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Capillary zone electrophoresis of aldose enantiomers: separation after derivatization with *S*-(−)-1-phenylethylamine☆

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Abstract

A method for the analysis of monosaccharide enantiomers of the aldose-type is described. Aminoalditols resulting from derivatization with *S*-(−)-1-phenylethylamine were subjected to capillary electrophoresis in a borate buffer. Standard on-column UV detection at 200 nm was applied. Studies of the dependence of separation selectivity and electrophoretic mobility on temperature, electrolyte composition and pH were carried out. Single-run resolution of all 16 derivatized aldohexoses and 3 out of 4 aldotetroses was achieved at pH 10.3 in a 50 m*M* borate buffer using acetonitrile as organic modifier.

1. Introduction

Although the biological pathways of sugar formation are well established, there is an alternative approach to sugar total synthesis, which recently has been investigated and discussed as a potential way for prebiotic sugar formation [2–5]. This alternative pathway encompasses the aldolization of glycol aldehyde, the first step of which opens a way to all four aldotetroses and the second step to all 16 aldohexoses. Studies on asymmetric induction in this reaction require–beyond analysis of the diastereomer distribution–consideration of the sugar enantiomer distribution. Development of an efficient analytical procedure, which allows fast and reliable identification of all 20 products together with detection of by-products, is a major prerequisite for successful work on this topic. Before developing the capillary electrophoretic method described in this paper analyses were performed using an HPLC method, which included several derivatization steps and was by far more time-consuming [4,5].

It is known that a certain number of naturally occurring L-sugars exist, e.g. L-(−)-galactose in agar or L-(−)-glucose in the leaves of jute. However, the data available suggest that systematic studies on sugar enantiomer distribution in nature are still far from being comprehensive. Therefore we believe that an efficient analytical technique for the separation of sugar enantiomers is of considerable importance in biological and chemical research.

Separation of neutral carbohydrates by electrophoresis is generally not feasible. In addition, sugars exhibit very low UV absorbance, because

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in solution, due to their tendency to form pyranose or furanose rings, only an extremely small percentage exists in the open-chain form with its easily detectable carbonyl group. Thus sugars are not ideal analytes for capillary zone electrophoresis. Even so, in recent years an increasing number of separation methods using this technique has been reported. One basis for this development has been the well-known fact that sugar molecules form negatively charged complexes with tetrahydroxyborate and that the stability and electrophoretic mobility of these complexes strongly depend on the configuration of the sugar hydroxy groups [6–8]. Using this approach Hofstetter-Kuhn et al. [9] showed that even underivatized sugars can be separated by capillary zone electrophoresis at elevated temperatures.

The low UV absorbance of underivatized sugars has been effectively overcome by the use of various derivatization reagents [10], the most common method being the reductive amination of the carbonyl group with an aromatic amine, such as 2-aminopyridine, *p*-aminobenzoic acid, ethyl-*p*-aminobenzoic acid or *p*-aminobenzonitrile [11,12]. When a chiral amine such as *S*-(−)-1-phenylethylamine is used for derivatization, sugar enantiomers are transformed into diastereomers and separation in an achiral environment may be achieved. This approach was first introduced for liquid chromatography and gas chromatography by Oshima et al. [13,14]. It has been successfully applied in the HPLC separation of various aldoses and–depending on the amine–ketoses of mono- and oligosaccharides. In gas chromatography further derivatization of the remaining hydroxy groups is unavoidable to render the analytes volatile and thus compatible with the technique.

Recently, Vorndran et al. [15] described a separation technique for sugars by capillary zone electrophoresis using a different approach based on indirect UV detection. The separation of several sugars and sugar acids was achieved by use of an UV-active buffer–in this case sorbic acid–as background electrolyte.

All these capillary electrophoretic methods do not take into account resolution of sugar enantiomers. Only recently a separation of sugar enantiomers using capillary electrophoresis has been reported [16]. Several monosaccharides were derivatized by reductive amination with 2-aminopyridine, 5-aminonaphthalene-2-sulfonic acid or 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid. Enantiomer discrimination was achieved by addition of various chiral additives to the electrolyte solution. Laser-induced fluorescence was used as detection method.

