ELIMINATION OF THE DEFICIENCIES AND EFFECTIVE UTILIZATION OF THE KhZh 1305 MICROCOLUMN LIQUID CHROMATOGRAPH

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The MSFP-1 microspectrophotometric attachment and a syringe micropump [1-5] are used for the separation of substances on the micro and ultramicro scales. The KhZh 1305 microcolumn liquid chromatograph has been designed on the basis of these instruments in Leningrad and its routine manufacture is being started up in Orlo.

A serious deficiency of the KhZh 1305 — the formation of bubbles of gas in the microcells — has prevented the high-quality chromatographic separation of substances. Preliminary degassing of the solutions and washing the microcells with ethanol has given a temporary effect.

We have established that the main cause of the formation of bubbles of gas is the heating of the cell compartment to 70-75°C by the heat liberated by the feed block for the DNU-65 lamp. In order to eliminate this heating the metal screen above the electronic blocks has been removed and the instrument has been operated with a open rear cover at 25°C. Under these conditions the temperature in the cell compartment did not rise above 40-42°C. In the course of a year, about 300 chromatographic determinations lasting from 30 min to 8 h have been performed. No bubbles of gas were formed in the microcells.

In order to normalize the temperature regime of the cell compartment in the instruments described, 3/5 or 4/5 of the surface of the wall of the cover and the upper part of the right side wall (wall of the cover) must be removed for ventilation openings. The cover of the Orlo KhZh 1305 must be raised from the frame of the instrument by 20 mm and fixed to four legs. To prevent liquid or foreign objects falling into the electronic blocks of the instruments, the screen must be set up at a height of 20 mm from the frame of the instrument in order to ensure ventilation.

The feed block of the DNU-65 lamp must be fitted with a microfan of the exhaust type which is used when the temperature above the electronic blocks rises. It has been established that in the operation of the KhZh 1305 the temperature should be 20-25°C. A strong circulation of air leading to fluctuation noise oscillations of the recorder pen is undesirable.

In installation and starting-up operations in the Orlo KhZh 1305 instruments, to ensure the ready passage of gas bubbles the dimensions of the microcells and grooves for the flow of eluent were increased, as a result of which the conditions for microcolumn chromatography (MCLC) were disturbed and effective MCLC with a microquantity of substance (10^{-6}-10^{-7} G) becomes impossible. The dimensions of the grooves for the flow of eluent and of the microcells must be reduced by installing a Teflon cell gasket designed for the purpose. To decrease the dead volumes that are undesirable for MCLC [3, 6, 7], the widening at the inlet and outlet of the microcolumns supplied in the Orlo KhZh 1305 kits must be excluded and capillary tubes with an internal diameter of 0.1 mm and of minimum length must be used to connect the microcolumn with the microcell and the microcell with the fraction collector.

For the gradient devices of the KhZh 1305, the internal diameter of the capillary tubes must be 0.1-0.3 mm. The tubes with an internal diameter of 1 mm supplied and used according to the recommendations of the technical description and instructions for the use of the KhZk 1305 are unsuitable for performing chromatography on the micro scale.

In the KhZh 1305, the armature of the modulator does not work satisfactorily. In the initial design the armature of the modulator was fixed to bearings [1]. In the standard

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production of the KhZh 1305, the designers have renounced the bearing apparently because of the existence of a high frictional force in it. Trouble-free working of the modulator can be achieved by changing its design: The body of the armature should have two tapered ends set in rubies, like the balance wheels of wrist-watches (the manufacture of parts in our watch industry has been brought to perfection).

It would be useful to supply the KhZh 1305 with an integrator in place of the UPRP-1 and with a refractometer, and to standardize the manufacture of the DNU-65 lamp.

All the polyethylene capillary tubes of the KhZh 1305 must be replaced by Teflon tubes, which are resistant to organic solvents.

Japanese workers using all the principles of the design and working of the MSFP-1 and KhZh 1305 microcolumn liquid chromatographs created by Soviet scientists [1-5] have made micro- and ultramicrocolumn high-performance liquid chromatographs in which Teflon capillary tubes have been used as connectors [8, 9]. They have performed the chromatographic separation of aromatic hydrocarbons [8-10] and glycosides [11] and have shown the possibility of using microcolumns for the separation of various classes of compounds.

