Journal of Chromatography A, 1413 (2015) 127–134

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Office Chromatography: Precise printing of sample solutions on miniaturized thin-layer phases and utilization for scanning Direct Analysis in Real Time mass spectrometry

Tim T. Häbe, Gertrud E. Morlock*

Interdisciplinary Research Center (IFZ) and Institute of Nutritional Science, Chair of Food Science, Justus Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany

ARTICLE INFO

Article history:
Received 28 June 2015
Received in revised form 31 July 2015
Accepted 3 August 2015
Available online 4 August 2015

Keywords:
Miniaturization
High-performance thin-layer chromatography
Ultrathin-layer chromatography
Bubble jet application
Digital image evaluation
DART-MS

ABSTRACT

Office Chromatography combines achievements in office technologies with miniaturized planar chromatography. In the life sciences, printing of materials became an accepted technique, whereas in separation science, the use of printers for chromatography is at its infancy. A bubble-jet printer was modified for exact application on miniaturized plates. Technical modifications included the removal of all unnecessary parts and the improvement of the positioning system, purge unit and sample supply system. Evaluation was performed via a slide scanner and image evaluation software. Printing of a food dye mixture solution ($n = 5$) led to a calculated mean deposition volume of 13 ± 1 nL/mm^2 per print-cycle. A mean determination coefficient (R^2; $n = 5$) of 0.9990 was obtained for application of increasing volumes, executed via increasing band widths of 50–200 μm (corresponding to 2–8 nL). Using larger band widths and multiple print jobs, deposition volumes of up to the microliter scale represented an alternative to cost-intensive standard equipment. After print, separation, detection and digital evaluation of five food dyes, mean R^2 ($n = 5$) were obtained between 0.9977 and 0.9995. The accuracy of printing was proven by mean recovery rates of 101–105% with repeatabilities of 3–7% (*%RSD*, $n = 5$). The transfer to nanostructured ultrathin-layer plates proved the synergetic potential of these fields of research. First, this modified printer was suited for printing of finely graduated scales of three preservatives for determination of the spatial resolution of scanning Direct Analysis in Real Time mass spectrometry.

1. Introduction

In life sciences, printing of materials became an accepted technique, *e.g.*, for printing of cells in medical surgery [1] like skin [2] and cartilage tissues [3], for lab-on-a-chip designs [4], for microfluidic design [5] and for evaluation of optimized ambient mass spectrometry (MS) interfaces for miniaturized materials [6,7]. Already in 2010, printing was transferred to the field of planar chromatography and used as a novel technique for precise sample application on miniaturized, ultrathin layers [8]. Planar chromatography is a separation technique routinely used to identify and quantify components in a wide range of sample matrices. One of its wide-spread and frequently used subcategories is thin-layer chromatography (TLC), which was permanently improved over the decades, with regard to instrumental process and stationary phases. Step-automatization and high-performance chromatographic layers merged to the subcategory high-performance thin-layer chromatography (HPTLC) with shorter separation times, higher resolution and better sensitivity along with an overall improved performance [9]. The current automated sample application technique in HPTLC is deposition by syringe as contact or spray-on application. The full analytical power of HPTLC is supported by automated devices, however, which are at present not capable of reproducible and precise handling of miniaturized plate dimensions for ultrathin-layer chromatography (UTLC).

UTLC, the latest subcategory of planar chromatography, started in 2001 with the introduction of the miniaturized UTLC layers with a 10 μm thick monolithic silica gel structure [10]. This sol-gel synthesis and other polymerization processes are still used to build up silica monoliths and styrenics-based monolithic layers with new characteristics [11,12]. Electrospinning is another process creating a nanofiber meshwork from a polymer solution, applied under high voltage on the carrier foil. A wide range of polymers and carbon nanofibers has been used to create differently shaped chromatographic fiber mats, recently shown with incorporated photoluminescence indicator [13–15]. The introduction of the

* Corresponding author.
E-mail address: Gertrud.Morlock@ernaehrung.uni-giessen.de (G.E. Morlock).

http://dx.doi.org/10.1016/j.chroma.2015.08.003
0021-9673/

computer-controlled glancing-angle deposition (GLAD) technique for the deposition of porous nanostructured thin films in 2008 blazed the trail to construct chromatographic structures like helices, spirals and vertical posts structured in the nanometer scale [16–18]. This flexibility in the three-dimensional design was further expanded by the carbon-nanotube-templated (CNT) microfabrication of porous silicon-carbon UTLC layers. These layers can be designed as herringbone CNT pattern with 3 μm wide SiO_2 nanowire hedges and 5 μm channels for a directed solvent flow on these 50 μm thick layers [7,19–21]. All these emerging miniaturized plate formats cannot be handled by common automated devices, like TLC sampler and automated development chambers [8]. Moreover, only small volumes of samples and mobile phase are required for these sensitive layers and can be applied and subsequently evaporated in the same device (*e.g.*, via a drying agent pad in its simplest form).