Here, we present a method which for the first time allows separation of all sixteen D- and L-aldohexoses in a single run using capillary zone electrophoresis (CZE). Discrimination of sugar enantiomers was achieved by reductive amination with enantiomerically pure (*S*)-(−)-1-phenylethylamine [13]. A standard on-column UV detector was used for detection at 200 nm. To optimize and validate the method extensive studies were carried out on the dependence of the electrophoretic mobility on various factors, such as temperature, electrolyte concentration, pH and others.

2. Experimental

2.1. Chemicals

Chemicals used for the preparation of the various buffers were of analytical grade. Sodium tetraborate and NaOH were obtained from E. Merck (Darmstadt, Germany). The reagents used for the reductive amination of the various aldohexoses and aldotetroses, *R*-(+)-1-phenylethylamine, *S*-(−)-1-phenylethylamine, *rac*-1-phenylethylamine and sodium cyanoborhydride, were also obtained from E. Merck.

Sugars were used in the best available quality without any further purification. Among the aldohexoses D-(+)-talose, L-(−)-talose, L-(−)-glucose, L-(−)-galactose, L-(−)-allose, D-(+)-mannose, L-(−)-mannose, D-(+)-altrose, D-(−)-gulose and L-(+)-gulose were obtained from Janssen Chimica (Neuss, Germany). L-(−)-Idose and D-(+)-idose were obtained from Sigma (Deisenhofen, Germany). D-(+)-Glucose and D-(+)-galactose were obtained from E.

Merck. D-(+)-Allose was obtained from Aldrich-Chemie (Steinheim, Germany). The aldotetrose D-(−)-erythrose was obtained from Aldrich-Chemie, and L-(+)-threose was obtained from Sigma.

2.2. Equipment

All separations were carried out using a Beckman P/ACE System 2210. The resulting electropherograms were analysed by Gold Software Version 7.12 SSC 2.00. For the photometric detection of the derivatized carbohydrates on-column UV detection was performed at 200 nm.

2.3. Electrophoretic conditions

All experiments were carried out with open-tube fused-silica capillaries (Beckman, 338472) of 50 μm I.D. (400 μm O.D.). Capillary lengths were 127, 107 or 77 cm and the UV–Vis detector was situated 7 cm from the cathodic end of the capillary. Temperature control during separation was achieved by liquid cooling (capillary cartridge coolant, Beckman, No. 359976). All samples were introduced into the capillary by pressure (3.45 kPa). Prior to separation the capillary was rinsed for 5 min with 0.1 *M* NaOH and for 10 min with the buffer solution.

2.4. Buffers

The buffers were prepared using HPLC-grade water (E. Merck). The pH was adjusted with 10 *M* NaOH or 10 *M* HCl (dissolved in HPLC-grade water). Organic modifiers, such as tetrahydrofuran (THF), methanol or acetonitrile were of HPLC quality (Carl Roth, Karlsruhe, Germany).

2.5. Reductive amination of aldohexoses and aldotetroses

A 1 *M* solution of each sugar was prepared in water (HPLC-grade quality) and stored at −20°C. The amines were dissolved in water (1 *M* solutions in HPLC-grade water). The pH of these solutions was adjusted to 6.5 with 10 *M* HCl. A solution of 9 mg of the reducing agent sodiumcyanoborohydride in 30 μl of water was prepared. The derivatization of the various sugars was carried out as follows: 12 μl of amine solution was added to 10 μl of the corresponding sugar solution. This reaction mixture was kept at 90°C for 10 min. The resulting solution of Schiff-base was treated with 4.5 μl sodiumcyanoborohydride solution at 90°C for 1 h to form a stable amine. After addition of 100 μl water the reaction mixture was stored at −20°C. Prior to sample introduction these solutions were further diluted.

3. Results and discussions

3.1. Derivatization

Derivatization of the analytes with the enantiomerically pure amine could be achieved under the conditions described above. Instead of a reaction temperature of 40°C and a reaction time of 3 h, as described by Oshima et al. [13], a reaction temperature of 90°C was applied in reductive amination with *S*-(−)-1-phenylethylamine. These conditions resulted in complete and efficient derivatization of the aldoses within 1 h. (In a preparative scale experiment D-(+)-glucose was derivatized with *R*-(+)-1-phenylethylamine as described in Section 2.5. and gave the corresponding aminoalditol in quantitative yield.) The time course of derivatization was studied using three selected sugars, namely D-(+)-glucose, L-(−)-glucose and L-(−)-galactose. As indicated in Fig. 1 reaction was completed after 20 min and no further change in peak areas was observed. Epimerization of the sugars during derivatization could be excluded since after derivatization of pure monosaccharides as described above only the peak corresponding to the pure diastereomer was detected in the electropherograms.

