Improving the signal-to-noise ratio in gel permeation chromatography by Hadamard encoding

Alexander F. Siegle, Oliver Trapp*

Organisch-Chemisches Institut, Ruprecht-Karls-Universität Heidelberg, Im Neuenheimer Feld 270, 69120, Heidelberg, Germany

ABSTRACT

Increasing sensitivity without further preconcentration steps is a major challenge in separation sciences. In this study a macro control approach that allows the implementation of Hadamard encoding on standard HPLC instrumentation was applied to the analysis of polysiloxane and polystyrene polymers by gel permeation chromatography. Here, the use of pseudo-random modulation sequences with 1023 elements (512 sample injections) improved the signal-to-noise ratio (S/N) by up to an order of magnitude. Comparison with conventional single injection confirmed the linearity and accuracy of the deconvolution process for the whole mass range of the column.

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1. Introduction

Gel permeation chromatography (GPC) is a liquid chromatographic technique that is widely used to separate and purify polymers and to determine their average molecular weight and molecular weight distribution [1]. It is also commonly applied in the quantification of macromolecules such as cyanobacterial glycogen [2], polysaccharides from seaweed [3], rotavirus-like particles [4] or naturally occurring *trans*-1,4-polyisoprene [5]. Compared to other modes of liquid chromatography sensitivity is often lower for GPC techniques, especially when coupled to refractive index or UV detection. Therefore it is desirable to develop methods for GPC that increase sensitivity of detection.

An information technology approach to increase sensitivity in separation sciences utilizes the modulation of the sample injection according to pseudo-random binary sequences. Decoding the obtained multiplexed data by application of the inverse Hadamard transform leads to significantly improved signal-to-noise ratios (S/N). This concept was first realized under the term correlation chromatography and employed in gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) [6–15]. Multi injection approaches based on pseudo-random modulation of the sample injection have also been applied to time-of-flight mass spectrometry [16–18], ion mobility spectrometry [19,20], capillary electrophoresis [21–25] and gas chromatography/mass spectrometry (GC/MS) [26–31].

Our group developed a macro control approach that allows performing pseudo-random injection sequences on standard HPLC instrumentation [32]. Since the success of the multi injection technique highly depends on the uniformity of the chromatographic performance in terms of parameters such as injection volume and retention times it proved advantageous to utilize commercially available HPLC systems. Besides the application in a conventional HPLC setup this approach has been employed for enhancement of signals in HPLC hyphenated with circular dichroism detection (HPLC-CD) [33]. Using structured modulation sequences and a deconvolution algorithm for the determination of analyte peak areas allowed rapid multisample analysis in GC [34,35] and HPLC [36].

In this study we report on the application of Hadamard encoding by the macro control approach to increase sensitivity in the GPC-UV analysis of polymer samples of varying complexity in order to demonstrate the applicability of this approach to other modes of separation in liquid chromatography.

2. Materials and methods

2.1. Chemicals and reagents

Tetrahydrofurane (THF, HPLC grade, 99.9%) without stabilizer was used as mobile phase for the experiments and purchased from Sigma-Aldrich. The polymer methyl vinyl polysiloxane (MVPS) which contained 4.5% vinyl groups was synthesized in our lab
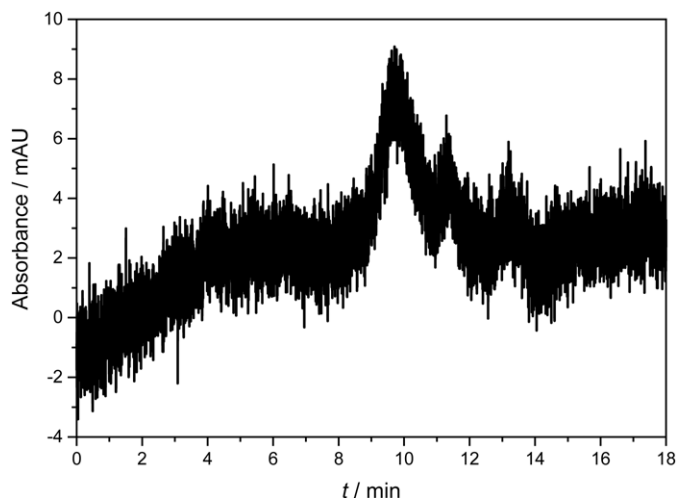
Fig. 1. Conventional chromatogram of a methyl vinyl polysiloxane sample. At the detection wavelength of $\lambda = 210$ nm excessive baseline noise can be observed.

[37]. A polysterene standard containing four polymers with peak molecular weights of $M_p = 67500$, 15700, 4870 and 1220 Da was purchased from PSS Polymer Standard Service (Mainz, Germany). The investigated polymers were dissolved in THF for the measurements to obtain concentrations of 19 mg/mL for the MVPS polymer and 10 and 100 μg/mL for the polystyrene polymers, respectively.

