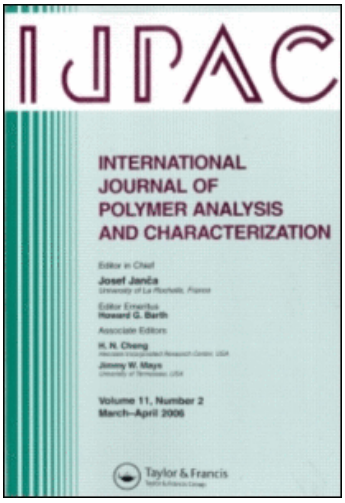


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J. Falkenhagen[a]; J. F. Friedrich[a]; G. Schulz[a]; R. -P. Krüger[a]; H. Much[a]; St. Weidner[a]
[a] Federal Institute for Materials Research and Testing, (BAM), Dept. Vl. 3, Berlin, Germany

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# Liquid Adsorption Chromatography *near* Critical Conditions of Adsorption Coupled with Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

J. FALKENHAGEN, J.F. FRIEDRICH, G. SCHULZ, R.-P. KRÜGER, H. MUCH and ST. WEIDNER*

*Federal Institute for Materials Research and Testing, (BAM), Dept. VI. 3, Unter den Eichen 87, D-12205 Berlin, Germany*

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Chemical heterogeneities and molecular weight distributions of poly(ethylene oxide) (PEO)-*co*-polymethylene (PM) model oligomers, which are relevant to the synthesis of commonly used tensides, were investigated. For analytical characterization, the well-known principle of liquid adsorption chromatography at 'critical conditions' (LACCC) was modified. Near the critical conditions of adsorption of the PEO unit, e.g., at slight adsorption conditions of PM, the copolymers could be separated according to their PM chain length. The eluates were separated and single fractions of each peak were continuously transferred onto the MALDI target by means of a commercially available device. Simultaneously, the MALDI matrix solution was continuously added with a second pump. This procedure offers the possibility of the formation of homogeneous matrix–polymer textures. By MALDI-MS a complete characterization of the chemical composition (PEO and PM chain length) of each peak could be achieved. The obtained MALDI mass spectra of the eluates at different retention times could be used for the molecular weight calibration of the LAC system. In this way, an additional application of SEC, as in conventional 2D-chromatography, was avoided by using the MALDI method as quasi chromatographic separation.

*Keywords:* Liquid adsorption chromatography; Matrix-assisted laser desorption/ionization mass spectrometry; Coupling methods, MALDI

* Corresponding author.

## INTRODUCTION

In recent years, the simultaneous characterization of chemical functionalities and molecular weight distribution of macromers and polymers has become more important. The most widely used method for polymer characterization is size exclusion chromatography (SEC) coupled with different detectors both for molecular weight and structural information. Nevertheless, chemical heterogeneities, that is, chemical composition and block length of copolymers, different end groups or different polymer architecture (stars, cycles, dendrimers, etc.), strongly superimpose and influence the molecular weight separation in SEC. Therefore, new methods of polymer characterization need to be established to elucidate chemical heterogeneities, as well as molecular weight distributions of complex polymers, simultaneously.

The principle of two-dimensional 'orthogonal' liquid adsorption chromatography is based both on the separation of polymers according to their molecular weight (SEC) and to functional groups (interaction chromatography, IC). This type of chromatography was introduced first by Pasch, Schulz, Much, Gorshkov and co-workers.[1–4]

In the first dimension of 2D-chromatography, the molecules are separated under 'critical conditions of adsorption' according to their chemical functionalities. At these chromatographic conditions the separation mechanism is characterized by a compensation of entropic and enthalpic contributions. The thermodynamic interpretation is given by the Gibbs–Helmholtz equation for the free enthalphy

$$\Delta G = \Delta H - T\Delta S \tag{1}$$

In contrast to the size exclusion mode of the chromatography with $\Delta S < 0$ and $\Delta H = 0$, the adsorption mode is characterized by enthalpic interactions between the stationary phase and polymer molecules. This can be expressed through $\Delta H < 0$ and $T\Delta S \ll \Delta H$. The compensation of enthalpic and entropic interactions of the repeating unit at the 'critical point of adsorption' leads to $\Delta G = 0$. A more detailed description can be found in Refs. [5–8].

