THE APPLICATION OF ULTRAVIOLET REFLECTANCE SPECTROSCOPY TO THIN-LAYER CHROMATOGRAPHY

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INTRODUCTION

The availability of improved instrumentation and techniques has resulted in an increased interest in the employment of spectral reflectance as a means of chemical analysis[1–5]. More recently a reliable method has been devised for the determination of the components of dye mixtures resolved on thin-layer plates by spectral reflectance[6], and a similar procedure has been applied successfully to the analysis of amino acids separated on chromatoplates[7,8]. Thus far, however, recourse to this technique has been restricted to the visible portion of the spectrum, and, hence, to the analysis of substances which are either colored or which can be converted to a colored species by the use of a suitable chromogenic reagent.

Because of the large number of substances having characteristic ultraviolet spectra that may be employed in their analysis, it was felt that the application of ultraviolet reflectance spectroscopy to thin-layer chromatography would prove to be an invaluable analytical tool, especially for those dealing with the pharmaceutical and biochemical sciences. The feasibility of this approach was ascertained by employing it to determine the composition of mixtures of aspirin (acetyl salicylic acid) and salicylic acid which had been separated on silica gel plates. This particular system was selected for study not only because the spectra of the two compounds were suited for the purpose at hand, but also because it presented no difficulties in the matter of locating the compounds at the conclusion of the resolution. Both appeared as yellowish-brown spots when the plates were dried.

EXPERIMENTAL

The aspirin and salicylic acid, which were of Merck U.S.P. and Chase U.S.P. purity, respectively, were dried over sulfuric acid for 24 h before use. 0.10 *M* stock solutions of the compounds in chloroform were used to prepare the dilution series employed in this study. Solutions were applied as spots by means of 5 and 10 μl micropipets. The 20 × 5 × 0.35 cm plates were coated with adsorbent by distributing a Merck silica gel G–water mixture (6:12) with a glass rod which rested on two thicknesses of masking tape affixed to the ends of the plates. This technique gave a uniform coating 0.5–0.7 mm thick. After the plates had been allowed to "set" for 20 min at room temperature, they were dried at 110° for 1 h and stored in a desiccator.

After spotting, the plates were dried by letting them stand at room temperature

for 10 min and then developed by the ascending technique with the use of a hexane–glacial acetic acid–chloroform mixture (85:15:10). R_F values observed for salicylic acid and aspirin were 0.35 and 0.2 respectively. Although in preparative work the spots may be removed from the chromatoplates directly after their development, in the analysis of the mixtures the plates were dried at a temperature of 90° in an oven for 2 h.

The reflectance spectra of the compounds were obtained with a Beckman Model DK-2 Spectrophotometer fitted with a standard reflectance attachment. All other measurements were made with a similarly equipped Beckman Model DU Spectrophotometer. The cells used to hold both the analytical samples and the reference material consisted of a circular quartz plate, which had a diameter of 22 mm, superimposed on a 40 × 40 × 1 mm piece of white paperboard. The quartz disk was held in place by means of a 40 × 40 × 3 mm plastic plate which was affixed to the backing paper with two pieces of masking tape. A circular window, 19 mm in diameter, in the upper surface of the plate opened into a concentric circular well, 24 mm in diameter, that was deep enough to accommodate the quartz disk. These data are presented schematically in Fig. 1, where a sketch of the assembled cell is also given.

