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Endgroup-based separation and quantitation of polyamide-6,6 by means of critical chromatography

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Abstract

Polyamide-6,6 is a polycondensation product from the two monomers adipic acid and 1,6-hexamethylenediamine. Depending on the reacted amount of these monomers, different ratios of amine and carboxylic acid endgroups can be formed. Besides linear chains, cyclic polyamides will also be formed. Using critical chromatography, polyamide-6,6 can be separated independently of molar mass. Retention is based solely on endgroup functionality. It is demonstrated that high-molecular-mass polyamide-6,6 ($M_w \approx 20\,000–30\,000$) can be separated using this approach. The separation was optimized by using different parameters, such as percentage modifier, temperature and pressure. The concentration of phosphoric acid was used for selective retention of the different endgroup functionalities. Using this property, a new method called critical gradient chromatography was performed where the mobile phase changes from a weak to a strong solvent with respect to the endgroup functionality, while retaining the critical conditions of the backbone unit. Quantification using UV detection is discussed.

Keywords: Critical chromatography; Gradient elution; Polyamide

1. Introduction

Polyamide-6,6 is the first commercially available polyamide, and is synthesized by polycondensation of the monomers adipic acid and 1,6-hexamethylenediamine (Fig. 1) [1]. Its molecular mass distribution can be determined using size-exclusion chromatography (SEC) [2]. Another important property of the polymer is its endgroup functionality. Besides the amount of carboxylic acids and primary amine endgroups, the total amount of cyclic structures which can be formed during intramolecular condensation reactions can influence the polymer performance. Titration is the most commonly applied technique to determine the amount of carboxylic acid and primary amine endgroups [3]. However, this technique does not distinguish between mono- or bifunctional carboxylic acid or amine chains, it does not give the possibility to determine deviating terminations of the chain and it does not account for possible cyclic molecules. A relatively new separation technique, which is known as critical chromatography, could fulfill these demands. The theoretical aspects of this separation technique were investigated intensively by Gorbunov and co-workers [4–8].

The distribution constant K of a molecule between the stationary and mobile phase, which also holds for a polymer, is given by

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$$K = \frac{c_s}{c_m} = k\phi = \left(\frac{t_r - t_0}{t_0}\right)\phi = e^{-\Delta G/RT} \quad (1)$$

where c_s and c_m are the concentration of the polymer in the stationary and mobile phase, respectively, k is the retention factor, ϕ is the phase ratio, t_r is the retention time, t_0 is the time of an unretained polymer with the same hydrodynamic volume, ΔG is the Gibbs free energy, R is the gas constant and T is the temperature.

According to the Martin rule the Gibbs free energy (ΔG) of a polymer is a summation of the Gibbs free energy of the endgroups and the backbone units [9]:

$$\Delta G_{polymer} = \Delta G_{endgroup1} + \Delta G_{endgroup2} + n\Delta G_{backbone\ unit} \quad (2)$$

Under critical conditions, polymer retention is independent of the number of backbone units, i.e. the Gibbs free energy term $n\Delta G_{backbone}$ becomes zero. This can be accomplished by compensating the enthalpic interaction effects (ΔH) with the entropic exclusion effects (ΔS) at a certain temperature T [10]:

$$\Delta G_{backbone\ unit} = \Delta H_{backbone\ unit} - T\Delta S_{backbone\ unit} = 0 \Rightarrow \Delta H_{backbone\ unit} = T\Delta S_{backbone\ unit} \quad (3)$$

Differences in the enthalpies of the endgroups under critical conditions are needed to obtain a good separation of synthetic linear macromolecules, with identical backbone units, but with deviating terminating functionalities.

To achieve this, one approach is the use of bare silica or polar-modified silica, which can interact with (polar) functional groups. Substantial interactions were obtained for the carboxylic endgroups [11] and for hydroxy endgroups of a polyester [12].

Another approach is the use of reversed-phase columns. Specific interactions can be predicted using available column test data. Different test procedures are available, such as, for example, the Engelhardt, the Walters, the Tanaka and the Galushko tests (see Refs. [13,14]). Although these tests were primarily developed to specify packing materials, functional group interactions with different molecules can also be quantified.

A large number of papers have been published demonstrating the possibilities of critical chromatography to separate polymers based on their endgroup functionality, independent of the number of repeating backbone units. Many of these investigations discuss the separation of low-molecular-mass oligomers with molecular masses which do not exceed 10 000 [15–19].

Critical chromatography is also used for higher-molecular-mass polymers. However, these kinds of separations are often not focussed on endgroup functionality but on deviating backbone units. When the Gibbs free energy of a certain backbone unit becomes zero, the remaining deviating backbone can be determined, as this part of the chain will promote exclusion or interaction, such as, for example, the grafting degree of polystyrene–graft-poly(ethylene oxide) [20] or tacticity of poly(ethylmethacrylate) [21]. Using this technique it is also possible to determine the chemical composition distribution of a block copolymer (of styrene and butadiene M_w = 100 000) [22] or to separate blends (different polyalkylacrylates, $M_w \approx 200\,000$) [23].

Problems with this technique have been reported. Philipsen et al. encountered problems in obtaining critical conditions of higher-molecular-mass polymers [24]. Berek et al. summarized the demands and problems with critical chromatography [25]. They observed recovery problems under critical conditions [26] and started to promote liquid chromatography under limiting conditions [27].

Critical conditions are often found after optimization of the mobile phase constituents and the column temperature [24,28]. However, other chromatographic conditions could also influence the critical conditions. It is known that, due to pressure,

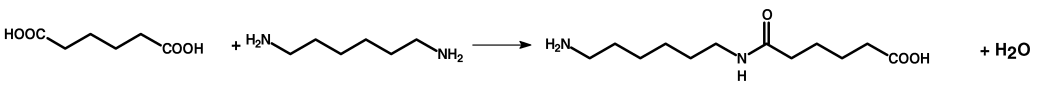


Fig. 1. Condensation of adipic acid and 1,6-hexamethylenediamine.

one of the mobile phase constituents can preferentially be adsorbed to the stationary phase [29]. For small molecules, this will influence retention factors to an extremely small extent. Retention factors of approximately 5 units for some low-molecular-mass aromatic compounds deviated roughly linearly 0.03–0.15 retention factor units due to steps of 100 bar pressure difference [30]. It can be anticipated that preferential adsorption of mobile phase constituents will influence high-molecular-mass polymers to a much greater extent. Besides percentage strong solvent, temperature and pressure, the choice of the stationary and mobile phase is also important.

Here we present a study of the critical separation of polyamide-6,6, to perform a separation based solely on differences in endgroup functionality. A normal-phase and a reversed-phase system were tested. A procedure is proposed to check recovery. Different parameters were used to obtain true critical conditions and to optimize the separation. Besides the role of the modifier concentration and stationary phase, temperature, flow (as a pressure regulator) and additive concentration were investigated to optimize the critical separation. Quantification using UV detection is also discussed.

