Use of Dyes for Better Reliability in a Microbiological Assay

Keyphrases ☐ Microbiological assay—seed layer dye ☐ Dye incorporation—agar plate seed layer ☐ Seed layer detection—dye method

Sir:

Microbiological assays by agar plate methods use a seed layer containing the test organism. This seed layer is usually poured onto a base layer, which has been solidified. Under normal conditions it is difficult to observe whether a particular plate has received the seed layer or not. To solve this problem, food dyes were evaluated for coloring the seed layer to permit visual assurance that a seed layer has been added to each plate.

FD & C Blue No. 1[1] was used for coloring the seed layer in penicillin assays. A 0.1% stock solution was prepared in sterile distilled water and used 1 ml./100 ml. of seed layer. The assays were done by the standard FDA procedure[2] except that all six cylinders in each of the three plates was filled with 0.05 unit/ml. of potassium penicillin G. Three plates without dye in the seed layer served as control. The results are given in Table I. Assays were performed by using *Sarcina lutea* ATCC 9341 as the test organism.

Table I—Effect of Blue Dye in Seed Layer on Zone Size in Penicillin Assay.

Plate No.	Zone Size, mm.[a] Normal Seed Layer	Zone Size, mm.[a] Seed Layer with Blue Dye
1	19.00	18.66
2	18.33	18.83
3	19.00	19.33
Av.	18.77	18.94

[a] Average of six zones per plate.

The zones obtained with dye in the seed layer are quite comparable with those obtained without dye. Moreover, the plates containing dye are a distinct blue, thus ensuring the presence of seed layer on top of the base layer. Finally, we found that the zones in the colored and uncolored plates were equally sharp. Colored seed layers should be useful in other microbiological assays; these are under evaluation.

S. G. Pathak
Davis R. Reese
Department of Microbiology
Philadelphia Pharmaceuticals and Cosmetics, Inc.
Philadelphia, PA 19131

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[1] H. Kohnsramm & Co., Inc.
[2] Procedures for detecting and measuring penicillin contamination in drugs, Department of Health, Education and Welfare, Food and Drug Administration, Bureau of Scientific Standards and Evaluation, Division of Antibiotics and Insulin Certification, Washington, D. C., October 1965.

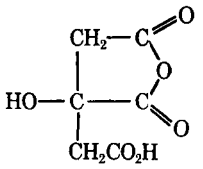
Synthesis and Isolation of Citric Acid Anhydride

Keyphrases ☐ Citric acid anhydride—synthesis, isolation ☐ NMR spectroscopy—structure

Sir:

We wish to report the synthesis, isolation, and characterization of a new crystalline derivative of citric acid. The compound which apparently has a melting point of 121–123° has been identified as the monomolecular unsymmetrical anhydride of citric acid. Several compounds are mentioned in *Chemical Abstracts* as citric anhydride, but upon further inquiry, it is found that these compounds are actually dehydrated citric acid, which is aconitic acid, or the anhydrous crystalline form of citric acid. Transient formation of a true anhydride of citric acid in aqueous solution was suggested (1–3) earlier but never isolated.

The method of preparation which has been found feasible in converting citric acid to its anhydride is based on interacting the solid acid with an excess of acetic anhydride. The critical aspect of the procedure is in controlling the reaction conditions so that neither acylation nor dehydration involving the alcohol group occurs. We have obtained relatively good yields by suspending finely powdered anhydrous citric acid in an excess of acetic anhydride in acetic acid and heating at 36–38° with good stirring. Based on its apparent equivalent weight as an acid in water, its NMR spectrum, elemental analysis, and other physical evidence, the crystalline anhydride recovered from the reaction mixture has the structure shown below (I).



I

The anhydride reacted readily with aniline to yield the expected monoanilides and hydrolyzed in water to yield citric acid. Further details on its chemistry will be presented later.

Since citric acid is so widely used in the food, drug and chemical industries, we feel that the anhydride may find similar utility. Some of the more apparent