Unfortunately L-(−)-altrose and L-(+)-erythrose were not commercially available. Therefore the available enantiomers D-(+)-altrose, D-(−)-erythrose were derivatized with *rac*-1-phenylethylamine, resulting in two diastereomer-

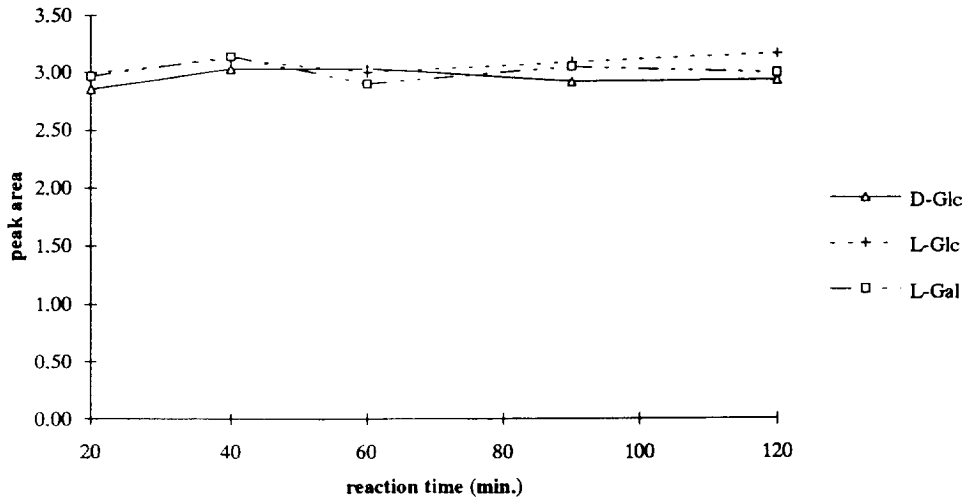

Fig. 1. Dependency of the peak area on the reaction time for the derivatization. Concentrations of the monosaccharides, *S*-(−)-1-phenylethylamine and sodium cyanoborhydride appear in Section 2.5. Reaction temperature, 90°C; capillary: fused-silica, L (total length) = 77 cm, l (lenght to detector) = 70 cm; carrier, 50 m*M* borate buffer; voltage, 30 kV; temperature, 20°C; UV detection, 200 nm; injection, 2.0 s by pressure (3.45 kPa).

ic products for each sugar. One of these products was the expected derivative of the sugar with *S*-(−)-1-phenylethylamine (1a and 2a in Fig. 2). The other diastereomer was the derivative of the sugar with *R*-(+)-1-phenylethylamine. Due to the achiral separation mechanism the diastereomers of D-sugars with *R*-(+)-1-phenylethylamine migrate with the same velocity as their enantiomers, which are the derivatives of the corresponding L-sugars with *S*-(−)-1-phenylethylamine (1b and 2b in Fig. 2). In Fig. 2 the derivatives of the non-available sugars L-(−)-

