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THE SEPARATION OF PURINE AND PYRIMIDINE BASES AND OF NUCLEOTIDES BY ION-EXCHANGE

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Waldo E. Cohn

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THE SEPARATION OF PURINE AND PYRIMIDINE BASES AND OF NUCLEOTIDES BY ION-EXCHANGE

Waldo E. Cohn

As a prelude to tracer studies on the enzymatic formation and degradation of nucleic acids, we have undertaken to develop ion-exchange techniques for the separation and analysis of the various bases, nucleosides and nucleotides at the milligram level. Although this technique is finding increasing application in biochemical studies for separations of these (8, 9) and other (1, 2, 6, 7) compounds, we were led independently to its use by virtue of our earlier experience with radioisotope separation by ion-exchange chromatography (4, 11). The present note describes briefly those methods which we have developed for the separation of each of the purine and pyrimidine bases and of the ribose nucleotides.

Of the five naturally occurring purine and pyrimidine bases, three (cytosine, guanine and adenine) exist as cations in solution of pH 4 and hence are able to combine with cation-exchangers. Since their affinities for the cation-exchanger differ, they may be eluted successively with such simple reagents as HCl or NaCl; this is demonstrated in Fig. 1. This procedure does not separate the nonionized bases, thymine and uracil. Since these will not usually be encountered together, no disadvantage attaches to this fact, but it is easy to separate them, as well as the other three substances, by resorting to anion-exchangers* and alkaline solutions as shown in Fig. 2.** Recoveries by both procedures, based on spectrophotometric absorption in the ultraviolet (260 - 265 mμ) are essentially quantitative. Identification of components was made by the shape of the ultraviolet absorption curve and by partition chromatography (3).***

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* The Dowex-A1 anion-exchanger, a strong base, was kindly furnished us by Dr. L. Matheson of the Dow Chemical Company, Midland, Michigan.

** These experiments were carried out by Mr. J. X. Khym.

*** We are indebted to Dr. C. E. Carter not only for the chromatographic analyses but also for continued encouragement and assistance during the course of these experiments.

Figure 1

Separation of Purine and Pyrimidine Bases by Cation-Exchange.

Exchanger: 8.1 cm. x 0.74 $cm.^2$ Dowex-50, ca. 300 mesh, H^+ form.

Eluting Solution: 2 N-HCl at 0.6 ml./min.

Test Material: 0.5 mg. Uracil, 1.0 mg. each of adenine, guanine, cytosine, in 7.5 ml. 2 N-HCl.

Recoveries: 98.5 - 101.5% (based on optical density at 260 mμ).

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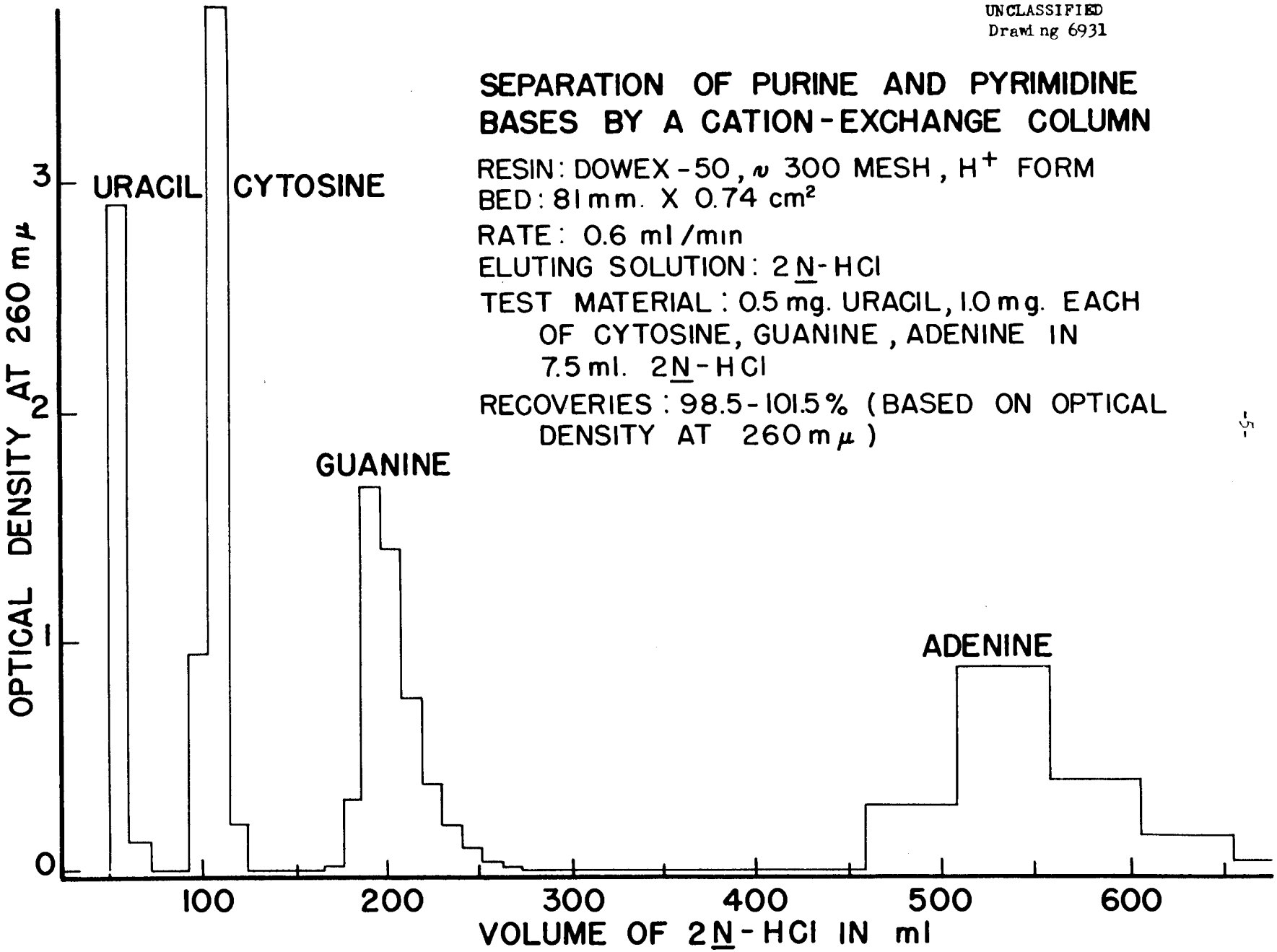


