proportional to the 5/2 power of E. The product of the peak tube power and the duty cycle gives the average tube power, a quantity to which the x-ray yield is related (see below).

The procedure for the measurement of x-ray intensity was as follows: The ion chambers were mounted in arbitrary positions near the break in the lead shielding around a wave-guide penetration; the chambers were not disturbed during a given set of measurements. Changes in the x-ray fields as registered by at least two different types of ion chambers were recorded for each step change in the PRF, the high voltage, or the RF output.

Although the linear rise in x-ray intensity with PRF was expected, the concomitant gradual rise in mean energy was not. This effect may have been caused by a gradual loading of the beam magnetic focusing fields that cause small changes in the internal x-ray scattering geometry.

The x-ray intensity rose with the 11.5th power of the high voltage E, a dependency that remains extraordinary (E^9) even when the intensity values are normalized to constant diode (video) power. There are two general high-voltage-dependent factors that could contribute to this effect: the collector (copper target) yield of x-rays, and the photoelectric effect (PE) in the copper structures and in the lead shielding. The yield factor is difficult to estimate because it depends on the collector beam-target geometry which cannot readily be determined inside a klystron; moreover, this geometry will vary with beam voltage in a fixed-focusing magnetic field. Finally, the relevant yield is only that yield *above* a certain photon energy, somewhere between 35 and 50 kev, that has a significant probability of penetrating the copper body or wave guide. By graphically integrating published spectral yield curves above an arbitrary cutoff value, one can obtain 3rd to 5th power dependencies on E (*3, 4*).

In order for the primary x-ray beam to reach the external ion chambers, it must channel along the copper drift tube and output cavity, through the wall of the output wave guide, and through a crack in the external lead shield (Fig. 3). The energy-dependent PE resonance absorption is the dominant photon interaction in these structures (*4*). Over a broad range of energy, the x-ray intensity transmitted through typical wall thicknesses of 0.2 to 0.4 cm can exhibit an E^5 dependence. Therefore, the observed high-voltage dependency may be explained in terms of the PE and the source yield factors.

In order to discuss the changes in x-radiation with relative RF output, it is necessary to list the effects of an impressed RF field on the electron beam in the body of a klystron (*5*). (i) The electron beam is velocity-modulated so that intense bunches of electrons arrive simultaneously at the microwave output cavity. If the electrons are initially at 120 kev, the bunch at the output cavity can contain electrons with energies between 90 and 150 kev. (ii) The electron beam is defocused, an effect that changes the x-ray source distribution within the klystron tube and the internal scattering geometry. (iii) The bunched electrons lose energy to the output cavity in crossing the cavity gap and arrive partially spent at the collector. (iv) Out-of-phase (unbunched) electrons are strongly accelerated in crossing the output cavity gap. This acceleration depends in a complicated fashion on the tube voltage, the gap transit time, tuning factors, and RF power-coupling factors. However, a typical S-band klystron output cavity gap of 2 cm could be charged with accelerating electric fields of the order of 100 kev/cm. The large increase in x-ray intensity and in mean energy with increasing RF output is evidence that effects (i), (ii), and especially (iv) more than compensate for effect (iii) on the x-ray yield.

I conclude that local x-ray shielding that is adequate for safe klystron operation at a given high voltage, PRF, and microwave power output may be entirely inadequate if there are significant increases in any of these operating parameters; and that x-ray intensity and mean energy measurements should prove to be a simple useful means of analyzing klystron function since the external x-radiation level is a sensitive indicator of output cavity tuning and power coupling factors.

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6. I thank E. Storm of the Los Alamos Scientific Laboratory, Los Alamos, New Mexico, for carrying out the energy-response calibrations of the PIC-6 ion chamber, C. Heon and N. Efremos of the M.I.T. Lincoln Laboratory, Bedford, Massachusetts, for operating the klystrons, D. Searl for assisting in the x-ray measurements, and A. Browne of the Lincoln Laboratory and R. V. Keating of the M.I.T. Laboratory for Nuclear Science, Cambridge, Massachusetts, for helpful discussions. Supported in part under AEC contract AT(30-1)-2098.