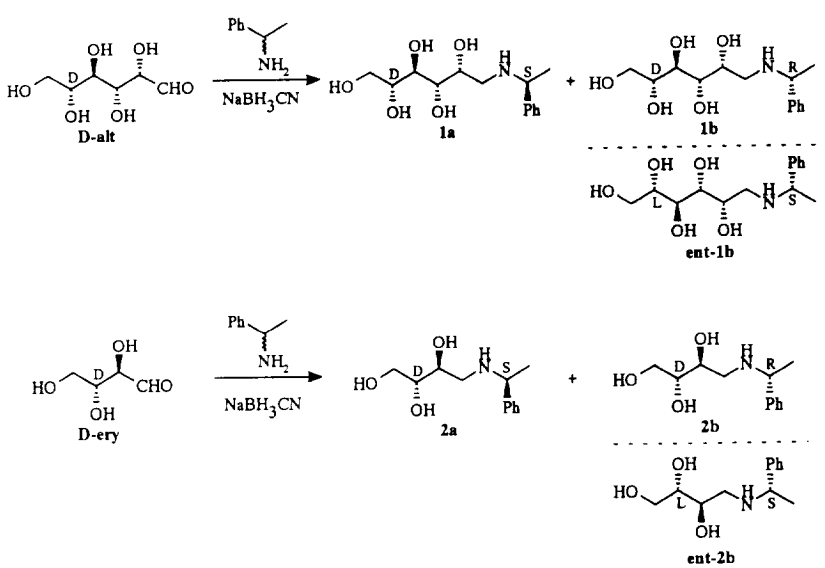

Fig. 2. Structures and derivatization reactions.

altrose and L-(+)-erythrose with *S*-(−)-1-phenylethylamine are specified as ent-1b and ent-2b.

In order to ensure that the derivatization reagent did not react preferably with one of the sugar enantiomers, a mixture of D-(+)- and L-(−)-galactose in a ratio of 1.8:1 was derivatized with *S*-(−)-1-phenylethylamine. Sodium cyanoborohydride was added to the reaction mixture after 2.5, 6, 8, 10 and 15 min, and after 1 h these solutions were diluted with water. The samples were analysed by capillary electrophoresis using the conditions described in Fig. 6. In the resulting electropherograms the ratios between D-(+)- and L-(−)-galactose ranged from 1.78:1 to 1.82:1, which clearly indicates that there is no preferable reaction of *S*-(−)-1-phenylethylamine with one of the sugar enantiomers.

3.2. Influence of pH and buffer concentration

First a mixture of 14 aldohexoses [no L-(−)-glucose and L-(−)-altrose] was derivatized with *S*-(−)-1-phenylethylamine as described above. To study the relationship between the pH-value and separation selectivity α ($\alpha = [t_m(D) - t_0]/[t_m(L) - t_0]$) of these sugar derivatives, the pH was increased from 8.3 to 10.8 with the borate concentration of the carrier electrolyte kept constant at 50 m*M*. For selected sugars the separation selectivities α as a function of pH are shown in Fig. 3. For some sugar derivatives increase in pH caused an increase in separation selectivity α, while for others a decrease in selectivity was observed. At the same time the electrophoretic mobility of the analytes was increased due to several factors such as enhanced complex formation with the tetrahydroxyborate anion at higher pH values. Configurational differences at carbon atoms C-2 to C-5 determine the stability of the borate complexes of the various carbohydrates. Selectivities, including differences in the dependence of the mobility on pH, strongly depend upon these complex stabilities. With the complex mixture of the 14 aldohexose-derivatives optimum selectivities were achieved at pH 10.3 (Fig. 4). Further increase in pH caused long separation times as well as a decrease in separation selectivity. With the exception of L-(−)-mannose/D-(+)-glucose and, as indicated in Table 1, D-(+)-mannose/L-(−)-glucose all sugar derivatives could be resolved.

The effect of borate concentration of the carrier electrolyte on the separation selectivity α