2.2. Instrumentation

Multiplexing GPC experiments were performed on an Agilent Infinity 1260 system equipped with a high performance autosampler model HiP-ALS SL+, a photodiode array detector (DAD), a refractive index detector (RID) and controlled by the ChemStation software package. Due to the experimental setup a delay of 0.25 min between the UV detector and the refractive index detector was observed for the elution time. In a multiplexing run the sample was injected according to pseudo-random n-bit Hadamard modulation sequences with $m = 2^n - 1$ sequence elements. Each sequence contains $2^n/2$ elements "1" which correspond to an injection and $2^n/2 - 1$ elements "0" for which no injection is performed. Modulation sequences were applied to the autosampler of the HPLC instrument by the execution of a macro as a pre-run command in ChemStation. The macro, its functionality and technical limitations of this approach have been described previously [32]. Separation of the MVPS sample and the polystyrene mixture were conducted on a PSS SDV analytical linear S column (particle size: 5 μm, 8 × 300 mm, 8 × 50 mm precolumn). THF was used as mobile phase with a flowrate of 1.0 mL/min. The column thermostat was set to 30 °C and the detection wavelength of the DAD was $\lambda = 210$ nm (MVPS) and $\lambda = 220$ nm (polystyrene), respectively. Typically an injection volume of 1 μL was used for the multi injection experiments, though the full range provided by the autosampler instrumentation can be utilized.

2.3. Data acquisition and deconvolution

The multiplexed and single injection data were acquired with a rate of 40 Hz (DAD) and 2.3 Hz (RID), respectively. Generation of the pseudo-random binary modulation sequences by use of a virtual shift register [38] and deconvolution of the multiplexed data by inverse Hadamard transform were performed using software written in Delphi (Embarcadero Technologies, South San Francisco, U.S.A.). The acquired multiplexed chromatograms were corrected
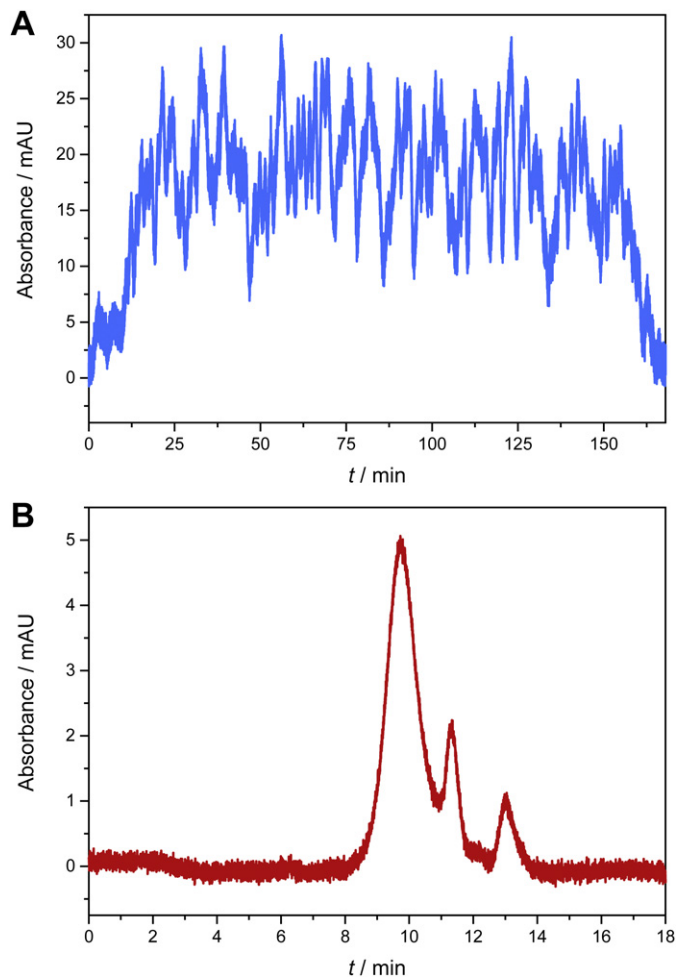

Fig. 2. Hadamard transform multiplexing experiment for the analysis of an MVPS polymer. Multiplexed chromatogram (A) obtained by modulation with $\Delta t = 18$ s according to a pseudo-random binary sequence with $m = 511$ elements (256 sample injections) and deconvoluted chromatogram (B) with significantly enhanced signal-to-noise ratio.
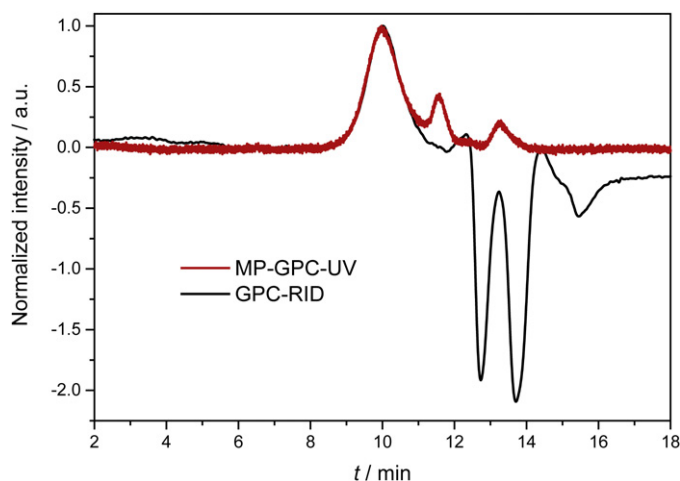

Fig. 3. Comparison of the UV chromatogram obtained by multiplexed UV analysis (MP-GPC-UV, red line) and the chromatogram obtained by conventional refractive index detection (GPC-RID, black line) shows good agreement for the MVPS peak at an elution time of 10 min (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)