2. Experimental

Two different chromatographic setups were used. The standard setup is a HP 1090 liquid chromatograph including a solvent delivery system, an autosampler, a column thermostat unit and a diode-array detector to measure the UV absorbance (Agilent, Waldbronn, Germany). Data collection was performed using Agilent A.08.01 software. If isocratic conditions were used, the mobile phase was always premixed to circumvent mixing problems.

The second chromatographic setup was used for the formic acid–1-propanol experiments and for extreme temperature-control experiments. The mobile phase was premixed and pumped using an Agilent 1100 quaternary pump, including a degasser and a control module.

The injector (Rheodyne, Cotati, CA, USA), equipped with a 55 μl loop, was mounted inside the column oven (Mistral, Spark, Emmen, Netherlands), which was connected to an Endurance autosampler (Spark). Approximately 6 m of 0.25 mm I.D. capillary tubing was used in this oven to thermostat the mobile phase before it reached the injector. UV detection was performed using a Linear 204 detector (Linear Instruments, Reno, NV, USA). The detector signal was collected with an X-Chrom/Windows NT 3.51 version 2.11b data management system (LAB-systems, Manchester, UK).

The mobile phase constituents used were hexafluoroisopropanol (HFIP; Biosolve, Valkenswaard, Netherlands), 10 m*M* phosphoric acid (made with phosphoric acid 85%, analytical-reagent grade; Baker, Deventer, Netherlands) in water (Milli-Q, Millipore, Milford, MA, USA), formic acid (Merck, Darmstadt, Germany) and 1-propanol (Baker). All stationary phases used (Nucleosil) were purchased as packed columns from Macherey–Nagel (Düren, Germany).

Two different kinds of polyamide-6,6 test samples were used for this investigation. First, a commercially available polyamide-6,6 sample was purchased from Aldrich (Milwaukee, WI, USA). Secondly, six polyamide-6,6 samples were specially prepared for this investigation to obtain different molecular mass polyamide-6,6 samples with different endgroup functionalities. These samples were also used to characterize the different peaks in the chromatogram. SEC curves were recorded similarly as described in Ref. [2].

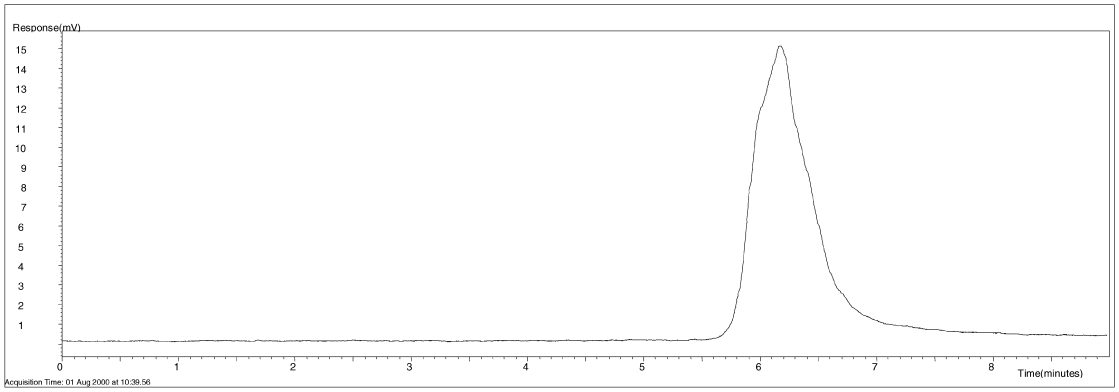
3. Results and discussion

3.1. Optimization

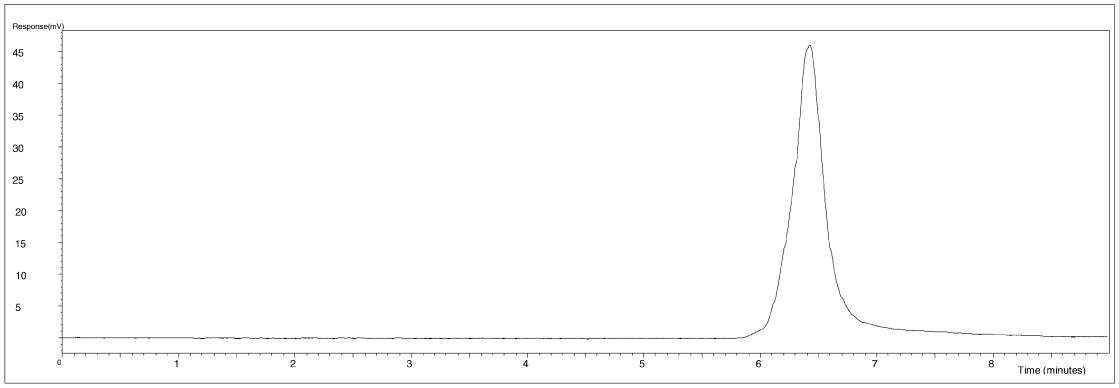
The critical conditions of oligomeric species can easily be approximated using two low-molecular-mass fractions with different molecular masses (e.g. $M_w = 750$ and $M_w = 1500$). The elution times of these samples must be equalized by adjusting the composition of the mobile phase (i.e. the percentage of the modifier concentration) as described elsewhere [28]. To obtain true critical conditions, a third high-molecular-mass polymer with well-defined properties is needed. Conditions often need to be slightly re-adjusted.

The chromatographic normal-phase conditions used for the critical separation of linear and cyclic

2.a PA-66 sample (M_w=3kD) with equal amounts of amine and acid terminating groups



