

J. Wannemacher¹, S. Jim², M. Taschuk², M. Brett^{2,3}, G. Morlock¹

¹Justus Liebig University Giessen, Germany

²University of Alberta, Edmonton, Canada

³NRC National Institute for Nanotechnology, Edmonton, Alberta, Canada

Introduction

Planar chromatographic techniques are applied successfully in numerous fields such as food, environmental and pharmaceutical analysis. [1, 2] Recently the trend towards miniaturization led to the introduction of monolithic UTLC media, which enable faster separations over shorter migration distances and reduce solvent consumption [3, 4]. Nanostructured thin films fabricated by glancing angle deposition (GLAD) are used for UTLC as well [5-11]. Despite developments in stationary phase design, silica gel is still the most widely used material for normal phase planar chromatography [12].

We tested GLAD films of four oxides (SiO₂, Al₂O₃, TiO₂, ZrO₂) in food dye and carotenoid separations and the effect of different film treatments (oxidation and UV-irradiation). Chromatographic performance was quantified using office chromatography and time-resolved UTLC-methods [13, 14]. Food dye separations were successfully hyphenated to ESI-MS.

Results and discussion

Film microstructure

Anisotropic serial bideposition (SBD) films were deposited (Fig. 1). For all oxides the nanoblade structure was similar, however column broadening and post density varied. The fanning angle depends on material melting point [15]. SiO₂ films exhibited better defined channels (Fig. 1). This might be one of the reasons for longer migration distances and higher mobile phase velocity on silica SBD films.

