konnten feststellen, dass bei der Durchsichtmessung die Streuung der Einzelwerte infolge der Faserstruktur des Papiers – auch bei Whatman Nr. I – sehr gross ist. Diese Streuung ist bei unserem Gerät wie auch beim Kugelreflektometer nicht vorhanden.

Des weiteren möchten wir mit unserer Mitteilung auf die Verwendungsmöglichkeiten sowohl des Pulfrich-Photometers in der genannten Modifikation für die Durchsichtmessung als auch des Kugelreflektometers in der Aufsichtmessung hinweisen.

Wir haben in unseren Abbildungen neben dem Elektrophoresestreifen die vier erhaltenen Kurven aufgezeichnet. Der Vergleich der einzelnen Kurven ergibt die Vorteile unseres Gerätes. Die Trennungen der einzelnen Eiweissfraktionen treten deutlich in Erscheinung, die « Extinktionswerte » werden mit gleich grossen Unterschieden in der Höhe, wenn nicht noch differenter hinsichtlich der Trennungsunterschiede, wiedergegeben. Die Verschiedenartigkeit der Kurvenformen ist nach unseren eingehenden Messungen durch das Meßsystem des jeweilig benutzten Gerätes bedingt.

H. RÖTTGER

Aus der Staatlichen Rheumaklinik und Rheumaforschungsanstalt des Staatsbades Elster, den 1. Februar 1953.

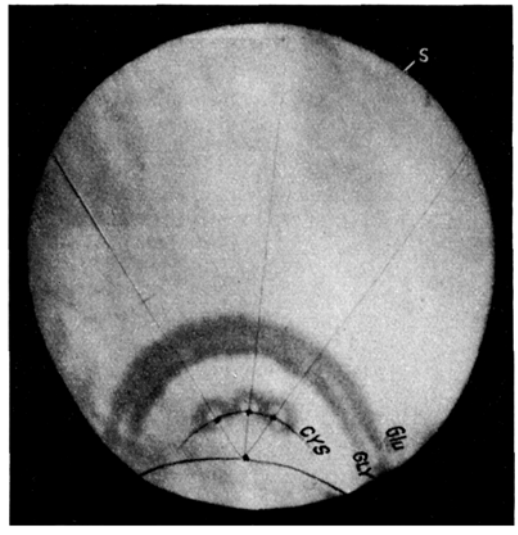
Summary

Four methods for the registration of paper electrophoresis are described and the results compared: globe-reflectometer, Pulfrichphotometer, quickphotometer an a new system of the photoreflectometer. The great sensitivity of this photoreflectometer makes it particularly suitable for the determination of the paper electrophoresis results.

A Modification in Horizontal Migration Paper Chromatography

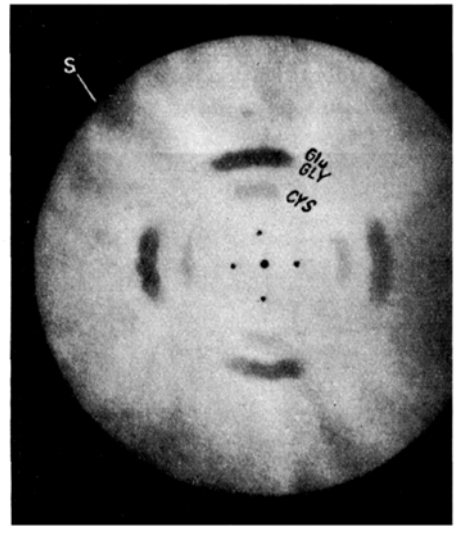
In view of the difficulty experienced in the separation and identification of substances having close R_f values, by the horizontal migration method of paper chromatography, GIRI and RAO[1] suggested the employment of either the multiple development technique of JEANES and co-workers[2], or of larger size filter-paper discs. From primary considerations, it would appear that better separations can be obtained if the mobile phase is made to travel a longer effective distance (thereby increasing the irrigating time) than in the conventional method with the wick at the centre. This could be achieved by shifting the position of the wick from the centre to a point as near the periphery of the filter disc as possible. To verify this, chromatograms were run with this modification and compared with those obtained in the usual way. The results obtained are described in this note.

WHATMAN No. 1 filter-paper discs of 18 cm diameter were used throughout and the temperature was maintained at 37 ± 1°C. The wick, made of WHATMAN No. 1, was inserted at a point about 0·5 cm away from the outer edge of the filter-paper, and the drops of mixture to be chromatographed were spotted on an arc of radius 1·5 cm with the wick as the centre. In this way the effective distance available was almost doubled. Except for this modification, the technique employed was identical with that of GIRI[1] or PROOM[3]. The time required to develop a chromatogram by the present method was about 5½ h.



1.—Modified method–n-Butanol-formic acid-water.

A chromatogram of a mixture of cystine, glycine and glutamic acids (R_f values 0·04, 0·15, and 0·20 respectively) in n-butanol-formic acid-water[1] (12:1:7) was run and is represented in Figure 1. The amino acids appeared to have travelled radially along lines drawn from the wick and were well separated.



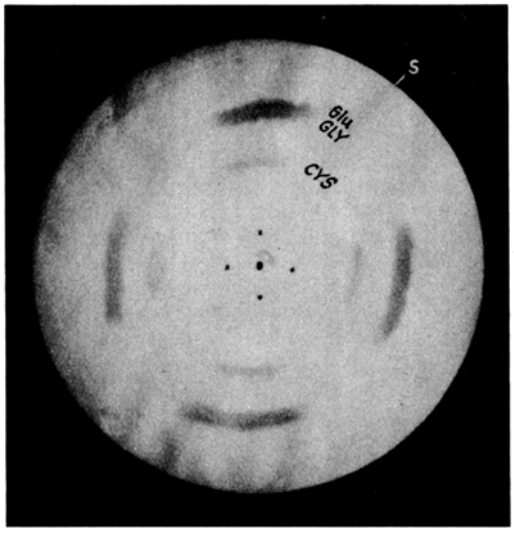
2.—Conventional method (irrigated once).

