THE CHROMATOGRAPHY OF NUCLEOTIDES, NUCLEOSIDES, AND PYRIMIDINES AND PURINES ON ACTIVATED CHARCOAL

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INTRODUCTION

It was found in certain experiments with radioisotope tracers in this laboratory that a better purification of uracil from radioactive contaminants could be achieved by a gradient elution of the uracil from charcoal. Further experiments demonstrated that this type of elution was applicable to other compounds of this type. Activated charcoal has been used as an adsorbent for pyrimidines and purines by numerous investigators[1–4]. However, no attempt was made to elute the adsorbed compounds selectively from the charcoal in those experiments. This paper describes a method for eluting the pyrimidines, purines, and their nucleosides and nucleotides selectively by a gradient elution procedure. The method is presented to show the feasibility of the chromatography of these compounds on activated charcoal. By judicious variation of the solvents, one may conceivably obtain better resolution of the adsorbed compounds.

MATERIALS

1. *Charcoal*

40 × 60 mesh Darco charcoal was used as the adsorbent in all of the experiments. This was made by grinding and sifting a larger particle size (20 × 40 mesh) Darco charcoal, and collecting only that fraction which goes through a 40 mesh sieve, but not through a 60 mesh sieve. This 40 × 60 mesh charcoal is then sedimented repeatedly in distilled water, each time sucking off the fine material, which fails to settle rapidly, with a water aspirator. This removes the fine charcoal particles which invaribly clog the column if not removed. The larger particles, which sediment rapidly, are allowed to stand 48 h in conc. aqueous HN_3–ethanol–H_2O (5:1:13, v/v/v) with one change of solvent. The charcoal is then washed repeatedly with distilled water until the water wash is neutral to litmus. The charcoal is stored in distilled water until ready for use. It should be mentioned that this charcoal is brittle, and care should be taken to avoid crushing the particles in this procedure.

2. *Solvents*

Gradient elution of the adsorbed compounds was carried out with aqueous NH_3, ethanol, *n*-propanol, and *n*-butanol. Reagent grades of concentrated ammonia and

ethanol were found to have a sufficiently low optical density in the range 240 mμ to 340 mμ without further purification. However, reagent grades of *n*-propanol and *n*-butanol were found to contain considerable amounts of aldehyde impurities, which absorb light in the wavelength range 240 mμ to 340 mμ. It was found that these aldehydes can be reduced to alcohols by treatment with sodium borohydride. The reduction is carried out by letting 400 ml of the alcohol and 6 g of sodium borohydride stand for 72 h in a cool place. During this time the mixture is swirled occasionally. Finally, the alcohol is distilled under vacuum in a flash evaporator at 40°. The results of such a treatment are shown in Table I.

TABLE I

OPTICAL DENSITY OF *n*-PROPANOL AND *n*-BUTANOL COMPARED WITH WATER

Wavelength (mμ)	*Optical density of*			
	Reagent n-propanol	*Reagent n-propanol treated with $NaBH_4$*	*Reagent n-butanol*	*Reagent n-butanol treated with $NaBH_4$*
220	1.58	0.481	∞	1.92
230	1.11	0.075	∞	0.750
240	0.542	0.024	∞	0.264
250	0.376	0.021	1.73	0.065
260	0.528	0.030	0.890	0.039
270	0.668	0.023	0.482	0.023
280	0.672	0.004	0.218	0.001
290	0.590	0.002	0.102	0.000
300	0.348	0.000	0.057	0.000
320	0.043	0.000	0.025	0.000

It was found that these two alcohols were sensitive to oxidation on standing. Therefore, it is necessary to flush them free of oxygen by bubbling nitrogen gas through the alcohol, and to store them in brown bottles. When stored in this way, the alcohols retain their low optical density for several months.

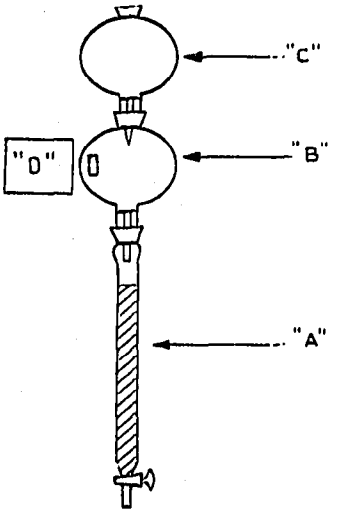

Fig. 1. Apparatus used for charcoal chromatography.

3. *Apparatus*

This consisted of a chromatography tube "A" and two 400 ml flasks, "B" and "C", all connected with ground glass joints as shown in Fig. 1. Flask "B" was stirred continuously with a magnetic stirrer "D". The solvent from flask "C" flowed through a fine capillary tip into flask "B".

