Separation of Tabletted Mixtures of Barbiturates, Aspirin, Phenacetin, Caffeine, Codeine, and Quinine by Ion-Exchange Paper Chromatography

By HAROLD V. STREET and S. K. NIYOGI

The resolution of a mixture of tablet fragments containing amobarbital, acetylsalicylic acid, acetophenetidin (phenacetin), caffeine, codeine, and quinine into its constituent parts by application of chromatography on modified cellulose ion-exchange papers is described. Horizontal circular and ascending cylindrical chromatography is employed. A complete qualitative analysis takes about 4 hours.

MODIFIED CELLULOSE ion-exchange paper sheets were first used for chromatography by Knight (1, 2) who described their use for the separation of mixtures of amino acids and of mixtures of metallic cations. Street and Niyogi (3–5) have described the use of these modified cellulose papers for chromatographic and ionophoretic separations of various toxic compounds.

Because chromatography on ion-exchange papers with aqueous "solvents" gives rise to a very rapidly moving "solvent" front, it was thought the procedure might be useful for a rapid identification of some of the tablets which are commonly encountered in toxicological analysis. In this type of work, it is necessary for the toxicologist to carry out an analysis of any fragments of tablets which may be found in, say, stomach contents or cups and drinking glasses.

The types of tablets most frequently encountered in toxicological analysis are those containing barbiturates or salicylates. These tablets may, in addition, contain acetophenetidin (phenacetin), caffeine, codeine, and quinine. Using mixtures containing equal proportions of these compounds, it is a relatively easy matter to separate and identify the components by chromatography on ion-exchange paper. However, in many of the tablets, the proportion of one compound to another is such that swamping of the minor component occurs. For example, in tablet A,[1] the ratio of acetylsalicylic acid to quinine is 48 to 1, so that if a chloroform extract of the tablet is applied directly to the paper, the quinine cannot be seen because of the presence of such a relatively large amount of salicylate which spreads over a large area of the paper and obliterates the quinine spot. If the amount applied is reduced so that the salicylic acid spot is of "normal" size, then the amount of quinine is too small to be detected.

When acetylsalicylic acid and quinine are present in equal amounts, no difficulty is encountered. It follows, therefore, that in order to detect the presence of quinine in tablet A, some preliminary differentiation procedure must be used, such as extraction with alkali to remove acetylsalicylic acid. This procedure will, of course, hydrolyze the aspirin to salicylic acid which, however, is easily recognized on the chromatogram by its bright blue fluorescence in 254 mμ light.

It has been found that by extraction of tablet fragments with chloroform and then extracting this chloroform with alkali, the two groups so obtained, namely neutral and acidic, can be applied to an anion-exchange paper and the various components identified. Quinine and codeine can be identified in the residue by chromatography on a cation-exchange paper. Horizontal circular chromatography as described by Kawerau (6) has been used with hand-cut, slotted, Whatman diethylaminoethyl cellulose ion-exchange paper, together with a simultaneous ascending "run" on Whatman cellulose citrate ion-exchange paper. The following account is a description of the procedures carried out for the identification of the compounds referred to above.

It is important to note that the work described here refers to modified cellulose ion-exchange paper and not to paper impregnated with ion-exchange resins.

EXPERIMENTAL

Materials and Reagents

Horizontal circular chromatography apparatus manufactured by Shandon Scientific Co., Cromwell Place, London, England. Whatman DE20 anion-exchange modified cellulose paper; the sheets were cut into circular disks of 26 cm. diameter, slots were cut as described by Kawerau (6). Whatman CT30 cation-exchange modified cellulose paper.

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[1] Anadin (International Chemical Co., Ltd.) tablets contain in each: aspirin, 4 gr.; phenacetin, 2 gr.; caffeine, 1/4 gr.; and quinine sulfate, 1/12 gr.

Ultraviolet lamp emitting 254 mμ radiation. Chloroform. Sodium hydroxide, 2.5% (w/v) and 10% (w/v) aqueous solutions. Hydrochloric acid, 5 *N* solution. Ammonia, 0.2 *N* and concentrated solutions. EDTA, 0.1 *M* solution of the disodium salt of ethylenediaminetetra acetic acid. Iodoplatinate reagent; mix together 5 ml. of 5% (w/v) platinic chloride, 45 ml. of 10% (w/v) potassium iodide, and 100 ml. of water.

