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Review

High-temperature liquid chromatography

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Abstract

The present state of the active use of elevated temperatures in liquid chromatography is reviewed, including the effects on retention, selectivity and efficiency. Separations in aqueous mobile phases as well as non-aqueous media are discussed, with particular emphasis on narrow-bore columns.

Keywords: Reviews; Temperature effects; Temperature programming

Contents

1. Introduction

Liquid chromatography (LC) at elevated temperatures has traditionally not been widely used, with the exception of size exclusion chromatography of polymers of low solubility, such as polyolefins, often requiring temperatures of 140–150 °C in halogenated aromatic solvents to be kept in solution. Also, in the early years of HPLC, ion-exchange chromatography was fairly often performed at elevated temperatures in order to increase the exchange rate and improve the column efficiency. With the development of more efficient packing materials, the use of higher temperatures decreased. In addition, the interest for using higher temperatures in aqueous media has been moderate due to fear of decomposition of analytes and stationary phases, and due to negative statements concerning the effects on selectivity. Thus, in 1996

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Zhu et al. reported that half of all HPLC systems were without means for column thermostatting [1].

Recently a more general acceptance of the importance of temperature as a variable in LC has taken place. This is based on the well-known decrease in viscosity of the mobile phase [2] and the increase in analyte diffusivity [3] at higher temperatures. The consequence of reduced viscosity is lowered back-pressure over the column, allowing higher speed or longer columns with higher plate numbers. The consequence of increased diffusivity is increased mass transfer, usually resulting in improved column efficiency [4].

Furthermore, recently much more stable stationary phases have been commercialized, and finally, the trend towards narrow bore columns has enabled chromatographers to use temperature in LC with improved results and fewer problems.

This review will cover the development in the last decade, also including some references to basic papers prior to that time.

In the literature, ''high-temperature'' is not an exactly defined term. Definitions as different as ''higher than room temperature'', ''higher than the boiling point of the mobile phase solvents'' and ''higher than 100 °C'' can be found. All papers which are seen as valid for anticipating the influence of temperatures well above room temperature have been included in the review. However, size exclusion at high temperature, which is a special topic for polymer chemists, is mentioned but not discussed. Temperature effects on separation of chiral compounds are not included, since this is more concerned with low than with high temperatures.

2. Influence of temperature on retention

The contribution of temperature to the retention is mainly given by the enthalpy term of the van't Hoff equation for the retention factor,

$$\ln k = -\Delta H/RT + \Delta S/R + \ln \beta$$

where ΔH is the enthalpy change associated with the transfer of the solute between phases, ΔS is the corresponding entropy change, R is the molar gas constant, T is the absolute temperature and β is the phase ratio of the column.

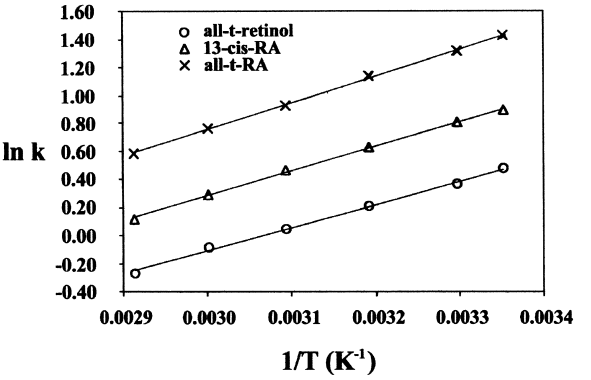

Fig. 1. Van't Hoff plot of the retention–temperature relationship of three retinoids, demonstrating reduced retention with increased temperature and no difference in selectivity within the temperature interval 25–70 °C. From Ref. [33], with permission.

If $1/T$ is plotted against ln k, in a van't Hoff plot, the enthalpy is given by the slope of the curve (Fig. 1). Typical enthalpy interactions in reversed-phase systems are about -10 to -15 kJ mol^{-1} for small molecules, while larger molecules usually have larger (negative) enthalpies. Since a solute with a large ΔH will be more affected by a temperature change than a solute with a small ΔH, changes in selectivity may take place at higher temperatures.

As a function of variations of the temperature, changes in retention can be caused either by changes in enthalpy or in entropy. Enthalpy changes which are not linear within a temperature range, can appear as a result of not linearly increasing adsorption enthalpy with increasing M_w, different desorption kinetics of different functional groups [5], dual retention mechanisms or temperature-related changes of the mobile phase [6].

Several studies have been performed in order to compare directly the effect of a change in the solvent composition with a change in temperature. For a series of compounds, Bowermaster and McNair [7] found that 1% increase in methanol concentration had approximately the same effect as a temperature increase of 4 °C.

Chen and Horváth [5] found a similar effect with alkylbenzenes in acetonitrile–water. These results on neutral analytes were later confirmed in another study with reversed-phase systems, showing similar proportions [8].

Increasing temperatures also reduce the viscosity of the mobile phase, which reduces the column backpressure. Since the enthalpy is a function of the internal energy ΔE, the partial molar volume ΔV_m and the pressure p, ln k can be given as:

$$\ln k = -\Delta E/RT - p\Delta V_m/RT + \Delta S/R + \ln \beta$$

Since ΔV_m is independent of temperature, it is possible to predict the effect of the temperature on the pressure dependence of ln k. The effect of pressure on retention has usually been neglected in LC, because the effects are small with small molecules. Recently the pressure effects on some proteins were studied at temperatures between 25 and 50 °C [9]. The retention was increased by up to 300% by increasing the pressure from 47 to 147 bar. The effect of a pressure increase of 100 bar on the change in partial molar volume, on the other hand, was only ~2%.

Many proteins and polypeptides show a different retention behaviour compared to smaller molecules and to many other macromolecules. Due to changes in the 3-dimensional structures and displacement of solvent molecules at the protein surface at higher temperature, both ΔH and ΔS may increase, resulting in increased retention with increasing temperature [9]. This increase was shown to be non-linear for insulins and possibly with a maximum at higher temperature (Fig. 2).

Since the effect of solvent strength usually is stronger than the effect of temperature on solute retention, direct comparisons between solvent gradients and temperature programs are usually not of major interest. The effects of solvent strength and temperature are often complementary [10]. Particularly for larger molecules, such as peptides and proteins, simultaneous optimization of temperature and gradient steepness has been considered a powerful and convenient means of controlling band spacing and retention [10,11]. In general, entropy-dominated separations are expected to benefit more from variations in temperature than from variations in solvent strength.

Asymmetric peak shapes of compounds with basic groups are occasionally seen with silica-based columns. This is usually caused by secondary interactions with residual silanol groups and sometimes also by slow kinetics. Fig. 3 demonstrates the ability of increased temperature in improving such a peak shape. It is not known whether the improved peak shape actually is caused by temperature-affected changes in pH or pK_a, improved kinetics or as more likely a combination of such effects.

