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Short Communication

Development of a specific device for densitometry of thin-layer chromatographic sheets in planar chromatography

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ABSTRACT

A specific device for densitometry of Empore TLC sheets is described and it is compared with two other devices using one or two glass plates as support. This comparison lie on a reading by densitometry at 225, 275 and 520 nm in reflectance and transmittance on silica gel and C_{18} sheets. The results of an application to the determination of aspartame are presented.

INTRODUCTION

The flexibility and easy cutting of Empore thin-layer chromatographic (TLC) sheets (Analytichem International, Harbor City, CA, USA) offer attractive features [1,2]. However, the distortion of the sheet during scanning densitometry is a drawback and leads to a risk of a decrease in the performance obtained in previous steps. This is the reason why scanning optimization has been carefully studied on new sheets: experiments have already been reported by Poole and co-workers using a support on glass plates [3] or a sheet of aluminium foil [4]. Hence, densitometry using a glass plate support on which a sheet is placed, or a "sandwich" configuration confining the sheet between two plates, has been carried out in comparison with the use of a new fixation system of the sheet. It is a rigid fixation, which is placed around the plate and allows it to be held firmly on its four sides. This system is composed of two elements: a pedestal fixed on a plane support and a double frame which can be opened and the superposed parts of which are fixed with a hinge and clips as a slide. Experiments were carried out in reflexion and transmittance, by scanning spectra and densitometry at 225–275 and 520 nm, with silica gel and C_{18} sheets with a fluorescence indicator. Application to the study of background noise on aspartame (tripeptided) was investigated.

The first results make the impossibility of using a "glass sandwich" obvious on account of the significant absorbance of the support due to the use of

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two glass plates. The results obtained with the new rigid device seem to be interesting because scanning densitometry in reflectance is made on a stretched, flat plate, and scanning densitometry in transmittance is not interfered with by the obstruction of a possible element as in scanning with a glass support. Moreover, this device is helpful in the manipulation and storage of plates.

EXPERIMENTAL

Chemical and reagents

Empore silica gel and reversed-phase TLC sheets with fluorescence indicator were obtained from Analytichem International. Ethanol, acetic acid, ninhydrin, 2,4,6-collidine and copper nitrate of analytical-reagent grade were supplied by E. Merck (Darmstadt, Germany). The aspartame used was the commercialy available product.

Instrumentation

The densitometric evaluations of plates after derivatization (for measurement of aspartame) and of plates without spots (for measurement of indirect interference of the support) were carried out with CD 60 TLC scanner from Desaga (Heidelberg, Germany), each with three steps. Spots of a 25 mg per 100 ml solution of aspartame dissolved in water–ethanol (10:90, v/v) were applied using 1-μl Microcaps.

Densitometry

The plates were scanned at 225, 275 and 520 nm in transmittance and reflectance for plates without spots and 225, 490 and 520 nm for plates after derivatization with ninhydrin. The monochromator band width was 10 nm and slit dimension of 0.1 x 1 mm was used. Aspartame was streaked on a reversed-phase (C_{18}) sheet and ninhydrin reagent for derivatization was applied [5] using overpressured derivatization [6] to avoid errors arising from spraying or dipping the sheets [7]. After OPD the sheets were heated at 100°C in a drying oven for 30 min. Aspartame appeared as a red spot. After cooling to room temperature the chromatograms were evaluated by densitometry (λ = 275, 490 and 520 nm; slit dimensions 0.2 × 3 mm; eight points per measurement; in the reflectance with deuterium and tungsten lamps).

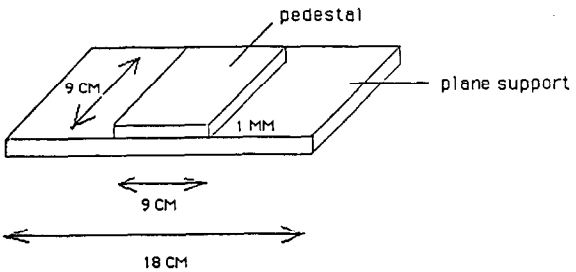

Fig. 1. Support for Empore TLC sheet (10 × 10 cm).

Description of the specific device for densitometry of sheets

The device is a rigid fixation made of plastic material, which is placed around the plate and allows it to be held firmly on its four sides. This system is composed of two elements: a pedestral fixed on a plane support (Fig. 1) and a double frame (Fig. 2) which can be opened and the superposed parts of which are fixed with a hinge and clips as a slide. In this case, no support is needed for scanning.

