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Short communication

Fibre optical scanning with high resolution in thin-layer chromatography

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Abstract

In this paper a high-performance thin-layer chromatography (HPTLC) scanner is presented in which a special fibre arrangement is used as HPTLC plate scanning interface. Measurements are taken with a set of 50 fibres at a distance of 400 to 500 μm above the HPTLC plate. Spatial resolutions on the HPTLC plate of better than 160 μm are possible. It takes less than 2 min to scan 450 spectra simultaneously in a range of 198 to 610 nm. The basic improvement of the item is the use of highly transparent glass fibres which provide excellent transmission at 200 nm and the use of a special fibre arrangement for plate illumination and detection. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Planar chromatography is a low-cost and simple separation technique which works well for the analysis of large sample numbers. Planar chromatography like high-performance thin-layer chromatography (HPTLC) is the method of choice for complex and dirty samples as impurities remain adsorbed at the point of application. This method offers a simple and economical alternative to other chromatographic techniques especially high-performance liquid chromatography (HPLC). Densitometric methods, where a scanner transforms the analog distribution of substances on a HPTLC plate into digital computer values, are today the most frequently used techniques for quantitative HPTLC. Commonly the plate is illuminated by a monochromatic light spot and the diffuse reflected light is measured.

Historically, densitometric scanning in thin-layer chromatography (TLC) for quantification purposes was first reported by Jork in 1962 [1], based on the theory of light remission, concisely presented in Ref. [2]. The first computer-controlled TLC scanning was reported by Ebel and Hocke in 1976 [3]. TLC or HPTLC scanning by use of optical fibres has been known for more than 30 years. In 1966, Hamman and Martin published the first paper describing a fibre device for TLC scanning in the range of 300 to 700 nm [4]. Two years later, the first quantitative TLC by use of a mixed bundle of glass fibres was reported by Beroza et al. [5] and an appropriate item was patented in 1982 [6]. The first fibre optic TLC scanner with a diode array detector was build by Bayerbach and Gauglitz in 1989 [7]. All papers dealing with glass fibre TLC scanners suffer from the

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inability of measurements below 300 nm [8] and this is the reason why TLC or HPTLC analysis by use of optical fibres at the present time is done only in the field of fluorescence measurements in a range over 400 nm [9–15]. Today modern optical fibres show good transparency at wavelengths below 300 nm making it possible to measure in the most interesting range for planar chromatography [16].

The purpose of our work is to improve the reliability, accuracy and reproducibility of planar chromatographic methods. Our aim is to develop a slit scanning densitometer which works reliably, shows a small resolution on HPTLC plate and is able to scan a track simultaneously at different wavelengths within a couple of seconds. We believe that only fibre assemblies in combination with diode array spectrometry meet all the demands.

2. Experimental

2.1. Apparatus

For direct spectrophotometry of HPTLC plates we designed an item which consists of a diode array spectrophotometer (J&M Aalen, Germany) working in a range of 197 to 612 nm with an average optical resolution of 0.8 nm. A laboratory-made reflection attachment of 50 identical optical fibres transports light of different wavelengths from a deuterium lamp to the HPTLC plate and back to the diode array detector. In this detection mode light source and detector are both placed above the surface of the HPTLC plate. For dense light intensities the light emitting and the light detecting fibres are arranged parallel to each other, because only in this arrangement the Lambert cosine law predicts an optimal response.

The HPTLC plate is placed horizontally on a mechanical stage which can be moved by use of two motors from Micropack (Stuttgart, Germany). The linear slide system works with constant velocity during reflection measurements. A laboratory-developed software was used for instrumental control and data collection. The whole device does not need any lenses, filters or slit-width adjustments.

Two realized optical fibre arrangements are shown in Fig. 1. Light from a deuterium lamp is transported in one set of fibres (25 fibres), either arranged in a separate line or fixed fibre by fibre between two reading channels. The reading channel is formed as a separate line (Fig. 1b) or fixed fibre by fibre alternately with the illuminating fibres (Fig. 1a). The one row fibre arrangement has a length of 5.5 mm and the double row array a length of nearly 3 mm. The illuminating fibres form a light spot on the HPTLC plate which depends on the numerical aperture of the fibres used. In the same way the reading fibres can only register spots of a certain size, which depends on the numerical aperture of these fibres.