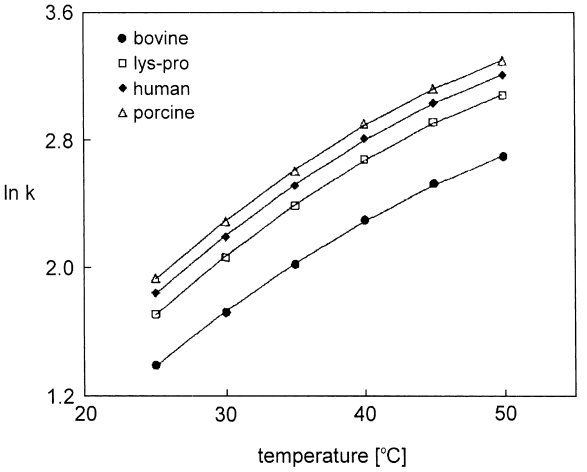

Fig. 2. Retention–temperature dependence of four insulin proteins, demonstrating increased retention with increasing temperature. From Ref. [9], with permission.

3. Influence of temperature on selectivity in reversed-phase systems

If the enthalpy difference of two solutes at different temperatures is related to a single retention mechanism (such as hydrophobic interactions in a homologous series), decreased selectivity is usually expected as a result of increasing temperature. Studies using small solutes in reversed-phase systems, with mostly simple hydrophobic interaction mechanisms, have demonstrated that elevated temperatures often lead to lower selectivity [6]. Using the solvation parameter model, the effect of temperature on selectivity was determined to be largest with compounds differing by size and by hydrogen-bond basicity [6]. In general the temperature effects were found to be significantly smaller than compositional changes in the mobile phase [6]. In another comparison between gradient elution and temperature programming of alkylbenzenes, similar retention

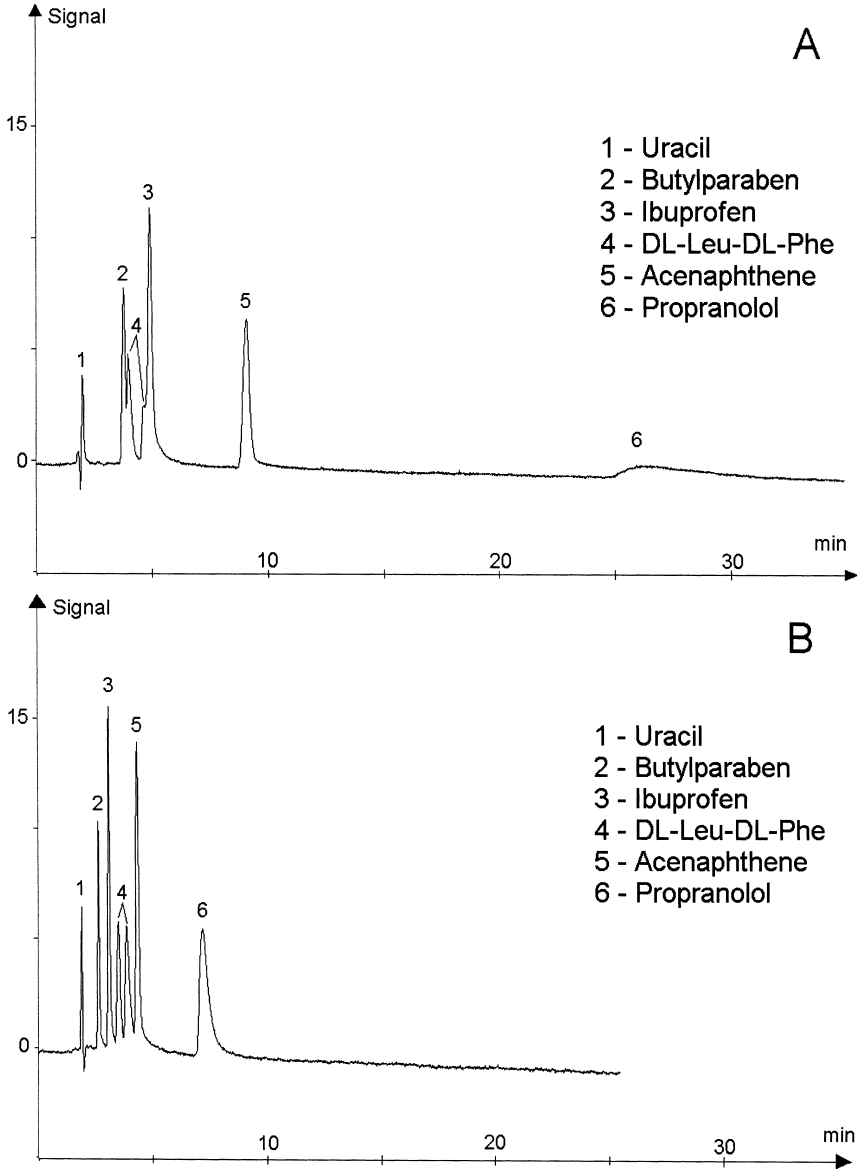

Fig. 3. Improvement of peak shape, analysis time and overall resolution by increasing the temperature from 10 °C (A) to 90 °C (B), in acetonitrile–water, pH 2 (70:30, v/v) on 3 μm Hypersil ODS. The elution order of ibuprofen and Leu–Phe was reversed. From Ref. [8], with permission.

trajectories were found with elution from 70 to 85% acetonitrile (with 6% increment per min) as with temperature increases of 30 °C/min from 30 to 120 °C [5].

In a series of papers, Zhu et al. [1,12–14] studied the combined use of solvent gradients and temperature for predicting separation of analytes by computer simulation. Although they defined high temperatures as above 35 °C and never went beyond 70 °C, their results are probably valid at higher temperatures too. Quantitative data on the effects of temperature versus solvent strength are not easily

extracted from the papers, but particularly on ionizable compounds there is little correlation between the two effects. Hydrophobic compounds with little substitution by polar functional groups gave the smallest selectivity effects on combined solvent and temperature variations [1]. Consequently, a combined variation of temperature and solvent strength was recommended as an effective first step in method development.