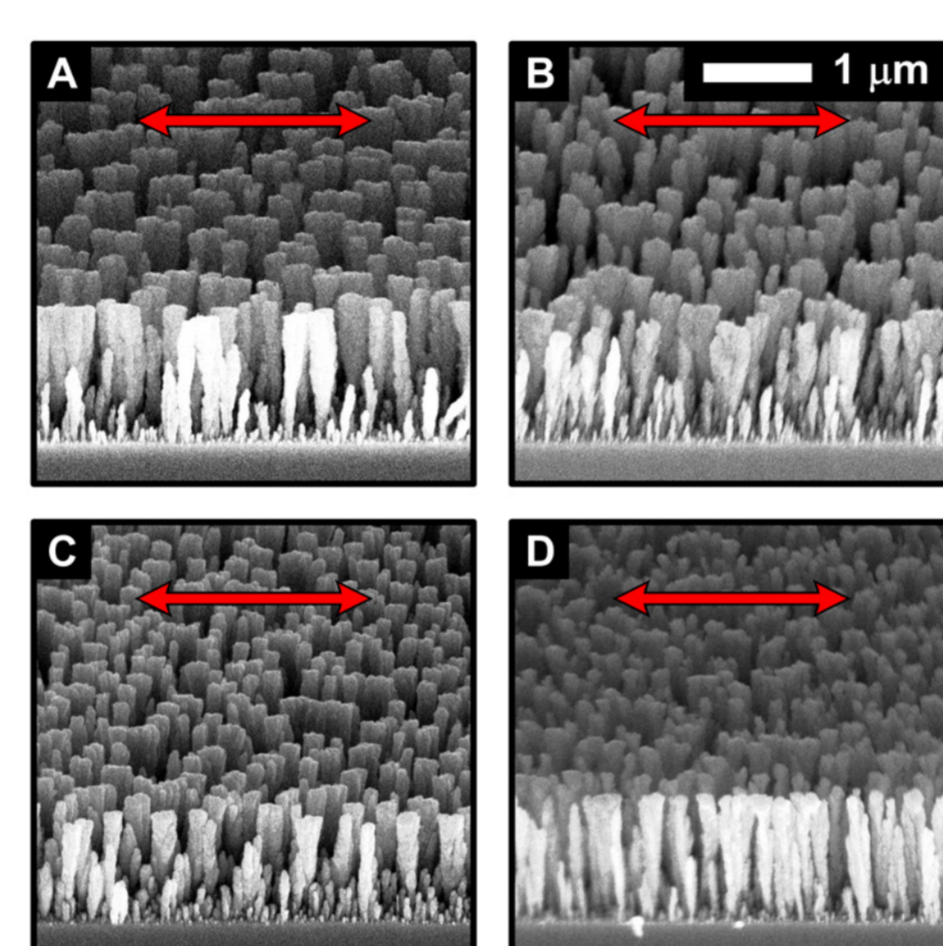


Fig. 1 Scanning Electron Micrographs of (A) silica, (B) alumina, (C) Titania and (D) zirconia films. The aligned nanoblade structures formed channels (arrows). [23]

Carotenoid separations

Retention on inorganic oxide surfaces is based on hydrogen-bonding, Brønsted acidity/basicity and Lewis acidity/basicity. While SiO₂ develops Lewis acid sites only when heated above 200°C [12, 16], the metal oxides used here have native Lewis acid sites [16]. Carotenoid separations were used to probe these characteristics (Fig. 2).

