Each unit weighs about 8 lb. and can be constructed in most laboratory shops for a cost of less than $12. for materials. The only similar cages that we have found in use were those reported by Moos, *et al.*(1), where plastic cages for solitary housing of mice for irradiation and longevity experiments were described. A photograph of the present cages, but no description of the units, appeared previously in a report by Burch (2). The caging units have been in use in our laboratory for about 3 years and have become an indispensable laboratory item.

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Circular Thin-Layer Chromatography of Tetracyclines

By GOVIND J. KAPADIA and G. SUBBA RAO

A simple circular thin-layer chromatographic method is described for the separation of hydrochlorides of tetracycline, oxytetracycline, and chlortetracycline employing three sequestering agents—viz., disodium ethylenediaminetetraacetate, tartaric acid, and oxalic acid. Better resolution has been achieved by the use of disodium ethylenediaminetetraacetate in the stationary phase and *n*-butanol saturated with water as the mobile phase.

ALTHOUGH PAPER PARTITION chromatography of tetracyclines has been reported by several workers (1–10), thin-layer chromatography (TLC) of these antibiotics has only been recently investigated. Nicolaus, Coronelli, and Binaghi (11) have studied the chromatography of several tetracyclines with various solvent mixtures by ascending TLC. They were, however, unsuccessful in resolving more than two tetracyclines by a single solvent system on one chromatogram.

The property of tetracyclines of forming chelate complexes with metallic ions suggests that such complexation might occur between these antibiotics and the metallic ions of the adsorbent and/or calcium sulfate added as the binder. In such a case, sequestering agents possibly could effect the separation of these compounds. The use of disodium ethylenediaminetetraacetate (EDTA) and oxalic acid has been promising in the chromatography of certain naturally occurring polyphenolic compounds that showed strong chelating property (12). These sequestering agents might also be employed in the resolution of tetracyclines by TLC.

This report presents the results of preliminary work in which three sequestering agents—*viz.*, EDTA, tartaric acid, and oxalic acid were used—and the separation of hydrochlorides of tetracycline, oxytetracycline, and chlortetracycline was studied. For the development of chromatograms the circular TLC technique of Bryant (13) was modified. The separation of the three antibiotics was achieved by employing all the three sequestering agents, although better separation was accomplished with EDTA.

EXPERIMENTAL

Apparatus.—The Desaga-Brinkmann apparatus for TLC (applicator model "S II") distributed by Brinkmann Instruments, Inc., Great Neck, Long Island, N. Y., was used for the preparation of plates. Glass plates (20 × 20 cm.) with 1/8 in. hole drilled at the center of each were utilized. Pyrex Petri dishes (diameter—14 cm., height—2.5 cm.) were employed as the solvent containers for the development of chromatograms. The adsorbent used was silica gel G (made according to Stahl by Merck, A. G., Darmstadt, Germany, and distributed by Brinkmann).

Preparation of Plates.—*Technique A.*—A slurry of 30 Gm. of silica gel G and 60 ml. of water was made in a dry mortar. This was poured into the applicator and coated over five plates which were then dried at room temperature for 15 minutes. The plates were further activated in an oven at 105–110° for 1 hour and stored in a vacuum desiccator. The adsorbent layer was kept constant at standard 250 μ thickness.

Technique B.—Nine grams of EDTA was dissolved in 60 ml. of water and, using 30 Gm. of silica

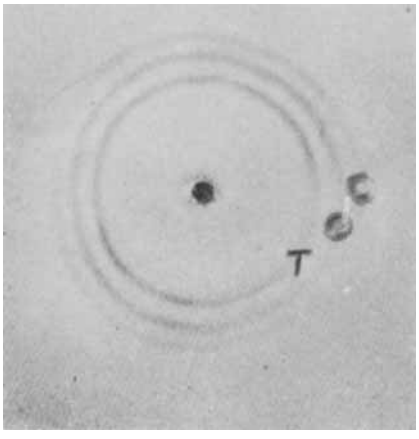

Fig. 1.—Separation of tetracycline (*T*), oxytetracycline (*O*), and chlortetracycline (*C*) hydrochlorides on silica gel G plate admixed with disodium ethylenediaminetetraacetate, using *n*-butanol saturated with water as the solvent.

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TABLE I. — R_f VALUES

Antibiotic	Solvent System BW/EDTA	BTW	BOW
Tetracycline HCl	0.23	0.26	0.38
Oxytetracycline HCl[a]	0.27	0.31	0.46
Chlortetracycline HCl	0.30	0.35	0.49

[a] Oxytetracycline base also resolved from the mixture of tetracycline and chlortetracycline hydrochlorides with the same average R_f value as that of its hydrochloride.

gel G, plates were prepared as in *Technique A*. In this case the plates were dried at room temperature for 30 minutes before placing in an oven.

Solvent Systems.—The organic phases of the following mixtures were used as the mobile solvents: *n*-butanol–oxalic acid–water (100 ml.: 5 Gm.: 100 ml.)—BOW, *n*-butanol–tartaric acid–water (100 ml.: 6 Gm.: 100 ml.)—BTW, and *n*-butanol saturated with water—BW.

The plates prepared by *Technique A* were developed by solvent systems BOW and BTW, those by *Technique B* were developed by solvent system BW.

Sample Application.—Any adhering adsorbent in the central hole of the plate was cleared by piercing through with a capillary. A solution of equal quantities of the hydrochlorides of the three tetracyclines in methanol was applied around the circumference of the hole using a lambda pipet so that 20 mcg. of each compound was deposited.

Development Technique.—A paper "wick" (length—$1^1/_8$ in., diameter—$^1/_8$ in.) was made by rolling a piece of Whatman No. 1 filter paper ($2^1/_2 \times$ 1 in.). This was inserted in the hole at the adsorbent side of the plate. The plate was then placed adsorbent side down over a Petri dish containing 50 ml. of developing solvent so that the "wick" dipped in it and the plate hole became the center of the dish. The hole on the clear side of the plate was covered with a vial. The movement of the solvent front was clearly visible; when it reached 6.5 cm. from the center, the plate was removed.

Detection.—The antibiotics were revealed on the chromatograms by spraying the plates with 5% methanolic solution of ferric chloride (4). The reagent gave dark grayish bands with each compound. Before spraying with ferric chloride, three yellowish-green fluorescent bands were visible under ultraviolet light. These bands were located at the same position where colored bands were seen after spraying with the chromogenic reagent. Identity of the bands was achieved by individually chromatographing each antibiotic.

In the chromatograms in which solvent systems BTW and BOW were employed, marked circular portions were observed. These were due to the organic acids used in the solvent mixtures as revealed by spraying 0.5% ethanolic Congo red solution. Blue circular areas of average radii 3.5 cm. and 4.5 cm. were seen with BTW and BOW systems, respectively, when average solvent movement was 6.5 cm. from the center. When sprayed with ferric chloride solution, these areas were a lighter yellow compared to the rest of the plate.

RESULTS

Figure 1 shows the separation of the three tetracyclines using BW solvent system and EDTA in the adsorbent. Table I lists the average R_f values of the compounds obtained with all the three solvent systems. The values were highest with BOW and lowest with BW/EDTA. In these two cases the differences in R_f values of oxytetracycline and chlortetracycline were the same. However, better separation was achieved with BW/EDTA where the bands obtained were sharp. With BOW the bands were broad and some spreading occurred. As a result of this the resolution was not as clear, although each of the two separated distinctly from the tetracycline. The spreading of the bands with BTW was much less, and a satisfactory separation of the antibiotics was observed. Comparison of chromatograms indicated that a better resolution of the compounds was achieved using BW/EDTA.

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