Solutes with basic groups experience strong effects of temperature on retention as well on peak shape, as seen in Fig. 3. In a recent study [8], both pH and mobile phase composition affected the selectivity differently at different temperatures, particularly of basic compounds. With the secondary amine propranolol, a 2 °C change in the column temperature had the same effect as 1% change in the acetonitrile concentration [8]. A multivariate experimental designed study demonstrated that variations of temperature within the 10–90 °C range were of approximately the same magnitude as pH variations within the pH 2–7 range, for the basic compounds amitriptyline, amphetamine and propranolol, with reversed-phase columns [8]. This means that, even if the temperature effects of basic compounds are a function of pH and difficult to predict [15], the resolution of neutral and basic compounds can be changed by manipulating solvent strength and temperature. A dipeptide had a reverse temperature effect at low pH in acetonitrile-based mobile phase, but not with methanol [8]. Reversed temperature effects (increased retention at elevated temperatures) are thought to be caused either by secondary interactions [15], reduced ionisation [15] or by reduced solubility in the mobile phase at higher temperatures. An example of a relatively rare reverse temperature program is shown in Fig. 4. The background for this chromatogram is the solubility of polyethylene glycols which decreases with increasing temperature [16].

In a theoretical analysis, Li [17] made a thorough thermodynamic analysis of the effect of temperature on selectivity for several complicated retention processes in reversed-phase LC. The selectivity of carboxylic acids was decreased at elevated temperatures with buffers with pK_a lower than the solute pK_a and increased with buffers of higher pK_a than the solute pK_a.

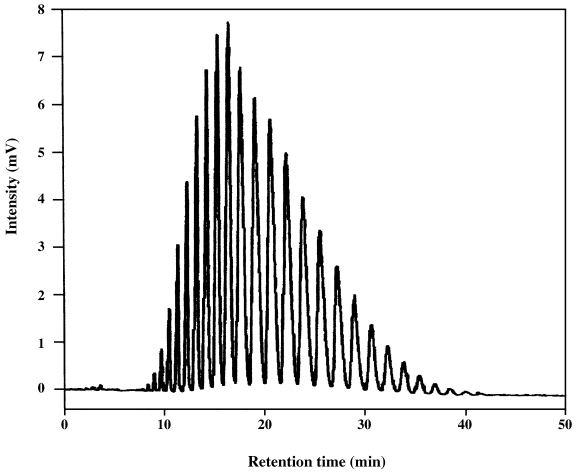

Fig. 4. Separation of PEG 1000 using a reverse temperature gradient from 80 to 25 °C on a 0.32 mm I.D.×30 cm fused-silica column packed with 3 μm Hypersil ODS. Detector, ELSD. From Ref. [16], with permission.

The selectivity of amine compounds was increased with all buffers at elevated temperatures, with a few exceptions [17]. In general, ionizable compounds can be expected to experience improved selectivity at elevated temperatures at high mobile phase pH [17]. This is in agreement with the experimental results [14] and is independent of the type of reversed-phase column.

For compounds with two ionizable groups, such as amino acids, the situation is more complex. With highly retained amino acids, more basic buffers are preferred at high temperatures. With less retained amino acids, more acidic buffers are preferred [17].

Solutes that experience two independent retention processes usually benefit from elevated temperatures as well. Examples are secondary retention effects of amines on silica-based columns and Lewis acid–base interactions of phenols and acids on zirconia-based columns [17].

Ion-pair chromatography of carboxylic acids is a special case. With low binding strength of the ion-pair complex and low retention enthalpy of the complex, the selectivity is expected to increase at elevated temperature. With high binding strength and high retention enthalpy, the selectivity is expected to decrease [17].

In a recent review article, Dolan [18] concluded

that temperature is now recognized as an important variable in controlling selectivity in RP-HPLC. Even if the main effect of increased temperature is decreased retention, peak reversals within relatively narrow temperature windows of polar and ionic compounds allow improved resolution of a variety of samples caused by changes in selectivity.

If the selectivity is reduced at high temperature, this does not necessarily lead to lower resolution. Improvements of the peak shapes of broad or asymmetric peaks by elevated temperatures may sometimes be more important than changes in the selectivity (see Fig. 3). Another example is the separation of 50 oligomers of a water-soluble copolymer of M_w 30 000 on a column with 2 μm nonporous particles [19]. The oligomers which were resolved at 100 °C with an acetonitrile solvent gradient, had no resolution at room temperature (Fig. 5).

3.1. Influence of temperature on column efficiency; OTLC

Open tubular (OT) columns have large advantages in gas chromatography and partially in supercritical fluid chromatography, but in general not in HPLC. This has been shown to be based on the much higher compressibility of a gas compared to a liquid [20], but is also related to the differences in diffusivity and viscosity. The viscosity of a liquid is decreased and the diffusivity is increased by elevated temperatures, resulting in improved mass transfer and increased column efficiency [21]. At very high temperatures, around 200 °C, the strongly reduced viscosity allowed long 50 μm I.D. open tubular columns [22–25] with up to one million theoretical plates (Fig. 6). With methylpolysiloxanes as stationary phases they concluded that temperatures above 150 °C resulted in too much degradation of the stationary phase with aqueous solvents [22]. Because of the low flow-rates, OTLC has been considered beneficial for coupling towards mass spectrometers [26]. Both linear and step temperature gradients have been compared in reversed-phase and normal-phase modes [27].

At lower temperatures, the reduced diffusivity requires smaller internal diameters in order to achieve the same plate height, resulting in increased backpressures due to the increased viscosity. This is a practical hindrance to open tubular HPLC, unless one accepts working with 5–10 μm I.D. columns at extremely high pressures.

3.2. Influence of temperature on column efficiency; packed columns

The reduced plate height ($h = H/d_p$) for packed columns can be given by the Knox equation,

$$h = A\nu^{1/3} + B/\nu + C\nu$$

where the reduced velocity, $\nu = \mu d_p/D_m$.

The effect of temperature on the A term is uncertain, but it is expected that elevated temperatures will improve the laminar flow and lateral mixing of analyte molecules from different flow channels, due to the increased diffusivity, although the improvements may not be significant [28]. The B term, the longitudinal diffusion, increases with increasing temperatures, and becomes significant at low linear flow-rates. The C term, which consists of mass transport between phases and diffusion inside the stationary phase, plus adsorption/desorption kinetics, will be reduced by increasing temperatures. The A, B and C coefficients can also be dependent on k. The coefficient A, which is a measure of how well the column is packed, is normally little affected [29]. Coefficient B reflects the geometry of the eluent in the column, and coefficient C reflects the efficiency of mass transfer between the mobile phase and the stationary phase. Both B and C can increase with increasing k [28,30,31].

Increasing the temperature from 25 to 65 °C on a zirconia-based packing improved the efficiency by 30% [32], while an increase from 25 to 70 °C on a silica-based packing improved the efficiency by 48% [33]. Increasing the temperature from 24 to 80 °C resulted in a 2.6-fold faster optimum linear velocity, and the reduced backpressure allowed twice as many theoretical plates, by using smaller particles or longer columns [21].

Thus, in general, elevated temperatures are beneficial for the column efficiency. There are almost no exceptions to this statement, such as when the reduced retention allows extracolumn dead volumes to become more prominent [34], or when higher longitudinal diffusion combined with low linear flow increases the B term too much.

