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## APPLICATION OF DIRECT SPECTROPHOTOMETRY TO THE ANALYSIS OF CHROMATOGRAMS

### II. EFFECTS OF SORBENT LAYER ON THE BATHOCHROMIC SHIFT OF THE ABSORPTION MAXIMUM OF A COMPOUND IN A THIN LAYER

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#### SUMMARY

Fundamental studies on the analysis of thin-layer chromatograms by direct spectrophotometry are described. Transmittance is more than seven times more sensitive than reflectance. The bathochromic shift of the principal absorption maximum of *l*-carvone in a silica gel thin layer is discussed.

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#### INTRODUCTION

Materials separated by thin-layer chromatography (TLC) can be quantified by either following the elution from the chromatographic sorbent or by carrying out an analysis directly on the chromatograms, for example by direct spectrophotometry<sup>1,2</sup>. Direct spectrophotometric procedures for quantitative analyses have been widely used and a number of applications of these methods to compounds separated by TLC for the analysis of complex mixtures of natural products have recently been described. Direct spectrophotometry can be applied to the analysis of the chromatograms if the poor reproducibility of the calibration curves can be overcome. Strictly quantitative analysis, however, has been paid relatively little attention, as pointed out also by TOUCHSTONE *et al.*<sup>3</sup>, and the factors that affect the results of the direct densitometry of TLC zones have not yet been systematically investigated.

In Part I<sup>4</sup>, the application of direct spectrophotometry to the quantitative analysis of paper chromatograms was discussed and the absorption spectrum of *l*-carvone on filter-paper treated with liquid paraffin was fairly sharp, corresponding with that on filter-paper that had not been treated with liquid paraffin. However, the complex problems of the bathochromic shift of the absorption spectrum due to the state of the sorbent layers remained as one of the major factors affecting the

precision of peak areas when direct spectrophotometry is used for quantitative chromatographic analysis.

In this paper, the bathochromic shift of the absorption maximum of *l*-carvone in a silica gel thin layer by direct spectrophotometry is discussed and the sensitivities in transmittance and reflectance spectrophotometry are also considered.

## EXPERIMENTAL

### Materials

*l*-Carvone (b.p. 80°/3 mm;  $n_D^{25}$  1.4937;  $d_4^{25}$  0.9514;  $[\alpha]_D^{25}$  -60.55°;  $\lambda_{\text{max}}^{\text{ethanol}}$  235 nm) was used as the sample. Stock ethanolic solutions of *l*-carvone suitable for TLC were usually prepared so as to contain 10–30  $\mu\text{g}/\mu\text{l}$  for the spectrophotometric procedure. All solvents were optically pure.

### Methods

All spectrophotometric measurements were carried out with a Shimadzu Model-50 (Japan) recording spectrophotometer equipped with end-on photomultiplier tubes for both transmittance and reflectance spectra. A deuterium lamp was used.

The quantitative study of *l*-carvone in a silica gel thin layer on quartz plates was carried out by measuring the transmittance and reflectance spectra. Samples were applied as spots on the chromatoplates with a Hamilton microsyringe and the plates were then placed in a vacuum desiccator in order to evaporate the solvent. The spots were positioned so as to be central in the light beam.

### Chromatoplates

A slurry of Silica Gel G (Merck, Kieselgel G; above 325 mesh) sorbent was spread on the quartz plate (35 × 25 × 1 mm) with a glass rod. A series of chromatoplates with different thicknesses of the layer were prepared, dried at 110° for 1 h and stored in a desiccator over dry silica gel until required for use.

## RESULTS

The comparison of the transmittance spectrum with the reflectance spectrum of each *l*-carvone (8.2  $\mu\text{g}$  per spot) in the thin layers of silica gel (above 325 mesh) with various thicknesses (78, 128 and 168  $\mu\text{m}$ ) in terms of absorbance is shown in Fig. 1.

The relationship between the bathochromic shift of each absorption maximum of *l*-carvone in the ultraviolet region and the thickness of the thin layer is shown in Fig. 2.

The relationship between the bathochromic shift of each absorption maximum of *l*-carvone (8.2  $\mu\text{g}$  per spot) in the ultraviolet region and the particle size (100–150, 200–250 and above 325 mesh) of the silica gel layers (60  $\mu\text{m}$  thick) is shown in Fig. 3.

The relationship between the bathochromic shift of each absorption maximum of *l*-carvone (8.2  $\mu\text{g}$  per spot) in the ultraviolet region and the time intervals (4, 8, 13, 17 and 22 min) between the spotting of the sample on the chromatoplate and the measurements is shown in Fig. 4.