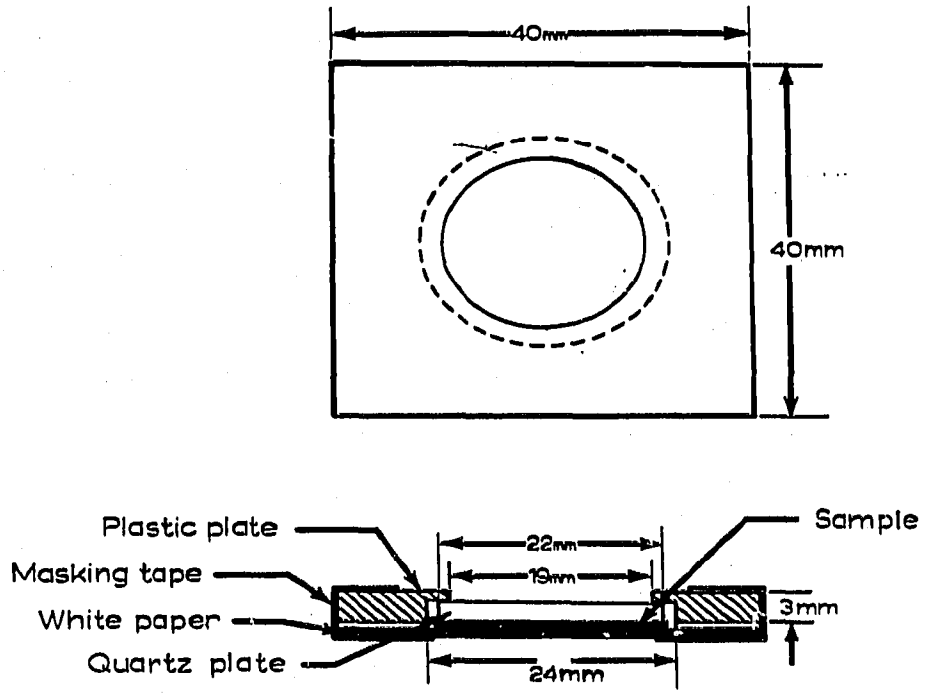

Fig. 1. Dimensions of cell elements and sketch of assembled cell.

The 70 mg which comprised the analytical samples were weighed to the nearest ±0.2 mg and ground in a small agate mortar for two periods of 20 sec each to insure homogeneity and uniform particle size. The material was then introduced into the cell and carefully compressed between the quartz disk and the paperboard by rotating the former until a thin layer having an approximate diameter of 22 mm and an approximate thickness of 20 mg/cm² was obtained. The reference standard in all cases consisted of silica gel adsorbent from the plate under investigation.

RESULTS AND DISCUSSION

Optimum conditions for drying of chromatoplates

The procedure employed in the drying of the chromatoplates following their development was decided upon after a study of the effect of drying times and temperatures upon the stabilities of salicylic acid and aspirin adsorbed on silica gel. When samples containing 5 μmoles were prepared according to the procedure given above and then subjected to different drying conditions, the reflectance spectra presented in Figs. 2 and 3 were obtained. As may be seen in Fig. 2, the general form

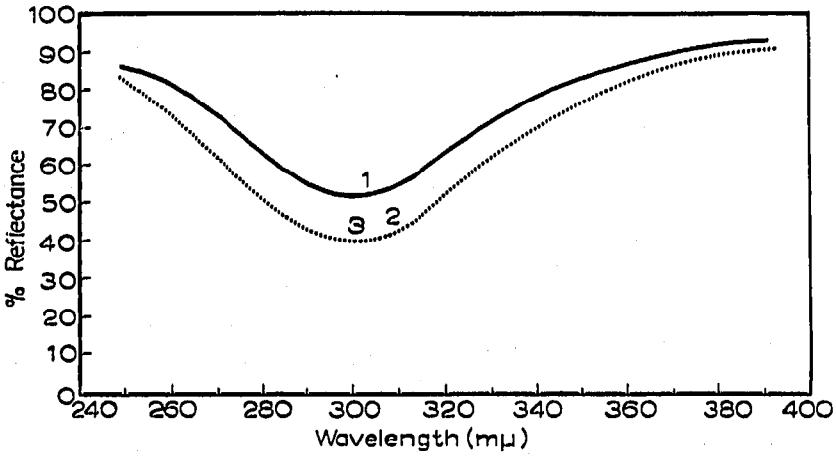

Fig. 2. Reflectance spectra obtained for salicylic acid adsorbed on silica gel G at indicated intervals after spotting: (1) After 15 min at room temperature; (2) after an additional hour at 90°; (3) after still another hour at 90°.

of the spectrum for salicylic acid, which exhibited an absorption maximum at 302 mμ, was unaffected by variations in drying time and temperature. The spectrum for aspirin, on the other hand, underwent substantial changes. A consideration of Fig. 3, which shows the aspirin spectra, discloses that the position of the absorption maximum