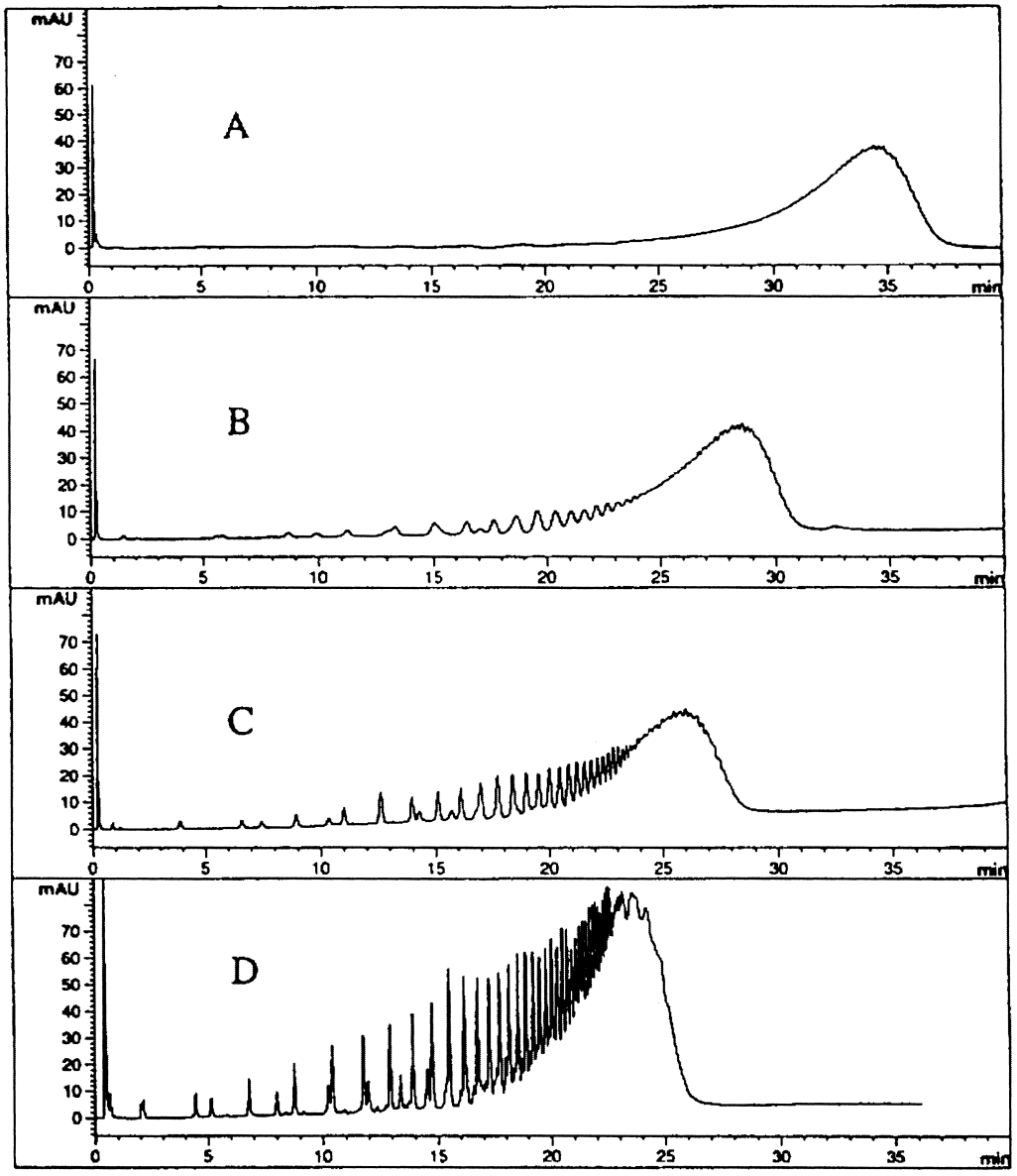

Fig. 5. Improved resolution of a water-soluble polymer of average M_w 30 000 by increasing temperature from 25 °C (A), to 50 °C (B), to 80 °C (C) and to 100 °C (D). From Ref. [19], with permission.

4. Temperature programs and isothermal separations

Few LC-instruments are currently equipped with a programmable oven [35], but with microbore columns or packed capillary columns temperature changes can be performed almost as in gas chromatography, as stated by Bowermaster and McNair almost 20 years ago [7]. Due to the lower thermal mass and increased thermal response, columns with small internal diameters are preferable both for changing the temperature in steps and for temperature gradients. Thus, the current trend towards narrow-bore columns is strengthened by the tempera-

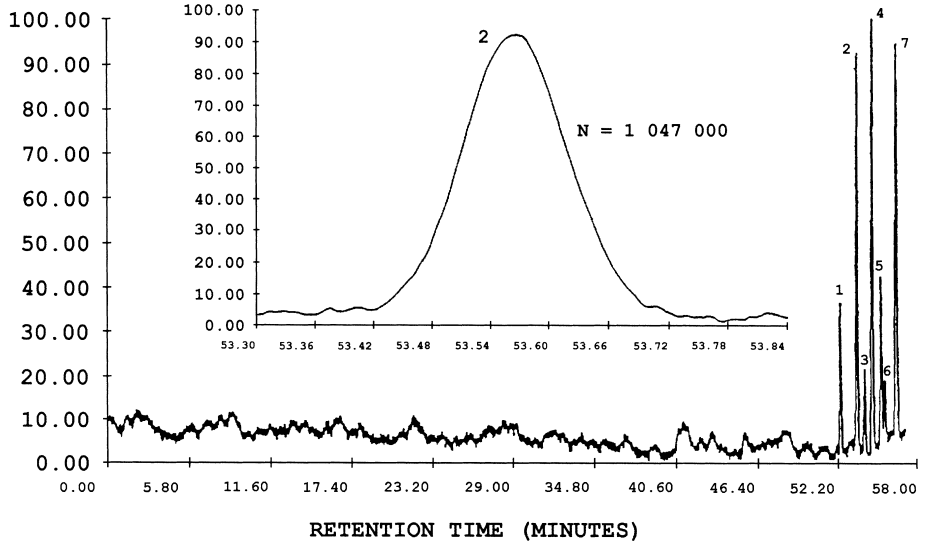

Fig. 6. Open tubular LC at 200 °C on a 20 m×51 μm I.D. SB-Methyl-100 column. Mobile phase, acetonitrile–water (50:50). Flow-rate, 0.65 μl/min. One million theoretical plates were measured on peak 2 (benzene). From Ref. [22], with permission.

ture argument as well. As a result of the growing interest in the active use of temperature in LC, some instrument manufacturers now offer programmable LC-ovens. In chromatography programs such as DryLab, temperature optimisation is included in the computer simulation software, reducing the number of steps required for experimental testing.