2.b PA-66 sample (M_w=8kD) with excess amine terminating groups



2.c PA-66 sample (M_w=3kD) with excess carboxylic acid terminating groups

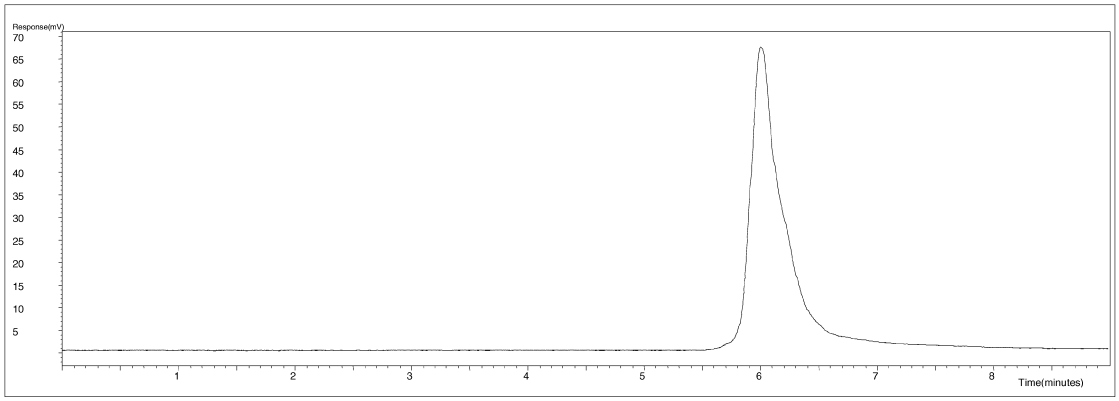


Fig. 2. Critical separation of low-molecular-mass polyamide-6,6. Column, 2×(250×4 mm) Nucleosil 50-5 (39 °C); flow, 0.75 ml/min; injection volume, 50 μl; polyamide concentration, 1 mg/ml; mobile phase, formic acid–1-propanol (80:20, w/w). Evaporative light scattering detection at 55 °C. 19 atm nebulisation pressure (1 atm = 101 325 Pa). (a) PA-66 sample (M_w 3000) with equal amounts of amine and acid terminating groups; (b) PA-66 sample (M_w 8000) with excess amine terminating groups; (c) PA-66 sample (M_w 3000) with excess carboxylic acid terminating groups.

polyamide-6 [28] were not applicable for functional endgroup separation of polyamide-6,6. Using such conditions, the separation of three low-molecular-mass polyamide-6,6 fractions, which differed significantly in endgroup functionality due to different initial ratios of both monomers during synthesis, is depicted in Fig. 2. No baseline separation of the different endgroup functionalities was obtained.

From different column test procedures we observed a very strong amine interaction with octadecyl-modified Nucleosil columns. Using this reversed-phase column and hexafluoroisopropanol and 10 m*M* phosphoric acid in water, baseline separation could be accomplished, as shown in Fig. 3 for different polyamide-6,6 samples. During optimization of this critical separation, the HFIP concentration is the first optimization parameter. Thereafter, temperature can be used as a first fine-tuning regulator to obtain critical conditions as given in Fig. 4. Increasing the temperature diminishes the interaction. The temperatures of some thermostating modules, such as, for example, the Mistral oven, are only adjustable to differences of 1 °C. The thermostating module of the HP1090 is adjustable to a tenth of a degree, which is more favourable for optimization of critical separations. Although different peaks could be observed on the Nucleosil 120-5C_{18} column at 42–43 °C, truely critical conditions could not be accomplished. At 43 °C the system was slightly entropically driven, while at 42 °C a slightly enthalpically driven system was observed.

Another optimization parameter for these kinds of high-molecular-mass polymers is the pressure drop across the column. Fig. 5 demonstrates the influence of pressure on a Nucleosil 300-5C_{18} column by adjusting the flow. Although, in principle, flow could influence column efficiency [height equivalent to theoretical plate (HETP) curve], the observed peak broadening cannot explain these small changes. As flow may also influence the real column temperature [31], we studied this effect using two different chromatographic systems. The temperature control of the HP1090 system is rather limited. Injection of the sample takes place before the column thermostating

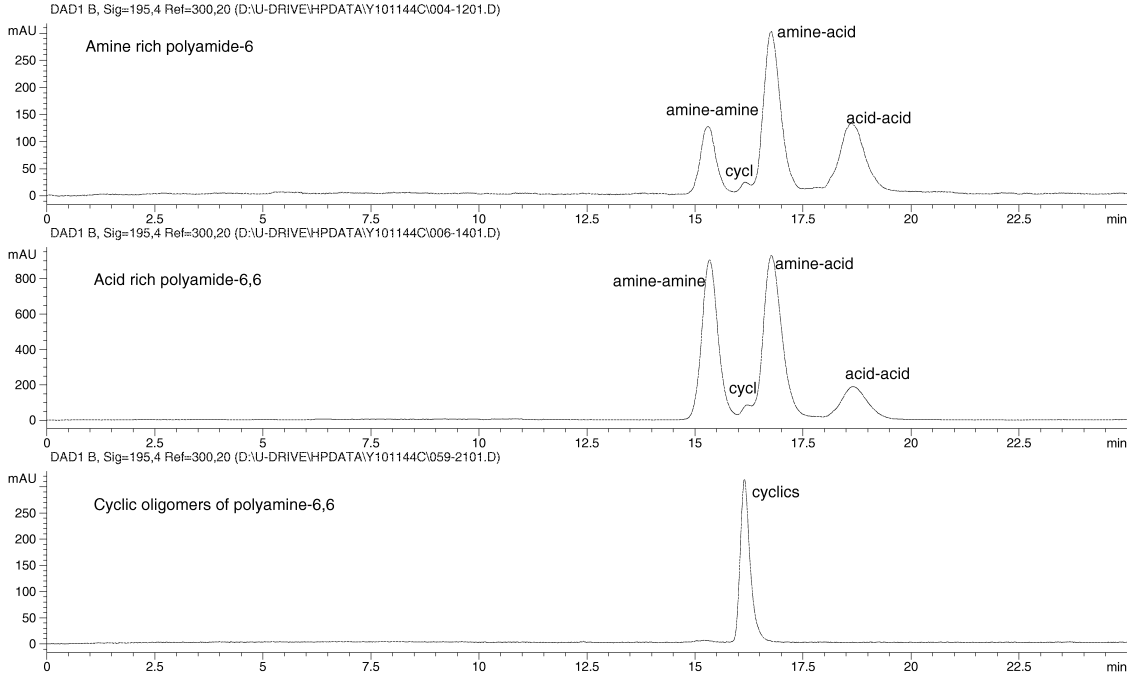


Fig. 3. Special polyamide-6,6 samples under critical conditions. Upper trace, primary amine-rich polyamide-6,6 (M_w = 10 000); middle trace, carboxylic acid-rich polyamide-6,6 (M_w = 3000); lower trace, cyclic oligomers of polyamide-6,6 (M_w = 700). Column, 2×(250×4) mm Nucleosil 300-5C_{18}. 89.5% (w/w) HFIP and 10.5% (w/w) 10 m*M* H_3PO_4; flow, 0.3 ml/min; column temperature, 39 °C.

