# Implementing a multivariate curve resolution method optimized by alternating least square (MCR-ALS) to deconvolute overlapping spectral polymer signals in SEC-DAD separations

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## INTRODUCTION

Peaks eluting from a size exclusion separation are often not completely baseline-separated, due to the inherent polydispersity of the polymer and low efficiency of the separation mechanism. Chemometrical deconvolution provides the possibility of calculating the contribution of each peak separately from the recorded spectrum[1]. Herefore, an in house developed MATLAB script dis-criminates between the different compounds based on their difference in UV-spectrum and retention time, using the entire 3D retention time-UV spectrum. The output of the script provides the calculated chromatograms of each compound as well as their respective UV-spectrum[2]. The latter can be used for peak identification, while quantitative calculations can be performed on the chromatographical peaks. This aproach allows for overlap in both rentention time as UV-spectrum, speeding up the analyses and extending the separation power of SEC separations. The applicability (both qualitative as quantitative) has been demonstrated on a mixture of three different polymer types.

## POLYMER STRUCTURES

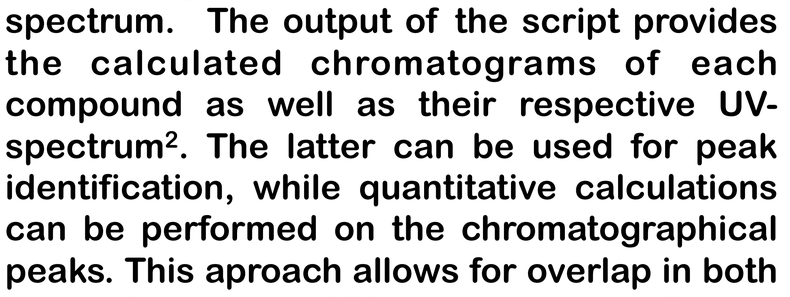


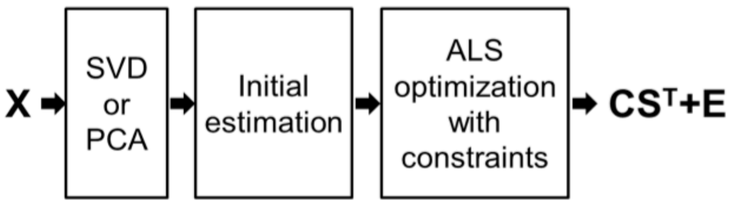
Figure 1: Left: structure of Polystyrene (PS), 41.400 g/mol; center: Poly ethoxyethyl acrylate (PEEA), 20.100 g/mol; right: poly methylmethacrylate (PMMA) 10.200 g/mol

## EXPERIMENTAL

| | |
|---|---|
| Instrumentation | Agilent 1100 LC system with autosampler |
| column | PIgel Mixed-D, 150 mm x 2 mm, 5 µm, in house packed (SEC) |
| column temperature | 30 °C |
| injection vol. | 3 µl |
| Flow rate, mobile phase | 50 µl/min, THF |
| Detection | Diode Array Detector (DAD): data collected from 190 nm up to 600 nm |

## DATA TREATMENT

The data matrix obtained from HPLC-DAD consists of two convoluted dimensions, i.e. the chromatographic profile and the spectro-metric profile. The deconvolution script results in the pure chromatographic and spectrometric profile from the bilinear data matrix X by applying certain constraints in the optimization process to obtain the pure chromatograms C, spectra S and the estimated resudual matrix E, as depicted below.




Schematic representation of the MCR-ALS method. SVD: singular value decomposition; PCA: principal component analysis[1].

## RESULTS & DISCUSSION

From the overlap of the pure spectra of the three polymers (Figure 2) can be concluded that quantification of PMMA is not possible because of the spectral overlap around 226 nm. Also, the spectra of the pure polymers and the obtained calculated spectra, after deconvolution of a mixture, are depicted. Almost complete overlap confirms the power of the deconvolution method.