Although the first attempts at temperature programming in LC are not recent, the lack of suitable equipment and of temperature-stable columns has slowed down progress. The attempts to use standard size (4.6 mm I.D.) LC-columns for temperature programs have added to this, creating radial temperature gradients in the column unless the solvent is preheated in the same oven. If the incoming solvent is cooler than the column wall, the fluid at the column center will be cooler than the fluid at the walls, resulting in peak broadening [36–38]. Thus, preheating the solvent has been recommended strongly [37–39]. With standard size columns, Wolcott et al. [39] suggested that the temperature difference between the mobile phase entering the column and the column should be less than 7 °C in order to avoid band broadening. However, whether the peak broadening is a problem or not depends on two things; firstly, the temperature difference between the incoming solvent and the column, and secondly the diameter of the column. With narrow-bore columns the thermal mismatch effects are reduced significantly [7,38] and can even be neglected [40] due to the fast heat transfer, solving the entire problem. With conventional HPLC-equipment, Thompson and Carr [41] recommended to use 2 mm I.D. columns, as a compromise between the reduced radial retention differences and the dead volumes of such instruments.

Another option with narrow-bore columns is sample focusing (reconcentration), which can be obtained with low temperature at the column inlet [42–45]. Fig. 7 demonstrates the use of a novel column oven concept with an incorporated cold spot allowing sample volumes to be increased by a factor of 10 000 by temperature-promoted solute enrichment on a packed capillary column. The cold inlet zone consisted of a 1.5 cm aluminium block mounted upon a Peltier element, while the hot temperature-programmable zone consisted of a 0.5 mm I.D. ceramic tube surrounded by coiled metal resistance wire connected to a PC-controlled power supply. The oven was designed to allow horizontal sliding of the capillary column inside the column oven housing, enabling rapid transportation of the column inlet between the

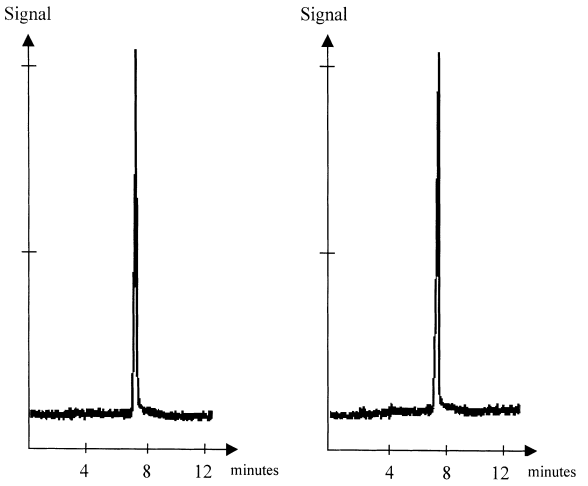

Fig. 7. Comparison of peak profiles when injecting 50 ng Irganox 1076 in acetonitrile in 0.05 μl and in 500 μl volume on a 0.32 mm I.D. column. The run (in acetonitrile) was started after completed injection when the capillary column was transferred from the cold zone to the hot zone. The temperatures were 0 °C in the cold zone and 90 °C in the hot zone. From Ref. [46], with permission.

two temperature zones. This design eliminates the time-consuming process of recooling the oven after a temperature gradient [46].

A rapid increase to the elution temperature requires a low thermal mass. Consequently, the only sensible thing to do with temperature programming is to use narrow-bore columns. The repeatability (RSD) of retention with temperature programs has been determined to 0.3–1.1% for 10–90 °C [47] and 2% for 40–110 °C programs [7]. Isothermal separations will benefit from the use of narrow-bore columns too, due to the much smaller equilibrium times at temperature changes.

Temperature programs (gradients) have demonstrated particular advantages in separation of homologs [48], as well as of relatively large compounds (Fig. 8). Also see the first review article on temperature control in LC [49].

Temperature programs (gradients) may have an advantage compared to solvent gradients with some detectors. With mass spectrometers, the mass-to-charge ratio and the abundance of fragment ions are expected to remain more constant with temperature programs [50]. However, one should be aware that there is a risk of increased column bleeding at high temperatures, increasing the background noise.

NMR-detection is also expected to benefit from replacing solvent gradients with temperature programs, since the chemical shifts will be less affected.

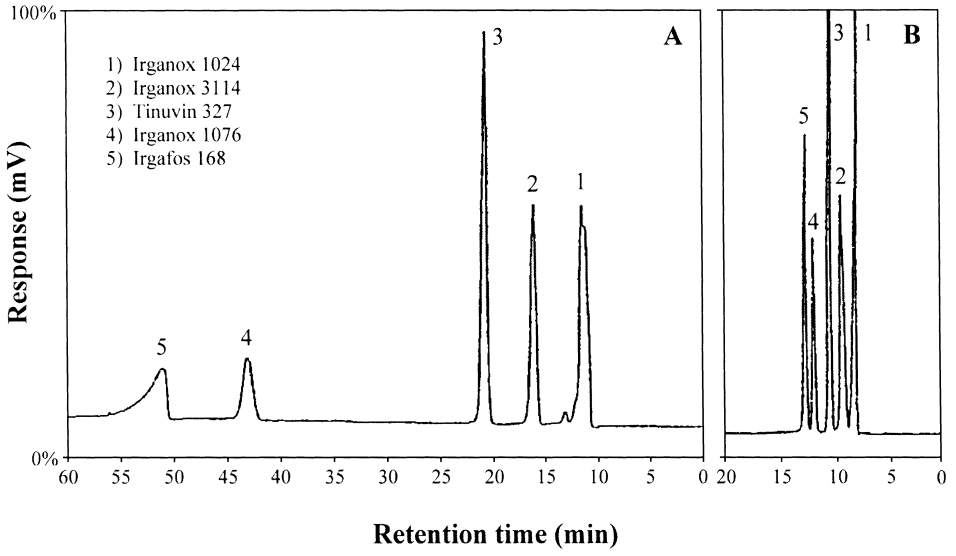

Fig. 8. Separation of polymer additives by isothermal elution at 50 °C (A) and by a temperature program from 100 to 150 °C (B). Column, 3 μm Hypersil ODS, 100 A, 0.32 mm I.D.×69 cm. Mobile phase, DMF–acetonitrile (10:90, v/v), 5 μl/min. UV-detection at 280 nm.

With the current trend towards micro-LC–NMR, with new microprobes for enhanced sensitivity, the use of temperature programs with LC–NMR will be examined in the near future.

With UV-detection and temperature programming, baseline problems can occur with some solvents due to temperature-dependent refractive indexes. Of the reversed-phase systems, the effects are small with methanol and acetonitrile–water mixtures, but larger with THF–water mixtures [51].