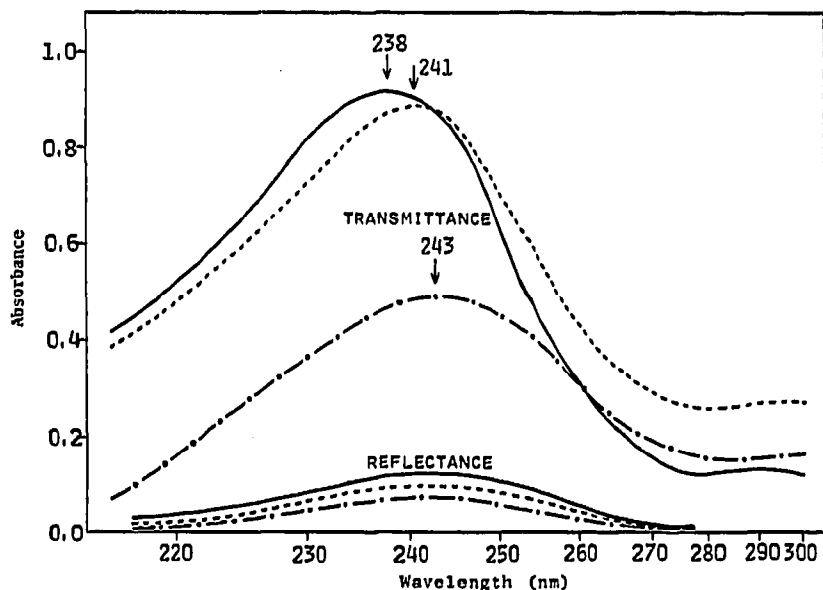


Fig. 1. Transmittance and reflectance spectra of *l*-carvone on silica gel layers of various thicknesses in terms of absorbance. Particle size of silica gel, above 325 mesh; amount of sample, 8.2  $\mu\text{g}$  per spot; layer thickness, — 78  $\mu\text{m}$ , - - - 128  $\mu\text{m}$ , - · - · 168  $\mu\text{m}$ .

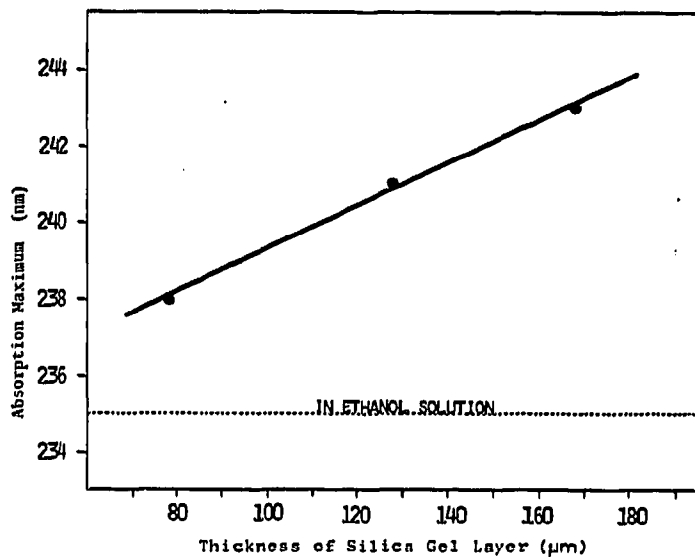


Fig. 2. Effect of the thickness of the silica gel layer on the bathochromic shift of each absorption maximum of *l*-carvone. Particle size of the silica gel, above 325 mesh; amount of sample, 8.2  $\mu\text{g}$  per spot.

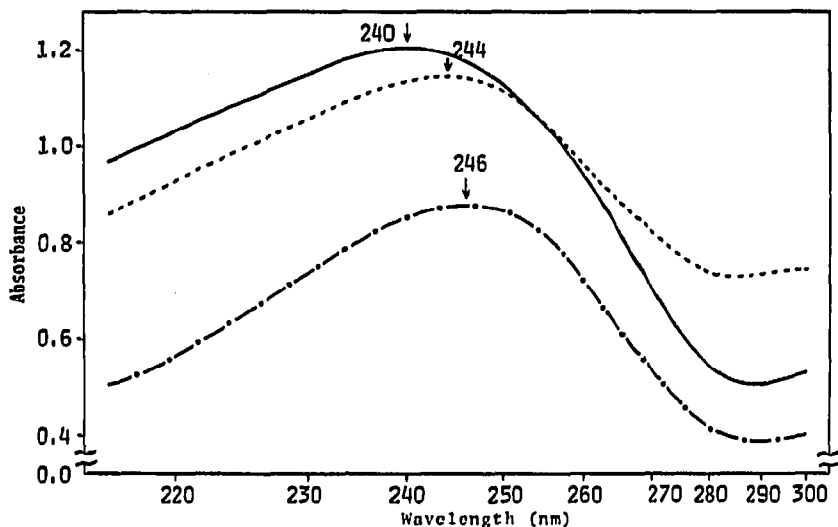


Fig. 3. Effect of particle size of the silica gel on the bathochromic shift of each absorption maximum of *l*-carvone. Layer thickness, 60  $\mu\text{m}$ ; amount of sample, 8.2  $\mu\text{g}$  per spot; particle size of silica gel, ——— above 325 mesh, - - - - 200-250 mesh, - · - · - 100-150 mesh.