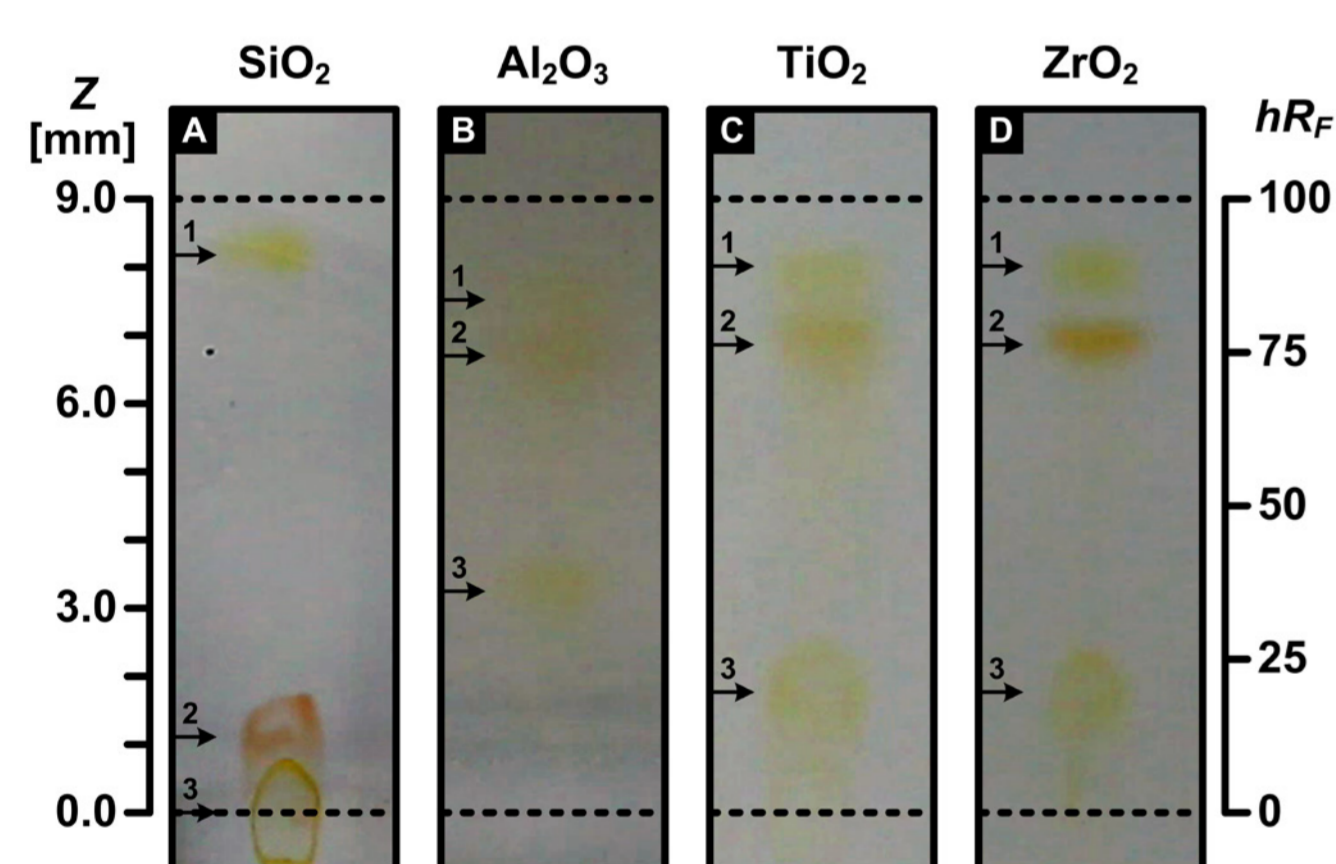


Fig. 2 Carotenoid separations on GLAD-thin films. 1: β-carotene, 2: canthaxanthin and 3: fucoxanthin. [23]

On SiO₂ films, the polar carotenoids were strongly retained, which suggests that retention is based mainly on hydrogen-bonding. On TiO₂ and ZrO₂ films, separations were similar with sharp separated dye spots. Al₂O₃ films gave poor results. β-Carotene retention was not as strong as anticipated. Therefore separating an alternative set of strong Lewis bases might give more insight into the metal oxides' chromatographic behavior.

Food dye separations and time-resolved figures of merit

Theoretical plate heights (H) below 5 μm and a detection limit (LOD) below 2 ng for tartrazine were achieved on GLAD films by combining time-resolved UTLC methods [14] and office chromatography [13] (Table 1).

Table 1 Chromatographic figures of merit [23]

GLAD-film (treatment)	SiO ₂ (untreated)	TiO ₂ (oxidized)	ZrO ₂ (oxidized)
Acid Red 14			
LOD [ng]	3 < LOD < 7	3 < LOD < 7	> 30
hR _F	89.1 +/- 0.1	86.0 +/- 0.1	90.5 +/- 0.1
H [μm]	3.3 +/- 0.1	2.5 +/- 0.1	2.3 +/- 0.1
Tartrazine			
LOD [ng]	< 2	< 2	< 2
hR _F	81.0 +/- 0.1	78.1 +/- 0.1	78.6 +/- 0.1
H [μm]	1.8 +/- 0.1	2.09 +/- 0.04	0.78 +/- 0.01

Food dye separations and post-deposition surface treatments

TiO₂ and ZrO₂ GLAD films are interesting alternatives to SiO₂ for GLAD-UTLC due to their high specific surface area [17]. As deposited TiO₂ and ZrO₂ films tend to be oxygen-deficient, so post-deposition oxidation heat treatments were used to produce stoichiometric TiO₂ and ZrO₂ films [17]. Titania's surface hydrophilicity increases upon UV-irradiation [18-20] and zirconia films exhibit photocatalytic activity [21, 22].

Experiments using the same mobile phase (ethyl acetate – methanol - water, 65:23:5) for all films showed that retention was greater on the metal oxide films than on silica. Therefore the water content of the mobile phase for TiO₂ and ZrO₂ was increased to obtain satisfying results.

The investigated oxidation heat treatment (200° C, 24 h), UV-irradiation (72 h) and a combination of both did not affect separations on SiO₂ films but had a significant effect on TiO₂ and ZrO₂-films (Fig. 3). Oxidation generally decreased analyte retention whereas UV-exposure increased retention. When both treatments were combined the effect of the oxidation heat treatment seemed more prominent.