The relationship between the densitometric response and the quantity of the separated substance contained in a spot, depends on the plate resolution of the scanner used. The size of the scanning slit should be as small as possible so that a concentration distribution within the area of the substance spot on the HPTLC plate can be regarded as approximately constant [17,18]. The measurement input of the new scanner is a function of a small overlapping area of the illuminated and detected spot. The resulting small overlapping area, the scanning slit, is mainly dependent on the distance of the fibre device from the plate. The checking of the distance of the fibre arrangement over the HPTLC plate is the only adjustment needed.

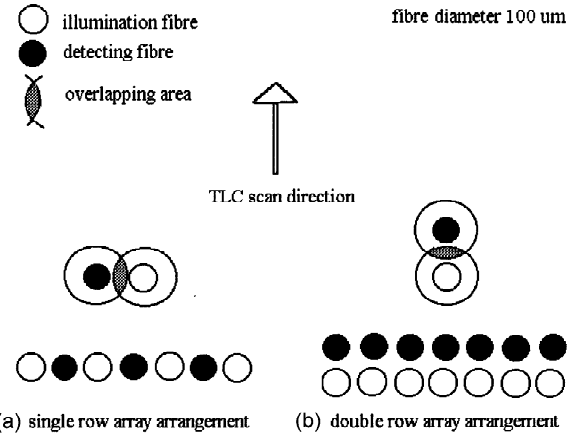

Fig. 1. Single row (1a) and double row fibre interface (1b) are related overlapping area.

2.2. Reagents and measurements

TLC plates were obtained from Merck and the dye test mixture was purchased from Camag. The dye test mixture contains the compounds ciba F II, indophenol, ariabel red, sudan blue II, sudan IV and dimethylamino-azobenzene. The test mixture was spotted dash-like (7 mm, 5 μl) on a silica gel 60 HPTLC plate by using a Camag Linomat IV device. The thin-layer plate was developed in a Camag horizontal developing chamber to a distance of 40 mm from the starting point, using toluene as the mobile phase. The first peak consists of decomposed dyes.

For the scan of the whole track 450 spectra in a range of 198 to 610 nm are measured over a distance of 35 mm. The first peak of the track is scanned over a distance of 0.75 mm. Each spectrum is measured within 0.5 s. All spectra are taken at a detection height of 450 μm over the HPTLC plate.

3. Results and discussion

3.1. Analytical characteristics

The instrumental response is measured as a function of the fibre detecting distance over the HPTLC plate. The light response is detected from plate surface up to 1000 μm in increments of 50 μm. The result is shown in Fig. 2. The light response increases in accordance with the detection distance due to the growing overlapping area. Nevertheless the light intensity also decreases with a declining measurement distance. These two effects counteract and result in a scope at which the intensity of the remission light is nearly constant. Measurements of a single row fibre arrangement at an average distance of 450 μm and of a double row array at an average distance of 500 μm show a large range of nearly 300 μm constant remission light.

3.2. Spectral resolution

The spectral resolution in the range of 200 to 620 nm is better than 1 nm per diode. The use of special fibre materials enables the device to detect a sufficient light intensity at 200 nm. The remission light

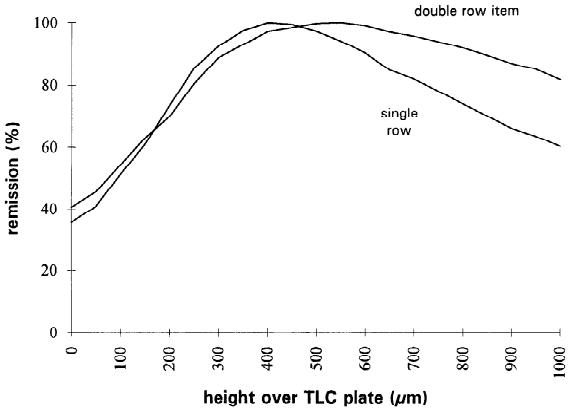

Fig. 2. Light response from HPTLC plate surface in increments of 50 μm for double row and single row fibre item.

intensity depends on the plate surface and show for silica gel material 1400 counts at 200 nm. Fig. 3 shows two densitograms of the dye mixture, measured at 248.6 nm and 198.5 nm. The densitogram at 198.5 nm underlines the good performance of the new scanner. The results verify that light fibre measurements from the HPTLC plate at around 200 nm of more than 10 bytes intensity resolution are possible.

