Quantitative Microscopic Ionophoresis and Chromatography*

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With 3 Figures

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Paper chromatography and ionophoresis have been very widely employed, particularly in biochemical and clinical work. Microscopic modifications are needed, where less than 1 µg of material is available, which still retain the simplicity and versatility of the conventional methods. If they could be applied to the pg scale (10⁻¹² g scale), the analysis of a single cell or cell region would then be possible.

A preliminary account has been given of a method a 100 millionfold or more as sensitive as paper strip techniques¹. Using mostly inorganic cation test mixtures, as little as 1.6–4.0 · 10⁻³ pg of each constituent can be quantitatively resolved in 15–180 sec run. The method lends itself to a number of possible variations, as well as to combination with interference microscopy.

Principle

The scale of the separation is greatly reduced. It is carried out under the microscope in very thin liquid films (0.01–10 µ) on an ordinary polished microscope slide or a “virgin” glass surface in a humid chamber. This surface acts as adsorbent and support instead of a porous solid or a gel slab. The thin horizontal film allows rapid condensation and evaporation of constituents and avoids thermal convection. It contains the developing acid and may also incorporate complex-forming agents to affect adsorption, ionic mobility or endosmosis. Two-beam reflexion

* “Microscopic ionophoresis and chromatography” will be briefly referred to as microphoresis when the mechanism is not specified, and simply as ionophoresis or chromatography when one mechanism predominates.
interferometry is used to observe the volume of the sample, film thickness and the zones. Test drop volumes are greatly reduced but not at the expense of increased concentration. The drops are delivered by a simple micropipette and micromanipulator, and their volumes calculated from the circular interference fringes after delivery. Cations are resolved by ionophoresis, chromatography, or a combination of both, with endosmosis here providing the solvent flow, and the film is then neutralized with ammonia vapour and rapidly dried. The zones are detected by a modification of the "breath figure" test observed interferometrically, and their areas taken as a measure of quantity. Zone contrast can be intensified by hydrophobic or hydrophilic producing reagents.

**Methods**

**General Description of Apparatus**

The design described here is adapted from a number of earlier versions used from 1949 onwards in the analysis of egg white and other test mixtures.

![Microphoresis Apparatus](image)

The microphoresis is carried out on a very clean microscope slide resting horizontally on acid-cleaned glass rod supports in a humid chamber (Fig. 1). Alternatively a freshly flattened, thin wall glass bulb is used, provided with small electrode cups (Fig. 1). In both cases, analysis is carried out on the lower surface. Atmospheric dust rapidly contaminates an upturned surface and might be analysed in this way. Inverting the surface provides simple and effective dust protection and is convenient for micromanipulation and humidity control.

The chamber A (Fig. 1) is constructed from "Pyrex" glass (J. A. Jobling & Co. Ltd., Sunderland, England) strips cemented together with "Araldite 1" cement (Aero Research Ltd., Duxford, Cambridge). It is closed by two sliding lids B and a central lid C, and is clamped to a 1-cm thick "Perspex" (Imperial Chemical Industries Ltd., London) platform E screwed to a microscope vernier stage F. The sides of the