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THE BEGINNINGS OF THIN-LAYER CHROMATOGRAPHY

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In 1938, as a post-graduate student at the Kharkov Chemistry and Pharmacy Institute, I worked on probation at the laboratory of physical chemistry in the Institute of Experimental Pharmacy (now the Kharkov Chemistry and Pharmacy Research Institute). The Head of the physical chemistry laboratory was N. A. IZMAILOV, aged 30, already known for his work on adsorption and physicochemical methods of analysis.

Fig. 1. N. A. IZMAILOV.

Fig. 2. M. S. SHRAIBER.

The main task of the Institute at that time was to improve the methods of control of pharmaceuticals. Working in the field of pharmaceutical analysis we encountered difficulties in the control of the so-called galenical preparations (tinctures, extracts, etc.) because of the lack of suitable methods of analysis. This impelled us to search for specific methods of analysis that could be used for the detection of physiologically active substances. Chromatography appeared especially promising in this respect. It was in this early period of development of chromatography that we became attracted to this refined method.

For the separation of complex mixtures that form part of pharmaceuticals, we used the method of column chromatography according to Tswett which made it possible to check the content of substances in vegetable materials and the quality of galenical preparations.

For the further analysis of substances after their separation by column chromatography, we used fluorimetry. For example, after the chromatographic separation of the substances in harmine tincture, by applying fluorimetry we could determine the harmine content accurately to within 2%, the concentration being $5 \cdot 10^{-9}$ g/l.

The first papers together with N. A. IZMAILOV were entitled "Harmine as a Fluorescent Indicator" and "Quantitative Determination of Some Alkaloids by Luminescence"[1,2].

However, despite the obvious advantages of the method, the combination of column chromatography with fluorimetry required too much time, which did not allow the use of this method for routine pharmaceutical analysis. Therefore, efforts were directed towards accelerating the separation of complex mixtures of substances. Our main idea was to take advantage of the high selectivity of the sorbents in combination with the separation of substances in a flat bed, as this had been made use of in capillary analysis, the early version of paper chromatography.

A very important factor in our work were Tswett's ideas about the analogy between the properties of the column of sorbent and a strip of paper[3,4]. It occurred to us to cut out of the column of adsorbent a thin (longitudinal) layer, which could be used as a strip of paper. We believed that carrying out the process in a thin layer would accelerate the process of separation.

Almost the very first experiments had favourable results. We then used the method of circular chromatography.

As a result of a number of subsequent experiments, a method of chromatographic adsorption analysis was developed, based on the separation of substances in a thin layer of adsorbent by using one drop of substance.

The results obtained with this method were similar to those obtained by column chromatography. The method enabled adequate results to be obtained by using one drop of the substance under study and only a small amount of adsorbent.

The time required for chromatographic analysis was significantly reduced. The method was also used for the preliminary testing of the properties of adsorbents, the mode of development and the properties of developing solvents.

Prior to column chromatography, we were probably the first to utilize the elution development version in thin-layer chromatography (TLC) and paper chromatography, which was developed from Tswett's idea that complete separation of the components of the mixture can be ensured only through the use of a dynamic method, *i.e.*, due to uneven migration of adsorption zones under the stream of pure solvent.

Therefore, in order to obtain better separation of substances after the stage of frontal TLC, at the position of application of the first drop of the solution we placed a drop of pure solvent and found that the separation was then more complete.

By means of this method, we studied flat-bed ultramicro-chromatograms of the following tinctures: absinthium, belladonna, capsicum, cinchona, foxglove, ipecacuanha, mint, rhubarb, strophantus, poison nut, valeriana, hellebore, lily of the valley, Spanish fly, cinnamon, etc.

Circular thin-layer chromatograms of different tinctures when viewed in ultra-

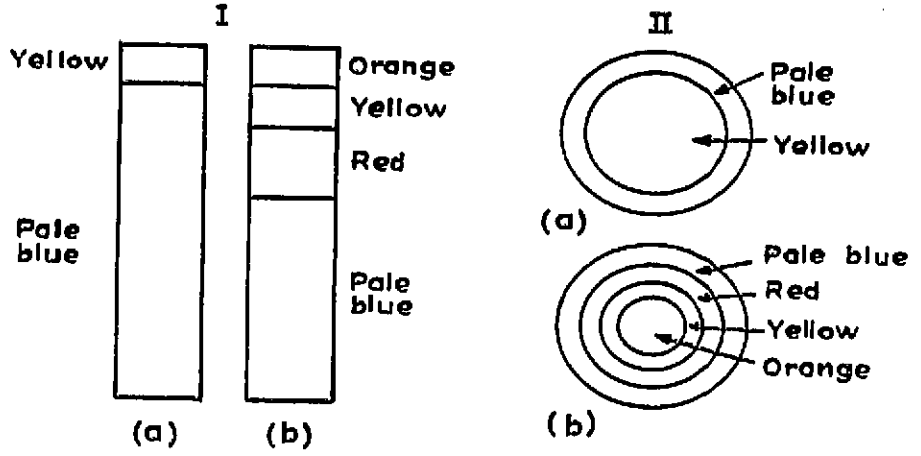

Fig. 3. Chromatograms of extract of belladonna. I, Chromatograms: (a) before washing; (b) after washing. II. Ultrachromatograms.

violet light exhibit such a bright and specific type of fluorescence that we considered this property to be suitable for the rapid analysis of pharmaceuticals. For this purpose, we prepared coloured drawings on paper of the zones obtained. These drawings could be used as a reference for the identification of tinctures. The tinctures were characterised by the number of zones and their positions and colours. The first two characteristics could be adequately resolved, but the indication of colours was vague. This was especially true for fluorescing colours. It was intended to compile a manual for the qualitative characterisation of galenical preparations by means of colour drawings of the zones, but the war interfered with our plans.

Nevertheless our paper[5], published in 1938, did not remain unnoticed. In 1941, CROWE[6], making reference to the abstract of our article, reported on the use of TLC in his laboratory for similar separations.

In a paper by MEINHARD AND HALL[7], our method is referred to under surface chromatography.

The use of TLC became common abroad mainly owing to the work of STAHL and coworkers in standardizing it*.

At present TLC is being successfully developed in the Kharkov Chemistry and Pharmacy Research Institute and is being used in studies on the composition of vegetable materials, the investigation of complex pharmaceuticals and for the quantitative determination of various classes of organic compounds (flavines, alkaloids, coumarins, cardiac glycosides, furanochromones, enzymes, etc.).

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* In his monograph[8], STAHL stressed the priority of Soviet scientists in the development of TLC, giving quotations and diagrams to illustrate their work. In a copy of it, he wrote: "I dedicate my book to Prof. N. IZMAILOV and M. SHRAIBER, pioneers of thin-layer chromatography". The original paper has been reprinted by PELICK *et al.*[9].

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