An important factor in MCLC is the production of narrow peaks, which is achieved mainly by decreasing the dead spaces and the volumes of the microcells and using the fine sorbents. In high-performance liquid chromatography (HPLC), as well, this principle is basic, but in HPLC the use of fine sorbents (grain size down to 5μm) greatly increases the working pressure if, to achieve high efficiency, chromatography is carried out at high linear rates of flow of the mobile phase. With a decrease in the dimensions of the columns this undesirable phenomenon — the rise in pressure — can be brought to a minimum. High pressures are usually created in working with large columns — 2 × 250 mm, 2 × 250 mm, 2 × 500 mm, and 1 × 3000 mm.

The fundamental idea of MCLC was shown by Soviet scientists in the investigation of nucleic acids on the micro and ultramicroscales [3-5, 12-16]. The great prospective possibilities and high efficiency of the MCLC method were shown.

Soviet, and later Japanese, workers have shown in practice the possibility of decreasing the static volume of the microcells to 0.01-0.05 μl (the microcell of the KhZh 1305 has V_{st} = 0.8 μl) and the dead volumes to 0.007-0.2 μl, which greatly raises the efficiency of MCLC [3, 9]. In the Orlo instruments, as already mentioned, to facilitate the displacement of the bubbles of gas the volumes of the microcells and the grooves for the passage of eluent are increased considerably on installation as compared with the design characteristics. This leads to a sharp fall in the efficiency of MCLC.

The initiators of MCLC, working on chromatographs with a MSFP-1 microspectrophotometer did not encounter the difficulties with bubbles of gas that have been described, since they selected as the optical section a SF-4, in which the feed block of the UV lamp is remote from the optical part. The armature of the modulator was borne in bearings [1].

In the KhZh 1305, the creation of the necessary temperature regime, a decrease in the volume of the microcells to the design figure (0.8 μl), and a decrease in the dead spaces and the diameter of the tubes for the gradient devices to a minimum will place the KhZh 1305 among instruments having no world analogs, since in the KhZh 1305 three important ideas of Soviet scientists have been embodied:

The microspectrophotometer of the KhZh 1305 works on the basis of the principle of double-beam spectrophotometry, which possesses a number of advantages over the traditional scheme [1];

the automatic device for changing wavelengths permits the recording of the spectrophotometric characteristics of the eluates with a cyclic change in wavelength without stopping chromatography. In HPLC, which has come into wide use, such characterization of the eluate at other wavelengths is performed by stopping chromatography, which raises new problems when working under a high pressure [17];

an increase in the efficiency of chromatography can be achieved by a proportional decrease in scale, i.e., in the volumes of ordinary chromatographic systems, with the simultaneous use of fine sorbents with no deleterious artifacts. This permits a saving in the use of sorbents, eluents, and other reagents and also makes it possible to perform analyses with micro and ultramicro amounts of substances (10^{-6}-10^{-9} g).

It must also be borne in mind that the cost of an HPLC column is from 600 to 1200 dollars and the preparation of a column for MCLC costs a few kopecks.

For MCLC we have used a fine fraction of the very cheap ground Dowex 1 × 8 resin (20-50 mesh) and have developed chromatographic methods for the separation (chromatography was performed at 25°C) of:

1) nucleoside 3'(2')-monophosphates of direct alkaline hydrolysates of the tissues of biological objects — time of chromatography (t.c.) 66 min;

2) nucleoside 5'-monophosphates — t.c. 27 min;

3) nucleoside 5'-mono-,-di-, and -triphosphates — t.c. 2.5 h;

4) a pyrimidyl-RNase hydrolysate of the high-molecular-weight rRNA of cotton seeds into isopliths — t.c. 5 h (column containing DEAE-cellulose); and

5) isopliths into individual oligonucleotides — t.c. 2.5 and 5 h.

For comparison we give literature information on the separation of substances by the HPLC method performed in columns filled with the strong anion-exchange resin Aminex A-28 (chromatography was performed at 90 and 70°C):

1) HPLC of nucleoside 3'(2')-monophosphates — t.c. 90 min;

2) HPLC of nucleoside 5'-monophoaphates — t.c. 75 min [18]; and

3) HPLC of nucleoside 5'-mono-, -di-, and -triphosphates — t.c. 2.5 h [19]. Another great advantage of MCLC is the use of polyethylene and Teflon connecting capillary tubes and glass pumps and columns which are relatively stable various conditions of chromatography. A disadvantage of HPLC is the low corrosion resistance of the chromatographic system because of the use of metal tubes, pumps, and columns in it.

Thus, the fastest possible elimination of the deficiencies of the KhZh 1305 and the improvement of the design of the instrument permit its wide use in various fields of chemistry, in molecular biology, and in medicine.

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