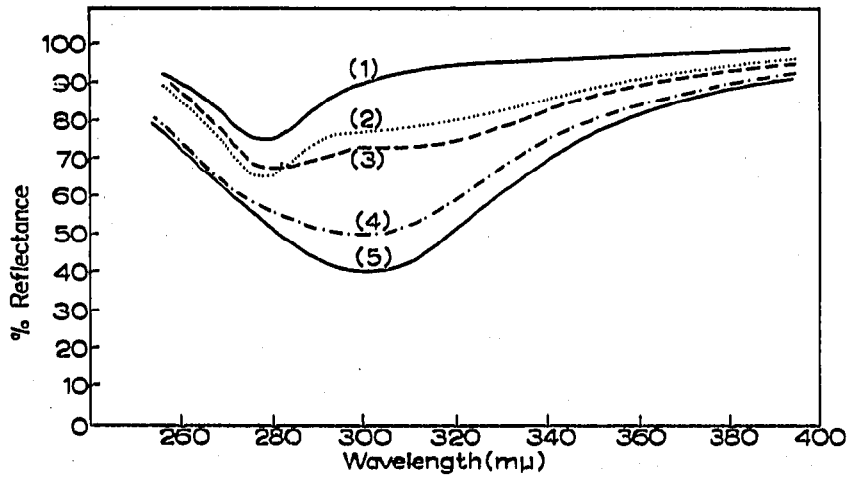

Fig. 3. Reflectance spectra obtained for aspirin adsorbed on silica gel G at indicated intervals after spotting: (1) After 15 min at room temperature; (2) after 2 more hours at room temperature; (3) after an additional 5 min at 90°; (4) after an additional 10 min at 90°; (5) after still another 10 min at 90°.

varied between 278 mμ and 302 mμ. Since aspirin is known to form salicylic acid upon hydrolysis and since the reflectance spectrum of the decomposition product of aspirin is identical with that obtained for salicylic acid, this shift of the absorption maximum may be ascribed to the conversion of aspirin into salicylic acid.

By making use of this conversion, it was possible to devise a more sensitive method for the determination of aspirin, since the decomposition product absorbs more strongly than aspirin itself. The experimental conditions under which the conversion was induced were selected after studying the effect of varying drying times and temperatures upon the % reflectance at 302 mμ—the absorption maximum for both salicylic acid and the decomposition product of aspirin—of 1 μmole of salicylic acid and 1 μmole of aspirin adsorbed on silica gel. A drying period of 2 h at 90° was found to be most convenient and expeditious.

Preparation of analytical samples

The precautions that had to be observed during the sample preparation were essentially the same as those suggested by FRODYMA AND FREI[6,7,9] for reflectance in the visible region of the spectrum. The optimum sample thickness for measurements with ultraviolet radiation was arrived at by determining the per cent reflectance at 302 mμ of samples consisting of 3 μmoles of salicylic acid per 70 mg of adsorbent but of different thicknesses. Constant reflectance readings were obtained when the sample thickness exceeded 16 mg/cm² with white backing paper, and 22 mg/cm² with brown plastic backing material. For values less than these, a gradual increase in per cent reflectance accompanied each increase in sample thickness. It was decided to employ a thickness of 18 mg/cm² in conjunction with white paperboard as this would allow for possible losses of material that might occur during the packing of the analytical sample in the cell. Inasmuch as the samples used in this study had a diameter of 22 mm, this thickness corresponded to an optimum sample size of 70 mg.

As was also the case with work in the visible region, the factors limiting the precision of the technique were associated with the packing of the sample in the cell. When the per cent reflectance at 302 mμ of samples containing from 0.5 to 2.0 μmoles of salicylic acid per 70 mg of silica gel was determined, the differences observed between readings for the same sample which had been repacked were of the same order as those obtained for replicate samples and in no case exceeded 0.6 reflectance units. Although uniformity of sample thickness was not as critical as in the visible, probably because the thickness was 2 mg/cm² in excess of the minimum needed for constancy of reflectance, the extent of the pressure applied when the material was compressed between the quartz disk and the backing paper did affect the reproducibility somewhat. When the pressure exerted was excessive, a decrease in the apparent reflectance of a salicylic acid–silica gel sample amounting to 1 to 2 % was noted. For pressures less than excessive, any derivations observed were within instrumental limitations.

