

Note

Modification of quantitative thin-layer chromatography by elution

TADASHI ENDO, AKIO KUWAHARA, HIDETOSHI TASAI and TATSUZO ISHIGAMI

Department of Chemistry, College of Science and Engineering, Aoyama Gakuin University, 6-16-1 Chitosedai, Setagaya-ku, Tokyo 157 (Japan)

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Thin-layer chromatography (TLC) is of interest as a method for quantitative micro-analysis, and many procedures¹ (planimetry^{2,3}, photodensitometry⁴⁻⁶, and photometry^{7,8}) have been developed. Although the spectrophotometric determination of eluted fractions is generally applicable, appreciable error ($\pm 4\%$) can arise in scraping off and eluting the spots, and some difficulties are encountered with polar samples of low solubility. Here we describe a technique for circumventing these difficulties, and also improving the accuracy of quantitative analysis of substances separated by TLC.

EXPERIMENTAL

Chromatograms were developed on Merck PLC silica gel F₂₅₄ pre-coated plates (5 × 1.2 cm; layer thickness 2 mm; Catalogue No. 5717/0012). The test mixture was brought into solution and applied to the start as a band from a 10- μ l hypodermic syringe; a gentle stream of air was used during sample application to keep the spots less than 2 mm in diameter. Generally, the concentration of the solution was 0.1 to 0.5% (w/v) and 4 to 12 μ l were applied. The chromatogram was developed by the ascending technique in a cylindrical tank lined with filter-paper to saturate the atmosphere with the developing solvent and so prevent the so-called edge-effects⁹. After development, the chromatogram was removed, and dried under reduced pressure for 15 min. The bands of substances being assayed were located in 254-nm radiation and outlined with use of a narrow metal spatula or a blunt pencil.

After the spots had been marked, the plate with the layer on it was divided into small pieces by using a glass-cutter. Each row of spots, and a magnetic-stirrer bar, were placed in a centrifuge tube, and the weight (W_1) of this tube was determined. A 1-ml portion of eluent was added to each tube, and the gel was scraped off the glass with a spatula. Approximately 9 ml of eluent were then added, and the mixtures were stirred for at least 1 h (even for extremely polar substances, stirring for 4 h was sufficient) by means of a rotating electromagnet.

After elution, fine adsorbent particles that could give rise to light scattering were separated by centrifuging (2800–3500 g; 20 min), and the centrifuge tube was re-weighed (W_2). The absorbance of the clear eluate was measured at the appropriate characteristic wavelength. A blank zone of adsorbent was taken from a clean TLC

plate at the same distance from the start as the sample spot had migrated, and this material was used to provide a blank absorbance. The apparent weight of substance in the spot was calculated from the molar absorption coefficient (ϵ) as follows.

$$W = \frac{A \cdot M \cdot W_s}{d \cdot \epsilon} \quad (1)$$

where:

- W = the apparent weight (mg) of substance in the spot,
 A = the absorbance,
 $W_s = W_2 - W_1$, the weight (g) of solvent used,
 M = the molecular weight of the substance, and
 d = the specific gravity of the solvent.

From eqn. 1, the extent of extraction (%) is given by $100W/W_0$ where W_0 represents the true weight of substance applied to the layer. Thus, the weight of substance in a mixture can be obtained from the extent of extraction and W .

RESULTS

Our method has the following features. We used a certain amount of water as co-solvent in the eluent, which led to standard deviations of less than 1%, even with such polar substances as 4-nitroaniline and an acylurea derivative (I), as shown in Table I. It appears that large deviations (*ca.* $\pm 4\%$) and extraction percentages higher than 100 in non-aqueous tetrahydrofuran or ethanol solution are attributable to the scattering of incident light by dispersed particles of silica gel. The co-existent water

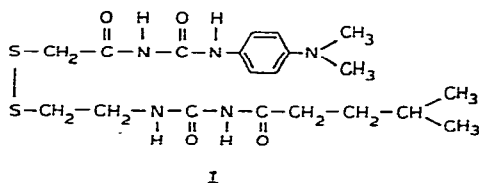


TABLE I.
PERCENTAGES OF EXTRACTION*

Compound	Eluent**	Extraction (%)***	Standard deviation (%)
I	100% Tetrahydrofuran	119.9	± 4.1
I	90% Tetrahydrofuran	104.8	± 2.0
I	80% Tetrahydrofuran	101.0	± 1.0
I	70% Tetrahydrofuran	86.0	± 2.0
4-Nitroaniline	100% Ethanol	107.3	± 4.3
4-Nitroaniline	90% Ethanol	105.3	± 0.9
4-Nitroaniline	80% Ethanol	100.5	± 0.8
4-Nitroaniline	50% Ethanol	98.4	± 1.2

* Calculated (see text).

** Percentage of organic solvent in mixture with water when appropriate.

*** Average of at least 5 values.

may assist in solubilizing these particles or, conversely, in preventing their dispersion in the solvent. Moreover, we found that the standard deviations increased slightly when the amount of water added as co-solvent exceeded a certain level.

We used 5×1.2 cm plates instead of the conventional 20×20 cm plates for reasons of economy, and preferred a layer thickness of 2.0 mm to the generally used 0.25 to 1.0 mm in order to prevent "creeping". The results in Table I show that our method is applicable to a wide variety of substances, including highly polar ones difficult to determine accurately by ordinary methods.

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