3.3. Spatial resolution on the HPTLC plate

The spatial resolution on the HPTLC plate de-

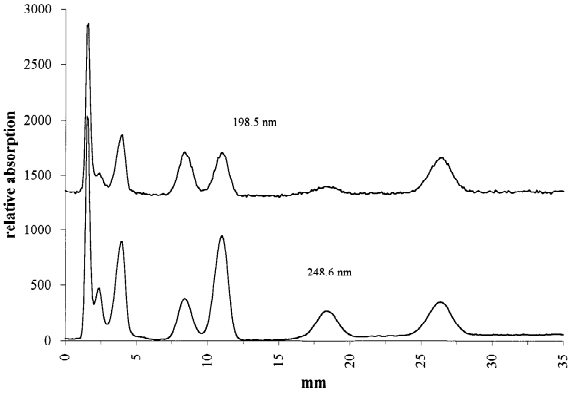

Fig. 3. Separation of ciba F II, indophenol, ariabel red, sudan blue II, sudan IV and dimethylamino-azobenzene, measured at 198.5 and 248.6 nm.

pends on the width of the scanning slit (the overlapping area) and therefore on the orientation of this overlapping area in relation to the direction of movement. Fig. 1 clearly shows that the theoretical spatial plate resolution increases if the double row array is used instead of the single row arrangement. To prove this aspect the first peak in Fig. 3 with a base width of 320 μm was scanned within 45 s at different fibre distances. For each scan 450 spectra are taken. Fig. 4 shows the twice measured dash as two peaks by use of the double row and the single row fibre arrangement. The single row scan is presented slightly shifted towards the double row scan. The single row result shows significant higher noise than the double row values. This lack in quality is due to the older production technique of the fibre used. It is clearly seen that the peak measured by use of the double row fibre arrangement is smaller than the same peak scanned with the single row item. This underlines the theoretical consideration that the double row arrangement should show a smaller scanning slit than the single row item. This results a better spatial resolution on the HPTLC plate in comparison to the single row fibre device.

For quantification of optical resolution on the HPTLC plate we measured the width of the detecting slit. The first peak of the chromatogram of Fig. 3 is scanned at different fibre distances by use of either the single or the double row item. The peak shape of the first dash on the HPTLC plate is assumed to be rectangular. The Gaussian shape of the detected densitogram must be caused by the non zero width of the scanning slit. The width of the slit can be calculated using the first derivative of the detected peak. The standard deviation of the first derivative of the detected peak is calculated according to Ref. [19].

A chromatographic peak is characterised by its height h and its standard deviation σ. The peak area is a function of these two values. If the base width of the peak is chosen as 4σ, 95% of the theoretical peak area is detected. A base width of 6σ includes more than 99% of the theoretical peak area.

The standard deviation of the first derivative of the leading peak side is determined as a 6σ base width. In Fig. 5 this peak width in μm is drawn against the fibre distances (in μm) over the HPTLC plate. A linear relationship for the single and the double row arrangement is observed. For the double row interface a slope of 0.0806 and an intercept of 101.4 and for the single row arrangement a slope of 0.131 and an intercept of 106.4 are calculated, respectively.

The strongly differing slopes show the measurement disadvantage of the single row fibre arrangement. Nevertheless, the determined slit widths and therefore the spatial resolution on plate for a 450 μm distance is calculated to 165 μm. The spatial resolution for the double row fibre interface at a

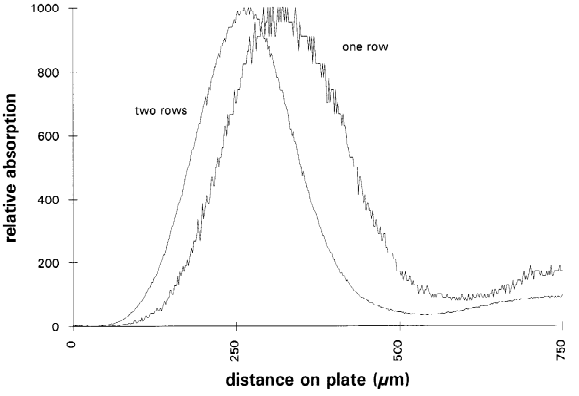

Fig. 4. A twice measured spot as two peaks by use of the double and single row fibre arrangement.

