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Analysis of higher polyamide-6 oligomers on a silica-based reversed-phase column with a gradient of formic acid as compared with hexafluoroisopropanol

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Abstract

The analysis of polyamide-6 oligomers and polymer is usually performed with expensive fluorinated alcohols like 2,2,2-trifluoroethanol (TFE) or 1,1,1,3,3,3-hexafluoroisopropanol (HFIP). Formic acid is well known as a mobile phase additive to adjust pH in reversed-phase high-performance liquid chromatography. However, formic acid is seldom used as a modifier to perform gradient elution chromatography on octadecyl-modified silica-based columns. Here we demonstrate the determination of cyclic and linear polyamide-6 oligomers using formic acid as a modifier on an octadecyl-modified silica-based column. This column was shown to be stable for more than 5000 column volumes, even when a mobile phase of 65–95% formic acid in water at a flow of 1 ml/min is applied. With formic acid under the conditions used (65–95% formic acid in water) the oligomers are retained on the column, while the polymer does not precipitate. In comparison, during adsorption and separation with a HFIP gradient, precipitation of the polymer occurs. The implications of the different separation mechanisms, i.e., adsorption vs. precipitation chromatography are discussed. Loadability is shown to be much better with the formic acid system. However, with formic acid as a modifier UV detection below 250 nm is not feasible. The less sensitive evaporative light scattering detector is used to detect the polyamide oligomers in the formic acid phase. In addition it is shown that capillary zone electrophoresis (CZE) with UV-absorbance detection using HFIP is an attractive combination as HFIP is UV-transparent and CZE allows low modifier consumption.

Keywords: Mobile phase composition; Polyamides; Nylon; Formic acid; Hexafluoroisopropanol

1. Introduction

Polyamide-6, also known as nylon-6, is a polycondensate of caprolactam. Although low-molecular-mass cyclic structures exist, the majority of the condensation reaction product is linear (Fig. 1) [1].

As the higher oligomers and the polymer itself are not soluble in common chromatographic solvents, the use of exotic mobile phase modifiers is necessary to determine the oligomers and polymer [2]. *m*-Cresol [3], benzylalcohol [4–6], *m*-cresol–chlorobenzene [7], hexamethylphosphorotriamide [8] and methyl chloride–dichloroacetic acid [9] have been used to determine the molecular mass distribution of polyamides. 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP), introduced by Drott, simplified the determination of the molecular mass distribution of the polyamide, as the analysis could be performed at room temperature

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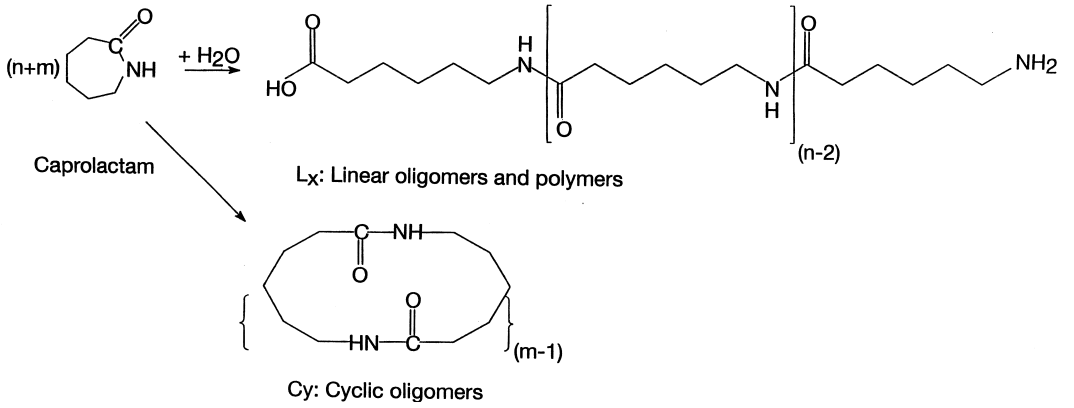

Fig. 1. Reaction scheme; formation of linear and cyclic structures.

[10]. Nowadays, HFIP is commonly used in size-exclusion chromatography (SEC) [11–17] and even special SEC columns for fluorinated alcohols are available.

Other fluorinated alcohols, like 2,2,2-trifluoroethanol (TFE) have been used in combination with SEC and reversed-phase high-performance liquid chromatography (RP-HPLC) to determine the oligomers of nylon-6 [18–23].