Procedure

Extraction.—A mixture of one-fifth of a tablet of tablet A, one-fifth of a compound tablet of codeine B.P., and 10 mg. of amobarbital was ground up in a mortar with 2 ml. of chloroform, and filtered. The mortar was rinsed with a further 2 ml. of chloroform which was poured through the filter. The filtrate was shaken vigorously with 0.5 ml. of 2.5% sodium hydroxide solution and the mixture centrifuged. The upper alkaline layer (solution I) was removed and set aside for chromatography. The chloroform layer was washed first with 4 ml. of water and then with 0.5 ml. of 5 *N* hydrochloric acid solution, and the mixture was centrifuged. These washings were discarded. The washed chloroform layer containing acetophenetidin and caffeine was set aside for chromatography (solution II).

The residue of the tablet fragments, after chloroform extraction, was shaken with 10 ml. of 10% sodium hydroxide solution and filtered. The filtrate was extracted by shaking with 10 ml. of chloroform. A 1-ml. portion of the separated chloroform layer was taken to dryness and redissolved in a drop or two of chloroform. This solution was used for chromatography (solution III).

Chromatography.—Solutions I and II were applied to two separate sectors of a circular sheet of Whatman DE20 ion-exchange paper. Chromatography was carried out in 0.2 *N* ammonia solution for 105 min. and the paper examined in 254 mμ light. The results of such a run are illustrated in Figs. 1 and 2.

Solution III was subjected to ascending chromatography on Whatman CT30 ion-exchange paper in 0.1 *M* EDTA for 18 minutes. The paper was examined, while wet, in 254 mμ light; it was then dried and stained with the iodoplatinate reagent to reveal the positions of the alkaloids. The results of this procedure are shown in Fig. 3.

DISCUSSION

The results show that with the DE20 paper, using dilute ammonia as solvent, barbiturate and salicylate can be separated in the alkali extract. Identification of the particular barbiturate can be carried out by the methods of Street (7, 8). Acetophenetidin and caffeine, both of which are present in the residual chloroform solution after alkali and acid extraction, can also be resolved on DE20 paper. Because there is a large amount of salicylate present in the tablet fragments, this compound is also seen in the "neutral" fraction. It does not interfere and is easily distinguished by its bright blue fluorescence in 254 mμ light.

It is interesting to note that in spite of the presence of a relatively large amount of acetophenetidin, this does not interfere with the resolution of acetophenetidin and caffeine in the "neutral" fraction. This

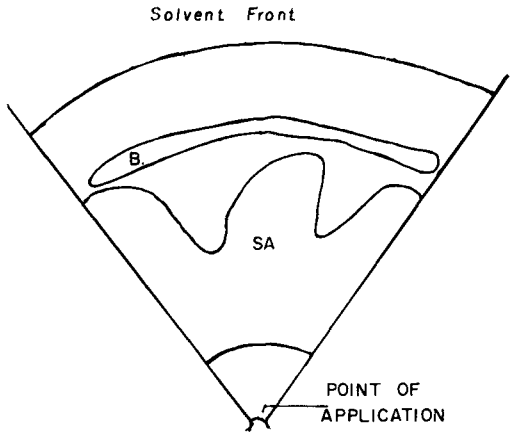

Fig. 1.—Horizontal circular chromatography of solution I on diethylaminoethyl cellulose ion-exchange paper in 0.2 *N* ammonia. Time of run, 105 min. SA, salicylate (blue fluorescent area); B, barbiturate (dark-purple absorbing area). Compounds revealed by inspection in 254 mμ light.