Figure 2

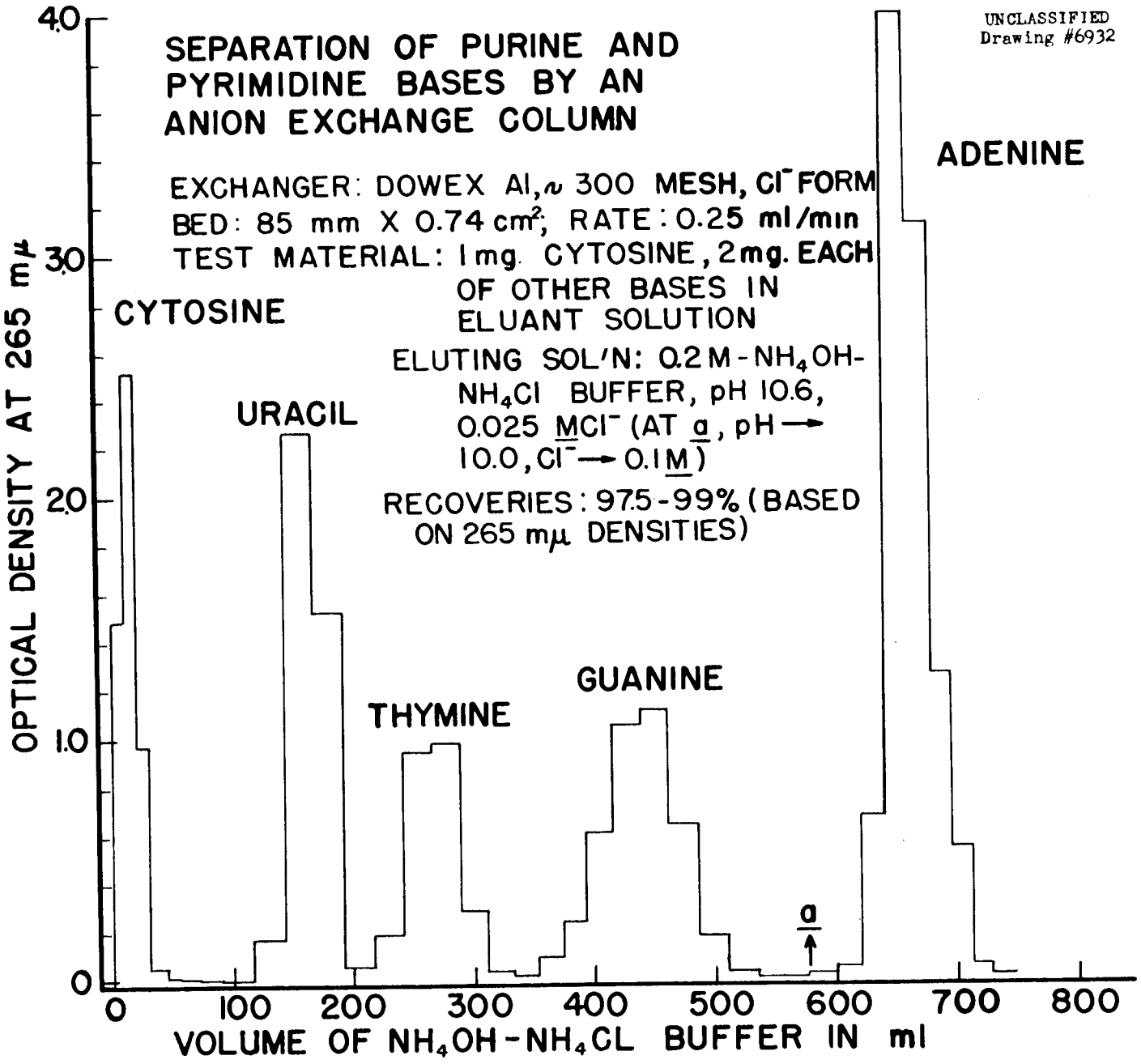
Separation of Purine and Pyrimidine Bases by Anion-Exchange.