Due to the given oversized instrumentation and the nonexistence of an automated online system in planar chromatography, comparable to high-performance liquid chromatography (HPLC) and gas chromatography (GC) systems, the field of planar chromatography was newly approached. A novel, interdisciplinary discipline was formed by the replacement of existing HPTLC equipment with simple office computer peripherals to combine achievements from print & media technology and miniaturized plate processing [8,22]. It was termed Office Chromatography (OC). This interdisciplinary concept was proven with slightly modified bubble-jet printers to apply analytes on UTLC plates [8,22]. The centerpiece of the modified thermal bubble-jet printer was the single printhead-chip based on ceramic inflow caverns, providing ink flow from the cartridges by capillary action. Containing two separate heating elements, 256 lined up jet nozzles per cavern vaporize and condensate a small amount of ink with a frequency of 10 kHz and more. The pulsation of these bubbles causes the ejection of a droplet (2.5 pL or 5.5 pL) per pulse at the tip of the jet nozzle and inverse refilling of the nozzle out of the cavern. During printing, multiple jet nozzles are activated simultaneously, according to the magnitude of the application area. For example, a water-soluble dye mixture solution was printed among others as 3.0 mm long and 0.2–0.6 mm wide bands (5.3 nL/mm^2; ranged 3.1–9.6 nL/band) with application linearities of $R^2 \geq 0.9988$ and precisions (%*RSD*) of 2.9–3.8% [22]. Or, different sugar and food dye solutions were printed as 3.0 mm long and 0.04–0.4 mm wide bands (23.0 nL/mm^2; ranged 3.0–30.0 nL) [23,24]. Since 2001, office scanners have been employed for the documentation and evaluation of chromatograms. Different TLC applications were demonstrated as for hydrazines (0.1–3.0 μg/zone; R^2 of 0.9987), staining solutions, Rhodamine B (0.35–1.75 μg/zone; R of 0.999), caffeine (0.2–0.8 μg/zone; R of 0.999), food dyes (30–203 ng/zone; R between 0.9952 and 0.9980) and aflatoxins (1–9 ng/zone) [25–29].

So far, quantitative studies were rarely performed on UTLC plates using a customized office printer in combination with a high-resolution office scanner in a precise miniaturized scale, disclosing the real performance for miniaturized planar chromatography [8,22]. Thus, in this study, an inexpensive high-resolution Drop-On-Demand printer [22] was further modified for precise quantitative application of sample solutions on different plate formats in a safe and standardized process. All unnecessary parts were removed for free access to the printing unit under operating conditions. As an alternative to professional TLC/HPTLC documentation systems, an unmodified slide scanner with transmitted light function was used for documentation and compared using image analysis. As a proof, performance data were presented for water-soluble food dye analysis. This food dye separation was also shown on CNT-UTLC plates [7] after sample application via the modified printer with a development time of 8 min. Applied for a spatial resolution study, first this modified printer was able to produce finely graduated scales for quantitative surface analysis via Direct Analysis in Real Time (DART) MS [6]. These printed scales or any other pattern can be used for the calibration of ambient surface analysis methods or for the application of an internal standard prior to quantitative surface analysis.

2. Experimental

2.1. Chemicals and materials

Azophloxin (E128; for microscopy), Crystal Ponceau 6R (E126; unspecified), Lissamine Green B (E142; 60%), Patent Blue V (E131; for microscopy), Tartrazine (E102; for microscopy), methyl-4-hydroxybenzoate (ME; ≥99.0%), ethyl-4-hydroxybenzoate (EE; ≥99.0%), and butyl-4-hydroxybenzoate (BE; ≥99.0%) were obtained from Sigma–Aldrich (Schnelldorf, Germany). Ammonium acetate, ethanol, ethyl acetate, glacial acetic acid and methanol (all HPLC grade) were purchased from Karl Roth (Karlsruhe, Germany). Ultrapure water was bidistilled prior to use (Destamat Bi 18E; Heraeus, Hanau, Germany). HPTLC plates silica gel 60, 10 cm × 10 cm (Merck Millipore, Darmstadt, Germany), and a CNT-UTLC plate 1.5 cm × 6.0 cm (obtained from the Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT, USA) were used prewashed by chromatography with methanol–water (4:1, v/v) and dried at 110 °C for 10 min [7,30].

2.2. Preparation of standard solutions

E102 (20 mg), E126 (30 mg), E128 (20 mg), E131 (20 mg) and E142 (10 mg) were dissolved in water–methanol (9:1, v/v) *ad* 50 mL supported by ultrasonication. Optional for spray-on application, the dye solution was diluted 1:10 (v/v) with methanol prior to application. Solutions of ME, EE, and BE were prepared in water–ethanol (4:1, v/v) at concentration levels of 2.0 mg/mL.

2.3. Conventional spray-on application as aerosol

The reference device for the bubble-jet printer was the Automatic TLC Sampler 4 (ATS 4) controlled via winCATS version 1.4.7 Planar Chromatography Manager (both CAMAG, Muttenz, Switzerland). Settings for sample application (0.3–1.5 μL) were: 6.0 mm band length, 9.0 mm track distance, 6.0 mm distance from the bottom plate edge and 100 nL/s dosage velocity.

2.4. Hardware modifications on the printer

A Pixma iP3000 (Canon, Krefeld, Germany) was used. The printer housing and the paper feed assembly were removed for free access to the plate and printhead under operating conditions. The power supply was installed external to reduce the voltage at the device to 24 V. The user interface circuit board was assembled at the rear side next to the logic circuit board. The sensors “cover open”, “paper presence”, “pick-up roller”, “paper size for duplex printing”, “paper end” and “paper output tray” for the housing and paper movement were removed and short-circuited on the circuit board appropriate to the CD-R printing mode. The optical sensor for the remaining ink amount in the cartridge was dismounted; values for “dot count” and “waste ink amount” were zeroed in the printer firmware on demand. By dismantling the printer down to the parts necessary for the CD-R printing mode, almost all parts of the paper feed system and the duplex unit were removed. The original CD-R print tray was replaced by a custom-built plate tray for the precise handling of all differently sized miniaturized plate-formats up to 10 cm × 10 cm (Fig. 1a and b). The remaining parts of the paper feed assembly consisted of two lateral axes and a small part of the transport slide (Fig. 1c). Optional, four solvent resistant guardrails were aligned to