Van der Maeden et al. demonstrated the separation of high-molecular-mass poly(ethylene terephthalate) oligomers with a water–HFIP gradient and UV-absorbance detection at 270 nm [24]. HFIP is also used in combination with gradient elution and detection at low wavelengths (200 and 230 nm) to determine the chemical composition distribution of a transamidated polyamide blend [25].

Some disadvantages of HFIP (price/performance and purity) made us look for alternative solvents which could be used in combination with gradient elution RPLC.

Formic acid is a very good and inexpensive solvent for polyamides at room temperature [1,26–28]. In HPLC it is a common additive for the aqueous mobile phase where it is used at low concentrations (typically 0.1–1%). Heukeshoven et al. demonstrated the use of high concentrations formic acid in the mobile phase to enhance solubility of poliovirus polypeptides, although they needed 2-propanol to elute the polypeptides [29]. We investigated the use of high concentrations formic acid (65–95% in water) as a gradient modifier in combination with an octadecyl-modified silica-based column and an evaporative light scattering detection (ELSD) system and compared it with HFIP with low-UV-wavelength detection to determine the higher linear oligomers (L6–L40) of polyamide-6.

2. Experimental

The polyamide-6 and the oligomeric samples were all synthesized at DSM. The HFIP method combined with UV detection was performed on an HP 1090 DR5 solvent delivery system (flow 0.2 ml/min) equipped with an autosampler with a 25-μl syringe (injected volume 5 μl) and a diode array detection (DAD, primary wavelength λ=200 nm) system, all from Hewlett-Packard (Waldbronn, Germany) and controlled by a Windows 95 workstation LC-3D version A.06.01. Mobile phase A contained 40% HFIP (Chemosyntia, Ingelmunster, Belgium) and 60% 10 m*M* phosphoric acid (made with phosphoric acid 85%, analytical-reagent grade, Baker, Deventer, The Netherlands) in water (Milli-Q, Millipore, Milford, MA, USA) and mobile phase B contained 85% HFIP and 15% 10 m*M* phosphoric acid in water. The column used was a 250×2.1 mm Zorbax SB300 C_{18} column (HP, Newport, DE, USA) at room temperature (RT).

The formic acid system, combined with ELSD was made up of an HP 1100 quaternary pump (Hewlett-Packard, flow 1.0 ml/min), a Midas autosampler (Spark, Emmen, The Netherlands) equipped

with a 250-μl syringe and a 20-μl fixed loop. Detection with the ELSD SEDEX 55 (Sedere, Vitry/Seine, France) system was performed with an optimized drift tube temperature of 55°C and 1.9 bar air front pressure. The detector signal was collected with an X-Chrom/Windows NT 3.51 version 2.11b data management system (LAB-systems, Manchester, UK).

Mobile phase A contained 65% formic acid (Merck, Darmstadt, Germany) and 35% water and mobile phase B contained 95% formic acid and 5% water. The column used was a 250×4.6 mm Zorbax SB300 C_{18} column at room temperature.

Both gradient timetables were identical. The initial 100% premixed mobile phase A was changed to 100% premixed mobile phase B in 240 min. The linear eluent velocity was in both systems 0.25 cm/s and the injected volume was approximately 1.5% of the column volume.

A fume hood should be used with both formic acid and HFIP containing HPLC systems. Acute health effects of formic acid and HFIP are comparable (Table 1). However, as long term health effects of HFIP are not well known, HFIP is considered to be very toxic.

Measurements of the number of theoretical plates of the 150×4.6 mm Zorbax SB300C_{18} column were performed with a test mixture (*p*-nitroaniline, nitrobenzene, 2-nitrotoluene, 1-chloro-3-nitrobenzene) on an HP1090 PV5 solvent delivery system equipped with an autosampler with a 25-μl syringe (injected volume 5 μl) and a DAD system (primary wavelength λ=278 nm), all from Hewlett-Packard and controlled by a Windows 95 workstation LC-3D version A.06.01. The mobile phase consisted of 50% acetonitrile (Merck) and 50% 10 m*M* phosphoric acid in water. The number of theoretical plates was calculated as the inverse slope of the squares of the retention times versus the squares of the corresponding standard deviation of the four peaks. The standard deviation was measured at half height.