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Droplet Countercurrent Chromatography

Abstract. *A new form of countercurrent chromatography, named droplet countercurrent chromatography, has been developed. This all-liquid separation technique is based on the partitioning of solute between a steady stream of droplets of moving phase and a column of surrounding stationary liquid phase. Milligram quantities of dinitrophenyl (DNP) amino acids were separated with an efficiency comparable to that of gas chromatography.*

Liquid-liquid chromatography and countercurrent distribution (*1*) are powerful analytical procedures for the purification and identification of a wide variety of compounds. These techniques have certain limitations, however. Adsorption of solute to the support used in liquid-liquid partition chromatography often causes peak tailing, and the capacity of this technique is low. Although solid supports are not used in countercurrent distribution, this technique is more cumbersome and generally has less resolving power but higher capacity. Recently, Ito and Bowman reported on a simple all-liquid microtechnique called countercurrent chromatography, which involves partitioning in a long helical tube in a centrifugal field and which has an efficiency comparable to that of

gas chromatography (*2*). In the course of efforts to scale up this procedure, we have developed a modification of countercurrent chromatography which we call droplet countercurrent chromatography. The method has a significantly higher capacity and is simpler to use than the parent method.

Droplet countercurrent chromatography is carried out by passing droplets of moving phase through a column of stationary phase. The moving phase may be either heavier or lighter than the stationary phase. When heavier, the moving phase is delivered at the top of the column, and, when lighter, through the bottom. The column (glass, Teflon, or metal may be used) is mounted perpendicularly and filled with stationary phase. The moving phase is introduced at the bottom through a tip chosen to give a steady stream of droplets about as large in diameter as the tubing. When a droplet reaches the top of the column it is delivered to the bottom of the next column through narrow-bore Teflon tubing. A small amount of stationary phase may also enter the Teflon tubing initially, but the effect is insignificant.

As the moving phase moves through the column, turbulence within the droplet promotes efficient partitioning of the solute between the two phases. The expected mixing of the stationary phase along the length of a column is minimized by the ascending series or stack of proper-sized droplets which serves to divide the stationary liquid into distinct segments.

With a given set of phases, generation of droplets of proper size is a function of the column bore, the interface of the immiscible liquids, the flow rate of the moving phase, and the diameter of the inlet tip. In general, small-bore columns (that is, less than 1.0 mm) produce plug flow in which the entire contents of the tube are displaced. If the bore is too large, then large droplets which form do not allow efficient partitioning of solute. If small droplets are introduced, some will coalesce into slower-moving larger droplets which move even more slowly. This can occur throughout the length of the column, sometimes with the result that large segments of the moving phase ascend the column, which obviously prohibits the efficient partitioning of solute. Introduction of the moving phase at too fast a rate is also deleterious, as the droplets abut one another and often coalesce into cylinders. The column walls should not have a high affinity for the moving phase. Thus, if the moving phase is aqueous, and the column is glass, the walls must be silylated to promote droplet formation; otherwise the liquid wets the walls and moves as a stream. A Beckman Accu-Flow pump, equipped with a side-arm reservoir for damping pulsations,