4.1. Separations in very hot water

A special case of high-temperature LC is chromatography in very hot water [52–61]. The dielectricity constant of water is reduced from 80 at 25 °C to 35 at 200 °C [52], giving the hot water more of the characteristics of an organic solvent. This allows the use of the flame ionisation detector [45,55,61,62] and offers a completely ''green'' LC-environment, for solutes and stationary phases that are stable in water at high temperatures. Silica-based stationary phases are expected to be rapidly degraded at such high temperatures, but in one report with pure water at temperatures up to 175 °C, the ODS columns were stable over a period of a few weeks [45]. Styrene–divinylbenzene polymers (PRP-1) were resistant at temperatures up to 225 °C [45]. Other resistant columns are either based on carbon, zirconia or other metal oxides. Polybutadiene-coated zirconia has been reported to be stable up to 200 °C [61] in water, although recent experiments with repeated temperature programs at 50–150 °C in aqueous phosphate solutions have demonstrated some reduction in the column efficiency as well as in the retention [63]. The phosphate is added to cover the Lewis acid sites on the zirconia backbone. In general, however, zirconia-based stationary phases appear to be advantageous for separations in ''superheated'' water [61,64,65]. Since pure water even at temperatures approaching 175–200 °C has been characterized as a relatively weak eluting solvent [45,58], it is not likely, however, that the need for organic solvents in LC will disappear in the near future.

4.2. Separations with non-aqueous systems

Non-aqueous systems have the advantage of being useful at higher temperatures than in aqueous systems, with less fear for hydrolysis of the stationary phase. The stationary phases can be reversed-phase materials or polar sorbents, for solutes which have little solubility in aqueous systems. The interactions may be of van der Waals type (on reversed-phase columns), or of polar type (on normal-phase columns with polar packings), or there may be no interactions, as in size exclusion chromatography. Size exclusion chromatography (SEC) is always done at isothermal conditions, never with temperature gradients. By increasing the temperature from 25 to 150 °C in an SEC-separation of polystyrenes, the column efficiency was increased by a factor of 4–6 and the analysis time was reduced by a factor of four [66]. Temperature programming of polymers in LC has also been named temperature gradient interaction chromatography (TGIC), mainly using reversed-phase columns [67–73], but recently also on silica columns [74]. Polyisoprenes of M_w 2000–200 000 and polystyrenes of M_w 10 000–1 500 000 were separated by temperature programs from below room temperature to 70 °C. The separation mechanism was confirmed to be adsorption, not precipitation–redissolution. This normal-phase system provided better overall separation of PI–PS mixtures than the similar reversed-phase system with C_{18} particles [68]. A van't Hoff plot of eight polystyrenes of different size demonstrate the impact of temperature on polymers of different molecular mass (Fig. 9).

A common problem with silica columns is reproducibility problems caused by variations in activity, which is why reversed-phase columns often are preferred.

With reversed-phase columns, the separations of polymers are mainly in accordance with molecular mass. Since the adsorption enthalpy for each monomer unit of polymers decreases with increasing mass, separation of individual compounds becomes increasingly difficult with increasing mass [74]. Separation of individual oligomers with M_w up to 10–20 000 has been demonstrated, such as in the separation of polymeric amines in Fig. 10.

In a separation of polystyrenes of M_w 2000, 5000, 10 000 and 17 500 on C_{18} particles with 10 nm pores, the four standard samples separated with a temperature program from 50 °C to 140 °C. At a constant temperature of 130 °C all the polystyrenes

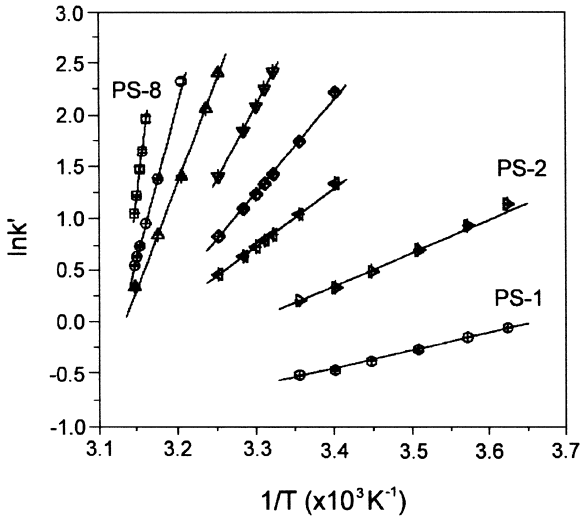

Fig. 9. Van't Hoff plot of polystyrenes, demonstrating the impact of temperature on the retention of compounds with different molecular mass. From Ref. [67], with permission.

coeluted (critical conditions), while at a constant temperature of 140 °C, the elution order was reversed with all compounds being excluded from the pores [75]. This demonstrates the feasibility of active use of temperature in LC, when even retention mechanisms can be controlled by the temperature.

5. Temperature and shape selectivity

Shape selectivity applies primarily to isomeric compounds with rigid, well-defined molecular shape. Typical examples are PAH, PCB, carotenoids and fullerenes. In general, the best separations of isomers are usually performed at reduced temperatures, but with some exceptions [76]. In a comparison between solvent and temperature effects, Snyder and Dolan [77] concluded that about 75% of the isomer pairs tested were best separated by long solvent gradients and low temperatures, while most of the remaining were better separated by long gradients and high temperatures.

The most dramatic temperature effects are with temperature-responsive stationary phases with reversible hydrophilic–lipophilic conformations. A well-known example is poly(*N*-isopropylacrylamide)-modified silicas [78,79].

However, this is considered to be outside the main scope of this review and will not be covered in more detail.

