A COMPARISON BETWEEN THIN-LAYER CHROMATOGRAPHY AND PAPER CHROMATOGRAPHY OF NUCLEIC ACID DERIVATIVES

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The feasibility of separating nucleic acid derivatives by thin-layer chromatography (TLC, Stahl, 1958, 1961) has been recently demonstrated (Randerath, 1961a, 1961b; Randerath and Struck, 1961). Instead of inorganic materials used preferentially in TLC[1], ECTEOLA cellulose and cellulose layers were used in these investigations. In contrast to silica gel or alumina layers which show a very strong absorption in the 260 mμ region, ECTEOLA cellulose and cellulose chromatoplates can be examined like paper chromatograms under an ultraviolet lamp. Moreover, the separations of nucleotides are much better on ECTEOLA cellulose and cellulose layers than on inorganic adsorbents.

This communication is dealing with the comparison of paper chromatography (PC) with TLC of nucleic acid components on cellulose chromatoplates (cellulose layer chromatography, CLC).

METHODS AND MATERIALS. CLC: Cellulose powder MN 300 G for TLC[2] containing plaster of Paris as a binder was employed. Preparation of the layer is very simple. A slurry is prepared by mixing 15 g. of the cellulose powder with 100 ml. of distilled water and shaking vigorously for 45 sec. The suspension obtained is poured into the apparatus described by Stahl (1958), and subsequently spread over the glass plates[3] which must be thoroughly clean. (Previous treatment with a detergent is recommended.) About ten plates, 10x20 cm size, are obtained.

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[1] e.g. Kieselgel G, Aluminiumoxid G, Merck, Darmstadt, Germany

[2] Macherey u. Nagel, Düren (Rheinland), Germany

[3] The complete equipment for TLC is commercially available (Desaga, Heidelberg, Germany)

The layers are allowed to dry overnight at room temperature. Cellulose layers, prepared in this way, are fixed on the glass plates more tightly than those described previously (Randerath and Struck, 1961). Plaster of Paris is interfering with separations of polyphosphates (Randerath, 1961a, 1961b) only if alkaline solvents are used.

PC: The following papers were used:

1. Whatman No.1[4] 2. Schleicher u. Schüll No.2043b[5]
3. Ederol No.202[6] 4. Macherey u. Nagel MN 263[2]

The papers were cut in the size of the glass plates (10x20 cm). For achieving comparable results, PC and CLC were carried out under exactly the same conditions. The samples (10^{-2} µmoles in each case unless otherwise stated) were applied from a pointed micropipet at a line 2,5 cm from the end of the plate or paper sheet in such a way that the starting spots had diameters of 3,0 $\pm$ 0,3 mm. After evaporation of the solvent in which the sample was applied (in most cases water) the plate or paper sheet was inserted into a chromatographic chamber filled with an appropriate solvent to a height of about 1,2 cm, the paper being attached to a glass rod. The chromatography was stopped after the solvent had traveled a distance of exactly 10 cm from the starting line. After drying (110°, 5 min.) the chromatograms were traced on cellophane sheets under a Mineralight lamp[7].

Solvents used:

Solvent 1: Distilled water. Nucleobases and nucleosides can be separated with water in PC (Tamm, Shapiro, Lipshitz and Chargaff, 1953) and in CLC (Randerath and Struck, 1961).

Solvent 2: Saturated ammonium sulfate - 1 M sodium acetate - isopropanol 80:18:2 (v/v). 2'-AMP, 3'-AMP, 2'-GMP and 3'-GMP are resolved in PC (Markham and Smith, 1951) and CLC.

Solvent 3: Tert. amylalcohol - formic acid - water 3:2:1 (v/v) (Michelson, 1959). This solvent gives excellent separations of nucleotides in CLC.

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[4] H. Reeve and Angel, London E.C.4, England

[5] C. Schleicher u. Schüll, Dassel, Krs. Einbeck, Germany

[6] J.C. Binzer, Hatzfeld/Eder, Germany

[7] Ultraviolet Products, Inc., San Gabriel, Calif., U.S.A.

Solvent 4: n-Butanol - acetone - acetic acid - 5% aqueous ammonia - water 3,5:2,5:1,5:1,5:1 (v/v). With this solvent nucleotides can be separated on paper, cellulose layers, and, in contrast to the solvent reported previously[8] (Randerath and Struck, 1961), on silica gel layers.

RESULTS AND DISCUSSION. Fig.1a and 2a show the cellulose layer chromatograms, Fig.1b and 2b the paper chromatograms, prepared under the same conditions. As can be seen by comparison, the spots on the chromatoplates are generally smaller; they appear less diffuse under a Mineralight lamp than those on the paper chromatograms.

Solvent 1: Nucleobases and their respective nucleosides are well separated within 45 min. on the cellulose layer (Fig.1a). On paper, however, they are scarcely resolved under the same conditions (Fig.1b).

Solvent 2: The four isomeric 2' and 3' purine nucleotides are separated within about 90 min. on cellulose chromatoplates (Fig. 2a), whereas on paper chromatograms (Fig.2b) only an incomplete resolution is achieved.

Solvent 3: In two hours an excellent separation of nucleoside mono-, di- and triphosphates can be effected by CLC, whereas on paper no resolution of nucleoside di- and triphosphates is obtained.

Solvent 4: Comparable separations of nucleotides are achieved within 90 min. (CLC), 150 min. (silica gel TLC), and 6-8 hours (PC). In general, separations are not as distinct as with solvent 3, partly due to some tailing, especially on the silica gel layers. This solvent does not separate guanosine mono-, di- and triphosphate.

R_F-values of nucleobases and nucleosides on cellulose layers (solvent 1) are reported elsewhere (Randerath and Struck, 1961). Table 1 lists the R_F-values of nucleotides (solvents 2-4).