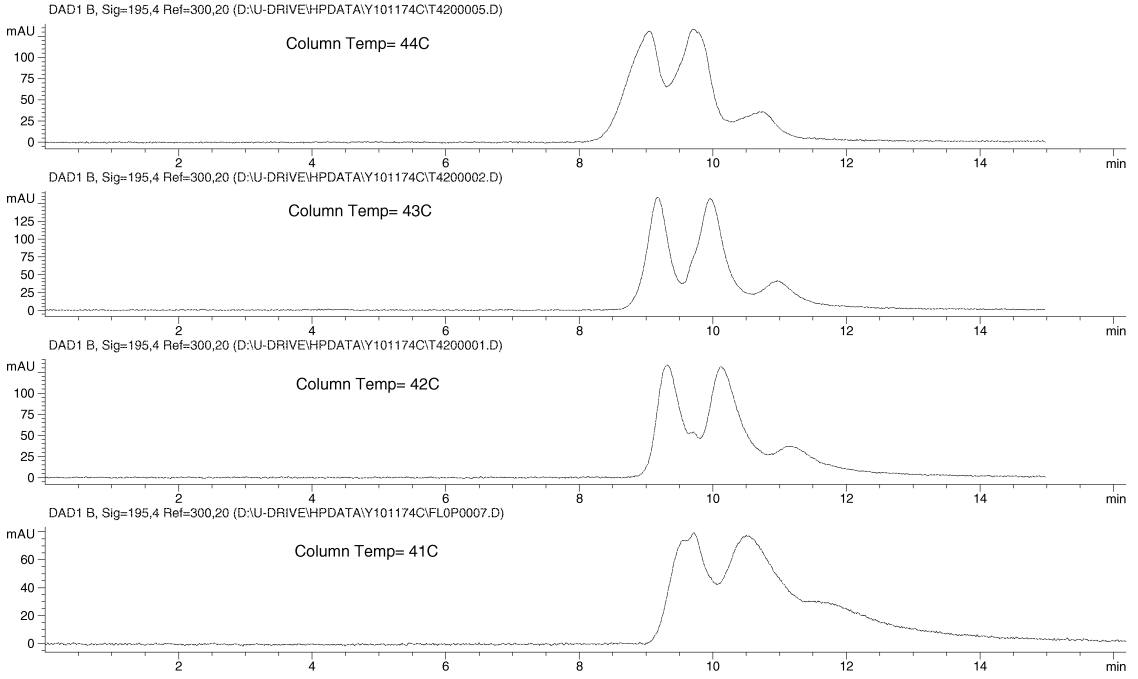


Fig. 4. Influence of temperature on the critical separation of polyamine-6,6 ($M_w \approx 30\,000$). Conditions: column, 2×(250× 4 mm) Nucleosil 120-5C_{18}; flow, 0.5 ml/min; mobile phase, 89.5% (w/w) HFIP and 10.5% 10 m*M* phosphoric acid; injection volume, 2.5 μl; $\Delta P = 295$ atm.

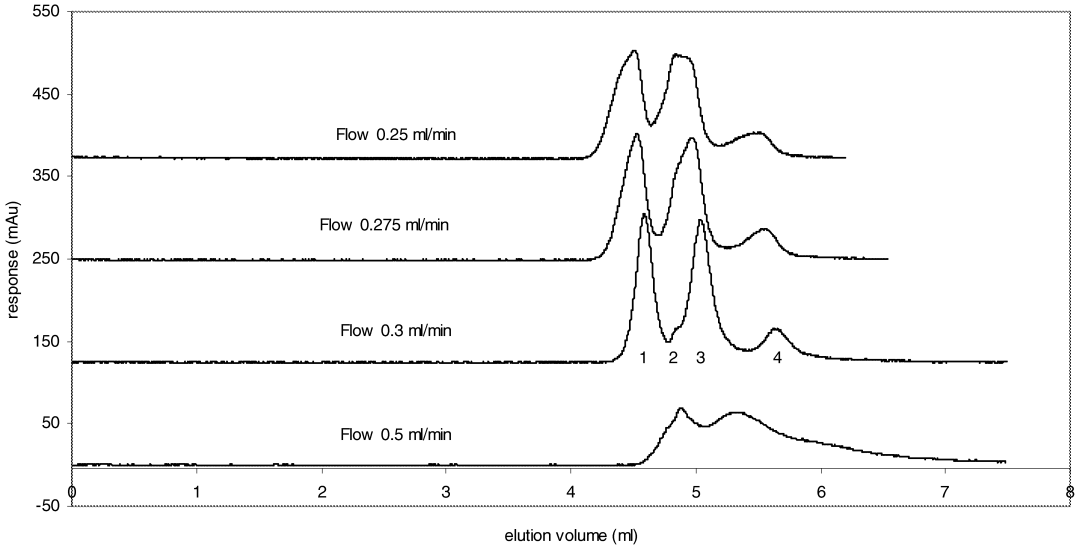


Fig. 5. Influence of flow on the critical separation. Conditions as in Fig. 4. Column temperature, 39 °C (peak 1, diacid; peak 2, cyclic; peak 3, amine–acid; peak 4, diamine).

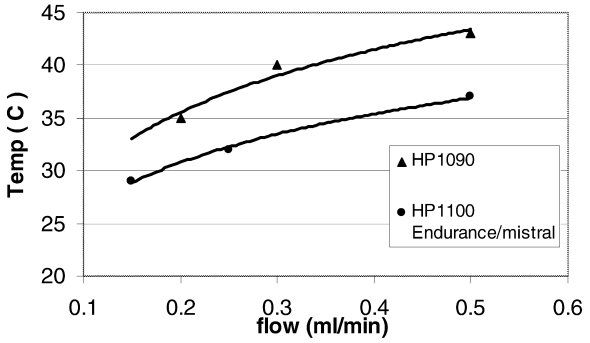


Fig. 6. Effect of the flow and temperature on the critical point of polyamide-6,6 on a Nucleosil 300-5C_{18} stationary phase and HFIP–10 m*M* H_3PO_4 as mobile phase.

module and the mobile phase is only preheated for a short interval to reach column temperature. A second chromatographic system (HP1100-Edurance/Mistral) was equipped with an injection module inside the column oven and the mobile phase was preheated in the same oven using approximately 6 m of 0.25 mm I.D. capillary tubing. An identical influence of the flow and temperature on the critical point was found for both systems, as shown in Fig. 6. Critical temperatures were not exactly the same for both systems, indicating small deviations in the thermostating modules. By decreasing the flow, critical conditions disappeared and reappeared after adding an extra column behind the detector, to create the original pressure drop across the first two columns. Fig. 7 shows a general optimization graph, interrelating the HFIP percentage, the temperature and the pressure (regulated by the flow). Under exclusion

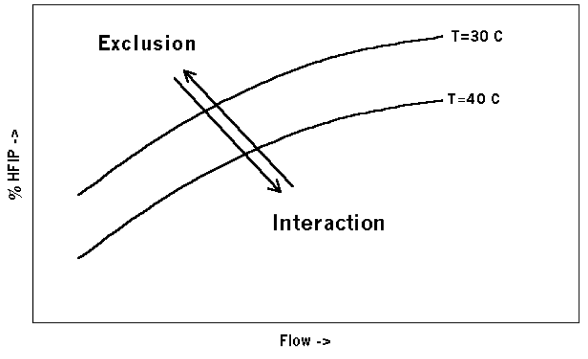


Fig. 7. General optimization chart, which interrelates the influence of the percentage of HFIP, the flow-rate (=pressure drop) and the column temperature on the critical point.

conditions, a decrease of the percentage of HFIP in the mobile phase, a decrease of the temperature or an increase of the flow (=pressure) will increase the interaction of the polymer with the stationary phase, which may result in critical conditions.