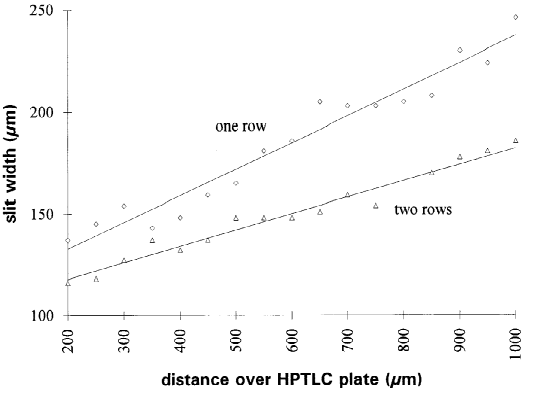

Fig. 5. Corrected split width (μm) drawn against the fibre distance (μm) over HPTLC plate.

distance of 500 μm is calculated to 141 μm. Resolutions of better than 100 μm can be determined using a 4σ peak width.

More interesting are the calculated intercepts of the two fibre arrangements which are both close to the 100 μm core diameter of the light transporting fibre. Probably the interpolated resolution on the HPTLC plate at zero distance is the fibre core diameter used. In the case of zero distance the overlapping area is zero and emission light must be transported through the surface layer of the plate into the detecting fibre core.

4. Conclusions

In conclusion, this new fibre arrangement meets all the demands of a modern HPTLC scanner. The innovation of the presented HPTLC analysis is the combination of a diode array detector, a new light fibre arrangement, stable plate illumination and adjustable formation of a very small scanning slit. Spatial resolutions of better than 160 μm on the HPTLC plate are possible. All these aspects show a substantial improvement of in situ quantitative densitometric analysis. The simultaneous registration at different wavelengths opens the way for chemometric evaluations to improve the accuracy and reliability of HPTLC analysis. Further investigations have to be done to work out the new scope of HPTLC analysis and to shorten registration time from 225 s (currently) to less than 60 s per track. Surely further investigations will show the practical advantages of this new fibre arrangement in comparison with commonly used devices.

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References

[1] H. Jork, Dtsch. Apotheker Z. 40 (1962) 1263.
[2] G. Kortüm, Reflexionsspektroskopie, Springer, Berlin, 1969.
[3] S. Ebel, J. Hocke, J. Chromatogr. 126 (1976) 449.
[4] B.L. Hamman, M.M. Martin, Anal. Biochem. 15 (1966) 305.
[5] M. Beroza, K.R. Hill, K.H. Norris, Anal. Chem. 40 (1968) 1608.
[6] Merck Pat., DE 3 247 355 A1 (1982).
[7] S. Bayerbach, G. Gauglitz, Fresenius Z. Anal. Chem. 335 (1989) 370.
[8] S. Ebel, W. Windmann, J. Planar Chromatogr. 4 (1991) 171.
[9] J. Strojek, S.A. Soper, K.L. Ratzlaff, T. Kuwana, Anal. Sci. 6 (1990) 121.
[10] A.N. Diaz, Anal. Chim. Acta 255 (1991) 297.
[11] A.N. Diaz, G. Paniagua, F.G. Sanchez, J. Chromatogr. A 655 (1993) 39.
[12] A.N. Diaz, F.G. Sanchez, Instr. Sci. Technol. 22 (1994) 273.
[13] N. Wu, C.W. Huie, Anal. Chem. 64 (1992) 2465.
[14] R.L. Aponte, J.A. Diaz, A.A. Perera, V.G. Dianz, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 687.
[15] R.M. Linares, J.H. Ayala, A. Gonzales, V. Gonzales, Anal. Lett. 31 (1998) 475.
[16] M. Huebner, H. Meyer, K.F. Klein, G. Hillrichs, M. Ruetting, M. Veidemanis, B. Spangenberg, J. Clarkin, G. Nelson, in: SPIE Proceedings (BIOS'00), San Jose, CA, January 2000, p. 42, Vol. 3911.
[17] V.A. Pollak, J. Chromatogr. 393 (1987) 143.
[18] W. Tausch, Messtechnik 2 (1972) 38.
[19] B. Spangenberg, Fresenius J. Anal. Chem. 360 (1998) 148.