Evaluation of HPLC column bleed by using an Ultraviolet and a Charged Aerosol Detector coupled to a high-temperature liquid chromatographic system

Thorsten Teutenberg[1], J. Paschlau[2], J. Tuerk[1], M. Holzhauser[1], T. K. Kiffmeyer[1]

[1]Institute of Energy and Environmental Technology (IUTA), Bliersheimer Straße 60, D-47229 Duisburg, Germany [2] ERC GmbH, Otto-Hahn-Straße 28-30, D-85521 Riemerling, Germany

1 Introduction

A major disadvantage for the routine application of high-temperature liquid chromatography is seen in the lack of temperature resistant stationary phase materials. If the column is heated to temperatures up to 200 °C, hydrolysis of bonded phase or dissolution of the support material may occur. Therefore, a simple and reliable detection technique should be employed to monitor the effluent if the stationary phase is heated to extreme temperatures. Ultraviolet detection is not always suitable for detecting particulate matter due to bonded-phase loss in a column under harsh conditions, because the signal depends on the analytes' molar absorptivity. Charged Aerosol Detection is a relatively new detection technique, where the eluent is nebulized with nitrogen and the droplets are dried to remove mobile phase, producing non-volatile analyte particles. A secondary stream of nitrogen becomes positively charged as it passes a high-voltage, platinum corona wire. This charge transfers to the opposing stream of analyte particles and is then transferred to a collector where it is measured by a highly sensitive electrometer. The signal intensity generated by a CAD is said to be directly proportional to the analyte concentration. This means that the detector response for a given column should be correlated to the amount of particulate matter per unit of time. In contrast to this, the UV signal is dependent on the molar extinction coefficient ε. This means that particles containing weak chromophores give a lower signal than those with a strong chromophoric system if UV detection is used. Therefore, the experiments conducted in this study should evaluate the usefulness of UV and CA detection for the on-line monitoring of HPLC column bleed.

2 Experimental

2.1 System set up

All experiments were carried out using a Shimadzu HPLC system as depicted in **Figure 1**. Furthermore, a homemade heating system (HT-HPLC 3) was used for controlling eluent and column temperature, which can be operated in temperature programmed mode. Pure deionized water was used as mobile phase. To keep the water in the liquid state a 500 psi back pressure regulator (GammaAnalysenTechnik, Bremerhaven, Germany) was used and connected behind the UVD.

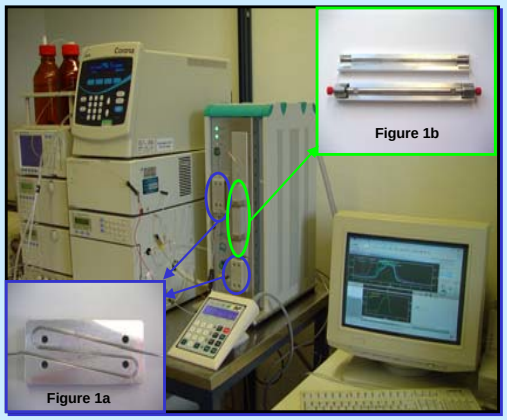

Figure 1: This illustration depicts the experimental set up of the whole HPLC system consisting of two LC-10AD$_{VP}$ pumps, a DGU-14 A degasser, a SIL 10AD$_{VP}$ autosampler, an SPD-M10A$_{VP}$ diode array detector (Shimadzu, Duisburg, Germany) and a Charged Aerosol Detector (ERC, GmbH, Riemerling, Germany). The CAD was installed directly behind the UV-DAD. The system was controlled via an SCL- 10AD$_{VP}$ controller. For data acquisition and analysis the Shimadzu Class VP software (version 6.12 SP3) was used.
The heating system was developed for high temperature liquid chromatography and consists of three modules, which can be independently controlled. The heating range of this system extends from room temperature to 225 °C with maximum heating rates of 40 °C/min. The system can be used for isothermal and temperature programmed operations. The first module is the eluent preheating unit. The stainless steel capillary is placed between two aluminium blocks (see insertion, Figure 1a). The second module is the column heating unit. The HPLC column is placed between two aluminium blocks. These blocks also enclose the column's end fittings (see insertion, Figure 1b). They have to be tailor made for different column types. The third module is for eluent cooling and is similar to the preheating unit.
Rapid cooling of the preheating and column unit is achieved by tap water from the laboratory, which can be directly connected to the heating system. Behind the heating block for eluent preheating and column heating is a copper tube where the water is flushed through. After a temperature programme is finished the water cooling is started automatically.