We also investigated the influence of the pore width on the critical conditions. Entilis et al. predicted that the critical conditions will not change with different pore widths and promoted small pore packings [4]. However, using different pore sizes of the stationary phase, Nucleosil *x*-C_{18} (x=50,120 or 300 Å) critical conditions did not appear under exactly the same conditions (Fig. 8). Column test results with low-molecular-mass analytes with different functionalities [13,14] also showed deviating interaction properties, concluding that not only is the pore diameter changed (see Table 1), which is consistent with other results [32], but by varying the pore size of a RP stationary phase, other properties may also change, such as, for example, the polarity. Using the 300 Å pore column, a nice separation is obtained between the cyclic structures (at 16.5 min), the linear chains with terminating dicarboxylic acids, the linear chains with terminating carboxylic acid amines and the linear chains with terminating amines (Fig. 8). With the 50 and 120 Å columns the cyclic compounds cannot be distinguished.

The main problem, which has not been discussed in the literature, is the possibility of manipulating the selectivity at the critical conditions. If a separation under the critical conditions is not satisfactory, it is very difficult to change a parameter to improve the selectivity to a major extent. Changing one parameter will almost automatically imply the loss of critical conditions, making it necessary to change another parameter at the same time. Although small improvements could be obtained, it can be anticipated that the selectivity will not change dramatically if, for instance, temperature and modifier concentration are changed while retaining the critical conditions. No selectivity changes can be observed in Fig. 6. The most obvious way to improve selectivity is to change the stationary phase, as demonstrated in Fig. 8, where, besides the stationary phase, other column properties also changed (see Table 1).

Another way to improve the separation efficiency is to change the mobile phase composition. As the interaction of the polyamide-6,6 series is probably

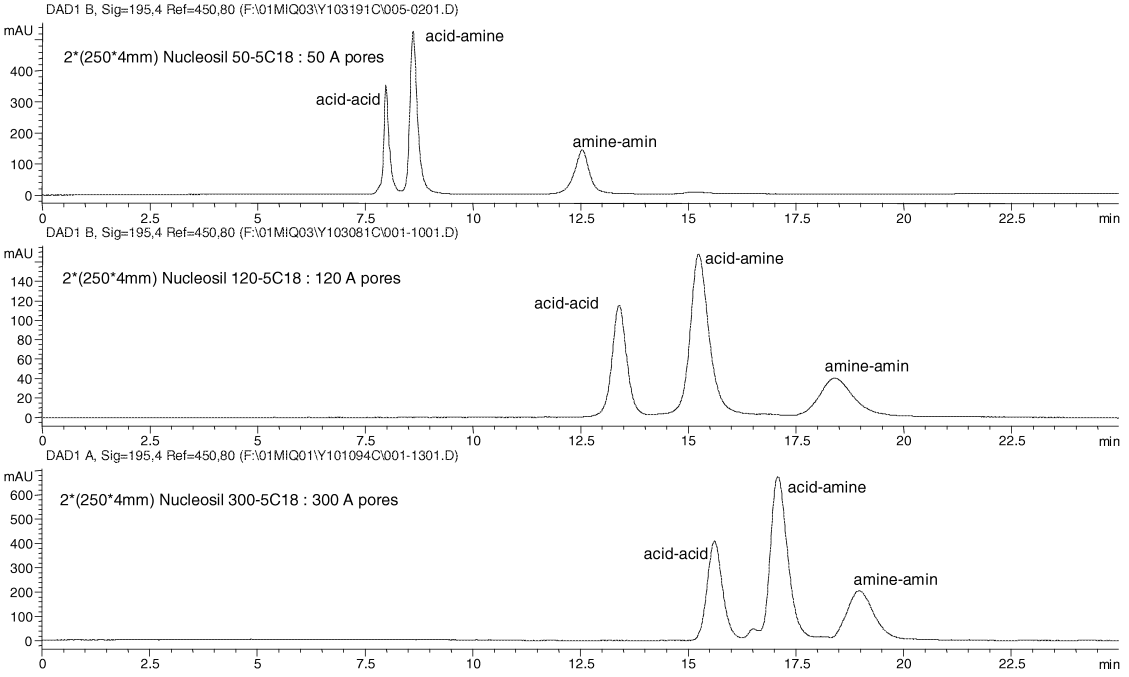


Fig. 8. Influence of different stationary phases on the critical conditions of polyamide-6,6 ($M_w = 3000$). Critical conditions for different stationary phases: (a) Upper trace: Nucleosil-50C_{18} (50 Å pores); column temperature, 42 °C; flow, 0.3 ml/min; mobile phase, HFIP–10 m*M* H_3PO_4 in water (85:15, w/w); $\Delta P = 180$ atm. (b) Middle trace: Nucleosil-120C_{18} (120 Å pores); column temperature, 42 °C; flow, 0.3 ml/min; mobile phase, HFIP–10 m*M* H_3PO_4 in water (88.5:11.5, w/w); $\Delta P = 145$ atm. (c) Lower trace: Nucleosil-300C_{18} (300 Å pores); column temperature, 39 °C; flow, 0.3 ml/min; mobile phase, HFIP–10 m*M* H_3PO_4 in water (89:11, w/w); $\Delta P = 170$ atm.

based on ion-exchange interactions of primary amines with silica-based cation-exchange sites on the stationary phase, the influence of the phosphoric acid concentration in the aqueous part of the stationary phase was investigated (Fig. 9). It is clearly demonstrated that the phosphoric acid concentration can be used to influence the selectivity of the different endgroup functional polymeric series, without losing the critical conditions. Using this feature, a phosphoric acid gradient can be applied, where the different polymeric series elute independently of the number of backbone units, but will be separated due

Table 1
Column test data using the Tanaka test

Test	Measure	Nucleosil 50-5C_{18}	Nucleosil 120-5C_{18}	Nucleosil 300-5C_{18}	Nucleosil 120-5C_8
Hydrophobicity	k_{amyl}/k_{butyl}	1.48	1.43	1.43	1.27
Amount alkyl chains	k_{amyl}	6.20	3.56	1.62	0.63
Steric	$k_{triph}/k_{o\text{-}terph}$	1.77	1.78	1.87	1.57
H-bound	k_{caf}/k_{fenol}	0.39	0.47	0.47	0.75
Cation-exchange pH >7	$k_{benzylamine}/k_{fenol}$	0.40	0.40	0.46	0.42
Cation-exchange pH <3	$k_{benzylamine}/k_{fenol}$	0.39	0.41	0.47	0.42
Anion-exchange pH >7	$k_{benzoic\ acid}/k_{fenol}$	−0.04	−0.06	−0.10	−0.16
Anion-exchange pH <3	$k_{benzoic\ acid}/k_{fenol}$	0.94	0.95	1.07	1.00

amyl, amylbenzene; butyl, butylbenzene; triph, triphenyl; *o*-terph, *o*-terphenyl; caf, caffeine.

