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Evaluation of active solvent modulation to enhance two-dimensional liquid chromatography for target analysis in polymeric matrices

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ABSTRACT

A new methodology is presented for two-dimensional liquid chromatography (2D-LC) separations of polymers. Active solvent modulation (ASM) was evaluated in its effectiveness to enhance solvent compatibility for both separation dimensions. As an example the determination of target compounds in epoxy resins was used. Ultra-high pressure size-exclusion chromatography was applied in the first dimension using THF as the solvent. The second dimension separation was operated in reversed-phase mode using an acetonitrile/water gradient. ASM prevents sample breakthrough in the second dimension and produces chromatograms that are of great peak shape and high resolution. It enables very sensitive determination of target components down to the low ppm level. The resulting high-speed 2D-LC method (10 min analysis time) showed good linearity ($R^2 > 0.9995$) and reproducibility (as low as 0.3–0.7% peak area RSD). ASM was also applied in comprehensive 2D-LC (SECxLC) mode for characterization of molecular weight and chemical composition distribution of a polymer blend consisting of epoxy novolac and phenol novolac. The SECxLC separation was executed at short run times (20 min). ASM technology can markedly enhance productivity in 2D-LC analysis for many complex sample matrices.

1. Introduction

Polymers constitute a class of compounds with significant heterogeneity. They typically exhibit distributions in more than one dimension, such as molecular weight distribution (MWD), functionality type distribution (FTD), chemical composition distribution (CCD), various branching distributions (BD) and more [1]. Polymers also contain various additives (e.g., inhibitors, flame retardants, plasticizers), intermediates and residual monomers. The complexity of a polymer sample renders its characterization a challenging task and very often separation methods are used for polymer analysis. Size-exclusion chromatography (SEC) is the most popular method and serves to determine MWD [1]. Gradient LC determines CCD. That is, two distinct methods are required to determine two attributes of the sample.

Two-dimensional liquid chromatography (2D-LC) is ideally suited to characterize polymers [2,3]. 2D-LC not only allows for the combination of two separation modes into one analysis but also for correlation between sample characteristics determined. E.g., by performing a gradient LC × SEC analysis one can correlate the CCD with MWD, or by coupling LC at the critical point of adsorption (LCCC) [4,5] with SEC allows correlation of FTD with MWD. Several such correlation experiments are presented in the literature. [2,6,7] They provide detailed insight into complex sample properties that could not be obtained from one-dimensional methods.

In 2D-LC, aliquots of ^{1}D effluents are transferred onto a ^{2}D column. Thus, the combination of highly complementary separation modes in a 2D-LC method can pose a considerable challenge if a solvent with high elution strength with respect to the ^{2}D column is preferred or even required for the ^{1}D separation. Strong solvents such as methanol, acetonitrile, tetrahydrofuran (THF) or chlorinated solvents (to name a few) are used to prevent irreversible adsorption on the ^{1}D column, which is often the case with polymeric sample matrices or natural products. Another example is to circumvent sample preparation and associated potential loss of target components. As such, productivity could be enhanced with a “dilute-and-shoot” approach. In both cases the strategy is to dissolve the sample in a strong solvent and to execute the ^{1}D separation at non-adsorptive conditions, as typically done in size exclusion chromatography (SEC). Strong eluents are also used in hydrophilic interaction liquid chromatography (HILIC) or normal-phase (NP) modes.

Injection of such strong solvents onto a ^{2}D column operated at reversed phase (RP) conditions can lead to severe peak distor-

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tion and a loss in resolution and sensitivity in ^{2}D. In fact, solvent strength related incompatibility between ^{1}D and ^{2}D is considered a major obstacle when developing 2D-LC methods [8]. The situation becomes even more severe when the volume of the sample transferred increases, which may be done in an attempt to increase sensitivity in ^{2}D. However, the opposite may occur with a large fraction of the solute remaining in the injection solvents and eluting within the dead volume. This phenomenon is called 'breakthrough' [9].

There are several strategies to increase the compatibility between ^{1}D and ^{2}D. One is to use an additional pump to deliver weak mobile phase in front of ^{2}D column via T-connection [10]. This approach leads to dilution of strong organic mobile phase and provides much better separations in ^{2}D. Schoenmakers and co-workers reported on various trap-and-release concepts [11], including modulation via temperature, solvent exchange and most recently via solid-phase extraction (SPE) [12]. Modulation via temperature has been executed by several research groups.[13,14] In all cases a significant signal intensity enhancement was observed, but the experimental/instrumental effort is relatively high.

Very recently a process termed Active Solvent Modulation (ASM) has been reported by Stoll et al. [15]. The ASM process dilutes the sample (^{1}D effluent) with weak mobile phase (with respect to the ^{2}D column) during the period of sample transfer from sampling loop onto the ^{2}D column, which leads to peak focusing on the column head and marked improvements in peak shape and sensitivity in ^{2}D compared with analyses where solvent compatibility precautions were not used [15]. ASM is simply a valve-based approach and does not require additional hardware nor tools such as SPE for solvent modulation. More details on ASM are provided in the experimental and results sections.

