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Time resolved chromatograms in ultra-thin layer chromatography

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ABSTRACT

Ultrathin-layer chromatography (UTLC) is a recently developed analytical method intended for compact, rapid separations of nanolitre analyte volumes. Optimizing this method's performance requires new measurement techniques compatible with the millimetre length scales and rapid separation dynamics observed in UTLC. We have designed, implemented and characterized a measurement system which records UTLC separations in full color with 32 μm spatial resolution and 33 ms temporal resolution. Our code analyzes multiple tracks per plate, filters analyte spots by color, and automatically generates time-resolved figures of merit. The instrument presented here captures a wealth of information from a UTLC separation, and should provide insight into UTLC physics and improved analytical performance.

1. Introduction

Ultrathin-layer chromatography (UTLC) offers many potential advantages over more traditional planar separation methods such as thin-layer chromatography (TLC) and high-performance TLC (HPTLC). Separations on monolithic silica gel UTLC plates (Merck) can occur quickly (<7 min) and over short distances (<30 mm) [1]. Such separations use lower analyte and reagent volumes and can be more sensitive [2,3]. UTLC enables parallel separation of densely spaced sample spots, making it ideal in areas such as food chemistry [4].

Glancing angle deposition (GLAD)-UTLC plates offer even quicker (<2 min) and shorter (<15 mm) separations [5,6] than Merck UTLC plates. Reactive ion etching of GLAD nanostructured thin film stationary phases enables tuning of chromatographic properties, and may be used to fabricate concentration zones that improve sensitivity and analyte resolution [7]. Excellent control over stationary phase morphology and the compatibility with post-processing techniques make GLAD a powerful platform for engineering high-performance UTLC separation media. However, full utilization of GLAD-UTLC microstructures requires a new perspective on the fundamental characterization of chromatographic physics. Manual plate operation and post-separation characterization may not always achieve the best possible chromatographic result given the enhanced speed of these separation media. Performance improvements may be achieved by incorporating real-time video analysis.

The concept of real-time analysis in TLC has been examined in recent studies in which multiple still images were obtained at defined times during the development. Lancaster et al. [8] demonstrated an improved signal to noise ratio of a single test dye by amalgamating snapshot images taken every 6 s on a TLC plate. In another study, Berezkin and Chausov [9] recorded and analyzed separations on circular TLC plates for every 1 cm movement of the mobile phase front. Zhang et al. [10] analyzed digital images taken several seconds apart during capillary-driven colored dye separations performed on particulate-packed microcapillaries to study dye movement and confirm TLC-like elution behaviors. However, these techniques have not yet been applied to miniaturized UTLC plates that achieve faster separations over shorter distances. Automated rapid development data collection provides more insight into plate performance and could eventually lead to analyte quantification improvements.

Quantitative TLC using charge-coupled device sensors has been studied extensively [11–17], in which a single plate image is taken at the end of the development. In much of this work, analytes were detected by measuring the intensity of UV fluorescence, UV absorption, visible light reflection, or visible light absorption. TLC performance quantification has also been performed using color model analysis, in particular chromaticity (saturation) measurement of analytes imaged under white light [18].

We use a customized video analysis approach to investigate time-resolved performance of a Merck UTLC plate. A consumer high definition video camera (1080p, 30 frames per second) provided

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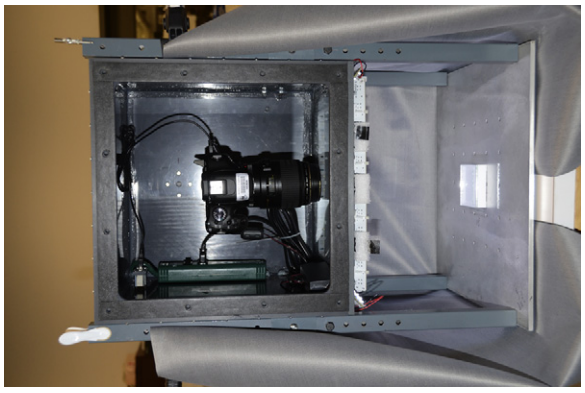

Fig. 1. Camera setup and apparatus used to take high-definition video of UTLC plate during development. A black cloth is draped around the system to control lighting during the development. The camera is isolated from the development chamber in order to protect the camera lens from any potentially corrosive vapors.

the frame rates and image resolutions necessary to characterize the UTLC's short development times and distances. A custom MATLAB script analyzes the video and reports figures of merit as functions of elution time.