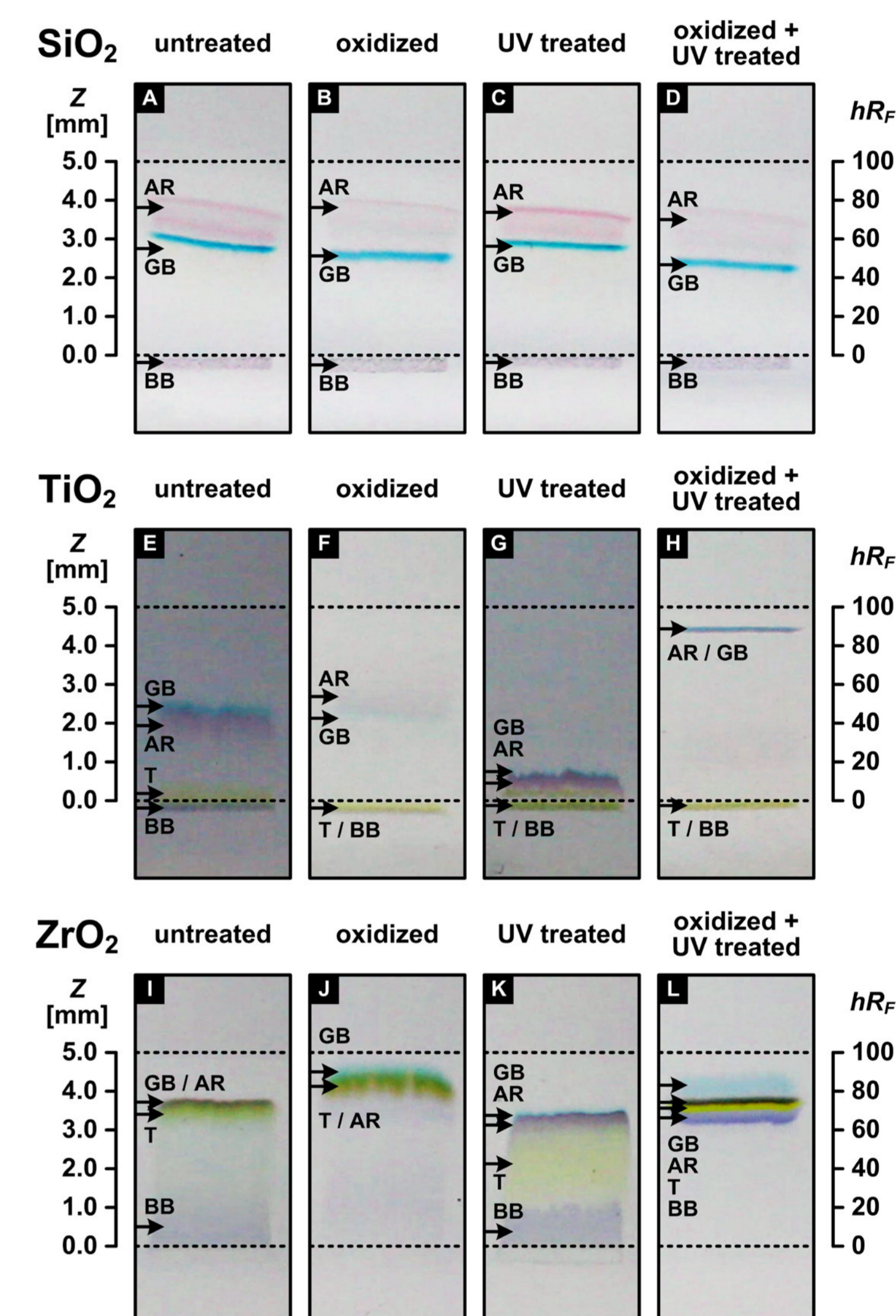


Fig. 3 Effects of different post-deposition treatments on separations of a food dye mix (AR: Acid Red 14, GB: Lissamine™ Green B, T: Tartrazine and BB: Brilliantblack BN) on (A–D) silica, (E–H) titania and (I–L) zirconia [23]

Hyphenation with ESI-MS

Separations of Tartrazine and Brilliant Black BN on TiO₂ and ZrO₂ films were successfully coupled to ESI-MS via the TLC-MS interface. Both dyes were easily identified by their characteristic ions (see Fig. 4). The successful hyphenation of GLAD-UTLC to ESI-MS demonstrates the possibility of analyte separation and mass spectrometric analysis with minimal solvent and time consumption.

