Inkjet application, chromatography and mass spectrometry of sugars on nanostructured thin films

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Introduction

One of the newest technologies for manufacturing layers in planar chromatography is ultrathin-layer chromatography (UTLC) [1] which started in 2001. Improvements like shorter migration distances, faster development, lower specific surface area (UTLC: 150 - 350 m^2/g, HPTLC: 500 m^2/g), and less solvent and sample use were already discussed in numerous papers [2-4]. Recent advancements in UTLC include office chromatography and glancing angle deposition (GLAD) nanostructured media [2, 4]. While typical planar chromatography instruments are optimized for TLC plates and inappropriate for UTLC, office chromatography employs print and media technologies and offers the possibility of a miniaturized one-click UTLC systems [4]. GLAD is a powerful platform for engineering thin films of numerous architectures in several materials [5, 6]. This work demonstrates the benefits of coupling office chromatography and ESI-MS with nanostructured GLAD-UTLC layers in practical sugar analysis [7].

Results and discussion

Method development

For the sugar separation on GLAD-UTLC films, the first derivatization on these films was demonstrated. The stationary phase used was a 5 µm silicon dioxide nanostructured thin film that was produced through GLAD. To optimize the stationary phase and the separation the density of the film was reduced. Application of sharp water-soluble sugar bands of 7 - 25 nL volumes was performed with a Canon inkjet printer.

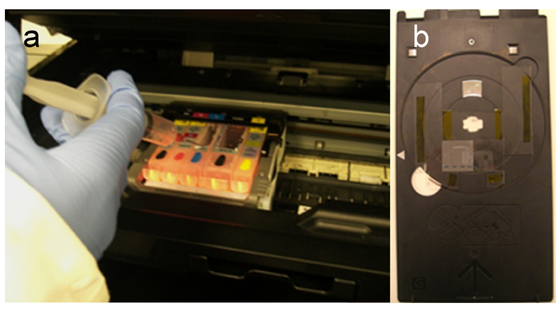

Fig. 1 Printer cartridge filling (a) and CD tray with GLAD-UTLC film (b)

The printer cartridges (with cut, open top and removed sponge) were filled with a syringe (Fig. 1). A computer program controlled the printer, which allowed deactivation of four of the five cartridges, the remaining one was used for sample dosage. The GLAD-UTLC film was placed on a prepared CD-tray (Fig. 2).

The plate was aligned with small glass pieces fixed on the CD-tray with double-sided tape. A pattern with four bands (3 mm x 0.1/0.2/0.3/0.4 mm) created using Adobe Illustrator. After drying the start zones, chromatography was performed in a special horizontal UTLC chamber [8]. The high sugar analyte polarities and low surface areas of GLAD film required a reduction in mobile phase polarity. The final mobile phase contained ethyl acetate, methanol, formic acid and water 8:2:1:0.5 (v/v/v/v). UTLC development took 4 min. The reduced specific surface area of the GLAD film required a reduction in mobile phased polarity. Sugars are very polar analytes, so in combination with a too polar mobile phase [8] they were not retarded on the GLAD films. So the mobile phase was adjusted. The GLAD-UTLC films were placed face down on the frit and aligned against a small glass spacer. Derivatization followed with β-naphthol reagent (Fig. 2). The films were derivatized by spraying. GLAD-UTLC chromatograms were documented with a camera [7]. For each track, a densitogram was produced showing the good separation of the four sugars.

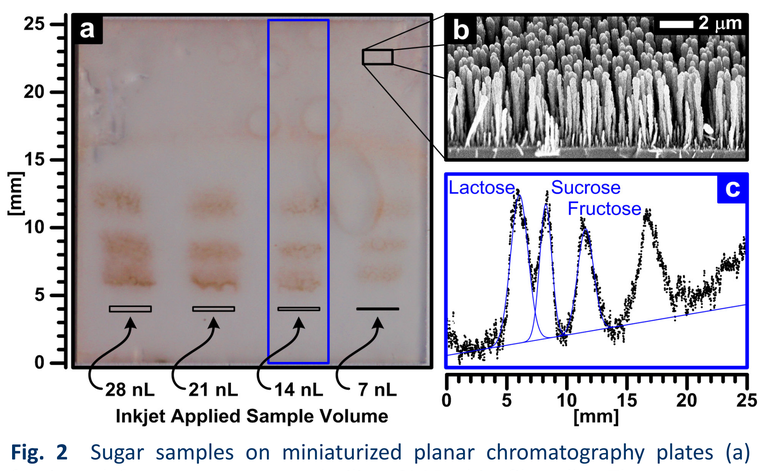

Fig. 2 Sugar samples on miniaturized planar chromatography plates (a) fabricated from nanostructured silica GLAD thin films (b). The separated sugars were derivatized by spraying with the β-naphthol reagent. Exemplarily, a densitogram of a track is shown (c; blue box: 26 mm x 47 mm).