Capillary zone electrophoretic experiments were performed on a Prince instrument (Lauerlabs, Emmen, The Netherlands). The capillary [60 cm (effective length 50 cm)×50 μm I.D.×365 μm O.D. fused-silica, J&W Scientific] was rinsed for 5 min at 2000 mbar with run buffer prior to hydrodynamic injection (0.1 min, 15 mbar, samples were dissolved in run buffer), after which during 120 min 15 kV was applied. UV detection was performed at 190 nm with a Spectra 200 (Spectra Physics, Reno, NV, USA). Run buffer was HFIP–25 m*M* H_3PO_4 in water (65:35).

3. Results

Formic acid is not often used as a modifier in silica-based RPLC, probably because it is considered to be an aggressive acid. However, its pK_a is not extremely low (pK_a=3.75) and, theoretically, the pH of the solution can never be less than 1.1. To test column stability, 5000 column volumes of 65% formic acid were pumped through the column. In Fig. 2a retention time stability under these test conditions is demonstrated. The stability appears reasonably good, as these high-molecular-mass oligomers are extremely sensitive to system instabilities (temperature and mobile phase compositions). Resolution is stable too (Fig. 2b), although the

Table 1
Comparison of formic acid and HFIP

	Formic acid	1,1,1,3,3,3-Hexafluoroisopropanol (HFIP)
Structure	HCOOH	CF_3–CHOH–CF_3
Price	15–100 US$/l	1500–3500 US$/l
m.p.↔b.p.	8↔101°C	−3↔58°C
Elution/adsorption of oligomers on Zorbax SB300 C_{18}	65% formic acid	40% HFIP
Elution of polyamide-6 on Zorbax SB300 C_{18}	95% formic acid	85% HFIP
Cloudpoint in water	60% formic acid	60% HFIP
MAC-(8 h)	6	? (very toxic)
LD 50 oral rat (mg/kg)	1100	1040
LC inhalation rat (mg/l/4 h)	13.6	7.4