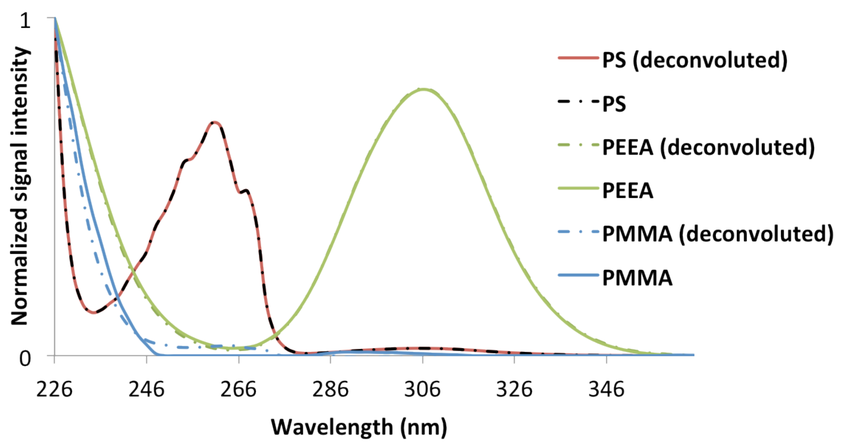



Figure 2: Overlayed spectra of PS, PEEA and PMMA before and after deconvolution

From the conventional multiple wavelength chromatogram in Figure 3 (a), one can observe that no baseline separated peaks could be obtained. Especially for PMMA, which does not exhibit a selective UV absorption at another wave-length than 229 nm, a clear overlap with PS and PEEA is present.

Figure 3 (b,c,d) shows the overlayed deconvolution results in which for each chromatogram the concentration of one component is altered. Successful baseline separated peaks are obtained. From the concentration deviation in Table 1, one observes a good accuracy and acceptable low influence of the alternating concentration component on his neighboring peak(s).

