Application of Biochemical Micromethods for the Investigation of Tropical Disease Pathogens

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Edited by
Frank Michal,
Scientific Working Group on Biomedical Sciences,
Special Programme for
Research and Training in Tropical Diseases

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Microcolumn High Performance Liquid Chromatography

G. I. Baram

Novosibirsk Institute of Organic Chemistry, Novosibirsk

Liquid column chromatography (LCC) is an important method for quantitative and qualitative analyses in biochemistry. Modern chromatographs provide the separation of complex mixtures in amounts of 10^{-8} -10^{-11} moles in one peak. The increase of sensitivity, which is important for work with biological materials, can be achieved by the use of special detectors which are usually rather expensive and complicated. An alternative and simple solution of the problem is to use microcolumns. The spreading of the substance zone during its movement along the column in this case is minimal and material reaches the detector at a high concentration.

Besides the higher sensitivity, microcolumn liquid chromatography (MCLC) has a number of important advantages compared with traditional LCC. First of all there is a considerable saving of expensive adsorbents and mobile phases. As the microcolumns can be operated at relatively low pressure, the pump and the sample injection systems are rather simple. The columns are easily packed, and therefore it is possible to choose any suitable stationary phase for the task involved.

The microcolumn chromatography methods are described in the literature (Knorre & Venkstern, 1973 and Ishii, et al., 1977).

The application of MCLC is demonstrated by the chromatography of chloroquine. Chloroquine diphosphate (Fig. 1) is a derivative of quinoline. It is well soluble in water at acidic and neutral pH. The solution of chloroquine in water has an absorbance maximum at the wavelength 330 nm with ε_M=15 000. Hence, chloroquine

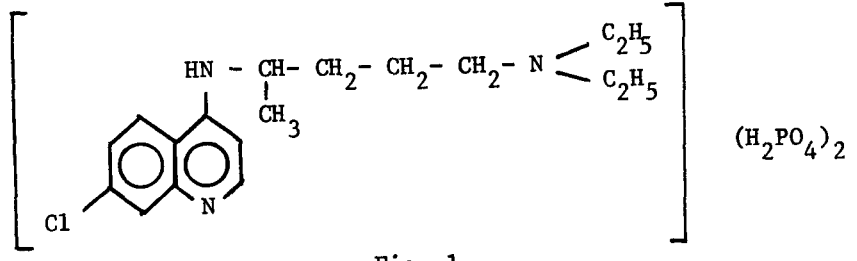

Fig. 1

may be detected during chromatography by absorption at 330 nm where nucleotides, peptides, sugars and many other compounds are not detected. The chloroquine free base is easily soluble in chloroform (ε_M^{330} = 12 000). Therefore, chloroquine and its metabolites may be also extracted by chloroform from alkalinized biological liquids and determined by chromatography on silica gel.