By the use of a valve, the eluted samples can be transfered on-line from the first chromatographic dimension (LACCC) into the second chromatographic dimension, in which the polymers are separated

according to their molecular weight by means of conventional SEC. The calibration of SEC requires polymer standards, which are not available for special polymers and copolymers, or the use of molecular weight-sensitive detectors, such as laser light scattering or viscometric detectors. However, the coupling of two different chromatographic systems is an expensive method and considerable efforts to determine the critical conditions of adsorption are needed.

For some polymer systems, the adsorption mode *near* critical conditions [$LAC_{near}CC$] (but still in the adsorption mode) can be used for improving simultaneous characterization of chemical and molecular weight heterogeneities. The separation conditions at $LAC_{near}CC$ can be obtained by changing the solvent composition at the 'critical' point of adsorption by the addition of a thermodynamically 'poor' solvent. This only requires the determination of 'critical' conditions of adsorption, approximately. The broadened peaks obtained in the $LAC_{near}$ CC mode are superimposed by a second distribution which characterizes the molecular weight of polymers possessing the same chemical composition. The eluate of a chromatographic run can be fractionated and single fractions can be transferred and deposited on-line onto the MALDI-MS targets.

In the last few years the use of matrix-assisted laser desorption/ ionization – time-of-flight – mass spectrometry (MALDI-TOF-MS) for simultaneous investigation of molecular weight distribution and chemical heterogeneities of polymers was shown. Few attempts of on-line- and semi on-line coupling of MALDI-MS with SEC (mostly for SEC calibration) were reported, that illustrate the applicability of this combination for polymer investigations.[4,9–13] By the use of MALDI-MS as a molecular weight sensitive detector in the $LAC_{near}CC$ mode, the determination of the molecular weight distributions and the chemical structure of polymers could be achieved simultaneously without an additional SEC analysis. Therefore, this combination of methods ($LAC_{near}CC$ coupled with MALDI-MS) does not represent an orthogonal 2D-chromatography, but more of a semi on-line LC-MS coupling. Due to the coupling of the quantitative aspects of LC detection with the accuracy of molecular weight determination of MALDI-TOF-MS, complete information of the structure of functional oligomers, macromers and, partially, of polymers, is possible.

## EXPERIMENTAL

For chromatographic investigations (LAC, $LAC_{near}CC$ and SEC), a Hewlett Packard HP 1090 M liquid chromatograph equipped with a Nucleosil 300 Å column with 5 μm pore size, 250 mm length and 4 mm i.d. was used. An evaporative light scattering detector (ELSD) SEDEX 45 (Sedere), and a refractive index detector, HP 1047A (Hewlett-Packard), were used. The chromatographic systems were kept at a constant temperature of 45°C. The eluent flow was adjusted at 0.5 mL/min.

The PEO-*co*-PM samples were obtained from Dr. Sobisch (ACA e.V., Berlin). Their structure can be expressed in terms of

$$HO-(CH_2CH_2-O)_x-(CH_2)_yH$$

The oligomers consist of diblocks, exclusively. Due to the mechanism of addition of ethylene oxide to alcohols, different (statistically distributed) PEO block lengths ($x$) were obtained. The length of the polymethylene unit ($y$) is determined by the length of the chosen alcohol ($C_8$–$C_{18}$).



### SEC Mode

By using pure methanol as the mobile phase and Nucleosil columns, the SEC mode was realized. The sample concentration was about 1 mg/mL.

### LACCC/$LAC_{near}CC$ Mode

The determination of 'critical conditions' of adsorption was performed starting with methanol as a thermodynamically 'good' solvent. Successive addition of water as a thermodynamically 'poor' solvent resulted in a change of the chromatographic mode to adsorption mode. The 'critical point of adsorption' was reached if samples with the same chemical composition, but different molecular weight, were eluted in one peak. For the investigated polymer system these 'critical solvent composition' (CSC) could be obtained by adjusting a methanol/water

mixture of 78.5/21.5 wt%. The $LAC_{near}CC$ mode was obtained by successive addition of water up to an amount of 26.3 wt% in the eluent. The composition of the mobile phase was adjusted by weighing of the single solvents.

