A simple uni-dimensional separation of nucleosides

In work connected with the analysis of nucleosides in pharmaceutical preparations we required a fast separation of adenosine, cytidine, guanosine, inosine and uridine from each other for purposes of subsequent quantitative determination.

On searching the literature twenty-one different separations were found using mainly buffered or alkaline butanol or isoamyl alcohol as partition systems or aqueous ammonia or buffers for adsorption chromatography on paper. However, none of the published separations gave good R_F differences for all five nucleosides, with the possible exception of when piperidine[1] was used, but no R_F value was quoted for inosine in this case.

As we wanted a rather fast separation, it was decided to try to improve on the adsorption systems already published. The direction in which this search was oriented was given by previous work of this laboratory[2], which had indicated that cellulose ion exchangers can function as adsorbents as well as exchangers and that the adsorbtive properties depended on the type and the polarity of the substituents chosen to confer ion exchange properties to the cellulose.

The R_F values of the five nucleosides on a number of ion exchange papers are given in Table I. Excellent separations with five well-defined spots were obtained with diethylaminoethylcellulose paper (Whatman DE-20) using water as solvent as shown in Fig. 1. The nucleosides are visible as dark spots under ultraviolet light. If placed on the paper in dilute HCl a light spot due to chloride ions is noted near the origin.

Resin-impregnated papers retained all the nucleosides strongly, with the exception of the weak base WB-2 paper which, however, did not separate all five nucleosides.

TABLE I

R_F VALUES OF NUCLEOSIDES ON CELLULOSE ION EXCHANGE PAPERS

Paper	*Solvent*	*Adenosine*	*Cytidine*	*Guanosine*	*Inosine*	*Uridine*
Whatman 3 MM (pure cellulose)	Water	0.48	0.32	0.52	0.70	0.77
Whatman AE 30 (aminoethyl-cellulose)	Water	0.61	0.79	0.18	0.15	0.42
Whatman DE 20 (diethylaminoethyl-cellulose)	Water	0.57	0.80	0.20	0.11	0.37
Macherey-Nagel strongly basic anion exchange paper (with quaternary ammonium groups)	Water	0.90	0.92	0.40	0.74	0.68
Whatman CM 50 paper (carboxymethyl-cellulose)	Water	0.64	0.68	0.67	0.86	0.90
Whatman CT 30 paper (cellulose citrate)	Water	0.13	0.15	0.40	0.70	0.85
Whatman P 20 paper (cellulose phosphate)	Water	0.02	0.02	0.04	0.34	0.84
Macherey-Nagel strongly acidic cation exchange paper (with sulphonic groups)	Water	0.15	0.14	0.22	0.46	0.68
Whatman DE 20	0.5 *N* HCl	all spots on the solvent front				
Whatman DE 20	0.5 *N* NH_4OH	0.52	0.77	0.28	0.35	0.69

We believe that the approach used here to achieve a paper chromatographic separation is novel in one respect, namely that usually the solvent mixture is altered to achieve suitable conditions while here we have altered the nature of the support,

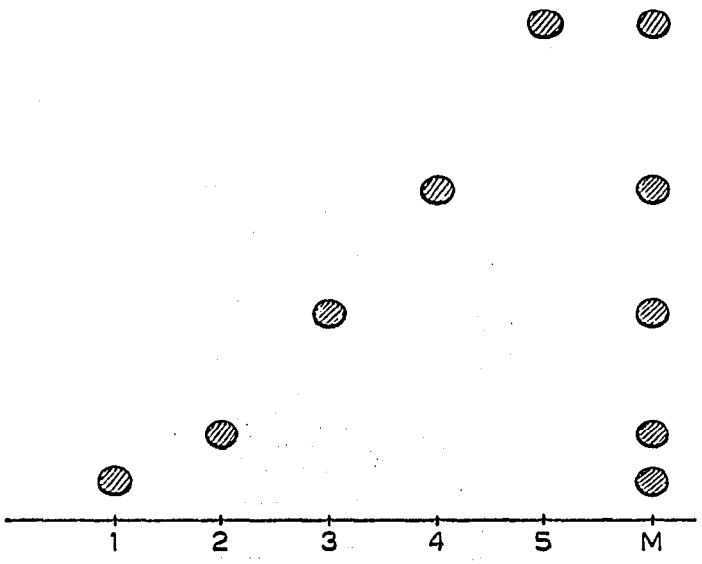

Fig. 1. Chromatogram (schematic) of nucleosides on Whatman DE 20 paper. (1) Inosine; (2) guanosine; (3) uridine; (4) adenosine; (5) cytidine; (M) a mixture of all five compounds.

rather in analogy to gas chromatographic procedures where the stationary phase is usually changed instead of the less important carrier gas.

Work is still in progress for adopting a quantitative procedure after separation and this will be published later.

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