2.2 Dependence of detector response on eluent temperature

The first experiment should evaluate the detector response dependence on eluent temperature for both Ultraviolet and Charged Aerosol Detection. A stainless steel capillary was placed in the system instead of a column. In subsequent experiments five different HPLC columns were then placed in the system and the baseline signal was monitored. For all experiments identical conditions referring to temperature programming were maintained. The temperature programme consisted of an isothermal hold up step for five minutes at 30 °C. After this, temperature was raised within five minutes from 30 °C to 200 °C and was then held constant for 10 minutes. The system was then cooled down to 30 °C and the baseline signal was monitored for another 20 minutes. The UV signal was monitored from 190 nm to 370 nm, although chromatograms depicted in this presentation are given at a wavelength of 254 nm. The eluent cooling temperature was kept constant at 30 °C to avoid any damage to the detector. While the UV detection cell is temperature resistant to at least 80 °C, eluent temperature should not be higher than 60 °C for the Charged Aerosol Detector. HPLC columns used in this study were from Phenomenex (Luna 5 µm C-18, 150 * 4.6 mm, Part No. 00F-4041-E0, Serial No. 113499-5), ZirChrom (CARB, 3 µm, 150 * 4.6 mm, Lot No. 36-147, Serial No. CARB110901C), Thermo (Hypercarb, 5 µm, 100 * 2.1 mm, Column No. 0945316W, Part No. 35005-102146), Polymer Laboratories (PLRP-S 100 A, 3 µm, 150 * 2.1 mm, Serial No. 3M-RPS1-124B-90), and ZirChrom (Prototype-CARB-TiO$_2$, 5 µm, 150 * 2.1 mm, Lot No. 32-143, Serial No. CARBTI011105M). All but the last column are commercially available.

3 Results and Discussion

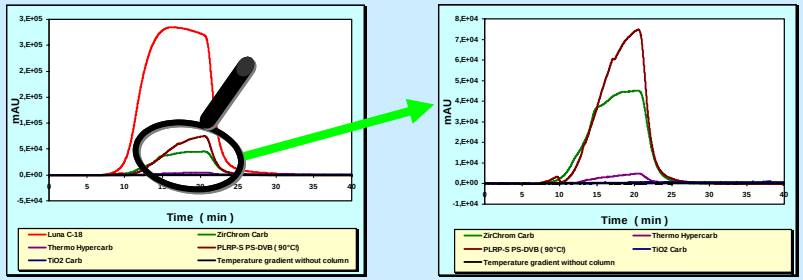

Figure 2: Signal intensity due to temperature programming for five different HPLC columns. Magnification on the right side does not include Luna C-18 column. **Detector: UVD at 254 nm**; flow rate of mobile phase (pure water): 0.5 mL/min; Temperature programme: 5 minutes at 30 °C, from 5 to 10 minutes from 30 °C to 200 °C, 10 minutes constant at 200 °C, then cooling down to 30 °C.

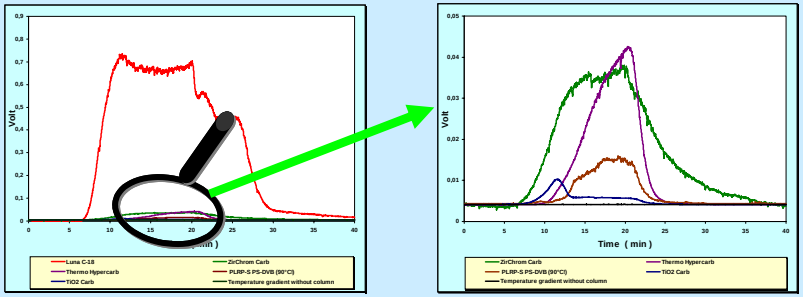

Figure 3: Signal intensity due to temperature programming for five different HPLC columns. Magnification on the right side does not include Luna C-18 column. **Detector: CAD**; flow rate of mobile phase (pure water): 0.5 mL/min; Temperature programme: 5 minutes at 30 °C, from 5 to 10 minutes from 30 °C to 200 °C, 10 minutes constant at 200 °C, then cooling down to 30 °C.

As becomes clear, the silica-based C-18 column gives the highest signal independent of the detection technique. This result is not surprising, because the silica support is readily hydrolyzed if the temperature is raised to 200 °C. In contrast to this, columns not consisting of silica gel show far less column bleed. If Charged Aerosol Detection is used, the column consisting of polystyrene divinylbenzene exhibits low bleed, whereas if UV detection is used the PS-DVB column has a steep rise in the baseline. If a carbon clad zirconium dioxide column is compared to a carbon clad titanium dioxide column, the bleed of the zirconium column is more pronounced than that of the titanium column. The carbon clad zirconium dioxide column was purchased three years ago, while the titanium column was a prototype column and delivered this year. Therefore, two explanations are possible: the process of carbonizing the support material could have been improved significantly by the manufacturer or the titanium support is more stable than the zirconium support. Comparing these two columns to the column consisting completely of graphitized carbon, it can be seen that the latter column exhibits stronger column bleed when Charged Aerosol Detection is used. This is in contrast to UV Detection and is unexpected as this phase consists of pure graphitized carbon.

4 Conclusions

Based on the experimental results it can be concluded that column bleed is significantly lower if columns designed for extreme pH-stability are used than for a conventional silica C-18 column. Furthermore, relative signal intensities of column bleed depend on the detection technique. At first sight, this might seem trivial but it should always be considered when interpreting results. For detecting HPLC column bleed, Charged Aerosol Detection offers a better alternative than Ultraviolet Detection. This is because peak area is not a function of the extinction coefficient of analytes. The prototype titanium dioxide carbon clad column shows the least bleed independent of the detection technique.

5 Acknowledgements

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