## MALDI-TOF-MS

Two different MALDI mass spectrometers, a Kratos Kompact MALDI III (Shimadzu, Germany) (for fractionation) and a Bruker Reflex III mass spectrometer (Bruker-Daltonik, Germany) (for investigation of mixture of samples), both operating at 20 KV acceleration voltage, were used. For ionization/desorption in both instruments, a UV laser working at a wavelength of 337 nm was applied. The laser pulse length was 3 ns. Typically, 100–200 transients were accumulated for one spectra. Sodium adduct ions were formed during ionization.



### Preparation of Matrix-Sample Spot

2,4,6-Trihydroxyacetophenone (THAP) (Aldrich, Germany) was used as the matrix. Matrix solutions with a concentration of 1 mg/mL (in tetrahydrofuran) were prepared and premixed with polymer solutions (1 mg/mL). The mass spectrometer was calibrated with different standard peptides, as well as by self-calibrating methods using polymers.

### Coupling of LAC with MALDI-TOF-MS

A commercially available interface LC 500 (Lab Connections; USA) was used for semi-on-line coupling of liquid chromatography and mass spectrometry. The matrix THAP, which was dissolved in THF (*ca.* 1 mg/mL), was added continuously through a mixing T-fitting to the eluent after the refractive index detector outlet by means of a secondary pump (Knauer, Germany). The flow rate of the added matrix solution was 0.2 mL/min. The resulting overall flow of 0.7 mL/min was focused onto the corresponding MALDI targets (Bruker or Shimadzu). Precoated targets (Lab Connentions), likewise available, were not used. The eluates, containing polymer, solvent and matrix, were sprayed onto the target and, simultaneously, the solvent was evaporated in a nitrogen gas stream at an elevated temperature of 170°C.

The temperature was carefully adjusted to avoid crystallization of matrix on the tip. The transfer system was controlled by software which enables the automatic assignment of sample spots to the corresponding retention times.

The evaporative light scattering detector (ELSD) was not used for these coupling experiments.

## RESULTS

The calibration curves for a polymer system in the three different modes of chromatography (SEC, LACCC and LAC) are exemplary shown in Figure 1. In the SEC mode, a typical S-shaped calibration curve can be obtained which expresses the separation according to the hydrodynamic volume of polymers beginning from high molecular weight molecules. In contrast to SEC, the LAC mode separates according to chemical functionality, which is superimposed by an additional molecular weight distribution. In the LACCC mode the molecules are only separated according to their functionalities independent of their molecular weight.



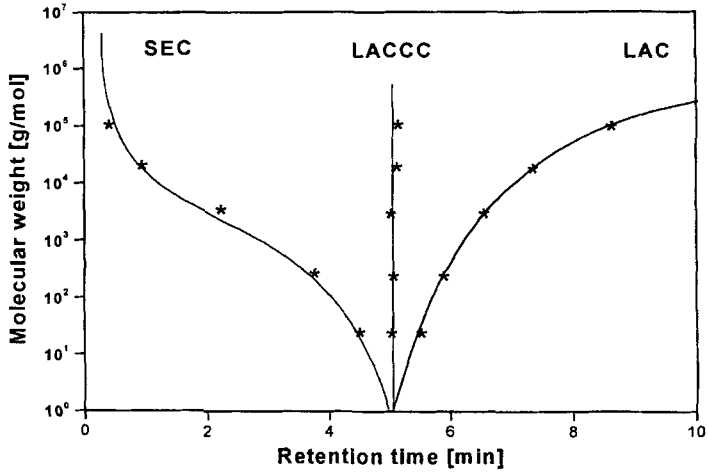


FIGURE 1 Three modes of chromatography: SEC: $\Delta S < 0$; LACCC: $\Delta G = 0$; and LAC: $\Delta H < 0$ in which $S$ is the entropy, $H$ is the enthalpy, and $G$ is the free enthalpy.