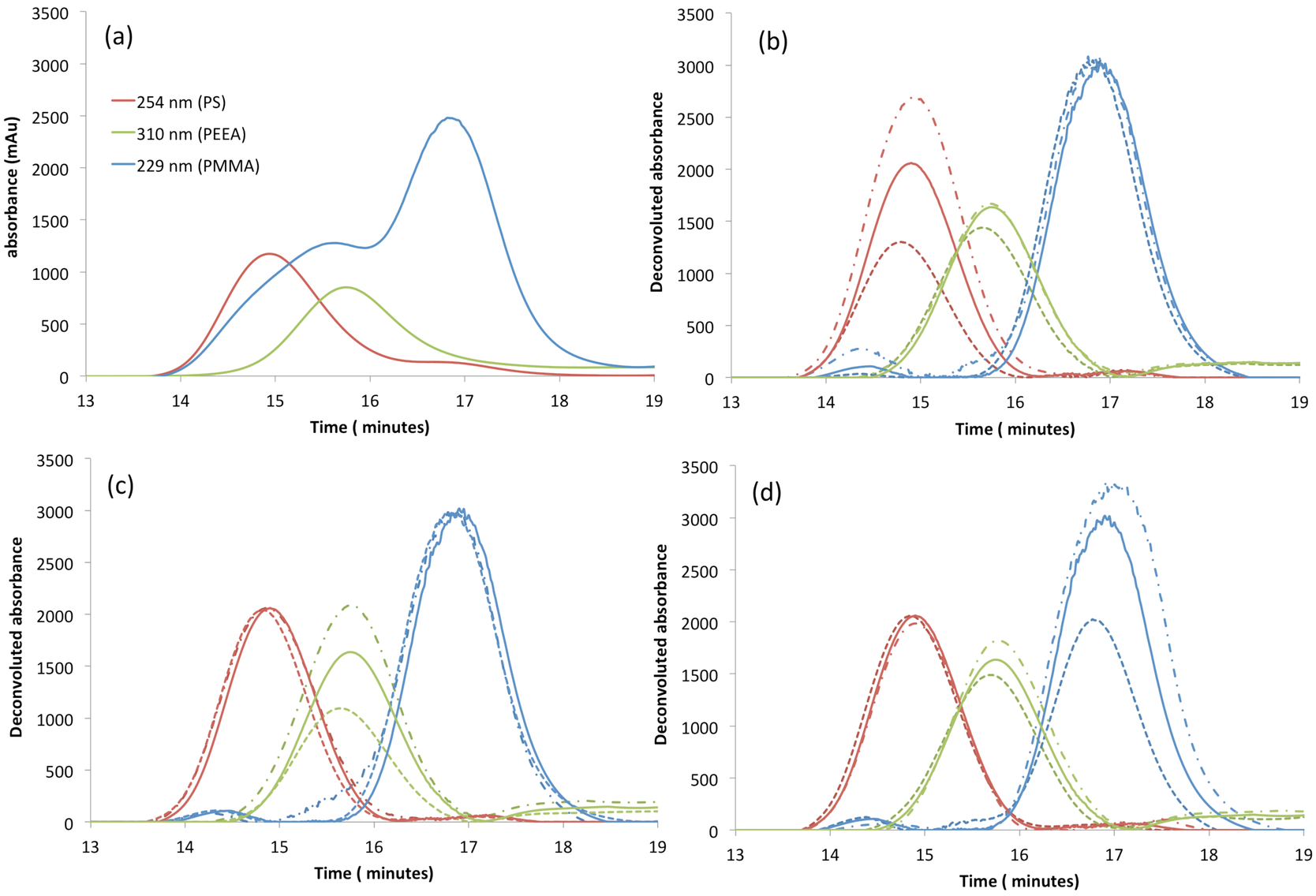



Figure 3: (a): Chromatograms recorded with a diode array detector (DAD) for each polymer at the optimal wavelength. (b), (c) & (d): overlays of the deconvoluted polymer mixtures, for which in each chromatogram one of the three polymer concentrations is altered (higher and lower in comparison with the three basic concentrations: 3,500 ; 6,000 and 10,000 µg/ml for PS, PEEA and PMMA, respectively. These basic concentrations are represented in all three chromatograms by the full lines. Exact composition and figures of merit of each chromatogram is presented in Table 1.

| | | Actual concentration (µg/ml) | Calculated concentration (µg/ml) | Standard deviation (%) | Concentration deviation (%) |
|---|---|---|---|---|---|
| (b) | **PS** | **2,000** | **2,085** | **6.71** | **+4.27** |
| | PEEA | 6,000 | 5,528 | **1.48** | **-7.87** |
| | PMMA | 10,000 | 10,399 | **1.44** | **+3.99** |
| | **PS** | **5,000** | **4,978** | **2.43** | **-0.44** |
| | PEEA | 6,000 | 6,229 | **1.02** | **+3.82** |
| | PMMA | 10,000 | 10,871 | **1.57** | **+8.71** |
| (c) | PS | 3,500 | 3,260 | **1.63** | **-6.85** |
| | **PEEA** | **4,500** | **4,293** | **0.81** | **-4.61** |
| | PMMA | 10,000 | 10,721 | **0.30** | **+7.21** |
| | PS | 3,500 | 3,756 | **2.75** | **+7.31** |
| | **PEEA** | **7,500** | **7,710** | **6.16** | **+2.82** |
| | PMMA | 10,000 | 10,802 | **1.24** | **+8.02** |
| (d) | PS | 3,500 | 3,592 | **4.54** | **+2.62** |
| | PEEA | 6,000 | 5,845 | **7.41** | **-2.59** |
| | **PMMA** | **7,000** | **6,704** | **1.57** | **+8.71** |
| | PS | 3,500 | 3,212 | **2.26** | **-8.21** |
| | PEEA | 6,000 | 6,617 | **1.01** | **+10.28** |
| | **PMMA** | **13,000** | **13,481** | **1.48** | **+3.68** |

Table 1: Composition of the chromatograms from Figure 3, actual prepared concentration, calculated concentration after deconvolution, standard deviation between the calculated and actual concentration, deviation on the calculated vs the actual concentration. A minimum 5-points calibration was carried out for each component separately.

## CONCLUSION

- Succesful baseline separated deconvolution of a mixture of three different polymers overlapping in both retention times and UV spectra.
- Good accuracy of the calculated concentrations under varying amounts of neighbouring polymers.
- Faster size exclusion chromatography (SEC)-analyses possible because overlap of retention time is allowed.
- Proof of concept of the deconvolution method and proof of applicability in the polymer analysis world.

References:
1 E Salvatore, M cocchi, A Marchetti, F Marini, A de Juan, Anal Chim Acta 2013, 761, 34-45
2 K Wilberg, J Chromatogr A, 2009, 1216, 7063-7070