For comparison the same mixture of amino acids, but in n-butanol-acetic acid-water (4:1:5) (corresponding R_f values being 0·25, 0·38 and 0·44), was run by irrigating the disc once (Fig. 2) and twice (Fig. 3) in the conventional way, and once with the wick near the periphery (Fig. 4). A close examination of the three chromatograms would show that the separation of glycine and glutamic acid is the best in Figure 4. Similarly the separation of lysine and histidine in n-butanol-

[1] K. V. GIRI and N. A. N. RAO, J. Ind. Inst. Sci. *34*, 95 (1952).
[2] A. JEANES, C. S. WISE, and R. J. DIMLER, Anal. chem. *23*, 415 (1951).
[3] H. PROOM and A. J. WOIWOD, J. Gen. Microbiol. *5*, 681 (1951).

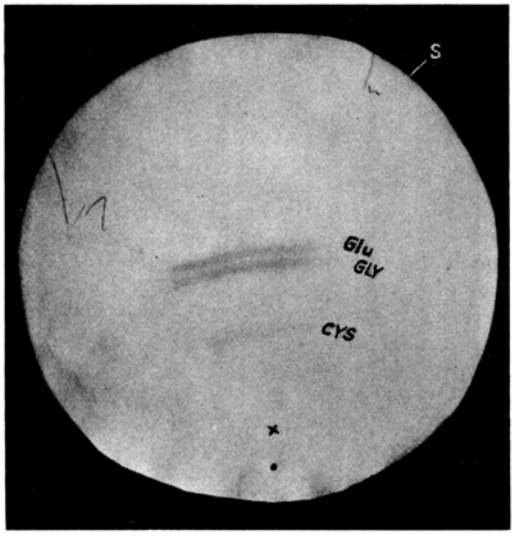
[1] L. F. WIGGINS and W. J. HOWARTH, Nature *170*, 279 (1952).

acetic acid-water (4:1:5) with R_f values of 0·31 and 0·38 respectively, can be easily achieved by the modified method, whereas this is not possible in the usual way.



3.—Conventional method (irrigated twice).

This method has been found useful in the identification of unknown substances by running the pure suspected amino acids simultaneously on either side of the unknown ones. Success has also been obtained in the separation of common sugars having close R_f values, e.g. lactose and maltose, R_f value 0·24 and 0·27 respectively, in butanol-acetic acid solvent as above at 22°C. Attempts are now being made to employ this method for the separation and identification of constituents of biological fluids.



4.—Modified method–n-Butanol-acetic acid-water. *CYS* = Cystine, *GLY* = Glycine, *GLU* = Glutamic acid, *S* = Solvent front, *X* = Position where the mixture of the amino acids is spotted. ● = Position of the wick.

R_f values for 24 amino acids and 13 common sugars have been calculated and, as expected, they were identical with those obtained by the conventional method. The maximum difference in a few cases was not more than 0·02.

It will thus be seen that with the modification suggested above, better separations are obtained with the 18 cm diameter filter paper disc by a single irrigation, thus saving time and material.

V. K. MOHAN RAO

Central Drug Research Institute, Lucknow, India, December 10, 1952.

Zusammenfassung

Bei horizontaler Papierchromatographie auf Rundfiltern wird das Trennungsvermögen grösser, wenn man die mobile Phase an einem Punkt in Peripherienähe zuführt und die zu chromatographierenden Substanzen auf einen um diesen Punkt gelegenen Kreisbogen aufträgt.

A New Nuclear Staining Method with a Vegetal Indicator Dye[1]: The "Sambucyanin"

In the course of investigations about some vegetal dyes, an extract from *Sambucus nigra* (L) berries has been used for the first time in histological technique. The plant is a very common *caprifoliacea* in Europe and Asia minor. A hydroalcoholic solution and a dry extract were prepared from bruised and retted berries, gathered in July and August, when fully ripened.

The hydroalcoholic solution (30% of alcohol) appears as a limpid liquid of deep red colour and contains, besides the dyestuff, traces of malic, tannic and tartaric acids, sugar and a colourless glucoside (Sambunigrin). The removal of these substances is not necessary for practical purposes, inasmuch as the solution itself is quite suitable for staining without further addition of mordants.

The solution, preserved at a low temperature, with the possible addition of some thymol crystals to avoid the development of mildews, keeps its own staining power unchanged for about two months.

After this period of time, the solution turns brown and greatly loses its staining power.

A dry extract can be prepared from hydroalcoholic extract, by means of low temperature vacuum evaporation suitable purification by repeated washing of the concentrate in ether-alcohol.

The dry extract appears as an amorphous hygroscopic blackish-red coloured powder which can be preserved active indefinitely at a low temperature. The hydroalcoholic solution can be prepared again from the dry extract. However, before using, it is advisable to allow this solution to "mature" for at least 48 h at room temperature in an uncovered container.

The dyestuff of *Sambucus nigra* is insoluble in absolute alcohol, ether, acetone, chloroform. It shows a characteristically strong red colour change in the presence of acids and a blue colour change in the presence of alkalis.

The potentiometer colour change showed the breakdowns as follows:

F. B. MALLORY: "It is doubtful, if the artificial dyes ever entirely replace the natural stains because the latter have certain valuable qualities of their own."