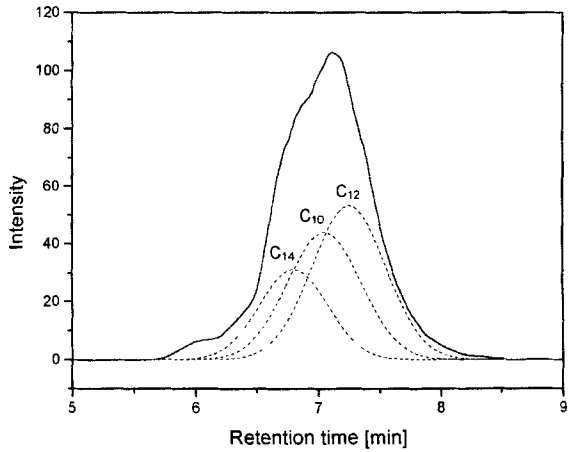


FIGURE 2 Chromatogram in the SEC mode of PEO-*co*-PM oligomers with PM chain lengths of $C_{10}$, $C_{12}$ and $C_{14}$ (and the corresponding 1 : 1 : 1 w/w/w mixture) using ELSD detection and 100% methanol as the eluent.



The SEC chromatogram of a 1 : 1 : 1 (w/w/w) mixture and corresponding PEO-*co*-PM oligomers with PM chain lengths of $C_{10}$, $C_{12}$ and $C_{14}$ is shown in Figure 2. A poorly resolved peak with shoulders at shorter retention times, i.e., higher molecular weight, was recorded. This figure illustrates the difficulties associated with the characterization of the molecular weight of samples differing by a few PEO as well $C_2$ units. Likewise, MALDI mass spectrometry could not elucidate the molecular weight or chemical heterogeneities of single components of the mixture. The MALDI mass spectrum, presented in Figure 3, shows three different molecular weight distributions with maxima at 2300, 1100 and 600 g/mol and different residual masses, corresponding to $C_{10}$, $C_{12}$ and $C_{14}$ 'end groups'. Additionally, another peculiarity of the MALDI analysis of mixtures becomes obvious. Although the mixture contains equal amounts of the PEO-*co*-PM oligomers, the signal intensity was completely different. It is known from previous investigations that different ionization probabilities of polymers, even if they differ slightly in structure, could strongly influence the intensity in MALDI mass spectra.[14,15] Therefore, a quantitative evaluation of MALDI analysis of mixtures is impossible in most cases.

In Figure 4 the LAC chromatogram of a mixture of PEO-*co*-PM oligomers at the 'CSC' is shown. The sharp peaks represent the

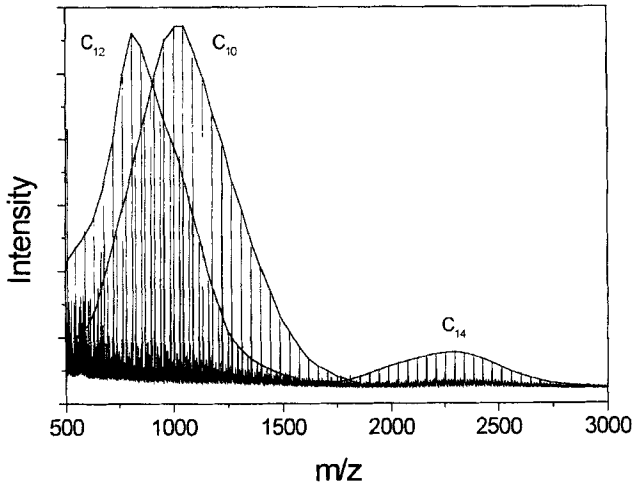


FIGURE 3 MALDI-TOF mass spectra of PEO-*co*-PM oligomers with PM chain lengths of $C_{10}$, $C_{12}$ and $C_{14}$ (1 : 1 : 1 w/w/w mixture) using THAP as the matrix.