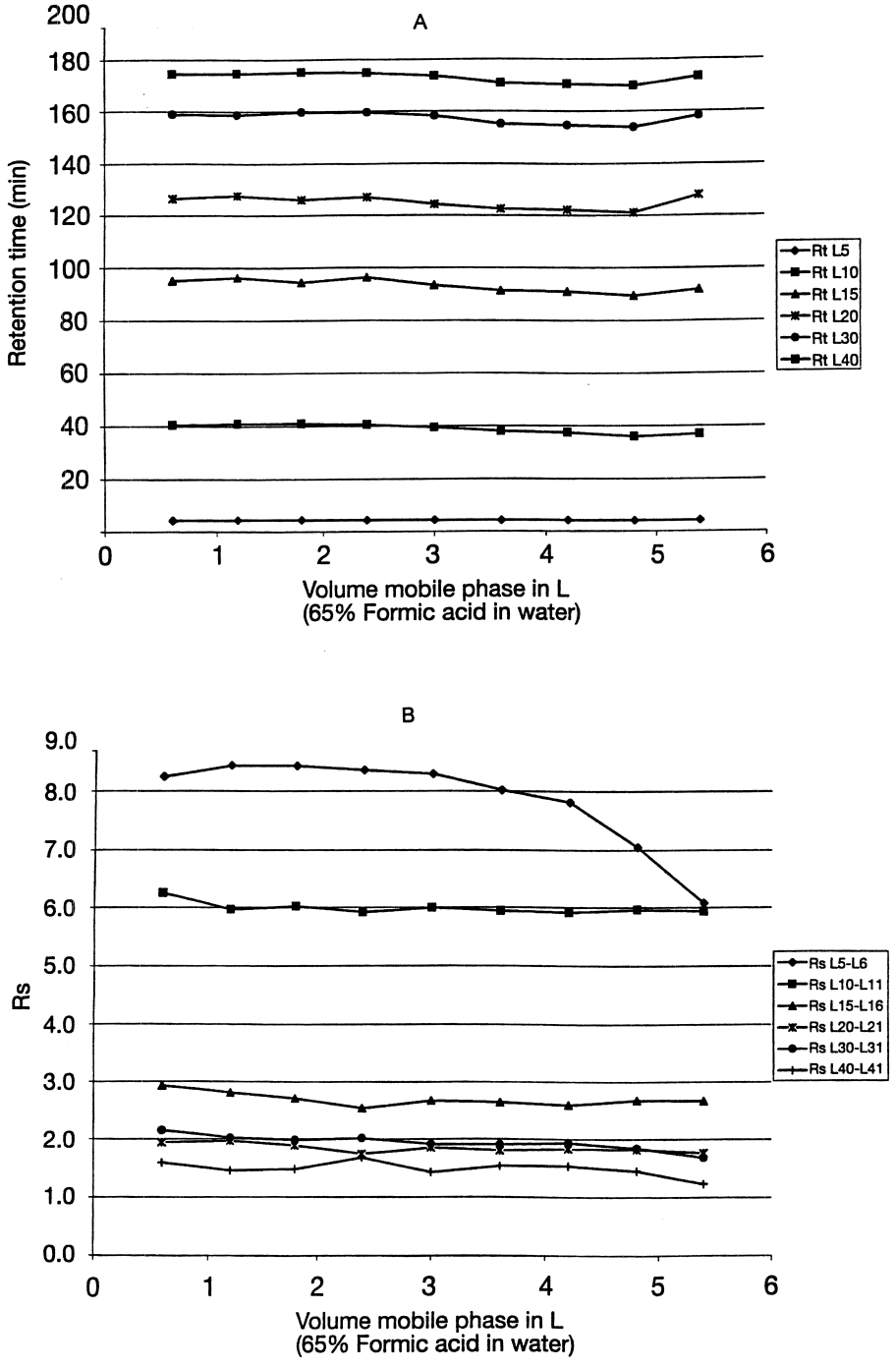

Fig. 2. Column stability. Retention time stability (a) and resolution stability (b) of the linear oligomers on a silica-based C_{18} column with 65% formic acid in water as the mobile phase.

resolution between the linear pentamer and hexamer decreased after approximately 3000 column volumes, which could be attributed to an injector seal problem. Number of theoretical plates in the column before and after this test was 12 000.

To compare formic acid with HFIP, some relevant data are given in Table 1 and Fig. 3. Formic acid is much less expensive than HFIP. To reduce costs of HFIP some special measures are taken. First, small internal diameter columns reduce modifier consumption. In practice, 2 mm internal diameter columns can be used in combination with common HPLC apparatus. Secondly, used HFIP can be purified by distillation as its boiling point (58°C) is low and no azeotropes are formed with water. Normally HFIP is doubly distilled and recoveries up to 80% are possible. Even with these precautionary measures, HFIP is economically unattractive.

In Fig. 4a–d representative chromatograms are given for a polyamide-6 (a, b) and a linear oligomeric (c, d) sample, both with a formic acid (a, c) and a HFIP (b, d) gradient.

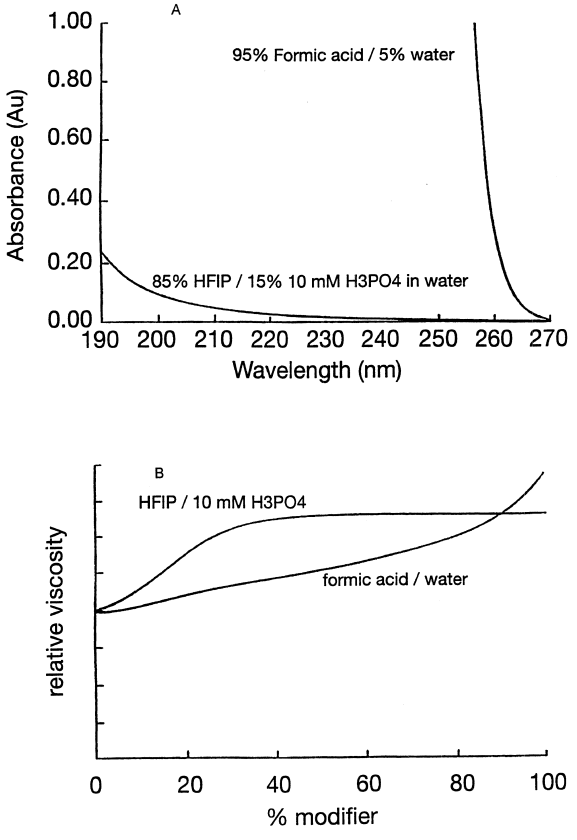

Fig. 3. UV spectra (a) and viscosity (b) of formic acid and HFIP.

Loadability is much better with formic acid as a modifier. Band spreading is worse with HFIP too. At the same oligoamide concentrations injected, peaks are narrower with formic acid (Fig. 4c–d), which may be caused by better wetting of the stationary phase. In Fig. 5 the peakwidth is given as a function of the injected concentration of the linear docosamer (L22). The addition of polyamide-6 to the injected sample does not influence peakwidth to a large extent.