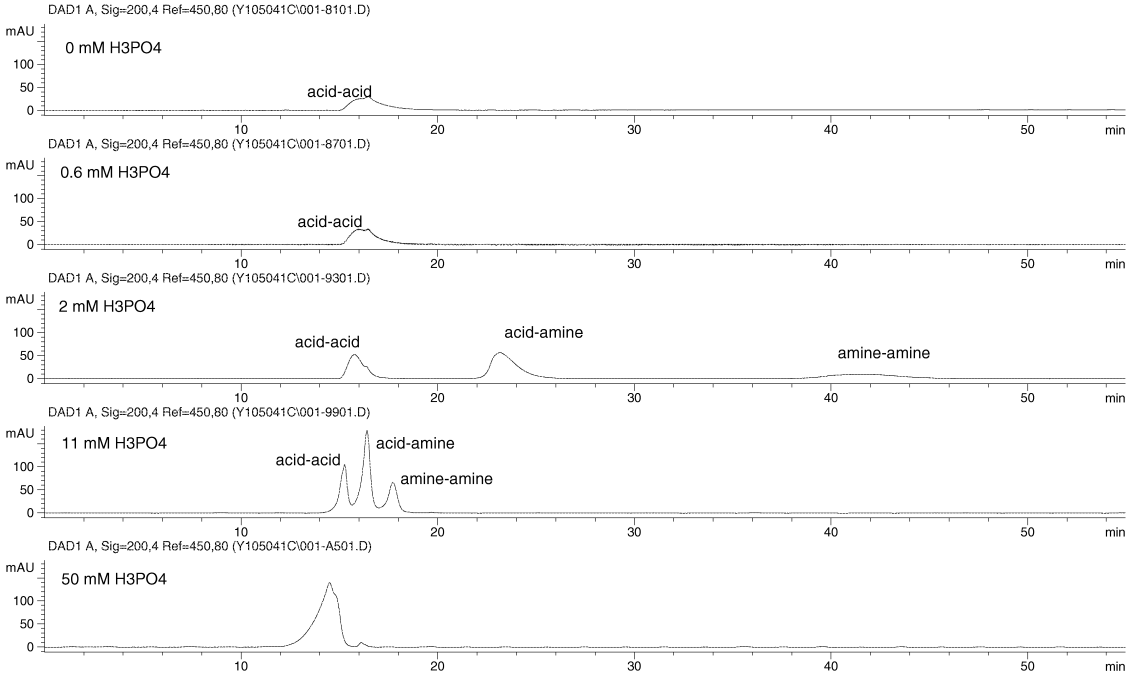


Fig. 9. Influence of the phosphoric acid concentration in the aqueous part of the mobile phase. Critical isocratic chromatographic conditions: HFIP–aqueous solution (84:16). The mobile phase is prepared by on-line mixing of three different stock solutions: (a) water, (b) 50 m*M* H_3PO_4 and (c) HFIP. The phosphoric acid concentration of the aqueous part is given in separate chromatograms. Injection, 1 μl containing 1–2.5 mg/ml PA-66 (M_w 3000) dissolved in HFIP–water (84:16, v/v). 84% (v/v) HFIP equals 89.5% (w/w) HFIP. Flow, 0.3 ml/min; column, 2×(250×4) mm Nucleosil 300-5C_{18}; detection performed at $\lambda = 200$ nm.

to a gradual decrease of interactions of the endgroup with the stationary phase (Fig. 10).

3.2. Strategy to check recovery

A chromatographic run during method development should consist of two consecutive parts to obtain critical conditions and to control recovery: first critical isocratic conditions followed by a gradient to full exclusion conditions.

An example of this approach is shown in Fig. 11. A low- and a high-molecular-mass polyamide-6,6 ($M_w = 3000$ and $M_w = 20\,000$) were injected onto a Nucleosil 120-5C_8 column and the conditions turned out to be near critical. The high-molecular-mass polymer with a specific endgroup eluted a few seconds faster than the low-molecular-mass polymer peak, indicating a slightly entropically driven system. Although the conditions were not exactly critical, a separation based on the different functionalities was obtained. As the system was slightly entropically driven it was very surprising that a significant signal was observed during the gradient step for the higher-molecular-mass polyamide-6,6 sample (at 20 min).

Polymers with deviating backbone units or very strong endgroup interactions could cause such a problem. However, as we observed this problem only on an octyl-modified stationary phase and not on an octadecyl-modified stationary phase this could not be the cause of the problem. Due to the shorter alkyl chains of the stationary phase, the concentration of HFIP in the mobile phase under critical conditions is smaller compared to that using a column with longer alkyl chains. Considering the mobile phase conditions (82%, w/w, HFIP) and that the cloudpoint of polyamide-6,6 is only 10% lower (72%, w/w, HFIP) it is postulated that the higher-molecular-mass polyamide-6,6 could precipitate on top of the column due to preferential adsorption of water to the stationary phase. This may indicate that preferential ad-