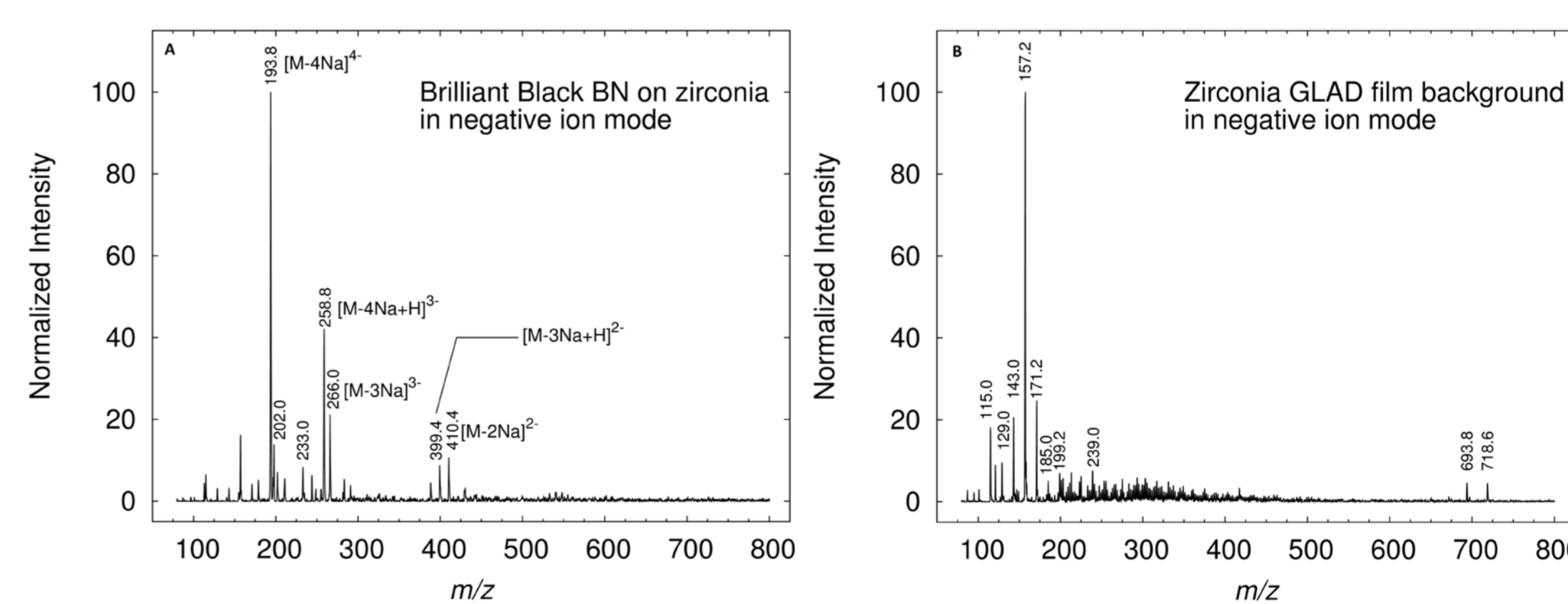


Fig. 4 Exemplary mass spectra recorded on zirconia in negative ion mode: (A) Brilliant Black BN and (B) zirconia film background [23]

Conclusions

We investigated the use of alternative inorganic oxides for miniaturized TLC. When combined with advanced sample application and imaging methods, low theoretical plate heights (< 5 μm) and detection limits (< 2ng) were achieved. Food dye separations on metal oxide GLAD films were successfully coupled with ESI-MS.

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IFZ Interdisziplinäres Forschungszentrum
Giessen Research Centre for
BioSystems, Land Use and Nutrition

