Table III. Isomerization and aromatization of limonene over CaO catalyst at 475 °C

% Products[a] Products	Run No. 1	Run No. 2	Run No. 3	Average and S.D.
Limonene	45.0	44.5	44.5	44.7 ± 0.2
α-Terpinene	5.5	6.0	6.0	5.8 ± 0.3
Terpinolene	3.5	3.5	3.0	3.3 ± 0.2
2,4(8)-Menthadiene	5.0	5.0	5.0	5.0 ± 0.0
γ-Terpinene	2.5	2.5	2.5	2.5 ± 0.0
p-Cymene	23.0	23.5	23.0	23.2 ± 0.2
p-Isopropenyltoluene	3.0	3.0	3.0	3.0 ± 0.0
Benzene	2.0	2.0	2.0	2.0 ± 0.0
Toluene	1.0	1.0	1.0	1.0 ± 0.0
m-Xylene	1.5	1.5	1.5	1.5 ± 0.2
Pyrolytic products	7.0	6.5	7.0	6.8 ± 0.2

[a] See footnote Table I.

tainty in measurement of small areas in the v.p. chromatograms[7].

Zusammenfassung. Es wird eine Apparatur zum Studium heterogener und pyrolytischer Reaktionen in Fliess-Systemen beschrieben, die für flüssige und gasförmige Ausgangsstoffe, Reaktionsprodukte sowie für deren Mischungen geeignet ist.

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Rapid Microestimation of Proteins by Membrane Chromatography on PVC Ultrafilters

In a previous communication we described a simple and rapid method for the quantitative estimation of proteins by radial chromatography on nitrocellulose membrane filters[1,2]. However, that technique had the following limitations: Low-molecular proteins (mol wt. below about 100,000) could be adsorbed to nitrocellulose only at acid pH values, e.g. 3.7, and for proteins having mol wt. below about 40,000 that estimation usually failed because of their insufficient adsorption on the carrier[3].

The limitations mentioned above seem to be surmountable in a simple way by using polyvinylchloride (PVC) membrane filters instead of nitrocellulose, since our preliminary experiments with PVC membranes[4] revealed that they adsorb proteins quite feasibly in a range of mol wt. about 12,000–400,000 and pH 3.7–9.06. The present paper brings more data confirming the above findings, reports new details of membrane chromatography on PVC and presents a series of calibration curves of various proteins.

Material and methods. PVC membranes Sartorius (Göttingen, Germany) SM 12801 were used in strips 3–4 × 10–20 mm, wetted in 40% aqueous ethanol and washed thoroughly by a 0.1 *M* phosphate buffer pH 7.2 which had been diluted with 0.9% NaCl 1:1. The same buffer was used for one-dimensional ascending chromatography as described in reference [4]. Bovine serum albumin, human γ-globulin and fibrinogen, ovalbumin, horse myoglobin[5] and bovine ribonuclease (Reanal) diluted in the developing buffer to 0.2% concentration were applied by means of a thin capillary calibrated by 1 μl[1,2] stepwise to the surface of a plexi glass slide in successive portions of about 0.3 μl. One calibrated capillary was used in all experiments. The droplets were quantitatively soaked into the starting edge of the wet membrane which stuck spontaneously to a supporting glass at the other end (Figure 1) and was then gently pressed with the fingers between that glass slide and a dry filter paper wick Whatman No. 1. The strips were then immediately developed for about 1–3 min until the flow marker, e.g. 10% potassium bichromate, reached the upper end. After staining the membranes with 0.5% amidoblack 10B in 5% trichloroacetic acid and destaining in water (Figure 2), the area of the protein layer was measured by means of

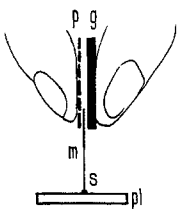

Fig. 1. Application of the sample to the PVC strip. p, dry filter paper wick; g, supporting glass plate; m, PVC membrane filter; (p, g and m are gently pressed together by fingers); s, sample; pl, plexi glass.

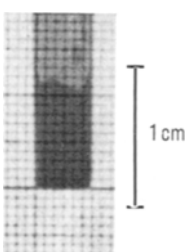

Fig. 2. Stained protein film developed chromatographically on a PVC membrane. 2 μl of 0.2% bovine serum albumin was developed in phosphate buffer pH 7.2 on PVC membrane SM 12801 for about 2 min. Stained with amido black 10 B. The transparent scale is placed upon the strip.

[1] T. I. Přistoupil, Nature *212*, 75 (1966).
[2] T. I. Přistoupil, M. Kramlová and J. Štěrbíková, J. Chromat. *34*, 370 (1968).
[3] T. I. Přistoupil, M. Kramlová and J. Štěrbíková, J. Chromat. *42*, 367 (1969).
[4] T. I. Přistoupil, J. Chromat., *49*, *550* (1970).
[5] Prepared in our Institute.

a transparent square millimeter scale (divided to 0.5 mm[1]) on both sides of the strip using a magnifying glass. The average value in mm² was then plotted against the absolute amount of the applied protein. The exact concentration of the pure protein standards was determined separately by dry weight and spectrophotometrically.