To study recovery, different amounts of an oligomeric mixture (L6–L50) were added to a nylon-6 solution. Recovery can be calculated with the use of this oligomeric mixture without the polyamide. The composition of this oligomeric mixture is calculated with the assumption of a constant contribution of the amide function to the UV absorbance [30]. It is well possible to integrate the peaks up to the linear tetracontamer (L40). In Tables 2 and 3 the recoveries are given. Compared to the formic acid gradient, the recovery of the HFIP gradient looks even better, despite the deviant mechanisms of precipitation versus adsorption. Precipitation of the polymer occurs at a concentration below 60% of each modifier. With formic acid at the conditions used (65–95% formic acid in water) the oligomers are retained on the column, while the polymer does not precipitate. In comparison, during adsorption and separation with a HFIP gradient (40–85% HFIP), precipitation of the polymer occurs. However, probably due to the good solubility of the higher oligomers (L6–L50) in 40% HFIP the recovery with a HFIP is at least as good as with the formic acid gradient.

HFIP is an attractive modifier as it is UV-transparent at low wavelengths (Fig. 3) although baseline elevation due to background absorption occurs (Fig. 6a). However, batch-to-batch quality is not constant and often not defined in terms of UV transmission. HFIP quality of batches from different manufactures show even more dissimilarities. Differences in background absorbance (λ=200 nm) of contaminations between $<$0.1 and $>$2.0 absorption units (AU) were found. Formic acid cannot be used in combination with UV detection beneath 250 nm (Fig. 2). ELSD is a good alternative, although quantification is less