The ASM approach was applied for determining small molecule target compounds in polymeric matrices. A synthetic sample of epoxy novolac fortified with known amounts of moderately polar and non-polar compounds was used to assess the effectiveness of ASM under demanding conditions. Size-exclusion chromatography was performed using THF in ^{1}D. 40 μL fractions of THF were transferred to reversed-phase ^{2}D separations. Peak focusing in ^{2}D in relation to ASM dilution times was investigated in detail. Linearity and reproducibility of the resulting ^{2}D separation was studied. Finally, the application of ASM for heart-cutting and comprehensive 2D-LC (LCxLC) analysis of polymer blends is presented.

2. Materials and methods

2.1. Chemicals and sample preparation

HPLC gradient-grade tetrahydrofuran (THF) and acetonitrile (ACN) was used (Merck, Germany). Water was from a Milli-Q system (Millipore, Germany).

Approximately 40 mg of an epoxy novolac was dissolved in 10 mL ACN. Target solutes (bisphenol-A, diglycidyl ether of bisphenol-A (DGEBA), salicylic acid, 2-phenoxy ethanol) were prepared at approximately 10 mg in 10 mL ACN. Bisphenol-A and DGEBA were spiked to epoxy novolac at levels ranging from 40 – 320 ppm and 2.5–20 ppm, respectively. For these spiking experiments a 1 wt-% solution of epoxy novolac was prepared.

The polymer blends were prepared as follows. Different amounts of 0.4 wt-% solution of a phenol novolac were mixed with a 1% solution of epoxy novolac to prepare blend ratios of 1:2.5, 1:5, 1:10, 1:20 and 1:40. The target concentration of epoxy novolac in the final blend solutions was 0.5 wt-%. This means the corresponding concentration of phenol novolac amounted to 0.2 wt-%, 0.1 wt-%, 0.05 wt-%, 0.025 wt-% and 0.0125 wt-%, respectively.

2.2. Experimental set-up

2.2.1. First Dimension

The first dimension of the 2D-LC system (Agilent Technologies) was composed as follows: A quaternary pump (G4204A); auto sampler (G4226A); column thermostat (G1316C); a diode array UV absorbance detector (DAD, G4212A) equipped with a standard flow cell (G4212-60008; 10 mm path length; V(σ) = 1.0 μL). Chromatograms were recorded using a 20 Hz acquisition rate. The column was a Waters APC XT 45 Å, 150 × 4.6 mm, 1.7 μm column (Waters Corporation), which was thermostatted at 40 °C. THF was used as the mobile phase in isocratic mode, and the flow rate was 0.75 mL/min. Typically a volume of 1 μL was injected, and detection was carried out at 280 nm.

2.2.2. Second Dimension

The second dimension of the system was composed as follows: A binary pump (G4220A) with the 35 μL JetWeaver mixer, column thermostat compartment (G7116B), and a diode array UV absorbance detector (DAD, G4212A) equipped with a 60 mm flow cell (G4212-60007, V(σ) = 4.0 μL) or a 10 mm flow cell (G4212-60008). Chromatograms were recorded using a 40 Hz acquisition rate. The columns were a Poroshell C_{18} 50 × 3 mm, 2.7 μm column (Agilent Technologies) or Ascentis Express C_{18} 50 × 3 mm, 2.7 μm column (Merck Sigma-Aldrich). All columns were thermostatted at 30 °C. Solvent A was water, and solvent B was ACN. The gradient elution program was: 10–10–30–80–10%B from 0–0.5–0.6–2.5–2.51 min. The total ^{2}D cycle time was 3.5 min, and the flow rate was 1 mL/min.

2.2.3. Interface

The two dimensions of the system were interconnected by the Multiple Heart Cutting (MHC) 2D-LC interface shown schematically in Fig. 1. MHC facilitates parking of ^{1}D aliquots in sampling loops of which twelve are distributed across two parking decks (A and B). Parking occurs in parallel to running ^{2}D cycles so that loops may be reused for parking. Details about operation of the MHC interface is reported elsewhere.[16,17] In contrast to the standard MHC interface (operated with Duo valve modulator [5067–4244]) the interface in Fig. 1 is configured with the new ASM valve that incorporates an additional flow path (ASM path). In the valve position shown in Fig. 1A, the ASM process is active. The ASM path is connected to the ^{2}D pump in parallel with the path through sampling loop-1 of MHC deck-B (flow indicated in red) in which an aliquot of ^{1}D effluent (blue) has been sampled previously. This causes the content of loop-1 to move towards the ^{2}D column. Since both paths reunite at the valve exit at port-X, the content of loop-1 is diluted with ^{2}D mobile phase prior to reaching the ^{2}D column (violet).

The elution strength of this sample (with respect to the ^{2}D column) depends on the flow rate through loop-1, through the ASM path and the solvent composition of the ^{2}D mobile phase pumped during the ASM period. The ^{2}D gradient typically initiates at weak elution strength conditions resulting in sample dilution, a weakening of the elution strength of the sample, and subsequently a focusing effect of the compound of interest on the ^{2}D column head [15]. Once the sample has been transferred onto the ^{2}D column, the ASM valve switches to the analysis position in Fig. 1B so that the remainder of the ^{2}D gradient is delivered through sampling loop-1. In the same way, all sampling loops on decks A and B are processed. Fig. 1C shows ASM position for cut #3 parked in loop-3 of deck A and Fig. 1D the final ^{2}D analysis position of cut #3. The ASM process is temporary because permanent dilution (i.e., fixed solvent modulation) can cause baseline disturbances with UV detection and would increase 2D-cycle time due to split ratio dependent flush out periods for the sampling loops [15].