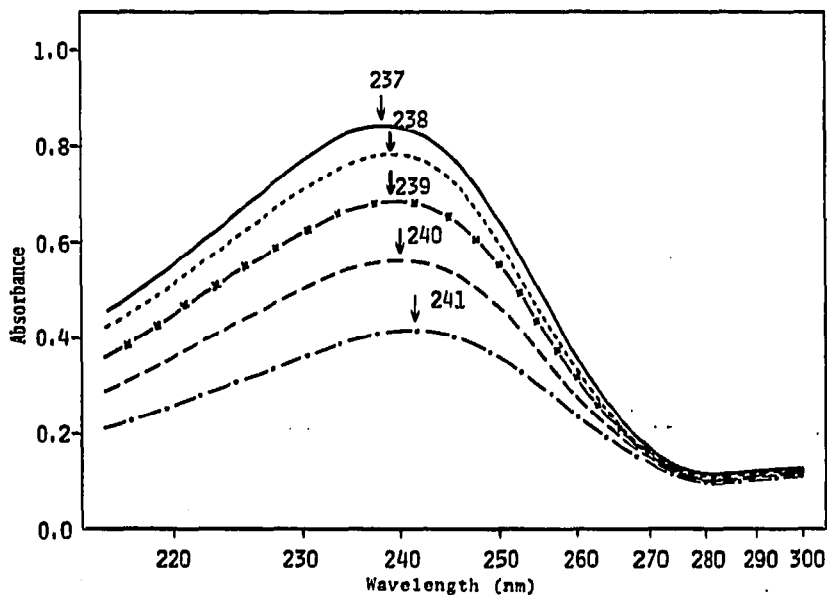


Fig. 4. Effect of the time intervals between spotting the samples and the measurements on the intensity of the transmittance spectrum in terms of absorbance and on the bathochromic shift of each absorption maximum of *l*-carvone. Layer thickness, 60  $\mu\text{m}$ ; particle size of silica gel, above 325 mesh; amount of sample, 8.2  $\mu\text{g}$  per spot; time intervals, ——— 22 min, - - - - 17 min, —×— 13 min, - - - - 8 min, - · - · - 4 min.

## DISCUSSION

*Sensitivities of transmittance and reflectance spectra in the ultraviolet region*

It can be seen in Fig. 1 that the reflectance spectra of *l*-carvone are characterized by a broad and generally flat appearance in comparison with the transmittance spectra. The transmittances are more than seven times more sensitive than the reflectances, which indicates that for quantitative analysis with the thin-layer plate, transmittance measurements are superior to reflectance measurements.

ZÜRCHER *et al.*<sup>5</sup> showed that reflectance spectroscopy is superior to the quenching technique and that the reproducibilities depend on the methods of layer preparation, application of materials, chromatography, drying and scanning. However, on theoretical grounds, GOLDMAN<sup>6</sup> indicated that quantitation by transmittance with the thin-layer plate was superior to reflectance. Also, TOUCHSTONE *et al.*<sup>3</sup> indicated that transmittance is more sensitive in determinations of the absorbances of the coloured zones than reflectance measurements for determining absorbance, fluorescence quenching and fluorometric modes of quantitation in TLC. Transmittance measurements are therefore more suitable than reflectance measurements for the quantitation of materials in the thin layers.

*Bathochromic shift of the principal absorption maximum*

Bathochromic shifts of the absorption maxima were observed<sup>4,7-12</sup> in the characteristic reflectance spectra in comparison with the transmittance spectra of the same materials in solutions. ZEITLIN AND NIIMOTO<sup>13</sup> found that a relatively constant bathochromic displacement took place when the reflectance spectra of acetone 2,4-dinitrophenyl hydrazone and 4-methyl-2-pentanone in the powdered states were measured.

It can be seen in Figs. 1 and 2 that the bathochromic shift of the principal absorption maximum of each *l*-carvone in silica gel thin layers is 3-8 nm in comparison with those of *l*-carvone in the ethanolic solutions. Therefore, the thickness of the layer itself is important. Furthermore, as shown by Fig. 3, the original particle size of the silica gel has some influence on the bathochromic shift.

It is important to operate at the absorption maximum for the quantitation of material separated by TLC. Therefore, before the quantitative analysis of materials in the thin layer can be carried out, it is necessary to consider the shift of the absorption maximum. DOSS<sup>14</sup> indicated that the reproducibility of fluorescence measurements in quantitative TLC depends mainly on the use of the same silica gel materials throughout, the careful adjustment of the light source, and the complete evaporation of the solvents from the adsorbent and the proposed silica gel thin layers with an aluminium foil-base.

As shown in Fig. 4, an investigation of the influence of the time intervals between spotting and the measurements on intensity indicates that the quantitative measurements should be made after allowing the sample spotted on the sorbent layer to stand for a minimum of 30 min in order to evaporate off all the solvent.

## CONCLUSIONS

Minor variations in the thickness of the sorbent layer and the conditions used for development of the plate apparently cause variations in the shape of chromatograms

which make it difficult to obtain reproducible results from plate to plate. It must be pointed out that the bathochromic shift of the principal absorption maximum can be reduced if the technique of preparing very thin layers is followed rigidly. A linear decrease in the layer thickness is useful in decreasing the shift of the absorption maximum. This can be achieved by using a stationary phase consisting of very thin layers of finely granulated sorbents.

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