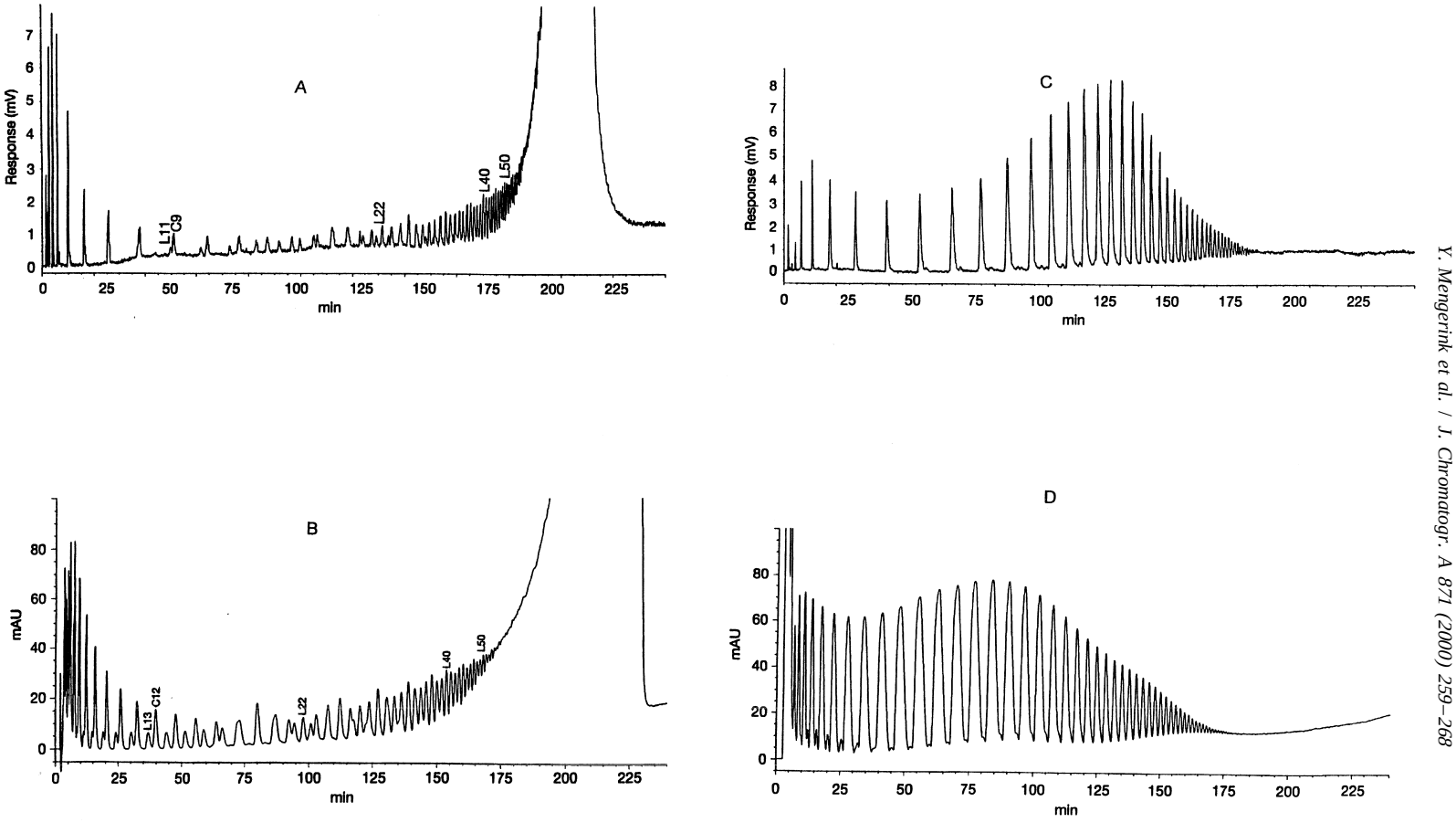

Fig. 4. Chromatograms of a representative polyamide-6 sample (a and b) and a linear oligomers standard (c and d). (a) 32.5 g/l PA-6 dissolved in formic acid–water (65:35), (b) 27 g/l PA-6 dissolved in HFIP–10 m*M* H_3PO_4 in water (65:35) and (c, d) 6 g/l linear oligomers (L6–L50) of PA-6 dissolved in formic acid–water (65:35). (a and c) Gradient elution with formic acid of 65 to 95% in water in 240 min, 20 μl injection on a 150×4.6 mm Zorbax 300SB C_{18} column and ELSD at 55°C. (b and d) Gradient elution with HFIP 40 to 85% in 10 m*M* phosphoric acid in 240 min, 5 μl injection on a 150×2.1 mm Zorbax 300SB C_{18} column and UV detection at 200 nm.

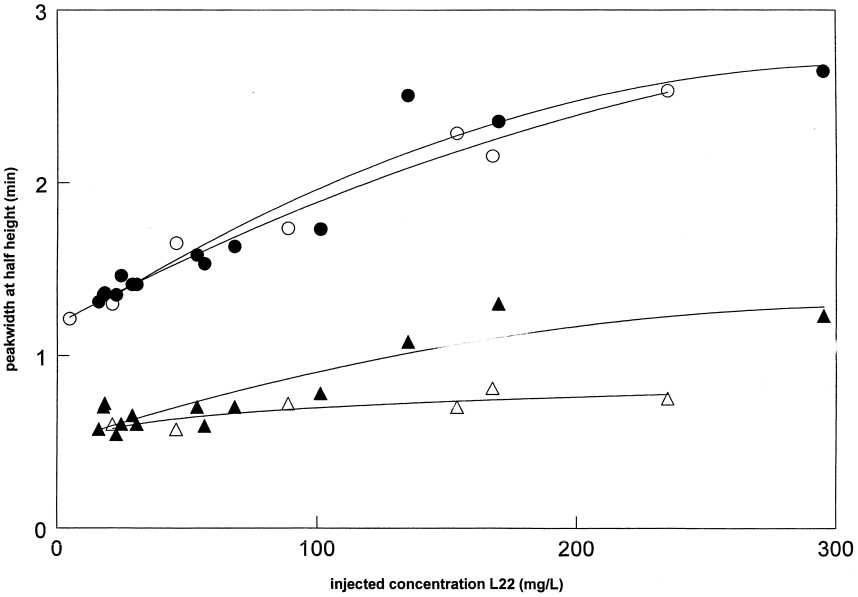

Fig. 5. Loadability of the linear oligomers of nylon-6, measured as the injected concentration of the linear docomer (L22) versus the peakwidth at half height. △ Formic acid gradient, ○ HFIP gradient. Filled markers addition of 2% polyamide-6 to the injected sample.

straightforward, as the calibration curve (concentration versus response) gives a logarithmic relation. Under optimized detection conditions, UV detection gives a better signal-to-noise ratio than ELSD, e.g., for the linear docosamer (L22) the limit of detection, determined as signal-to-noise=3, is approximately 5 mg/l with ELSD and 1 mg/l with UV detection at 200 nm.

However, the detectability of the oligomers during a formic acid gradient can be improved as preconcentration of the sample on the top of the column is applicable, since the formic acid concentration of the injected solution and the starting conditions of the gradient are identical (Fig. 6b). An injection volume of 100 μl appears optimal. At these volumes the system gets already overloaded when high concentrations of oligomers/polymers are injected, resulting in a rapid decrease of column performance.