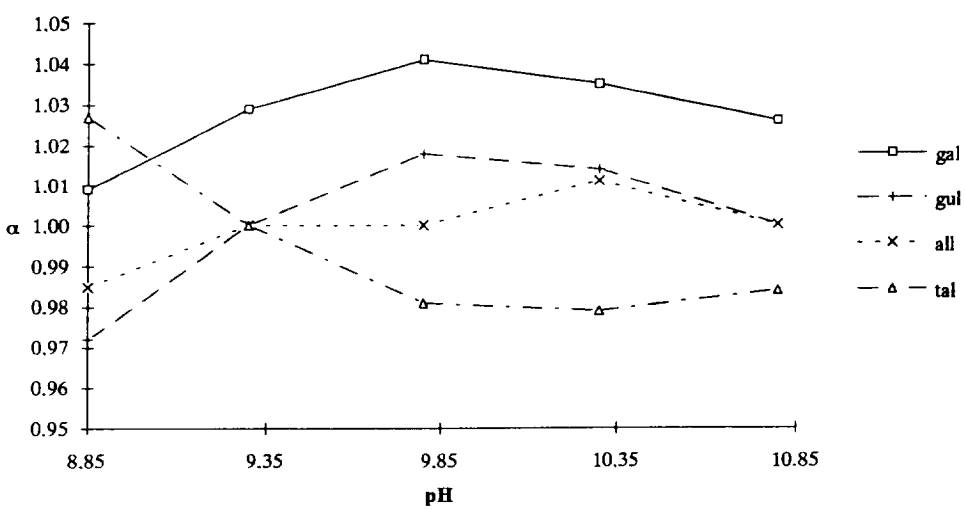

Fig. 3. Dependency of separation selectivity α on pH for selected aminoalditols. Capillary, fused-silica, L = 107 cm, l = 100 cm; carrier, 50-m*M* borate buffer; voltage, 30 kV; temperature, 25°C; UV detection, 200 nm.

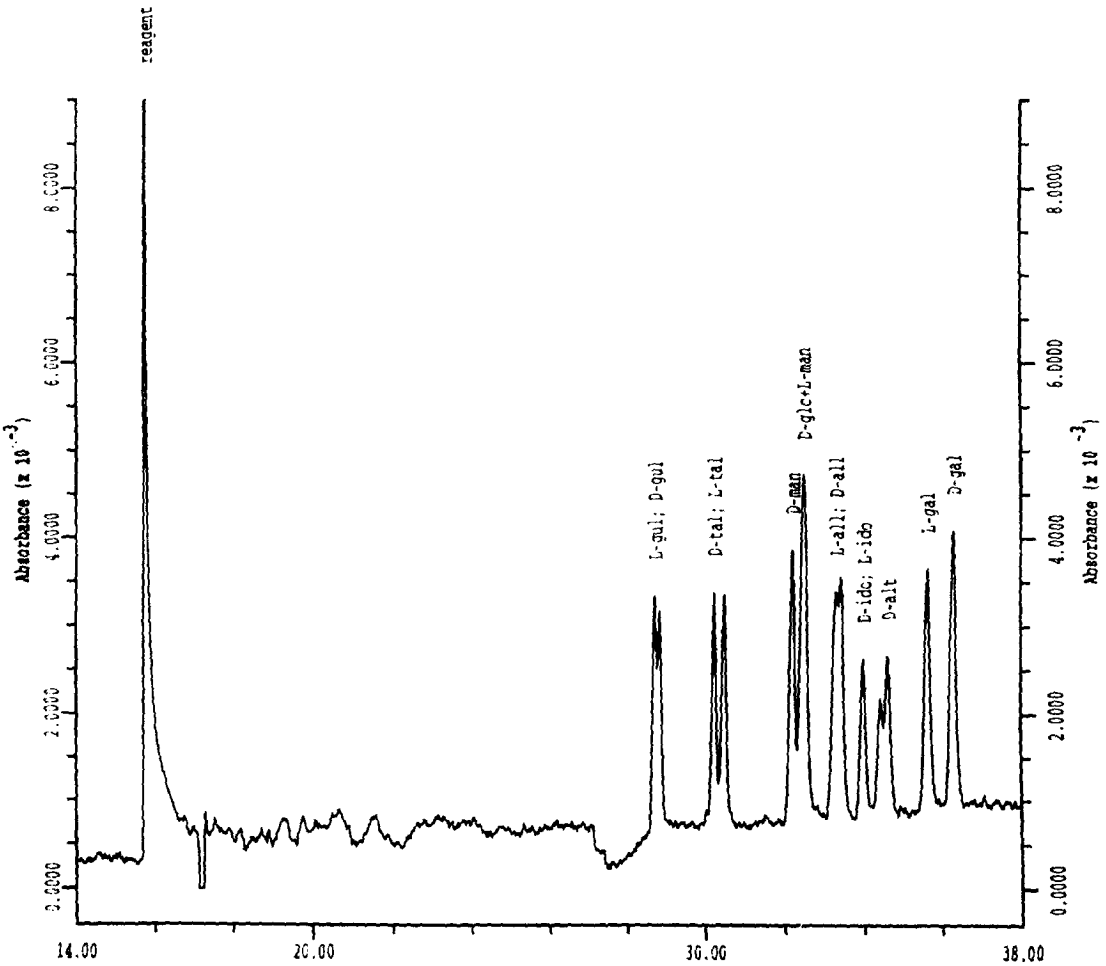

Fig. 4. Separation of a mixture of 14 aldohexoses [no L-(−)-altrose and L-(−)-glucose] derivatized with *S*-(−)-1-phenylethylamine. Capillary, fused-silica, L = 107 cm, l = 100 cm; carrier, 50 m*M* borate, pH 10.3; voltage, 30 kV; temperature, 25°C; UV detection, 200 nm; injection, 3.0 s by pressure (3.45 kPa).