Similar results were obtained with all papers tested. In each case separations were better on cellulose chromatoplates than on paper chromatograms. The rate of movement of the solvent is only of minor importance; separations on extremely "slow" papers (MN 263) are not much better than those on "fast" papers.

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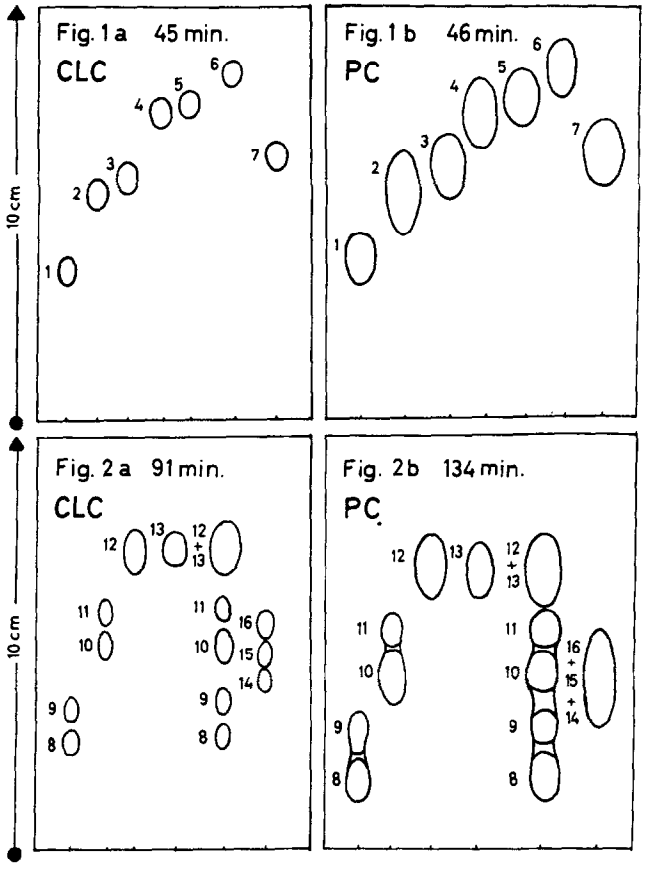
[8] n-Butanol - acetone - acetic acid - 5% aqueous ammonia - water 4,5:1,5:1:1:2 (v/v)



List of Compounds.

1 = Adenine
2 = Adenosine
3 = Hypoxanthine
4 = Inosine
5 = Uracil
6 = Uridine
7 = 6-Chloropurine
8 = 3'-AMP
9 = 2'-AMP
10 = 3'-GMP
11 = 2'-GMP
12 = Cytidylic acids
13 = Uridylic acids
14 = 5'-AMP
15 = ADP
16 = ATP
17 = 5'-CMP
18 = CDP
19 = CTP
20 = 5'-UMP
21 = UDP
22 = UTP
23 = UDP-glucose

Legend to Figures.

Fig.1a, 2a and 3 represent cellulose layer chromatograms (cellulose powder MN 300 G), Fig.1b and 2b are the corresponding paper chromatograms. Papers: Ederol No.202 (Fig.1b), Schleicher u. Schüll No.2043b (Fig.2b). Details in text. The quantities spotted on the starting line were 10^{-2} µmoles of each compound, except for the isomeric mixtures of adenylic and guanylic acids ($1,5 \times 10^{-2}$ µmoles each), and of cytidylic and uridylic acids (3×10^{-2} µmoles each). The compounds are listed above.

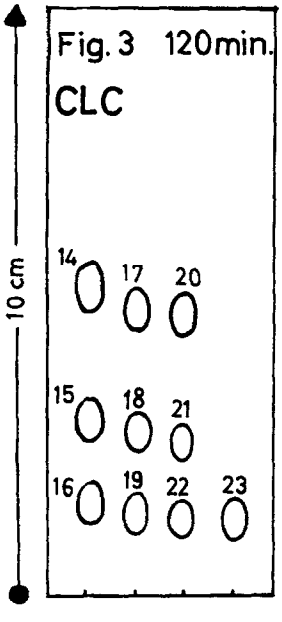


Fig.3

Table 1

R_F-values of nucleotides (CLC)

Compound	Solvents 2	3	4
5'-AMP	0,41	0,52	0,35
ADP	0,48	0,29	0,17
ATP	0,56	0,16	0,08
5'-CMP		0,48	0,30
CDP		0,27	0,13
CTP		0,13	0,07
5'-UMP		0,47	0,30
UDP		0,26	0,14
UTP		0,13	0,08
2'-AMP	0,35		
3'-AMP	0,28		
2'-GMP	0,58		
3'-GMP	0,49		
Uridylic acids	0,73		
Cytidylic acids	0,73		
UDP-glucose		0,12	0,13

It should be mentioned that the weight of the cellulose layer is less than that of the paper sheets (cellulose layer: about 4 mg./cm^2, papers: 8-12 mg./cm^2). The differences between CLC and PC are presumably due to the different microstructures of the cellulose powder and the paper.

Chemically modified cellulose (e.g. ECTEOLA cellulose) may be employed in cases of more complicated separations.

SUMMARY. Solvents are given for the chromatography of nucleobases, nucleosides and nucleotides on cellulose layers. A separation of the four isomeric 2' and 3' purine nucleotides can be achieved in about 90 min. with the Markham and Smith solvent. It is demonstrated that cellulose layer chromatography (CLC) of nucleic acid derivatives is superior to paper chromatography when compared under identical conditions. Analogous results are obtained when CLC and paper chromatography of amino acids are compared (Randerath, 1961c). Thus, the superiority of CLC towards PC may well be a general phenomenon.

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