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This Week's Citation Classic

Randerath K & Randerath E. Ion-exchange chromatography of nucleotides on poly(ethyleneimine)-cellulose thin layers. *J. Chromatography* **16**:111-25, 1964. [J. C. Warren Labs., Huntington Memorial Hosp., Harvard Univ., and Biochem. Res. Dept., Harvard Med. Sch. at Massachusetts General Hosp., Boston, MA]

This paper showed that a great number of naturally occurring ring mononucleotides can be separated and identified by poly(ethyleneimine)-cellulose thin-layer chromatography. R_F data for 33 compounds are given, and the factors are discussed which influence the mobility under different elution conditions. The method is compared with other present techniques for separating nucleotides. [The *SCI*® indicates that this paper has been cited over 280 times since 1964.]

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"In 1961, while working in F. Cramer's laboratory at the Technische Hochschule in Darmstadt, Germany, I extensively used paper and ion-exchange column chromatography to separate nucleotides. Thin-layer chromatography of lipophilic compounds on silica gel had just then become popular. Hypothesizing that ion-exchange thin layers might give more rapid separations of small amounts of nucleotides than paper or columns, I first attempted to prepare thin layers of ion-exchange materials. ECTEOLA- and DEAE-cellulose layers were soon found to provide rapid separations of nucleotides,[1] but the properties of layers of such chemically substituted ion-exchanges varied somewhat from batch to batch. I therefore explored the possibility of preparing anion-exchange thin layers by incorporating defined cationic chemicals into cellulose thin layers, in analogy to the preparation of reversed-phase thin layers by impregnating silica gel with lipids Cellulose layers containing lipophilic amines such as n-tetradecylamine were found to give reproducible nucleotides separations but because of the hydrophobicity of such layers the separations were slow. Hydrophilic amines appeared preferable, therefore, but how could one prevent such compounds from migrating with the aqueous mobile phase during chromatography? The idea occurred to me that hydrophilic amines of sufficiently high molecular weights might be physically trapped in thin-layer powders and I immediately started searching for such a material. Only a single compound, poly-ethyleneimine (PEI, mol. weight 30,000) was commercially available. I remember my disappointment at the low molecular weight of PEI but since the price was only about 4 deutsche marks (then $1), I ordered it anyway. Without knowing that PEI is adsorbed strongly to cellulose (which is the basis for its extensive use in the paper industry), I made a series of cellulose thin layers containing different amounts of PEI.

"I remember my amazement and disbelief when on Saturday, July 28, 1962, I first observed how a mixture of AMP, ADP, and ATP, giving rise to spots smaller than the capital letters in this article, separated completely on a PEI-cellulose thin layer in less than five minutes by development in 1 M NaCl solution. Clearly, this technique had a higher resolving power for nucleotides than any then existing technique.

"During 1963, Erika Randerath and I carried out a systematic study of nucleotide separations on PEI-cellulose thin layers. This work was detailed in this highly cited paper, an earlier version of which had been rejected by another journal. This and our subsequent work on nucleotide separations was done at the Massachusetts General Hospital in Boston where we had moved early in 1963 to join research groups led by H.M. Kalckar and PC. Zamecnik. A review of this work was published subsequently. [2] It is particularly gratifying that the first full paper written after our immigration to the US has become a *Citation Classic*. I think this may be due to the fact that the paper introduced a powerful, yet simple and inexpensive separation technique, which soon became indispensable to many investigators in nucleotide and nucleic acid research."

1. **Randerath K.** Thin-layer chromatography of nudeolides *Angew. Chem. Int. Ed.* **1**:435-9, 1962.
2. **Randerath K & Randcrath E.** Thin-layer separation methods for nucleic acid derivatives. *Meth. Enzymol.* **12A**:.323-47, 1967.