Exchanger: 8.5 cm. x 0.74 $cm.^2$ Dowex A1, ca. 300 mesh, Cl^- form.

Eluting Solution: 0.2 M-NH_4OH-NH_4Cl Buffer, pH 10.6, 0.025 M-Cl^- (at a, pH changed to 10.0, Cl^- to 0.1 M), at 0.25 ml./min.

Test Material: 1 mg. cytosine, 2 mg. each of other bases, in 10 ml. eluting solution.

Recoveries: 97.5 - 99% (based on optical density at 265 mμ).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Drawing #6932
SEPARATION OF PURINE AND PYRIMIDINE BASES BY AN ANION EXCHANGE COLUMN
EXCHANGER: DOWEX A1, ~ 300 MESH, Cl⁻ FORM
BED: 85 mm X 0.74 cm²; RATE: 0.25 ml/min
TEST MATERIAL: 1 mg. CYTOSINE, 2 mg. EACH OF OTHER BASES IN ELUANT SOLUTION
ELUTING SOL'N: 0.2 M-NH4OH-NH4Cl BUFFER, pH 10.6, 0.025 MCl⁻ (AT a, pH → 10.0, Cl⁻ → 0.1 M)
RECOVERIES: 97.5-99% (BASED ON 265 mμ DENSITIES)
CYTOSINE
URACIL
THYMINE
GUANINE
ADENINE
a
OPTICAL DENSITY AT 265 mμ
4.0
3.0
2.0
1.0
0
0
100
200
300
400
500
600
700
800
VOLUME OF NH4OH-NH4CL BUFFER IN ml

The nucleotides of yeast nucleic acid (uridylic, cytidylic, guanylic and adenylic acid) exhibit to some degree the basic properties of the purine or pyrimidine constituent; this is modified by both size and the negative phosphate group with the result that the exchanger-to-ion bond is considerably weaker. Weak acids have been found to be the most practical eluting agents and a separation by means of dilute acetic acid is demonstrated in Fig. 3. Recovery of the starting material, based upon absorption at 260 mμ, varies from 85 to 100%. In the absence of pure starting materials (the commercial preparations showing 5 - 50% impurity by these methods), it is difficult to do better than this without an extensive series of preparations of pure compounds. It is possible that there is a significant amount of acid hydrolysis of purine nucleotides occurring during their stay in the exchanger bed but we have not been able to prove this conclusively. Regardless of this possibility, the method as it stands will isolate the bulk of each constituent in spectrophotometrically pure form.

Concentration and metathesis of the large volumes collected in the separations is easily achieved (5, 10), where evaporation is undesirable, by recycling the effluent through the same column, washing with water to remove residual acid or base, then eluting with a reagent which removes the adsorbed ion rapidly (e.g., NH_4OH in the acid separations).

Although the separations reported are all at or near the milligram level, we have been able to effect practical separations on the same size column on up to nearly 100 mg. amounts. The lower limit is, of course, set by the limit of detection. At the higher levels, overlapping of bands becomes more prominent due to the relationship between the concentration of an ion and its distribution coefficient (12).

The details of these and related separations, with examples of their application to specific problems, will be reported elsewhere.

Figure 3

Separation of Mononucleotides of Yeast Nucleic Acid by Cation-Exchange.

Exchanger: 24 cm. x 0.74 $cm.^2$ Dowex-50, ca. 300 mesh, H^+ form,

Eluting Agent: 0.1 M acetic acid at 0.15 ml./min.

Test Material: 100 mg. mixed nucleotides, recovered from their Ba salts following 0.6 N-$Ba(OH)_2$ hydrolysis of yeast nucleic acid (conversion of nucleic acid to mononucleotides not quantitative), in 10 ml. 0.1 M acetic acid.

Recoveries: 85 - 100% (based on optical density at 260 mμ).

Cross-contaminated Material: 0.25% at A, 0.4% at B, 1.0% at C.