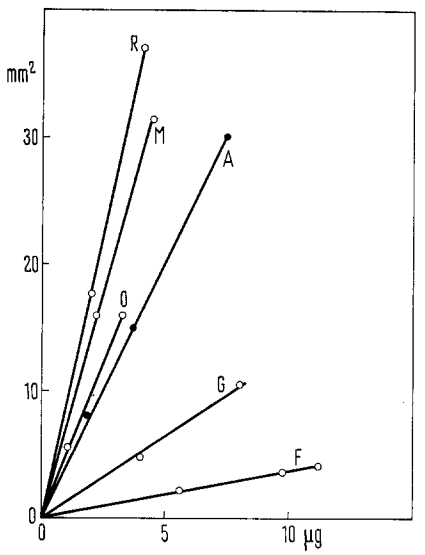

Fig. 3. Calibration curves of proteins. R, ribonuclease; M, myoglobin; O, ovalbumin; A, serumalbumin; G, gammaglobulin; F, fibrinogen. PVC membrane filters Sartorius SM 12801 and 0.1 *M* phosphate buffer with 0.9% NaCl were used.

Results. The calibration curves (Figure 3) were different for individual proteins and all were linear in the given region with a deviation of the mean not exceeding 3%. This fact which confirmed all our previous experiences in this field[1,2,4], makes it possible and reasonable to construct the calibration curve for a given protein (or a standard protein mixture, e.g. serum) by measuring exactly only one dot, i.e. the area corresponding to a given amount of protein and then drawing a straight line through that dot and the zero point of the scale. To ensure regular results it is necessary to avoid the presence of detergents, e.g. the Tweens, of high-molecular polyethyleneglycols[4] and of nonstandard proteins.

It is possible to conclude that this new microtechnique is very accurate, easy to perform, inexpensive and rapid, each estimation taking only a few minutes. It is suitable both for routine or for special ultra-micro estimation of soluble proteins in a broad range of molecular weights and at physiological pH values.

Zusammenfassung. Chromatographische Entwicklung von Proteinen auf PVC-Membranfiltern und Flächenbestimmung des adsorbierten Films zur raschen Proteinanalyse wurden beschrieben.

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A Capacitance Indicator for Infinitesimal Muscular Movements

This report concerns a new way of registering very small muscular movements in several places at the same time, without any mechanical connections between the object and the recording device, by means of a capacitance movement indicator. The simultaneous use of 4 indicators on a Langendorff heart preparation gives a more complete picture of muscular displacements at a higher sensitivity and a higher frequency than is ordinarily achieved with classical methods of registration.

An existing capacitance bridge method was modified, using the heart as one of the two plates in a condenser[1].

The device is very small (20 × 15 × 5 cm), constructed of inexpensive discrete components and operated by a 9 V battery. The sensitive device of the capacitance movement indicator is a bridge with a transformer fed by a 12 kc/s oscillator. 2 arms in the bridge are formed by the transformer and the other 2 by the condensers C_1 and C_2, whose common point is connected to the earth (Figure 1). The bridge is balanced when the 2 condensers are equal. The capacity of one of them can be changed, by heart movements when the heart, which constitutes one of the condenser C_2 plates, produces a change in the distance between these plates. This will also be the case when the earth point, which is the same as the heart and one of the condenser C_2 plates, moves. The bridge is then unbalanced and by measuring this unbalance one may obtain a relative measurement of the movements. Signals due to the unbalance in the bridge are tapped from the center of the transformer and after amplification they are fed to a phase sensitive detector. Changes in the capacity of C_1 or C_2 cause the signals from the detector to change level, in one direction by an increase and in the opposite by a decrease. The upper registration limit of the device is determined by the filter condensers in the detector, which in the present apparatus permit frequencies as high as 1500 Hz. After the detector the signals pass another set of filters which are chosen to suit the frequencies in the pattern to be registered and here an upper limit of about 350 Hz is chosen. In these experiments an Ink-Jet oscillograph with an upper limit of about 700 Hz was used. The amplitude of the deflections on the recorder depends on the sensitivity of the capacitance movement indicator and the distance to the object. The sensitive plate with its device has to be balanced with an oscilloscope each time its distance to the object is varied. The sensitive endpoint T_2 can be arranged in different ways depending on the size of the object and its movements. A closer approach of the earthed heart towards the sensitive plate can be registered as a positive or negative deflection on the recorder. Geometrically the plate can be of any shape and of any metal. It can be formed as a plate, or a ball, but the cut off wire of the conductor alone will suffice. The conductor to the sensitive plate has to be earthed in order to avoid disturbances. As the device measures its relations towards earth, the cable should preferably be of low capacitance type (about 30 pf/m).

Three types of model experiments were made, 1 with an eccentric plate, 1 with a micrometer screw and 1 with

[1] The author wishes to acknowledge the advice and assistance of Prof. H. HERTZ, Prof. L. STIGMARK and Mr. I. JONSSON of the Lund Institute of Technology.