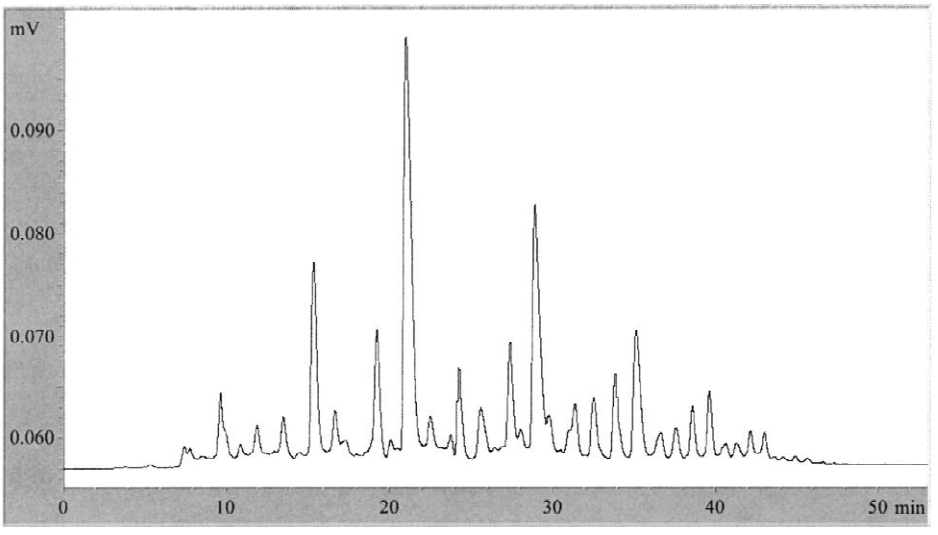

Fig. 10. Separation of Chimassorb 2020, an oligomeric hindered amine light stabilizer with a M_w up to about 15 000. Column, 0.32 mm I.D.×35 cm, 3 μm Hypersil ODS, 100 A. Temperature program, 30 °C for 2 min, then 2 °C/min to 140 °C. Mobile phase, ethyl acetate–acetonitrile–triethylamine (40:50:10, v/v), 5 μl/min. Detector, ELSD. From Ref. [80], with permission.

6. Practical implications of high-temperature LC

Most implications have already been discussed, but a few things are left to be mentioned. At high temperatures, close to or above the boiling point of the constituents of the mobile phase, a backpressure regulator is needed to avoid boiling within the column. With the larger size columns this can be a needle valve. With microbore columns this is just a narrow-bore outlet tube.

Fundamental studies on temperature effects on retention should preferably be performed at constant pressure, also using a backpressure regulator, since the retention of some compounds can be strongly influenced by pressure [9].

The stability of the stationary phases must always be kept in mind. At constant temperatures above 100 °C, in aqueous mobile phases, the most commonly used silica-based C_{18} phases today were rapidly degraded over time [80]. At temperatures below 70 °C, most reversed-phase materials experience few problems, while some packings can be used with programs to 90 °C also without problems [80].

What about the safety aspect of flammable solvents in a hot oven? With narrow bore columns, the potential small leaks are not expected to cause any problems. With standard size columns, the risks are increasing with the solvent volumes. On the other hand, the polymer industry has used size exclusion on 7–8 mm I.D. columns at high temperatures for decades without problems.

In order to use high temperatures, the analytes need to be thermally stable during the chromatographic run. However, with fast analyses and short residence times it is possible to analyze relatively unstable compounds and complex molecules with high-temperature LC [81].

7. Conclusions

Let there be no doubt on the magnitude of temperature effects versus solvent strength on solute retention: with few exceptions solvent strength affects the retention more than temperature. However, combining solvent strength and temperature can have a very powerful effect particularly on analysis time and selectivity, but also on column efficiency. With narrow-bore columns the low thermal mass assures rapid response with both temperature steps and temperature gradients. There are still limitations on the use of silica-based stationary phases in aqueous media at high temperatures, but more columns which are resistant towards hydrolysis become available all the time.

References

[1] P.L. Zhu, J.W. Dolan, L.R. Snyder, N.M. Djordjevic, D.W. Hill, J.-T. Lin, L.C. Sander, L. Van Heukelem, J. Chromatogr. A 756 (1996) 63.
[2] H. Chen, C. Horwath, Anal. Methods Instrum. 1 (1993) 213.
[3] C.R. Wilke, P. Chang, AIChE J. 1 (1955) 264.
[4] J.H. Knox, J. Chromatogr. Sci. 15 (1977) 352.
[5] M.H. Chen, C. Horváth, J. Chromatogr. A 788 (1997) 51.
[6] D. Bolliet, C.F. Poole, Analyst 123 (1998) 295.
[7] J. Bowermaster, H. McNair, J. Chromatogr. Sci. 22 (1984) 165.
[8] J.V. Tran, P. Molander, T. Greibrokk, E. Lundanes, J. Sep. Sci. 24 (2001) 930.
[9] P. Szabelski, A. Cavazzini, K. Kaczmarski, X. Liu, J. Van Horn, G. Guiochon, J. Chromatogr. A 950 (2002) 41.
[10] W.S. Hancock, R.C. Chloupek, J.J. Kirkland, L.R. Snyder, J. Chromatogr. A 686 (1994) 31.
[11] R.C. Chloupek, W.S. Hancock, B.A. Marchylo, J.J. Kirkland, B.E. Boyes, L.R. Snyder, J. Chromatogr. A 686 (1994) 45.
[12] P.L. Zhu, L.R. Snyder, J.W. Dolan, N.M. Djordjevic, D.W. Hill, L.C. Sander, T.J. Waeghe, J. Chromatogr. A 756 (1996) 21.
[13] P.L. Zhu, J.W. Dolan, L.R. Snyder, J. Chromatogr. A 756 (1996) 41.
[14] P.L. Zhu, J.W. Dolan, L.R. Snyder, D.W. Hill, L. Van Heukelem, J. Chromatogr. A 756 (1996) 51.
[15] D.V. McCalley, J. Chromatogr. A 902 (2000) 311.
[16] T. Andersen, P. Molander, R. Trones, D.R. Hegna, T. Greibrokk, J. Chromatogr. A 918 (2001) 221.
[17] J. Li, Anal. Chim. Acta 369 (1998) 21.
[18] J.W. Dolan, J. Chromatogr. A 965 (2002) 195.
[19] J. Bullock, J. Chromatogr. A 694 (1995) 415.
[20] G. Guiochon, Anal. Chem. 53 (1981) 1318.
[21] G. Sheng, Y. Shen, M.L. Lee, J. Microcol. Sep. 9 (1997) 63.
[22] G. Liu, N.M. Djordjevic, F. Erni, J. Chromatogr. 592 (1992) 239.
[23] G. Liu, N.M. Djordjevic, F. Erni, J. Chromatogr. 598 (1992) 153.
[24] G. Liu, L. Svensson, N.M. Djordjevic, F. Erni, J. Chromatogr. 633 (1993) 26.
[25] G. Liu, N.M. Djordjevic, F. Erni, Chromatographia 38 (1994) 313.