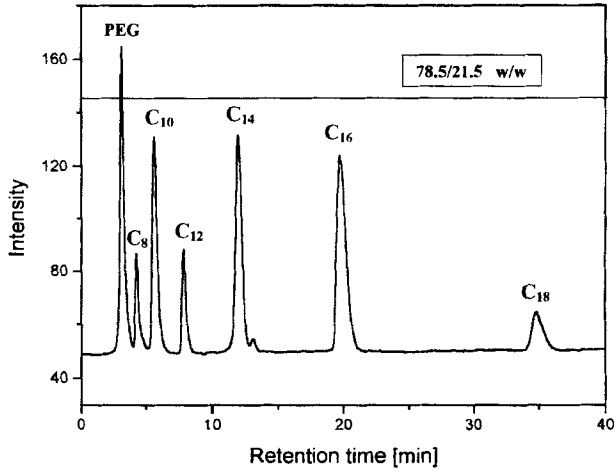


FIGURE 4 LAC chromatogram of PEO-*co*-PM oligomers at the 'critical' point of adsorption (methanol/water; 78.5/21.5 wt%) using ELSD detection.

oligomers that were separated according to their 'end groups' (PM chain length), independent of the molecular weight of the PEO. At a higher concentration of water in the eluent (> 21.5 wt%), the separation conditions were changed into the adsorption region *near* the

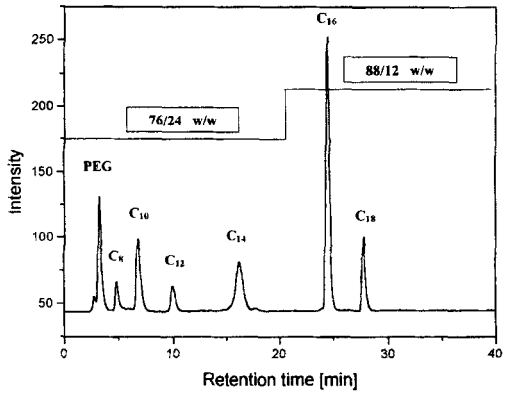


FIGURE 5 LAC chromatogram of PEO-*co*-PM oligomers in LAC mode *near* the 'critical' point of adsorption (methanol/water; 76.0/24.0 wt%); from 0–20 min and 88.0/12.0 wt%; from 20 min retention time) using ELSD detection.



'critical' point (see Figure 5). At this point, the peaks became significantly broader and, simultaneously, the retention time increases. To accelerate the elution of PM with chain lengths more than 14, the composition of the solvent had to be changed to a higher water concentration at 20 min retention time. However, the broadening of peaks was not sufficient for MALDI fractionation. Hence, the amount of water was further increased up to 26.3 wt%. At this methanol/water eluent ratio, the chromatograms of $C_{10}$, $C_{12}$ and $C_{14}$ PEO-*co*-PM oligomers are shown in Figure 6. Relative broad peaks were obtained. The samples eluted within 3 min ($C_{10}$), 4 min ($C_{12}$) and 8 min ($C_{14}$). This enables fractionation of the chromatographic run with sufficiently high resolution. The points in Figure 6 ($C_{12}$) represent single fractions separated for MALDI analysis. A further increase of water concentration (> 30 wt%) resulted in broad, not baseline-resolved peaks. Futhermore, this could contaminate the columns, because higher molecular weight compounds may not be eluted.

The eluates were continuously sprayed (together with the matrix solution) onto the MALDI target. MALDI mass spectra of clearly defined positions on the target were recorded. The results of the fractionation of $C_{12}$ sample and their subsequent MALDI analysis are shown in Figure 7. The spectra are characterized by peak pattern with different maxima, but the same repeat unit of 44 g/mol, which is

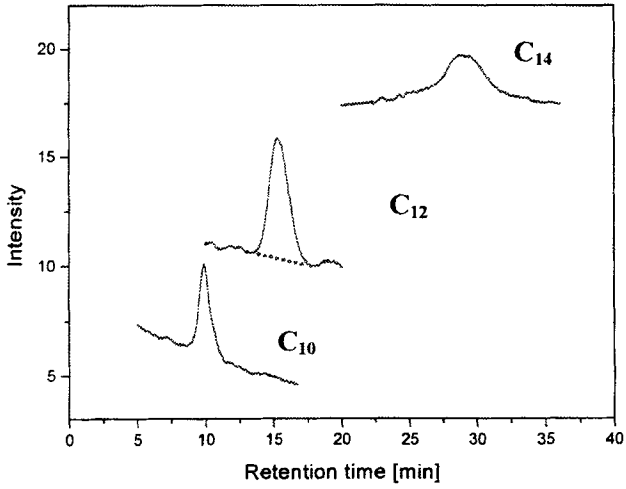


FIGURE 6 LAC chromatogram of PEO-*co*-PM oligomers with PM chain length of $C_{10}$, $C_{12}$ and $C_{14}$ in LAC mode *near* 'critical' point of adsorption (methanol/water; 73.7/26.3 wt%) using refractive index detection



characteristic for poly(ethylene oxide) (PEO). A second distribution of PM homologous (repeat units of 14 or 28, depending on the process of synthesis) could not be found. Thus, the separation of PEO-*co*-PM oligomers in the $LAC_{near}CC$ mode according to PM chain length could be exclusively proved by means of MALDI-TOF-MS.