was studied at a constant pH-value of 10.3 and increasing buffer concentrations from 25 m*M* to 100 m*M* (Fig. 5). An increase of borate concentration led to a reduction of the electroosmotic flow and, as a consequence, to very long separation times. Although an increase in separation selectivity could be achieved at higher borate concentration the two diastereomers of D-(+)-glucose and L-(−)-allose could not be separated. At pH 8.8 an increase of buffer concentration up to 200 m*M* improved resolution and decreased time of analysis. However, the results obtained at pH 10.3 using a 50-m*M* borate buffer could not be reached.

3.3. Temperature control

Hoffstetter-Kuhn et al. [9] in their electrophoretic separation of underivatized carbohydrate–borate complexes obtained narrow peaks and thus acceptable selectivities at a temperature of 60°C. According to their study formation of borate complexes with underivatized sugars is a complex reaction, because there are six different

Table 1
Migration time of sugar derivatives depending on the concentration of the organic modifier acetonitrile

Sugar derivative	Migration time (min)				
	0% AN	9% AN	13% AN	17% AN	23% AN
L-Gul	28.7	35.2	38.5	41.3	49.0
D-Gul	28.8	35.4	38.7	41.6	49.3
D-Tal	30.1	37.9	41.8	45.2	54.5
L-Tal	30.5	38.3	42.3	45.8	55.2
L-Glc	32.1	41.0	45.4	49.4	60.2
D-Glc	32.5	41.3	45.7	49.7	60.7
D-Man	32.1	41.3	45.7	50.1	61.5
L-Man	32.5	41.7	46.3	50.5	61.9
D-Ido	34.0	43.0	47.8	52.2	63.5
L-Ido	34.4	43.2	48.3	52.8	64.2
L-All	33.2	43.2	47.8	52.3	64.8
D-All	33.4	43.6	48.3	52.8	65.6
D-Alt	34.6	44.9	50.3	55.3	68.9
L-Alt	35.0	45.4	50.8	56.0	69.7
L-Gal	35.6	46.3	51.9	57.4	71.9
D-Gal	36.3	47.2	52.9	58.5	73.5

AN = acetonitrile. Capillary: fused-silica, L (total length) = 107 cm, l (length to detector) = 100 cm; carrier, 50 m*M* borate, pH 10.3; voltage, 30 kV; temperature, 25°C; UV detection, 200 nm; injection: 3.0 s by pressure (3.45 kPa).

structures for a sugar molecule dissolved in water: the α- and β-pyranose rings, the α- and β-furanose rings, the open-chain form and the hydrated open-chain form. Since at higher temperature the equilibrium for complexation with the tetrahydroxyborate anion is reached faster,