[26] L.M. Nyholm, P.J.R. Sjoeberg, K.E. Markides, J. Chromatogr. A 755 (1996) 153.
[27] K. Ryan, N.M. Djordjevic, F. Erni, J. Liq. Chromatogr. Relat. Techn. 19 (1996) 2089.
[28] J. Li, P.W. Carr, Anal. Chem. 69 (1997) 837.
[29] J.H. Knox, J. Chromatogr. Sci. 15 (1977) 352.
[30] J.H. Knox, H.P. Scott, J. Chromatogr. 282 (1983) 297.
[31] R.W. Stout, J.J. De Stefano, L.R. Snyder, J. Chromatogr. 282 (1983) 263.
[32] J.W. Dolan, L.R. Snyder, R.G. Wolcott, P. Haber, T. Baczek, R. Kaliszan, J. Chromatogr. A 857 (1999) 41.
[33] P. Molander, T.E. Gundersen, C. Haas, T. Greibrokk, R. Blomhoff, E. Lundanes, J. Chromatogr. A 847 (1999) 59.
[34] P. Molander, R. Trones, K. Haugland, T. Greibrokk, Analyst 124 (1999) 1137.
[35] T. Greibrokk, Anal. Chem. 74 (2002) 374A.
[36] H. Poppe, J.C. Kraak, J.F.K. Huber, J.H.M. van den Berg, Chromatographia 14 (1981) 515.
[37] N.M. Djordjevic, P.W.J. Fowler, F. Houdiere, J. Microcol. Sep. 11 (1999) 403.
[38] J.D. Thompson, J.S. Brown, P.W. Carr, Anal. Chem. 73 (2001) 3340.
[39] R.G. Wolcott, J.W. Dolan, L.R. Snyder, S.R. Bakalyar, M.A. Arnold, J.A. Nichols, J. Chromatogr. A 869 (2000) 211.
[40] T. Greibrokk, T. Andersen, J. Sep. Sci. 24 (2001) 899.
[41] J.D. Thompson, P.W. Carr, Anal. Chem. 74 (2002) 4150.
[42] P. Molander, K. Haugland, D.R. Hegna, E. Ommundsen, E. Lundanes, T. Greibrokk, J. Chromatogr. A 864 (1999) 103.
[43] P. Molander, A. Holm, E. Lundanes, E. Ommundsen, T. Greibrokk, J. High Resolut. Chromatogr. 23 (2000) 653.
[44] P. Molander, A. Thomassen, E. Lundanes, G. Fladseth, S. Thorud, Y. Thomassen, T. Greibrokk, J. Sep. Sci. 24 (2001) 947.
[45] B.A. Ingelse, H.-G. Janssen, C.A. Cramers, J. High Resolut. Chromatogr. 21 (1998) 613.
[46] A. Holm, P. Molander, E. Lundanes, T. Greibrokk, J. Sep. Sci., submitted for publication.
[47] P. Molander, K. Haugland, G. Fladseth, E. Lundanes, S. Thorud, Y. Thomassen, T. Greibrokk, J. Chromatogr. A 892 (2000) 67.
[48] R. Trones, T. Andersen, T. Greibrokk, D.R. Hegna, J. Chromatogr. A 874 (2000) 65.
[49] B. Ooms, LC–GC 14 (1996) 306.
[50] J.S. Yoo, J.T. Watson, V.L. McGuffin, J. Microcol. Sep. 4 (1992) 349.
[51] G. Openhaim, E. Grushka, J. Chromatogr. A 942 (2001) 63.
[52] Y. Yang, M. Belghazi, A. Lagadec, D.J. Miller, S. Hawthorne, J. Chromatogr. A 810 (1998) 149.
[53] R.M. Smith, R.J. Burgess, Anal. Commun. 33 (1996) 327.
[54] D.J. Miller, S. Hawthorne, Anal. Chem. 69 (1997) 623.
[55] R.M. Smith, R. Burgess, J. Chromatogr. A 785 (1997) 49.
[56] T.B. Young, S.T. Ecker, R.E. Synovec, N.T. Hawley, J.P. Lomber, C.M. Wei, Talanta 45 (1998) 1189.
[57] T.M. Pawlowski, C.F. Poole, Anal. Commun. 36 (1999) 71.
[58] R.M. Smith, O. Chienthavorn, I.D. Wilson, B. Wright, S.D. Taylor, Anal. Chem. 71 (1999) 4493.
[59] B. Yan, J. Zhao, J.S. Brown, J. Blackwell, P.W. Carr, Anal. Chem. 72 (2000) 1253.
[60] S.M. Fields, C.Q. Ye, D.D. Zhang, B.R. Branch, X.J. Zhang, N. Okafo, J. Chromatogr. A 913 (2001) 197.
[61] C.A. Bruckner, S.T. Ecker, R.E. Synovec, Anal. Chem. 69 (1997) 3465.
[62] T. Greibrokk, E. Lundanes, R. Trones, P. Molander, L. Roed, I.L. Skuland, T. Andersen, I. Bruheim, B. Jachwitz, in: J.F. Parcher, T.L. Chester (Eds.), Unified Chromatography, ACS Symposium Series, ACS, Washington, DC, 2000, p. 120.
[63] T. Andersen, N.T. Nguyen, unpublished results.
[64] J. Zhao, P.W. Carr, Anal. Chem. 72 (2000) 302.
[65] T.S. Kephart, P.K. Dasgupta, Anal. Chim. Acta 414 (2000) 71.
[66] C.N. Renn, R.E. Synovec, Anal. Chem. 64 (1992) 479.
[67] W. Lee, D. Cho, B.O. Chun, T. Chang, M. Ree, J. Chromatogr. A 910 (2001) 51.
[68] H.C. Lee, T. Chang, Macromolecules 29 (1996) 7294.
[69] H.C. Lee, W. Lee, T. Chang, J.S. Yoon, D.J. Frater, J.W. Mays, Macromolecules 31 (1998) 4114.
[70] H.C. Lee, T. Chang, S. Harville, J.W. Mays, Macromolecules 31 (1998) 690.
[71] W. Lee, H.C. Lee, T. Park, T. Chang, J.Y. Chang, Polymer 40 (1999) 7227.
[72] T. Chang, H.C. Lee, W. Lee, S. Park, C. Ko, Macromol. Chem. Phys. 200 (1999) 2188.
[73] W. Lee, H.C. Lee, T. Park, T. Chang, K.H. Chae, Macromol. Chem. Phys. 201 (2000) 320.
[74] W. Lee, D. Cho, B.O. Chun, T. Chang, M. Ree, J. Chromatogr. A 910 (2001) 51.
[75] I. Bruheim, P. Molander, M. Theodorsen, E. Ommundsen, E. Lundanes, T. Greibrokk, Chromatographia 53 (2001) 266.
[76] L.C. Sander, S.A. Wise, J. Sep. Sci. 24 (2001) 910.
[77] L.R. Snyder, J.W. Dolan, J. Chromatogr. A 892 (2000) 107.
[78] H. Kanazawa, Y. Kashiwase, K. Yamamoto, Y. Matsushima, A. Kikuchi, Y. Sakurai, T. Okano, Anal. Chem. 69 (1997) 823.
[79] Y.X. Ong, J.Q. Wang, Z.X. Su, D.Y. Chen, Chromatographia 54 (2001) 208.
[80] T. Andersen, unpublished results.
[81] J.D. Thompson, P.W. Carr, Anal. Chem. 74 (2002) 1017.