The sample-matrix preparation using the automatic interface was very homogeneous and, additionally, the same laser power was applied. Therefore, the intensity of the obtained spectra could be compared directly. However, the ratio of sample and matrix concentration was not equal. For further investigations this ratio should be kept constant using a pump control, which varies the flow of the matrix pump depending on the intensity of the concentration detector.

The molecular weight at peak maximum was used to create a calibration curve, which is shown for $C_{10}$, $C_{12}$ and $C_{14}$ in Figure 8. In comparison to Figure 1, the steep slopes of the calibration curves presented in Figure 8 indicate, that the retention in the LAC mode results in near 'critical conditions'. However, the longer the PM blocks, the more the adsorption effects appear. This becomes obvious looking at the decreased slope of the calibration curves at the higher molecular weight PM tails. A better chromatographic separation should be

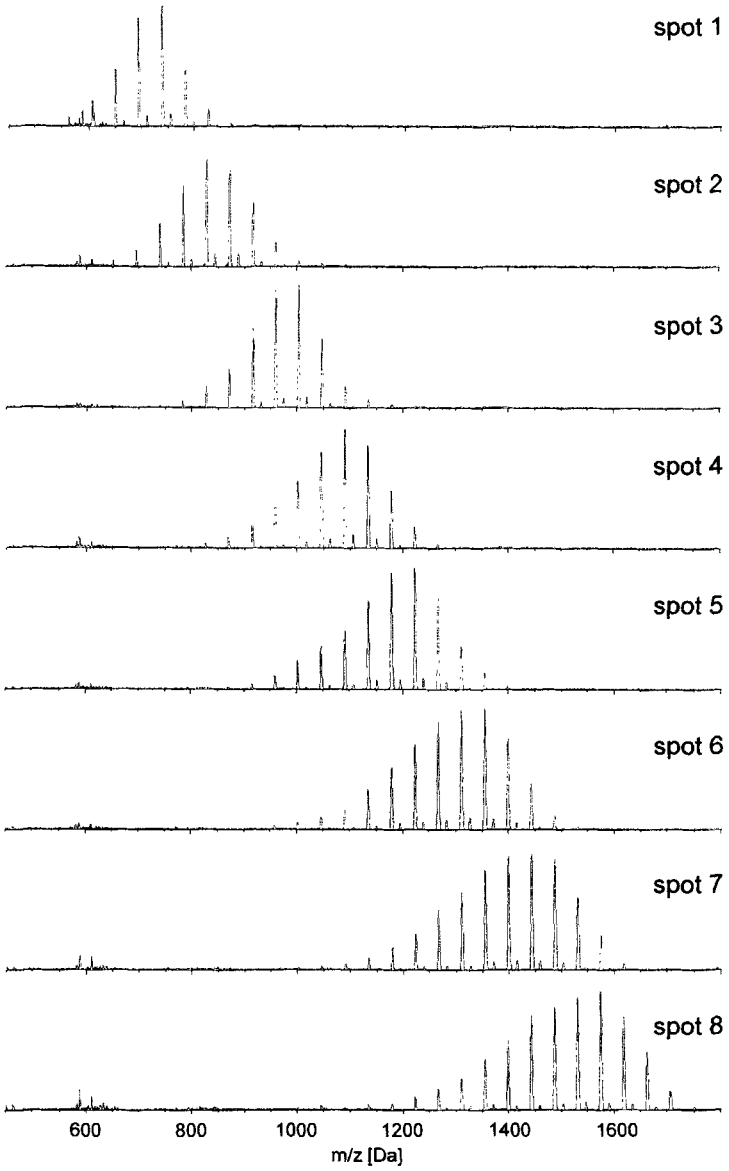


FIGURE 7 MALDI-TOF mass spectra of PEO-*co*-PM ($C_{12}$) oligomer fractions obtained by $LAC_{near}CC$ (matrix: THAP, on-line deposition of sample and matrix simultaneously).