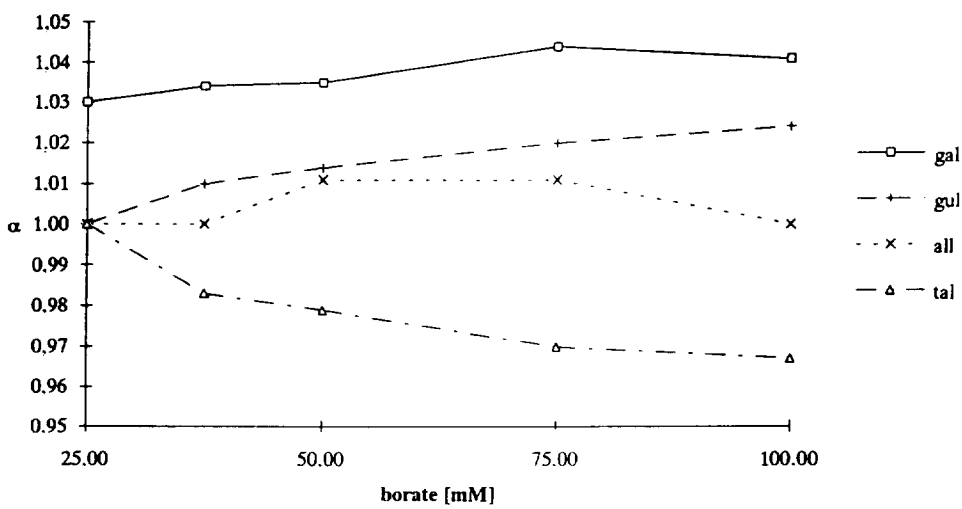

Fig. 5. Dependency of separation selectivity α on borate concentration for selected aminoalditols. Capillary, fused-silica, L = 107 cm, l = 100 cm; carrier, borate, pH 10.3; voltage, 30 kV; temperature, 25°C; UV detection, 200 nm.

narrower peaks are obtained. In addition, an increase in temperature results in higher proportions of the open-chain carbonyl form and due to the decreased viscosity a larger volume is introduced into the capillary. All these effects explain the improvement both in selectivity and in efficiency of the separation.

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In contrast to the underivatized carbohydrates, our attempts to run the electrophoretic separation at higher temperatures resulted in a dramatic loss of resolution and in broad peaks. P. Jandik and G. Bonn had observed the same effect with carbohydrates derivatized with *p*-aminobenzoic acid [17]. Reductive amination reduces the number of possible solution isomers to the open-chain form and thus the equilibrium for complexation with the tetrahydroxyborate anion is less affected by an increase in temperature. It may be assumed that the observed loss of