PROCEDURE

The compounds were adsorbed on a 1.5 × 15 cm column of Darco charcoal (40 × 60 mesh) in tube "A" by slowly running (0.25 ml/min) an acidic solution (pH 2.0) of the compounds through the column. The column was then washed with distilled water

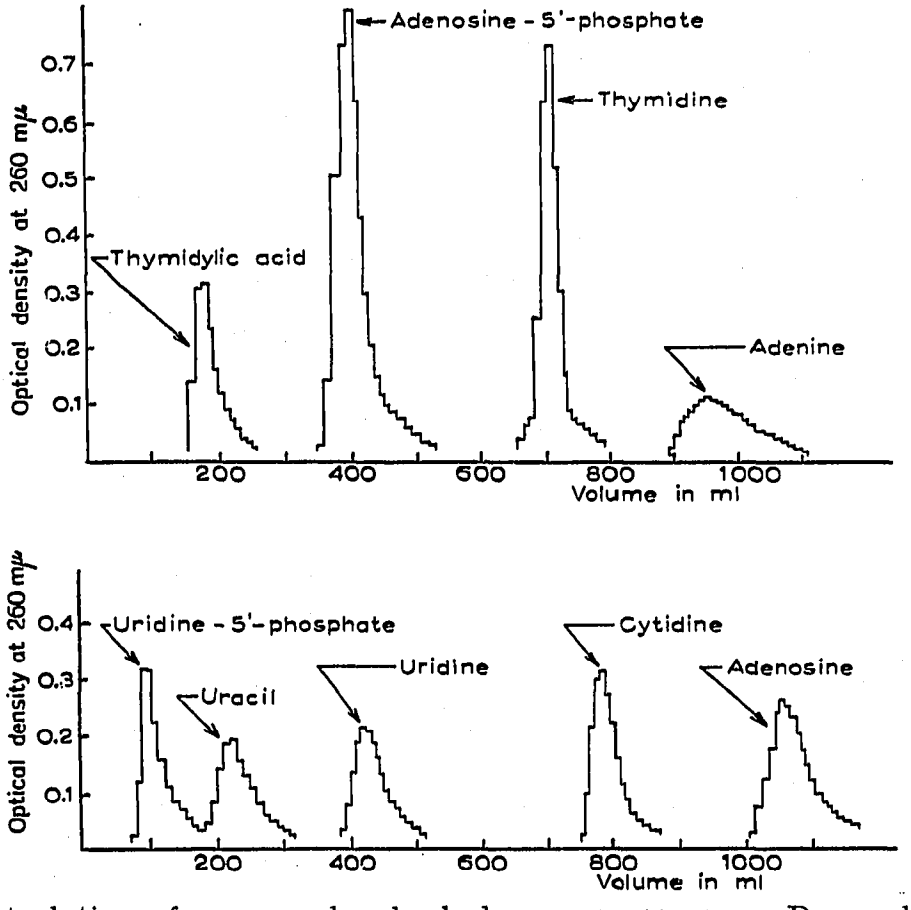

Fig. 2. Gradient elution of compounds adsorbed on a 1.5 × 15 cm Darco charcoal column.

until the water wash was neutral to litmus. The elution of the adsorbed compounds was carried out as follows.

A volume of 16 ml of water was added above the charcoal column in tube "A", and a volume of 405 ml of water was added to the mixing flask "B". The volumes and concentrations of solvents added to the top flask "C" were as follows (the solution in the mixing flask "B" was not changed between new solvents):

First 300 ml: conc. aqueous NH_3–ethanol–H_2O (5:1:13, v/v/v)

Second 300 ml: conc. aqueous NH_3–ethanol–H_2O (5:5:13, v/v/v)

Third 300 ml: conc. aqueous NH_3–*n*-propanol–H_2O (16:11:26, v/v/v)

Fourth 300 ml: conc. aqueous NH_3–*n*-propanol–*n*-butanol–H_2O (14:12:7:21, v/v/v/v)

A flow rate of 0.25 ml/min was used throughout the elution.

RESULTS

The elution peaks for some pyrimidines and purines and their nucleosides and nucleotides are shown in Fig. 2. The percentage recovery after adsorbing a known quantity of the compound on the charcoal was calculated from the area under the curve and the ε_{260} value for the compound in alkaline solution. The precentage of recovery for each compound is shown in Table II.

TABLE II

RECOVERY OF COMPOUNDS FROM A 1.5 × 15 cm CHARCOAL COLUMN

Compound	*Micromoles adsorbed*	*% Recovery*
Uridine-5′-phosphate	2	88
Uracil	3	98
Uridine	2	97
Cytidine	3	77
Adenosine	2	72
Adenine	2	73
Thymidylic acid	3	65
Adenosine-5′-phosphate	3	95
Thymidine	3	99

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SUMMARY

A method has been described for the chromatography of purines, pyrimidines, and their nucleosides and nucleotides on activated charcoal. Spectrophotometric grades of *n*-butanol and *n*-propanol, used in the elution procedure, were prepared by treatment of the alcohols with sodium borohydride. In our laboratory charcoal chromatography proved more effective than other methods for purifying the acid-soluble uracil from rat liver slices in ^{14}C-radioisotope experiments.

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