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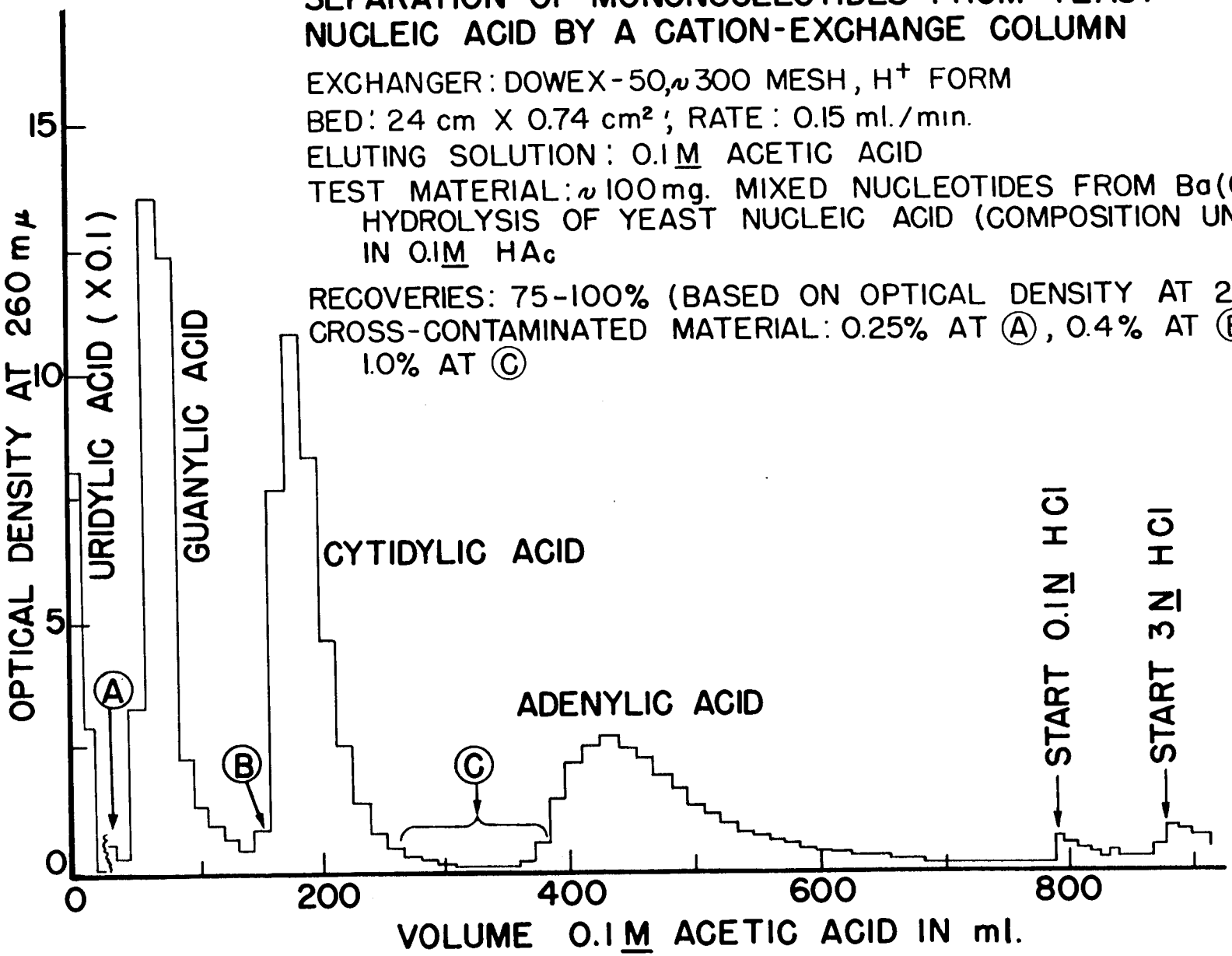
Drawing #6933

SEPARATION OF MONONUCLEOTIDES FROM YEAST NUCLEIC ACID BY A CATION-EXCHANGE COLUMN

EXCHANGER: DOWEX-50, ~300 MESH, H^+ FORM
BED: 24 cm X 0.74 cm^2; RATE: 0.15 ml./min.
ELUTING SOLUTION: 0.1 M ACETIC ACID
TEST MATERIAL: ~100 mg. MIXED NUCLEOTIDES FROM $Ba(OH)_2$ HYDROLYSIS OF YEAST NUCLEIC ACID (COMPOSITION UNKNOWN) IN 0.1 M HAc
RECOVERIES: 75-100% (BASED ON OPTICAL DENSITY AT 260 mμ)
CROSS-CONTAMINATED MATERIAL: 0.25% AT Ⓐ, 0.4% AT Ⓑ, 1.0% AT Ⓒ



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