To improve the performance of the HFIP system, a ternary system could be suggested. However, the

Table 2
Average recovery of oligomers L6–L40 at different additions

Concentration of polyamide-6 (g/l)	Concentration of oligomers L6–L50 added (g/l)	Average recovery L6–L40	
		HFIP gradient (%, w/w)	Formic acid gradient (%, w/w)
16	0.05	106	102
22	0.2	101	90
16	1.0	100	92
21	1.2	103	92
23	2.0	98	90
19	2.6	101	90
23	7.2	82	97
32	2.5	99	83
39	0.4	101	95

Table 3
Average recovery of additions for different oligomers

Group of oligomers	Average recovery of different additions	
	HFIP gradient (%, w/w)	Formic acid gradient (%, w/w)
L6–L10	106	118
L11–L15	110	108
L15–L20	95	91
L20–L25	87	77
L25–L30	88	78
L30–L35	98	85
L35–L40	110	89

usability of most common UV-transparent co-modifiers is questionable. Acetonitrile is not miscible at most water–HFIP compositions, although all binary combinations mix. Both methanol and ethanol forms azeotropes with HFIP–water, making the distillation to recycle HFIP troublesome.

Another way to improve performance is the use of capillary zone electrophoresis (CZE), which can be used to determine the linear oligomers selectively. At low pH the endoosmotic flow is negligible and the linear polyamide molecules bear a positive charge on