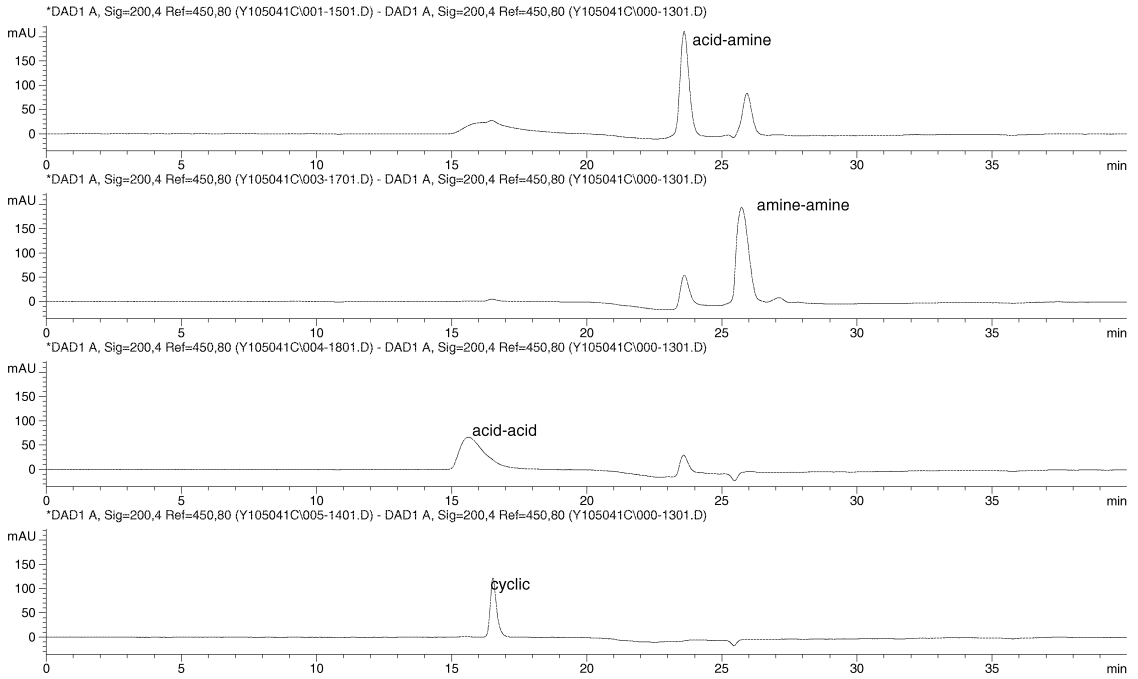


Fig. 10. Critical gradient chromatography. Conditions: HFIP–water (84:16, v/v). The mobile phase is prepared by on-line mixing of three different stock solutions: (a) water, (b) 50 m*M* H_3PO_4 and (c) HFIP. The phosphoric acid concentration of the aqueous part is changed gradually from 0 to 50 m*M* in 40 min. Injection, 1 μl 1–2.5 mg/ml M_w 3000 PA-66 (first trace), M_w 8000 amine-rich PA-66 (second trace), M_w 3000 acid-rich PA-66 (third trace) and M_w 678 cyclic PA-66 (fourth trace) dissolved in HFIP–water (84:16, v/v). 84% (v/v) HFIP equals 89.5% (w/w) HFIP. Flow, 0.3 ml/min; column, 2×(250×4) mm Nucleosil 300-5C_{18}; detection performed at $\lambda = 200$ nm.

sorption can be a limiting factor, especially if critical conditions approach cloudpoint conditions.

3.3. Quantification

Seven different polyamide-6,6 samples were separated under the optimized critical conditions. A small influence of the molecular mass on the total UV detector response can be expected when detecting linear polyamide chains [33]. As the amide function is the only UV-absorbing group, the monomers adipic acid and hexamethylene diamine will not contribute to the total detector response; a linear dimer (the reaction product of Fig. 1) will only absorb UV light due to one amide function. The UV absorbance of a linear polyamide-6,6 chain can be estimated using the equivalent absorption coefficient of an amide function:

$$\varepsilon'_L \approx \frac{(n-1)\varepsilon_{amide}}{131 + 113(n-1)} \quad (4)$$

where ε'_L is the absorption coefficient of a linear polyamide-6,6 chain with n backbone units in AU g^{-1} l m^{-1} and ε_{amide} is the UV absorbance of an amide function in AU $equiv^{-1}$ l m^{-1}; 131 and 113 are the average molecular masses of a backbone unit with (131) and without water (113) in g mol^{-1}.

Due to this deviation the number of amide functions is not exactly equal to the number of backbone units, but for polyamides with a molecular mass greater than 1000 this effect is smaller than 10% (Fig. 12).

Nevertheless, if the molecular mass distribution of the polyamide is not significantly influenced by the terminating endgroups, the ratio of the endgroup functionality of the linear chains can be calculated directly from the UV response. Table 2 compares the

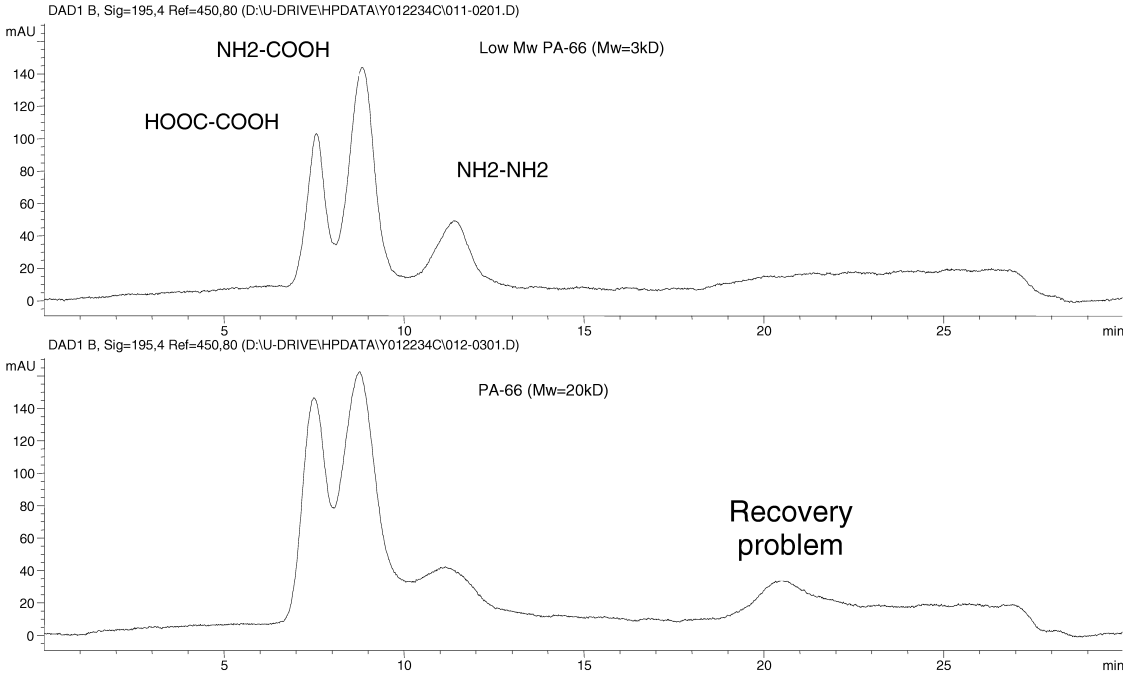


Fig. 11. Determining a recovery problem using isocratic and subsequent gradient elution. Critical conditions: 3×(125×2.1) mm Nucleosil 120-5C_8 (23 °C); flow, 0.2 ml/min; $\Delta P = 200$ atm; mobile phase gradient, $t_{0\ min} = t_{5\ min}$ = HFIP–10 m*M* H_3PO_4 (82:18, w/w), $t_{15\ min}$ = HFIP–10 m*M* H_3PO_4 (92:8, w/w). Injection, 1 μl.