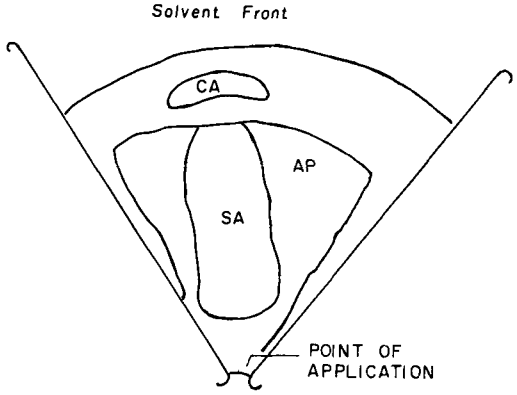

Fig. 2.—Horizontal circular chromatography of solution II on diethylaminoethyl cellulose ion-exchange paper in 0.2 *N* ammonia. Time of run, 105 min. SA, salicylate (blue fluorescent area); AP, acetophenetidin (dark-purple absorbing area); CA, caffeine (dark-purple absorbing area). Compounds revealed by inspection in 254 mμ light.

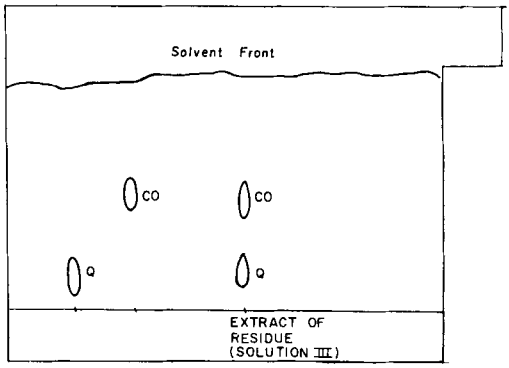

Fig. 3.—Ascending cylindrical chromatography of solution III on cellulose citrate ion-exchange paper in 0.1 *M* EDTA. Time of run, 18 minutes. Q, quinine; CO, codeine. Compounds revealed by dipping the paper in the iodoplatinate reagent.

means that it is possible to apply a large amount of this fraction to the paper.

Codeine and quinine are extracted by chloroform from the residue of tablet fragments after addition of alkali. These two alkaloids are clearly resolved on CT30 paper in about 20 minutes. Some quinine is also present in the initial chloroform extract of the tablets but is removed from the "neutral" fraction by washing with dilute acid.

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Diffusion of Sodium Salicylate and Salicylic Acid within Hydrophilic Ointments

Measurement with a New Diffusion Cell

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A cell has been designed to measure diffusion of medicaments within dermatologic vehicles to eliminate the influence of an external medium which differs from the vehicle. The cell consisted of two Lucite plastic compartments separated by a cellophane membrane. A medium containing a medicament was placed in one compartment and diffusion occurred into the other compartment which contained the same vehicle without the medication. The cell was used in a study of the diffusion of sodium salicylate and salicylic acid in various components of hydrophilic ointments made with three nonionic surface-active agents: Tween 40, Atlas G-7596-J, and Brij 35.

IN VITRO investigations of the efficiency of medicated dermatologic vehicles have usually utilized a system in which the drug diffuses from the vehicle into a dissimilar medium. Events which occur in this external medium provide the data for the evaluation of the vehicle. Workers in this field have noted the desirability for additional knowledge of diffusion within the base and its physicochemical properties (1–3). It is possible that in many reported experiments the results obtained have been more dependent upon the rate of diffusion in the external medium than upon the rate of diffusion in the vehicle being tested. Fuller, *et al.* (1), suggested that the rate of absorption of a drug from a locally applied vehicle will depend on the rate of the slowest of the processes involved in the transfer of medicament to the tissue fluids. Thus, *in vitro*, the limiting influence could occur in the external medium, at the interface, or in the preparation containing the medicament. One significant physical property is diffusion of drug molecules within the vehicle.

A cell has been developed which makes possible the measurement of diffusion of a drug within its vehicle and it has been applied to diffusion measurements of salicylic acid and sodium salicylate in hydrophilic ointments prepared with three different emulsifiers. Studies involved media of increasing complexity from simple aqueous solutions to fluid emulsions to semisolid hydrophilic ointments.

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EXPERIMENTAL

The Diffusion Cell.—Since free diffusion studies in liquid media require rather elaborate methods to avoid boundary disturbances due to the effect of convection currents, vibration, and gross movement of the apparatus, Geddes (4) suggested separation of the phases by a porous diaphragm to eliminate practically all boundary disturbance. Preliminary studies indicated that methods which brought