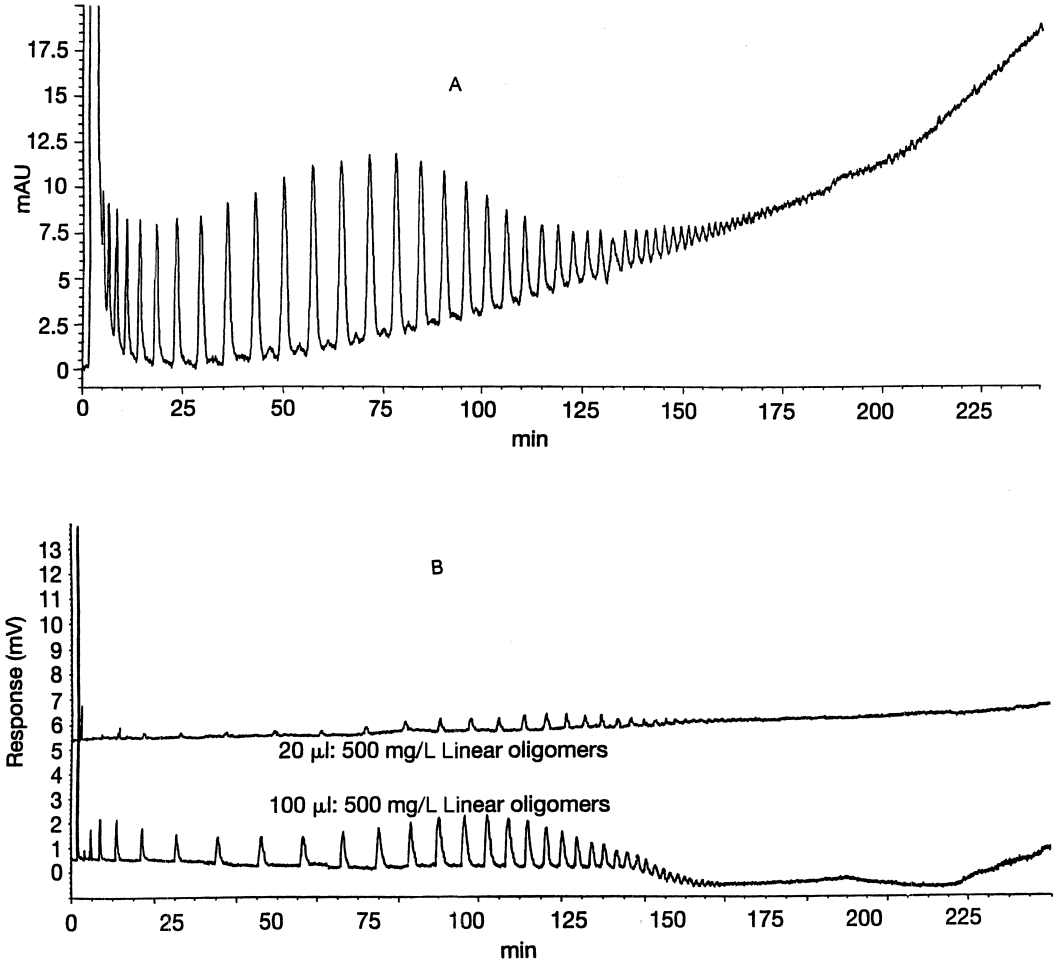

Fig. 6. Detectability of oligomers of nylon-6. Chromatograms of a linear oligomers standard (500 mg/l in formic acid–water, 65:35) (a) 5 µl injection with HFIP as the mobile phase, (b) 20 µl (upper trace) to 100 µl (lower trace) injection with formic acid as the mobile phase. (a) Gradient elution with HFIP 40 to 85% in 10 m*M* phosphoric acid in 240 min, column: 150×2.1 mm Zorbax 300SB C_{18} at RT and UV detection at 200 nm and (b) gradient elution with formic acid 65 to 95% in water in 240 min, column: 150×2.1 mm Zorbax 300SB C_{18} at RT and ELSD at 55°C.

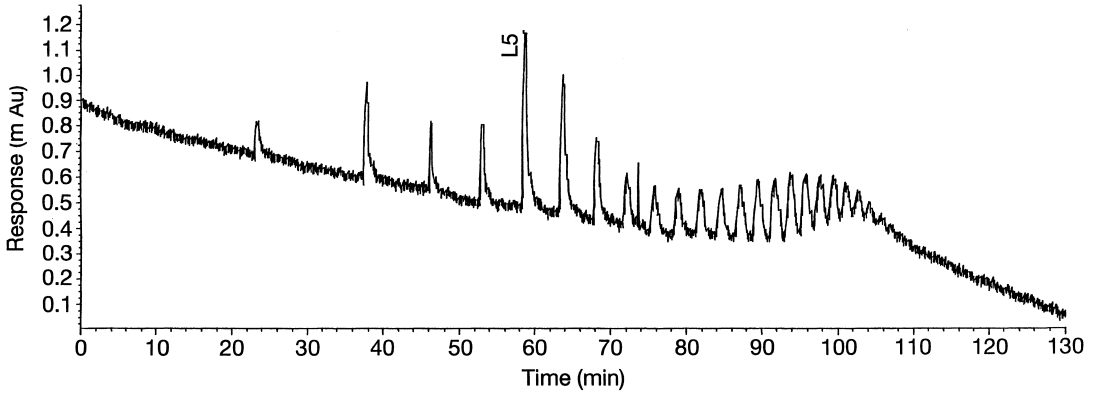

Fig. 7. Capillary zone electrophoreses of a linear oligomers standard 1 mg/ml dissolved in HFIP–25 m*M* H_3PO_4 (65:35). Run buffer: HFIP–25 m*M* H_3PO_4 (65:35). Applied voltage 15 kV, capillary 60 cm (effective length 50 cm)×50 μm fused-silica (J&W Scientific). Hydrodynamic injection 0.1 min and 15 mbar.

the amine endgroup. Due to the difference in charge-to-mass ratio, they can be separated (Fig. 7). HFIP and UV detection form an attractive combination in CZE, as HFIP is UV-transparent and CZE allows low modifier consumption.

4. Conclusions

High concentrations of formic acid can be used on C_{18}-modified RP columns. Compared to expensive fluorinated alcohols, formic acid is an important alternative to separate the higher oligomers of polyamide-6 as octadecyl-modified silica-based columns are stable for at least 5000 column volumes at 65–95% formic acid in water.

Loadability is much better with formic acid than with HFIP. Band spreading is worse with HFIP too. At the same concentration injected, the peaks with the HFIP gradient are twice as broad as with the formic acid gradient.

As HFIP is a strong modifier, the oligomers elute under HFIP conditions where precipitation of the polymer occurs. With formic acid as a modifier, the oligomers elute and the polyamide-6 does not precipitate on the column. However, this thus not influences recovery of the linear oligomers from the linear hexamer (L6) up to the linear tetracontamer (L40).

HFIP does not absorb much UV energy at low wavelengths, making UV detection at 200 nm feasible. To detect the oligoamides with formic acid as the mobile phase, the less sensitive ELSD system has to be used. Fortunately, preconcentration, to improve detectability, is applicable here. Due to the non-linear relation between concentration and response, calibration with ELSD is less straightforward.

It is shown that CZE in aqueous H_3PO_4 with HFIP using UV detection at 190 nm can be an attractive alternative for the selective separation of the linear oligomers of nylon-6.

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