Milk chocolate analysis

The miniaturized technique was applied to actual food samples such as chocolate (Fig. 3). The dissolved sample solution was diluted to a total sugar concentration of 2.3 % to avoid the risk of nozzle clogging due to the very high sugar concentration. This sample was printed with descending mass loadings (Fig. 4).

Fig. 3 Chocolate sample [9]

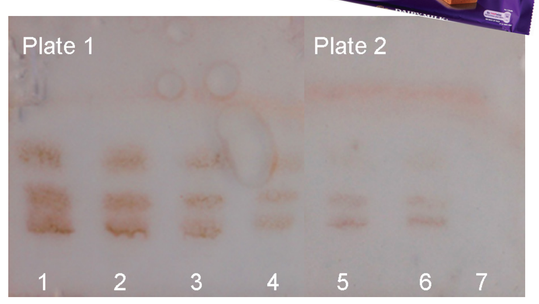

Fig. 4 Chocolate sample separation on two 5 mm VP 86° SiO_2 plates with descending mass loadings (660-70 ng/band; tracks 1–6; track 7 below LOD)

Hyphenation of GLAD films and ESI-MS

The zone of interest was tightly fixed with the circular cutting edge of the elution head (TLC-MS Interface). A glucose solution was applied on the GLAD-UTLC phase, dried and used for recording of mass spectra without development and derivatization. This measurement provided two hexose signals in both ion modes (Fig. 5).

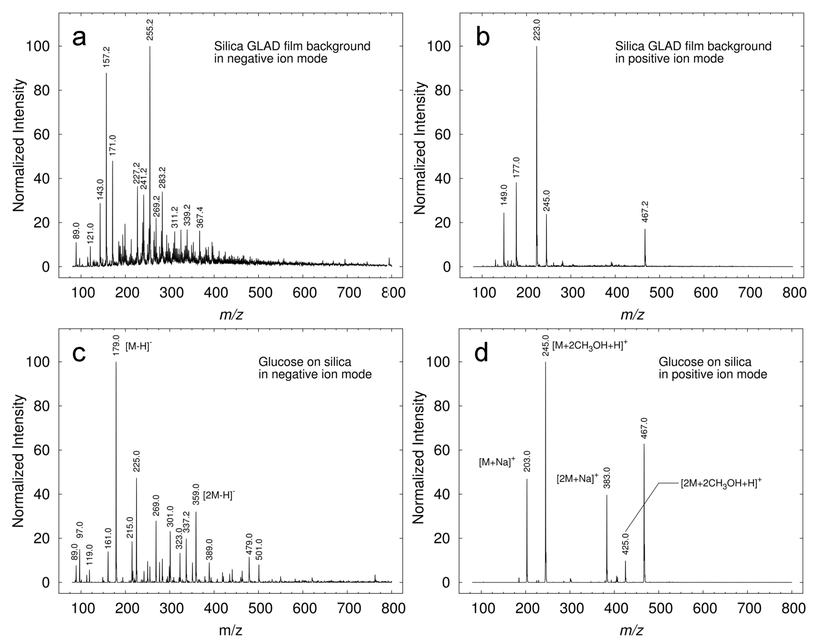

Fig. 5 Mass signals on the GLAD-UTLC 5 µm VP SiO_2 layers: background (a, b) and glucose zone (c, d)

In the negative ion mode, the base peak was the deprotonated hexose molecule at *m/z* 179 $[Glc\text{-}H]^-$, but also its deprotonated dimer was evident at *m/z* 359 $[2Glc\text{-}H]^-$. In the positive ion mode the respective sodium adduct of the hexose molecule at *m/z* 203 $[Glc+Na]^+$ and *m/z* 383 $[2Glc+Na]^+$ were clearly visible. The measured background of the silicon dioxide GLAD-UTLC layers showed background signals, which could repeatedly be found in the spectra in both ionization modes. Further on, the applied glucose solution was derivatized with the 2-naphthol reagent. The reagent background was measured and subtracted from the glucose derivative spectrum. The resulting mass spectrum showed several signals and it was possible to identify glucose in the positive ion mode at *m/z* 181 $[Glc+H]^+$ and *m/z* 361 $[2Glc+H]^+$, however, not the derivatization product.

Conclusions

A first successful separation and derivatization of a real sample demonstrated the potential of GLAD-UTLC layers in food chemistry and confirms its integration into the office chromatography concept. Underivatized sugars were detected through the use of mass spectrometry, demonstrating the possibility of coupling the advantages of miniaturized GLAD-UTLC layers with MS.

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