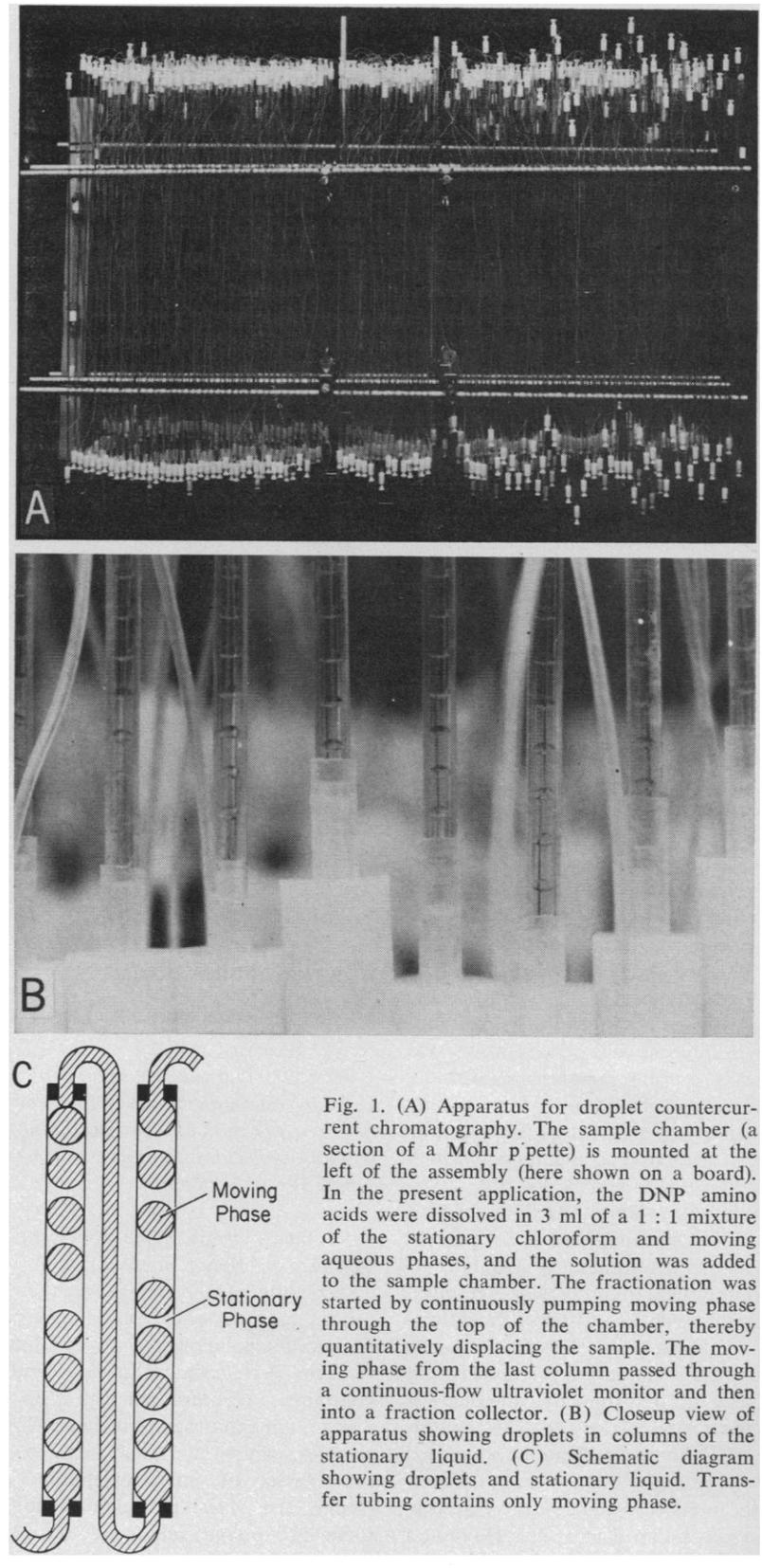

Fig. 1. (A) Apparatus for droplet countercurrent chromatography. The sample chamber (a section of a Mohr pipette) is mounted at the left of the assembly (here shown on a board). In the present application, the DNP amino acids were dissolved in 3 ml of a 1 : 1 mixture of the stationary chloroform and moving aqueous phases, and the solution was added to the sample chamber. The fractionation was started by continuously pumping moving phase through the top of the chamber, thereby quantitatively displacing the sample. The moving phase from the last column passed through a continuous-flow ultraviolet monitor and then into a fraction collector. (B) Closeup view of apparatus showing droplets in columns of the stationary liquid. (C) Schematic diagram showing droplets and stationary liquid. Transfer tubing contains only moving phase.

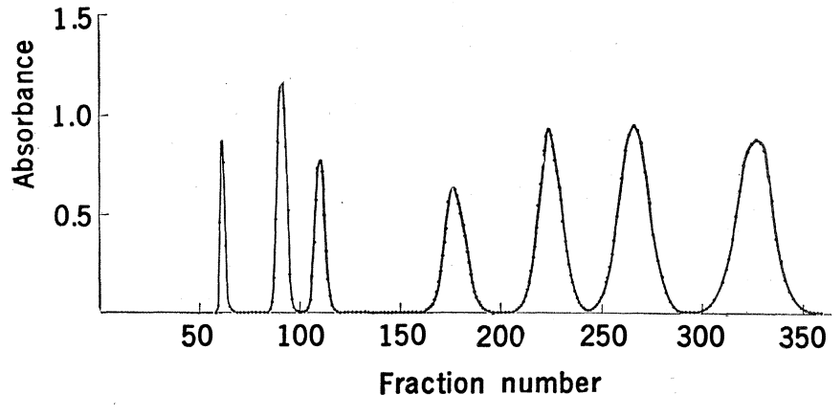

Fig. 2. Separation of DNP amino acids. Peaks (partition coefficients are shown in parentheses) in order of elution are: dinitrophenyl-L-ornithine (> 100), dinitrophenyl-L-serine (3.8), dinitrophenyl-L-threonine (2.4), *N,N'*-di(dinitrophenyl)cystine (0.94), dinitrophenyl-β-alanine (0.71), dinitrophenyl-L-alanine (0.56), and dinitrophenyl-L-proline (0.45).

can be used to deliver a constant flow of droplets. The rate is set at the highest flow compatible with a steady generation of discrete droplets. The highest permissible flow gives droplets which are barely separated.