2. Materials and methods

2.1. Detection method

Our detection method is *in situ*, using a high-definition video camera (Canon EOS Rebel T2i digital single lens reflex; capable of capturing 1920 × 1080 pixel frames at 30 frames per second) with a dedicated macro lens (Canon EF 100 mm f/2.8 USM) operating in the visible spectrum. The camera utilizes a CMOS image sensor and applies variable bit rate MPEG-4 AVC/H.264 video compression with 4:2:0 subsampling. The camera is oriented normal to the UTLC plates and contained in a custom PVC enclosure to protect the plastic camera body from organic solvent vapor (Fig. 1). A 10 in. square glass window was sealed to the bottom of the enclosure and placed directly between the camera and the horizontal development chamber (Desaga H-Chamber, 50 mm × 50 mm, Sarstedt, Nümbrecht, Germany). An adjustable stand for the enclosure was used to obtain the highest possible quality spatial resolution (32 μm, measured with 1951 Air Force Test Pattern, Thorlabs R3L3S1N), in which all UTLC separation tracks could be contained within the camera's field of view. A black cloth was draped around the enclosure to prevent exterior lighting from entering the camera. A direct USB feed-through connection was used to transfer video data to a computer's hard drive without breaking the seal. Two white LED strips (5500 K, theledlight.com, Inc., Carson City, NV) illuminated the development chamber from opposite sides of the enclosure, perpendicular to the elution direction at ∼45° to plate normal. Each strip consisted of 12 LED's with luminous intensity of 5000 mcd per LED. Illuminating from only these two sides reduced any unwanted reflections and shadows from the horizontal development chamber surface.

2.2. Preparation and spotting technique

To evaluate our detection method, dye separations performed on a UTLC plate (Merck, Darmstadt, Germany) were analyzed. The analyte was undiluted Test Dye Mixture III (CAMAG, Muttenz, Switzerland). In the order of elution, this undiluted mixture comprised of 3 mg of N,N-dimethylaminobenzene (*DY* – dimethyl yellow), 0.5 mg of sudan IV (*OR* – oracet red), 2 mg of sudan blue II (*SB*), 1.5 mg of ariabel red (*AR*), 4 mg of indophenol (*IP*), and 2 mg of ciba F-II (*OV* – oracet violet) per mL of toluene. All plates were hand-spotted using a 30 gauge blunt end stainless steel needle (I&J Fisnar, Wayne, New Jersey, USA) dipped into the diluted dye mixture. The needle was pressed against the UTLC plate for approximately 1 s.

2.3. UTLC plate development and frame extraction

Toluene (≥99.5%, Sigma–Aldrich Canada, Oakville, Canada) was used as the mobile phase for developing the Merck UTLC plate. We filled the conditioning trough with toluene to improve vapor phase saturation within the horizontal development chamber. A porous glass frit dipped into the chamber reservoir supplied mobile phase to the sorbent layer.

The macro lens was focussed before development by placing into the chamber a glass piece with markings scratched into its underside. The camera lens' focus and depth of field were manually adjusted through the Canon EOS Utility computer interface (Version 2.8.1.0, Canon Inc.) to ensure that the image of the scratched markings appeared clear and sharp. System parameters such as lighting intensity, camera aperture size (F3.2), ISO setting (400), and shutter speed (1/60) were all optimized and kept constant during the course of the video. After starting the video recording, the spotted UTLC plate was developed in the horizontal chamber until the mobile phase front reached the end of the plate, removed, and then dried under a warm air stream. This procedure records the full UTLC development, capturing the set of all possible development times and ensuring the best separation performance is recorded for each analyte component.

Following development, the video data were downloaded directly to the computer's hard drive. Ten full-resolution (1920 × 1080 pixels) video frames per second were extracted from the separation video using ZoomBrowser EX software (Version 6.5.1.15, Canon Inc.). In this case (Merck UTLC plate with development duration in the minutes), we deemed it unnecessary to extract the full 30 frames per second. Any time-dependent features of interest could easily be obtained using 10 frames per second. The first extracted frame marked the instant that the mobile phase entered the stationary phase; the last extracted frame was taken as the moment before the plate was manually removed from the chamber.

2.4. Identification and analysis of dye components

Custom programming code written for MATLAB (Version 7.8 R2009a, MathWorks Inc.) was used to generate time resolved chromatograms from each chromatography video. Analysis involved four main steps, outlined briefly here and described in full detail in the Section 3. Prior to analysis all pixels within each extracted frame were converted from RGB (red, green, blue) to HSV (hue, saturation, value of brightness); we use the HSV color model [19] throughout this work. First, each analyte was assigned a particular hue range, where hue is constrained over the interval of 1–360°. Second, a recipe file was created that contains development information from the UTLC video such as mobile front flow constant, analyte hue ranges, and coordinates of the separation tracks and initial spot locations. Third, color-filtered chromatograms were generated and Gaussian fits applied for each analyte (for every track from each extracted video frame). Migration distances and peak widths of each analyte were calculated from the fits. Lastly, all relevant figures of merit (retention factors, theoretical plate numbers, separation numbers, and resolution between zones) were generated, and reported as functions of development time.