the technique and the flexibility it offers.

D. Screening In Forensic Science

The Finnish group of Pelander has developed an OPLC technique for high-confidence identification of drug abuse substances in urine samples.[23] The authors take an innovative 2D approach to the problem, developing each sample twice: once in an acidic solvent and then in a basic solvent mixture. Fifteen samples and Rf correction standards are applied to each column.[24]After scanning densitometry of each separation, two corrected Rf values

are used to identify a particular drug substance from among more than 200 library compounds. A graphical 2D representation of their data is given in Figure 9, together with chromatograms from the analysis of a urine sample containing codeine. In zones on the map where two or more products show nearly identical Rf data, it is possible to differentiate between them by their UV spectra using scanning UV densitometry on the column. The success of this technique relies on the high peak capacity of the columns and the choice of two solvent systems with a low mutual correlation between them. In clinical and forensic toxicology the use of this low cost, rapid screening technique allows the group to perform routine high-throughput analysis, while more sophisticated instrumentation (LC–MS and GC–MS) is dedicated to other tasks (i.e., low-dose target compounds). Other specific assays for opiates, alkaloids (poppy) [27] and cannabinoids[28] have also been developed using OPLC.

E. Preparative Isolation:

OPLC is also semi-preparative chromatography allowing the isolation of more than 200 mg per run on a 20 20 HTSorbTM column.[25] It has been shown that the injection volume can attain more than 20% of the column volume while still maintaining adequate resolution for fractionation. OPLC has also been used for fractionation of metabolites of a radiolabelled pharmaceutical from tissue extracts during ADME studies.[26] Preparative OPLC columns 20 20 cm and 500 µm thick have only recently become available (11 µm particle size). Method development performed on a 5 cm wide column can be scaled directly to a 20 cm wide preparative column by simply adjusting the flow-rate. OPLC is also being explored as a substitute for solid-phase extraction prior to NMR studies.[27] The interest lies in the possibility to visualize the sorbent bed and to know when the molecules of interest have been eluted from the column. This is not a trivial matter in this application, as the molecules are eluted with deuterated solvents.

F. Bioactive Molecule Research:

Bacterial and fungal toxins and their metabolic by-products in food products are known to cause serious health problems in humans and animals and, therefore, require regulatory monitoring. Several publications relate methods for the detection and assay of mycotoxin metabolites in food stuffs,[29] such as rice,[30] wheat[31], fish[32] and corn.[33] The authors propose a screening method for aflatoxins with a detection limit below 0.1 ng which requires minimal preparation, high sample throughput and low operating costs.[26,32] Similarly, OPLC methods have also been developed for some peptide cyanobacterial toxins (mirocystins and nodularins).[34] Bioautography is a particular on-column detection method that has also been applied in combination with OPLC in the search for bioactive substances from plant sources (pharmacognosy) and in toxic substance detection.[35] The OPLC column serves a double purpose: as the separation medium and as a mechanical support for cell culture. If a bioactive substance is present on the column, cell growth is modified (i.e., antibiotics inhibit proliferation, leaving cell-free zones on the column). The active substance can then be isolated and characterized. It is interesting to note that Tyihak et al. have taken this OPLC–bioautography technique a step further to explore cellular defence mechanisms.[36] OPLC, with its open planar column format and high peak capacity, is the only high-resolution, forced flow chromatographic technique capable of rapid, high-throughput screening with bioautography.

IV. CONCLUSION & FUTURE DEVELOPMENTS

Future Developments/Perspectives in OPLC

OPLC is coming of age. Its rudimentary beginnings, some 25 years ago, have led to innovative, high-performance products. The commercialization of modern, high-efficiency columns has eliminated the need for analysts to prepare their own from TLC plates, making the technique even more accessible. The open-heart column in OPLC provides several unique possibilities for product detection, including standard on-line techniques such as UV, radiation, ELS, ESI-MS and NMR. Off-line detection methods include densitometry via a fluorescent indicator, via the UV chromophores of molecules on the column or via the addition of colorimetric reagents to reveal the presence of specific molecules (particularly those that do not have a chromophore). ESI-MS and MALDI-MS have been demonstrated with TLC and, therefore, should also be applicable to OPLC columns. Today, practical solutions are available for several sample types. An increasing number of publications in the field show that several

groups have adopted the technique, more often as a complement to HPLC, as it has all the required properties for preparative isolation, semi- quantitative screening and product profiling (fingerprinting).FEW technology has recently been integrated into routine instruments. This is also a fundamental part of a unique multiple sample injection/detection system which allows simultaneous, parallel purification of 4 or 8 samples in a single run using the un segmented flat columns presented here.[28] This opens the avenue for new applications in the field of high- throughput screening, combinatorial chemistry and microscale preparative isolation after parallel synthesis. 2D protein analysis could also become a viable application for proteomics research on these 2D flat columns; active research is underway in this direction. These and many other exciting possibilities are motivating innovations that only flat column technology can provide. For these and many other reasons, OPLC merits its place in modern research laboratories next to classic column chromatography instruments.

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