Figure 1 shows an assembly used to separate dinitrophenyl (DNP) amino acids. It consists of 300 silylated glass columns of standard Pyrex tubing (0.6 mm in wall thickness and 3 mm in outside diameter) 60 cm long connected by AWG 24 standard-wall Teflon tubing (*3*). The system has a capacity of about 460 ml (excluding the volume of moving phase in the Teflon tubing, which is 80 ml). Solvents [in this case, chloroform, acetic acid, and O.1*N* HCl, 2 : 2 : 1 (by volume)] were mixed and allowed to equilibrate in a separatory funnel. The chloroform layer (stationary phase) was pumped into the assembly. The DNP amino acids, 2 to 10 mg each, were dissolved in 3 ml of a 1:1 mixture of both phases, and this solution was placed in a 5-ml sample tube (for example, a modified 5-ml Mohr pipette with suitable high-pressure fittings). The aqueous phase was then pumped through the top of this tube at a flow rate of 16 ml/hour (10.2 atm). Fractions, each 3 ml, were collected, and their absorbances were determined at 350 nm.

Symmetrical, completely resolved peaks were obtained for each component in the mixture (Fig. 2). The efficiency of the separation calculated from the peak of dinitrophenyl alanine was 900 theoretical plates (*4*). The analysis time of 70 hours could not be shortened with the present design without decreasing the number of columns, as moving phase was pumped at a near maximum rate. Although 30 mg of mixture was used in the present study, 300 mg have been separated with little loss in resolution. The capacity of the technique may be increased by the use of columns of larger bore, but it is not yet known to what extent this modification affects resolution. Resolution is, of course, proportional to the number of columns used; the necessary number of columns depends on the separation desired.

Droplet countercurrent chromatography, which may be compared to the classic separation technique of countercurrent distribution (*1*), has high resolving power, compactness, simplicity of design, and ease of operation. It would appear to be well suited to the separation of milligram quantities of peptides, ribonucleic acids, lipids, and similar substances.

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3. The Teflon tubing connecting the columns passed through a Delrin fitting which with suitable tubing adapters secured it to the glass tubing. The base of the fitting was fitted over the glass tubing which was then flanged. A sleeve of polyethylene tubing (2.92 mm, inside diameter; 3.73 mm, outside diameter) approximately 1 cm long was placed over the glass to adapt the Delrin to the flange. Inserted into the glass tubing was a 1-cm length of AWG No. 18 standard-wall Teflon tubing containing a polyethylene tip (0.76, inside diameter; 1.22 mm, outside diameter) for the production of droplets of suitable size. The Teflon transfer tubing passed through the male threaded head of the Delrin fitting and into the Teflon adapter in the glass tubing. The transfer tubing had a tendency to slip out under high pressure. It was held in place with a short sleeve of shrinkable Teflon 1203 Penntube WTF. When shrunk, the sleeve bound to the transfer tubing and provided an anchor to the male Delrin fitting.
4. We calculated the number of theoretical plates using the formula
$$N = (4R/W)^2$$
where N is the number of theoretical plates, R is the elution volume, and W is the peak width volume. The elution volume does not include the volume of the moving phase in the Teflon tubing, as this volume is merely in transit and not involved in the partitioning process.

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Macroglobulin Structure: Variable Sequence of Light and Heavy Chains

Abstract. *The variable regions of the light and heavy chains on the same macroglobulin (immunoglobulin M) molecule are no more related in amino acid sequence than are the variable regions of the light and heavy chains of different immunoglobulin molecules. Subgroups of μ chains are similar in their variable sequence to subgroups of γ chains.*

In the 5 years since the primary structure of Bence Jones proteins was first reported (*1, 2*) it has been established that the polypeptide chains of all immunoglobulins are divided into a variable NH_2-terminal region and a constant COOH-terminal region. In the κ and λ antigenic classes of Bence Jones proteins, which are equivalent to the two classes of immunoglobulin light chains, the variable region comprises some 110 amino acid residues or approximately the first half of the molecule, and the constant region constitutes the second half (*3*). Many changes in sequence occur in the variable region of different light chains of the same antigenic class, but none or only one or two occur in the constant region. Studies of the sequence of μ and γ heavy chains have shown a similar division into a variable region and a constant region (*4–10*). Although the variable region is about the same length as in light chains,