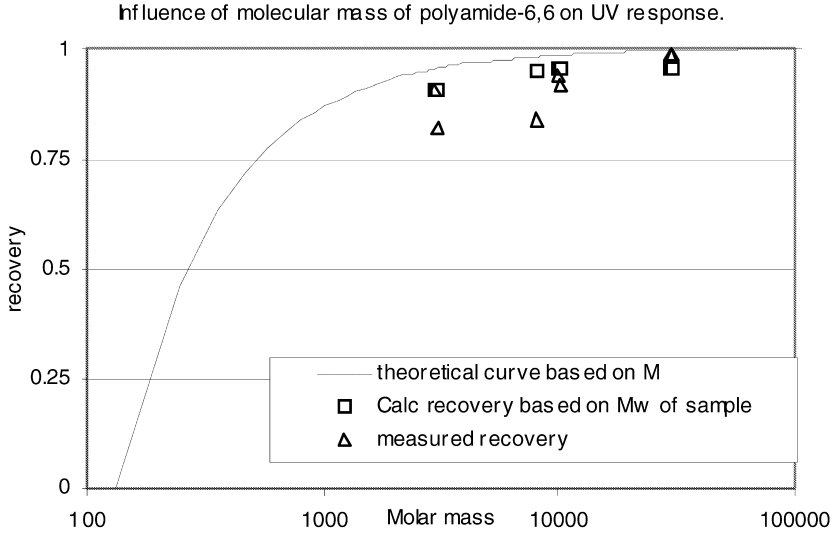


Fig. 12. Influence of molecular mass of polyamide-6,6 on the relative response, defined as the relative UV absorbance divided by the injected mass.

Table 2
Calculated results, using the PA-66 sample (Aldrich) as a calibration sample (total area 100%)

PA-66	M_w ($\times 10^{-3}$)	M_{inj} (μg)	Rec. calc. (%)	Rec. meas. (%)	Acid–acid (%, w/w)	Acid–amine (%, w/w)	Amine–amine (%, w/w)	Cyclic (%, w/w)
1 Acid/amine	3	7.28	91	91	23	43	22	2
2 Acid/amine	25	8.65	96	98	32	42	20	5
3 Amine rich	8	5.78	95	84	1	14	68	1
4 Amine rich	10	3.10	96	94	17	44	31	2
5 Acid rich	3	6.98	91	82	63	16	1	1
6 Acid rich	10	10.9	96	92	36	41	13	2
7 PA-66	30	2.37	96	99	37	44	13	5
(Aldrich)	30	5.78	96	101	37	43	16	5
	30	10.9	96	99	37	43	15	4
Average					37	43	15	5

Rec. is calculated recovery (calc.; using the SEC curve and Eq. (4), linear monomers not included!) and measured recovery (meas.); M_{inj} is the injected mass.

results for seven different polyamide-6,6 samples. The high-molecular-mass PA-66 (30 000) is used as an external standard by dividing the total area by the injected mass. Three different concentrations were injected, as depicted in Fig. 13. For the amine-rich and acid-rich low-molecular-mass polyamide-6,6, recovery was 10% less than expected from the calculated recovery. This could be due to an excess of unreacted monomers (which were used to prepare these low-molecular-mass polyamides) or to the presence of other low-molecular-mass components, such as, for example, water.

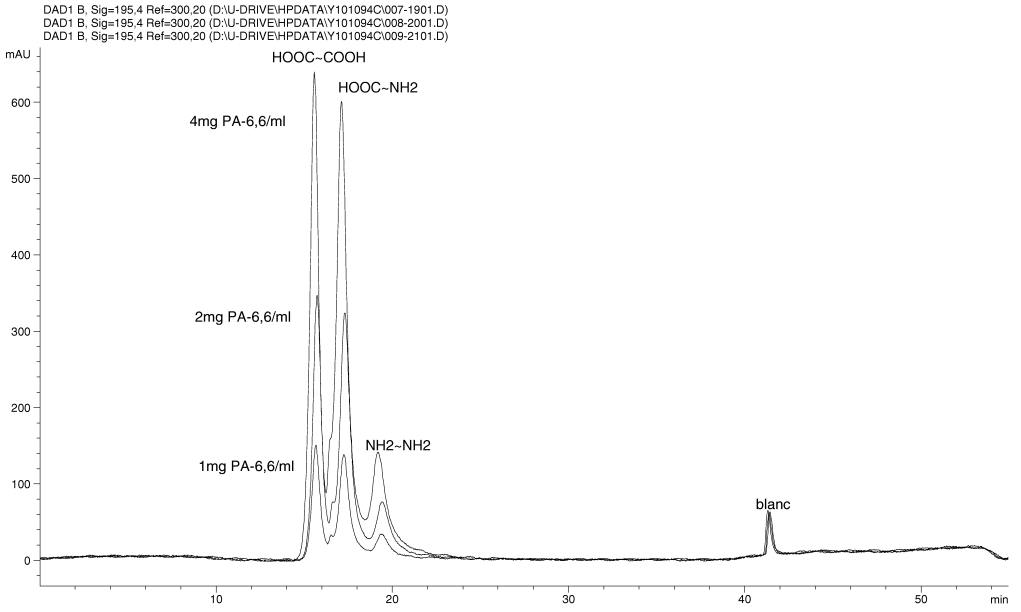


Fig. 13. 2.5 μl injection of three different concentrations of polyamide-6,6 (M_w = 30 000) of approx. 1.2 and 4 mg/ml. Mobile phase timetable: $t_{0\ min}$ = HFIP–10 m*M* H_3PO_4 (89.5:10.5, w/w), $t_{20\ min}$ = idem $t_{0\ min}$, $t_{25\ min}$ HFPP–10 m*M* H_3PO_4 (95:5, w/w), $t_{35\ min}$ idem as $t_{25\ min}$, $t_{36\ min}$ idem as $t_{0\ min}$. Flow, 0.3 ml/min; column temperature, 39 °C.

4. Conclusions

We have demonstrated that a baseline separation of polyamide-6,6 based solely on endgroup functionality can be accomplished. Optimization was performed using the percentage of HFIP, the column temperature and the flow-rate, which regulated the pressure. Under critical conditions the flow-rate cannot be optimized separately, because the critical conditions shift due to the preferential adsorption of one of the mobile phase constituents. Critical gradient chromatography can be used to increase resolution while retaining the critical conditions. Using a gradient step after the isocratic conditions, recovery can be checked.

UV detection worked well for the quantification of higher-molecular-mass distributions. However, for lower-molecular-mass distributions ($n < 10$) this may result in accuracy problems.

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