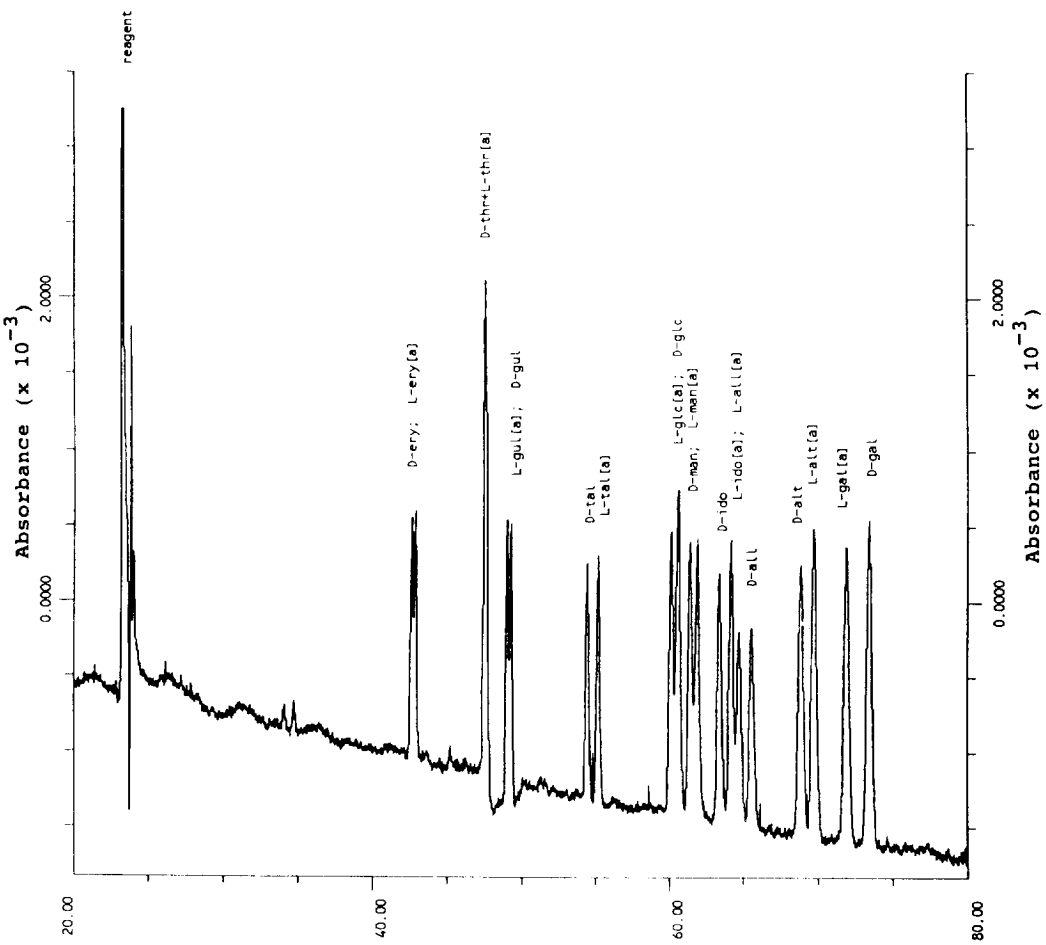

Fig. 6. Separation of a mixture of 8 D-aldohexoses and 2 aldotetroses derivatized with *rac*-1-phenylethylamine. Capillary: fused-silica, L = 107 cm, l = 100 cm; carrier, 50 m*M* borate, pH 10.3 and 23% acetonitrile; voltage, 30 kV; temperature, 25°C; UV detection, 200 nm; injection, 3.0 s by pressure (3.45 kPa). In the electropherogram the signals of derivatives of D-sugars with *R*-(+)-1-phenylethylamine are assigned as L-sugars marked with [a]. They are enantiomers of the derivatives of L-sugars and *S*-(−)-1-phenylethylamine and have the same electrophoretic mobility in the separation system.

resolution for derivatized carbohydrates is caused by larger longitudinal diffusion of the analytes.

3.4. Further modifications

To obtain resolution of all 16 aldohexoses further modifications of the buffer were attempted. It is well known that various organic modifiers are capable to reduce the electroosmotic flow to a certain extent. With the common organic additives THF, methanol and acetonitrile the strongest decrease of the electroosmotic flow is effected by acetonitrile, followed by THF and methanol. Methanol was added up to 30% to the buffer solution and THF up to 5%, but both of these solvents did not lead to an increase in resolution. Acetonitrile was added to the electrolyte up to 30%. Best results were obtained by addition of 23% of acetonitrile to the carrier electrolyte. Although electroosmotic flow velocity was strongly reduced resulting in a considerable increase of migration times, this effect was compensated by an improvement in resolution. Table 1 shows migration times of all aldohexose-derivatives with increasing percentage of acetonitrile. The selectivity α for derivatized sugar enantiomers is hardly affected by the addition of the organic modifier. The increasing amount of organic modifier led to a separation of the diastereomeric sugar derivatives of L-(−)-glucose/D-(+)-mannose and D-(+)-glucose/L-(−)-mannose, which in a 50 m*M* borate buffer, pH 10.3 had the same electrophoretic mobilities. An amount of 9% acetonitrile in the electrolyte resulted in a separation of the derivatives L-(−)-glucose and L-(−)-mannose, whereas the derivatives of D-(+)-glucose and D-(+)-mannose under these conditions had the same mobility. With addition of more acetonitrile all four diastereomers could be separated. A similar effect was observed for the sugar derivatives of D-(+)-/L-(−)-idose and D-(+)-/L-(−)-allose. Here an increasing amount of acetonitrile in the electrolyte led to an inversion of the electrophoretic mobility of the sugar derivatives of D-(+)-/L-(−)-allose and D-(+)-/L-(−)-idose. Fig. 6 shows the electropherogram of a standard mixture of 8 D-aldohexoses and 2 aldotetroses derivatized with *rac*-1-phenylethylamine. With the exception of the diastereomers corresponding to D-(−)- and L-(+)-threose all sugar derivatives could be resolved. The reproducibility of migration times in the electrolyte system used for this separation was 0.9% ($n = 6$). Without the organic modifier the reproducibility of migration times is 0.3%.

4. Conclusions

The capillary zone electrophoretic method presented in this paper allows easy and efficient analysis of sugar enantiomers after derivatization with *S*-(−)-1-phenylethylamine. In contrast to GC or HPLC methods [13,14] there is no need for further purification or derivatization of the aminoalditols. All 16 aldohexoses could be resolved in a single run. From a mixture of aldohexoses and aldotetroses 19 out of 20 products were separated in one run.

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