RESULTS AND DISCUSSION

The densitometric measurements of the EMPORE TLC sheets were carried out under conditions identical with those used for the commercially available glass-supported sorbent layers, in three steps: (A) the sheet was placed on a glass plate support; (B) the sheet was placed between two glass-plates ("Sandwich" configuration); and (C) the sheet was fixed with the new specific device.

Spectra of the same sheet (without spots) in steps A, B and C were recorded and adsorbances were measured at 225, 275 and 520 nm. All the spectra

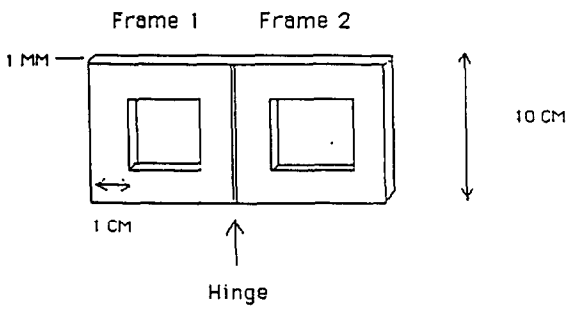

Fig. 2. Frame for Empore TLC sheet (10 × 10 cm).

TABLE I

ABSORBANCES AT 225, 275 AND 520 nm OF EMPORE SHEETS (REVERSED PHASE AND SILICA GEL) IN REFLECTANCE AND TRANSMITTANCE USING STEPS A, B and C

Absorbances expressed in milliabsorbance units, mean results of five measurements.

Mode	Step	Reflectance			Transmittance		
		225 nm	275 nm	520 nm	225 nm	275 nm	520 nm
Reversed phase	C	–[a]	160	550	5250	6300	5000
	A	–	225	580	5100	6250	5100
	B	2700	4400	650	4800	6300	5000
Silica gel	C	–	20	645	5750	6200	4700
	A	–	10	645	5800	6150	4750
	B	3100	4500	700	6300	8000	4750

[a] No absorbance.

TABLE II

STUDY OF NOISE OF 225, 275 AND 520 nm OF EMPORE SHEETS (REVERSED PHASE AND SILICA GEL) IN REFLECTANCE AND TRANSMITTANCE, B AND C *VERSUS* A

Values represent the % increase or decrease in absorbance in of steps B and C *versus* step A, mean results of five measurements.

Mode	Step	Reflectance			Transmittance		
		225 nm	275 nm	520 nm	225 nm	275 nm	520 nm
Reversed phase	C/A	−0.9	−0.5	+0.1	−3.1	−10.6	−1.1
	B/A	+11	+15.4	+11,2	+11.2	+14.0	+0.3
Silica gel	C/A	+0.7	+0.7	+0.7	−12.6	−2.2	−3.8
	B/A	+10	+14.8	+10.8	+3.3	−0.3	+0.2

TABLE III

SIGNAL-TO-NOISE RATIO OF NINHYDRIN-DERIVATIZED ASPARTAME AT 275, 490 AND 520 nm IN STEPS A, B AND C

Mean results of eight measurements.

Step	Signal-to-noise ratio		
	275 nm	490 nm	520 nm
C	2.9	5.1	3.7
A	2.5	3.05	3.46
B	2.42	1.0	2.0

are identical with respect to qualitative data (aspect, shape, ...); only quantitative values of the absorbance differ with the steps used (Table I). The results show a high noise level in reflectance with the sandwich configuration (B). In transmittance, A, B and C seem to be identical. Chromatograms were recorded at 225, 275 and 520 nm to compare the noise using steps A, B and C. Results are expressed as the percentage of increasing (+) or decreasing (−) noise *versus* step A as a reference (Table II). Results show the lowest noise using the new specific device (C) and the greatest noise with the sandwich configuration (B). It is likely that these differences

are essentially characteristic of the glass plate support. To confirm these data, a signal-to-noise ratio study was performed in the reflectance on aspartame after ninhydrin derivatization [5]. The results are given in Table III. The signal-to-noise ratios were found to be essentially identical except at 490 nm, the classical wavelength for evaluating ninhydrin-derivatized aspartame by densitometry [5]. In this case results clearly favour step C.

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