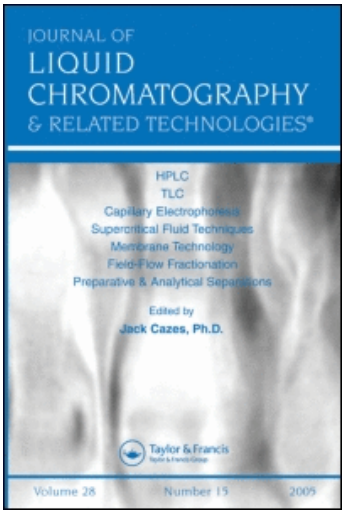

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HIGH PERFORMANCE THIN-FILM CHROMATOGRAPHY IN BIOLOGICAL STUDIES

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The conventional thin-layer chromatography /TLC/ method of Egon Stahl had not been significantly modified for a long time. But lately much attention was paid to the optimization of the rapidity and sensitivity of the method. The advances in column, gas and liquid chromatography very much contributed to it. In TLC, like in column chromatography, the use of very fine and homogeneous sorbent fractions was very promising. It appeared especially efficient in liquid chromatography where diffusion coefficients are low.

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The use of fine sorbent fractions resulted in the drastic increase of the resolution power and decrease of the analysis duration as well as in 3-4 times shortening of the layer length. For the analytical resolution the layer about 100 /um in thickness /like in conventional TLC/ was used. That brought some researchers to the conclusion that the optimal diameter of sorbent particles was 5-7 /um and that the use of finer sorbent particles should decrease the resolution efficiency. We have shown /1-5/ that the use of still finer sorbent fractions coupled with the appropriate reduction of a layer thickness and the utilized length results in the constant increase of the resolution efficiency, rapidity and sensitivity. However, the sorbent layer area should be large enough not to require the use of complex microanalytical devices. Besides, for the full realization of the efficiency one needs further and further minimization of a starting spot size.

It has been shown that the optimal layer thickness is from 10 to 15 /um, its optimal length is 2-3 cm, and the optimal mean diameter of sorbent particles is 1-2 /um. Layers prepared on microscope glasses, 1x3 inches, are very handy. Using such a plate it is possible to obtain 2 two-dimensional and 2 or 3 unidimensional chromatograms simultaneously.

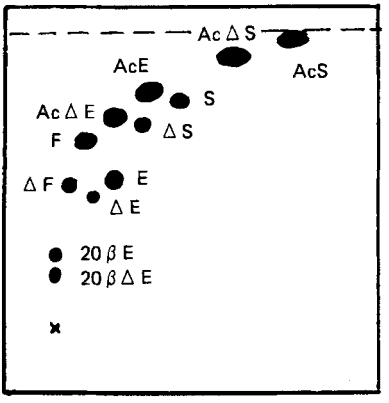

Fig.1. Two-dimensional chromatogram of steroids obtained on silica gel layer 1.5x 1.5 cm

The resolution power is perfectly illustrated by a two-dimensional chromatogram of steroids on a 1.5x1.5 cm silica gel layer /Fig. 1/.

The major problems in the work with ultra-fine sorbent fractions is the necessity of keeping the definite balance 1/ between the interaction of sorbent particles and a glass plate and 2/ between the particles themselves, because of the higher value of free surface energy. Therefore, a precise quantity of sorbent applied per a unit of a layer is a must. For different sorts of silica gel and its fractions the optimal quantity of sorbent varies within the range from

0.8 to 1.2 mg per cm^2 of support material which is by an order of magnitude less than for MERK HPTLC plates and by 1.5 order of magnitude less than for conventional TLC plates. Due to the higher surface energy of ultra-fine sorbent fractions the layers may be prepared without a binder or adding 1-2% of gypsum. The mechanical solidity of the layer is perfect. The disregard of the optimal conditions results in specific defects, e.g. regularly alternating sorbent "waves", "Liesegang ring"-like defects, rents, etc. Attention should be paid to the possible partial aggregation of particles which occurs upon the long-time storage of fractions, evet wet. Layers are formed mainly around such aggregations which makes the plates unfit for work /"speckled" plates/. To avoid this, a short-time ultrasound treatment of fractions prior to the preparation of layers is recommended /11/. The preparation of layers under optimal conditions is an extremely simple and highly efficient process. Coating of several hundreds of plates which will retain their resolution power for at least 8 years takes an assistant a day.

Based on the above-mentioned characteristics, such sorbent layers could rather be named "sorbent films". Therefore, the term "high performance thin-film chromatograohy" /HPTFC/ seems justified for the method of analysis on thus prepared sorbent.