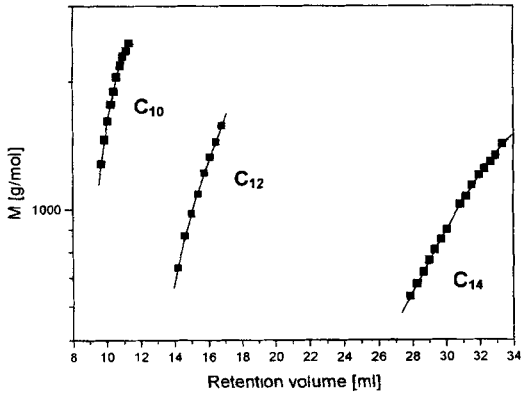


FIGURE 8 Calibration curves for $C_{10}$, $C_{12}$ and $C_{14}$ (molecular weight versus retention time) obtained in the LAC mode coupled with MALDI-TOF-MS (peak maxima from mass spectra were used).



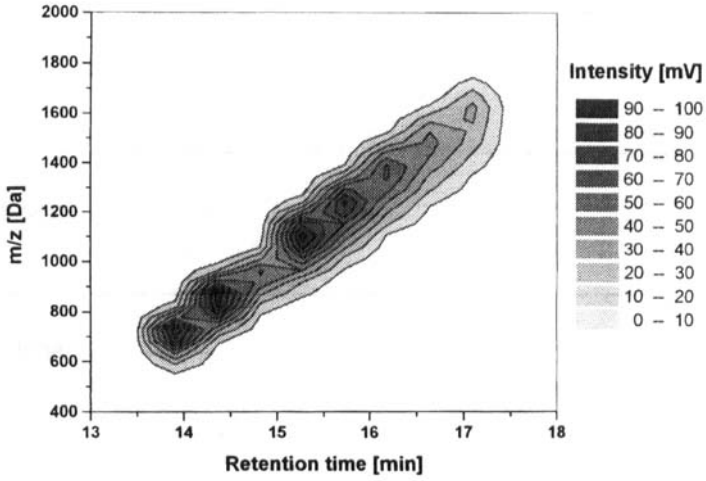


FIGURE 9 3-D contour plot of retention time (obtained by $LAC_{near}CC$) versus molecular weight (obtained by MALDI-MS) of PEO-*co*-PM ($C_{12}$) oligomer (third dimension is the intensity of mass spectra peaks).

obtained by appling a gradient flow in $LAC_{near}CC$. This will be examinated in future studies.

The combination of retention times obtained by $LAC_{near}CC$ with intensities from MALDI mass spectra can be used to construct a three-dimensional contour plot (Figure 9). This plot (only for $C_{12}$)

clearly illustrates the possibilities of coupling of $LAC_{near}CC$ with MALDI mass spectrometry to determine differences in the chemical structure, as well as molecular weight within a relatively short time.

## CONCLUSIONS

The modification of liquid adsorption chromatography at 'critical conditions' through changing elution conditions from the '*at* critical conditions' (LACCC) mode into the '*near* critical conditions' ($LAC_{near}CC$) mode was investigated. Additionally, matrix-assited laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) as a molecular weight sensitive detector for the simultaneous characterization of molecular and chemical heterogeneities of copolymers was applied. For the investigated PEO-*co*-PM oligomer system, the time-consuming evaluation of separation conditions in the two-dimensional chromatography was simplified. Separation in the LAC mode and additional fractionation of samples provided narrow distributed polymer fractions which results in better ionization conditions for MALDI-TOF-MS. Chemical and molecular weight distributions can be characterized completely by means of MALDI-TOF-MS as a detector for LAC without any SEC calibration standards. Especially, for low-molecular weight copolymer systems, MALDI-TOF-MS can substitute for SEC. Quantitative information about the chemical heterogeneity obtained by LAC detection methods are combined with additional quantitive information about the molecular weight distribution.



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