The determination of aspirin and salicylic acid

Calibration curves were prepared for both aspirin and salicylic acid by applying different concentrations of the two compounds to thin-layer plates which were then developed, dried, and prepared for the measurement of reflectance according to the method outlined in the experimental section. In both cases the absorbance at 302 mμ

was determined and then plotted as a function of the square root of the concentration. Three of the plots obtained with salicylic acid and two obtained with aspirin are presented in Fig. 4. Although the data graphed there were gathered at different times and with the use of different dilution series of the two compounds, the five plots are remarkably similar and almost coincide in the concentration range below 3.0 μmoles. This suggests the possibility of employing a single calibration curve for the determination of both substances. The close coincidence of the plots obtained for a single compound also indicates that it is unnecessary to run a set of standards alongside each unknown in routine analysis.

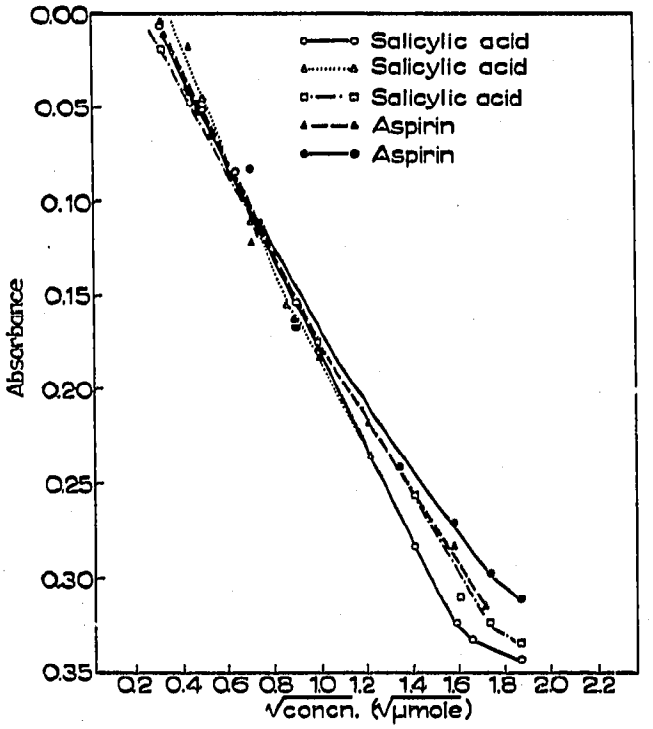

Fig. 4. Absorbance at 302 mμ of salicylic acid (trial 1, O—O; trial 2, △···△; trial 3, □·—·□) and aspirin (trial 1, ●—●; trial 2, ▲– – –▲) adsorbed on silica gel as a function of the square root of concentration.

The precision that can be anticipated when aspirin and salicylic acid are resolved on chromatoplates and then analyzed by means of ultraviolet reflectance spectroscopy was determined by running through the procedure outlined above with four one μmole replicates of each compound. This standard deviation of the reflectance readings obtained for these different samples was found to be 0.37 reflectance units for salicylic acid and 0.47 reflectance units for aspirin. There was no difference between any pair of readings which exceeded 0.8 reflectance units with salicylic acid and 1.1 reflectance units with aspirin.

CONCLUSIONS

The components of salicylic acid–aspirin mixtures resolved on thin-layer plates can be determined by carrying out ultraviolet spectral reflectance measurements on spots removed from the dried plates and packed in an appropriate cell. Calibration

curves obtained for the two compounds by means of this procedure were so similar and so reproducible that for routine analyses a single curve that needed to be checked only periodically sufficed for the analysis of both salicylic acid and aspirin. The method provided quantitative data having a standard deviation amounting to 0.37 reflectance units for salicylic acid and 0.47 reflectance units for aspirin. A linear relationship between absorbance and the square root of the concentration was observed with spots containing up to 3.0 μmoles of either compound.

This technique should be applicable to the analysis of a large number of complex organic compounds which absorb in the ultraviolet and which are resolvable on thin-layer plates. Especially promising is the employment of this technique in conjunction with another involving the incorporation of fluorescent and phosphorescent materials in the adsorbent layer[10–13].

SUMMARY

A method which can be employed for both preparative and analytical purposes was devised for the resolution of salicylic acid–aspirin mixtures on chromatoplates. Ultraviolet reflectance spectroscopy was then used to determine the amounts of resolved material by carrying out measurements on spots removed from the dried plates and packed in a suitable cell.

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