It is convenient to prepare sorbents for HPTFC by method of sedimentation in appropriate solvents, e.g. in methanol - chloroform mixture for alumina /4/ and in water for silica gel /6/. For example Kieselgel 60 MERK is ground in a mill and sedimented in water in cylinders, 25 cm in hight. Fractions sedimented within the intervals of 3-6, 6-12, 12-24 and 24-48 hours are taken. The finer particles, usually making up 1-2% of the initial amount of Kieselgel, are removed. The coarser particles are ground again. Usually 4 final fractions make up 5%. For example, the grinding and fractionation of 940 g Kieselgel 60 MERK for preparative TLC yielded 42.4 g final fractions. The 1^{st} fraction /3-6 h/ was enough for preparing 335 plates for HPTFC using microscope glass as a support material, the 2^{nd} fraction, for 733 plates; the 3^{rd} for 830; and the 4^{th}, for 487 plates. Such a number of plates is adequate for obtaining more than 5000 two-dimensional chromatograms. With conventional TLC technique, 10000 times more sorbent would be spent. For the preparation of HPTFC plates carefully washed glasses are submerged into 1 ml suspension containing the necessary quantity of the sorbent and previously treated with ultrasound and then dried for a night.

HPTFC does not require special equipment. Plates are developed in glass chambers previously described /2/. Zones are detected by spraying using a usual spray.

Starting spots are applied by thin glass or metal capillaries. An analysis on 2 cm layer is usually done for the time from 2 to 10 min. In some cases HPTFC method yields chromatographic zones with diameters of several tenths of a millimeter. A layer thickness being 10-15 /um, the increase of sensitivity by 1-1.5 order of magnitude compared with conventional TLC is possible. For example, when analysing pyruvic acid in the form of quinoxalones we achieved the sensitivity of several 10^{-12} moles. After the resolution the plates were sprayed with 10% water solution of phosphoric acid, and zones of quinoxalones were visualized by intensive yellow-green luminescence. The technique may be used successfully for the group analysis of -ketoacids /7-10/- the major intermediates of the tricarboxylic acid cycle.

A high sensitivity of HPTFC allows the identification of components of complex mixtures chromatographed on conventional TLC layers. In a previous work /11/ a mixture of several dozens of substances - products of hydrocortisone microbial transformation was separated by a two-dimensional technique using "Silufol" plates. Some steroid zones were eluated from a layer and then identified by HPTFC method on alumina and silica gel layers in various solvent systems.

Mechanically solid films prepared of ultra-fine sorbent fractions may be successfully used for some

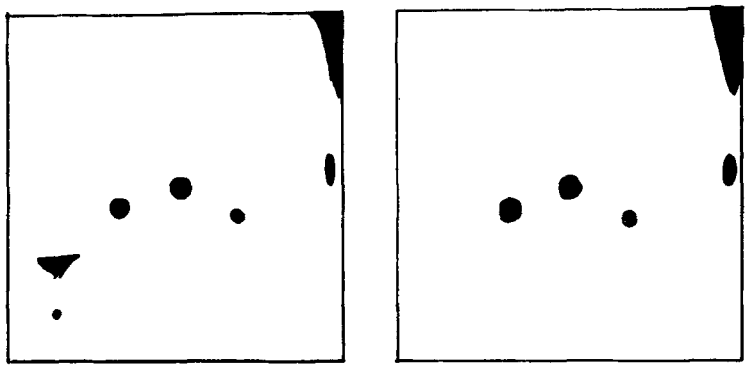

Fig.2. HPTFC of phospholipids of yeast mitochondria.
I - the extraction on a layer,
II- the analysis of previously extracted mitochondria.
Silica gel layer 2x2 cm.

analyses hardly done by conventional TLC method. Figure 2 presents chromatograms of phospholipids of mitochondria of the yeasts Endomyces magnusii, obtained by 2 different ways. In the 1st case water suspension of mitochondria was applied to a silica gel layer and then twice extracted on the layer by a two-fold development of the plate for a length of 3-4 mm with chloroform-methanol mixture /1:1/. In the 2nd case mitochondria were previously extracted according to Folch. Though both results were analogous, the 1st procedure is convenient for operating with microgram quantities of biological material.

A high analysis rate of HPTFC method makes it efficient for the regulation of high rate chemical pro-

cesses. It allows the control of the completeness of the reaction producing the derivatives for gas chromatography, e.g. fatty acid methylation or fatty hydroxyacid acetylation. In many cases the method, properly instrumentalized for the quantitative interpretation of thin-film chromatograms, is not inferior to gas chromatography in sensitivity and analysis rate.

Often the densitometry is not done immediately after obtaining a chromatogram and many substances are decomposed in air leading to misrepresentation of quantitative results. To do away with this inconveniency, the densitometry of negatives after photographing in visible or UV-light is recommended. This procedure was successfully used for the analysis of nucleosides and nitrous bases of nucleic acids /12-13/.

HPTFC method is an efficient analytical means, especially, for biochemical research. It solves the most of analytical problems in the studies of biological and environmental objects /5, 14, 15/.

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