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Chiral separation by capillary electrophoresis with oligosaccharides

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ABSTRACT

Maltodextrins, *i.e.*, mixtures of linear α-(1–4)-linked D-glucose polymers, were found to be effective as chiral electrolyte modifiers to perform direct, rapid separations by capillary electrophoresis of racemic mixtures of 2-arylpropionic acid non-steroidal anti-inflammatory compounds and coumarinic anticoagulant drugs, and also diastereomeric cefalosporin antibiotics. Enantioselectivity seemed to be dependent on an as yet unidentified combination of variables.

INTRODUCTION

The importance of enantiomeric differences in biological processes has been well recognized. Numerous chiral drugs have been shown to display stereoselectivity in their pharmacokinetic behaviour and/or pharmacological action [1,2]. In environmental sciences also enantioselectivity has been demonstrated, *e.g.*, in the enantiospecific activity of chiral pesticides [3]. Knowledge of these differences has influenced decision making and in some instances this has led to a re-thinking of drug development, where the use of pure enantiomeric drugs was shown to be an advantage or even a necessity. In view of these recent considerations and the ensuing regulatory requirements, the availability of easily accessable chiral separation techniques has become increasingly important.

Chiral separations for analytical purposes have been achieved using either gas chromatography (GC) or high-performance liquid chromatography (HPLC), both generally requiring derivatization with a chiral reagent in order to form diastereoisomers which can subsequently be separated by conventional chromatographic methods. Direct chiral GC and HPLC separations have been performed using chiral stationary phases. Apart from optical asymmetry generated by chemical synthesis (*e.g.*, L-valine-*tert.*-butylamine coupled to polysiloxane for chiral GC, helical polymethacrylates adsorbed on silanized silica for chiral HPLC), various natural products have been used, in either modified or native form, to obtain chirality for HPLC: polysaccharides (*e.g.*, cellulose or amylose) or proteins (*e.g.*, albumin, α-1 acid glycoprotein). In addition, direct separation of enantiomers by HPLC has also been demonstrated using chiral mobile phase additives, *e.g.*, chiral counter ions in ion-pair chromatography. All these methods require either a cumbersome sample preparation or a long column equilibration time and a long analysis time. None of them, except for recent advances based on the development of microbore HPLC [4], are convenient enough to allow high sample throughputs. Therefore, improving the available analytical methodology has grown into a major issue for the development of chiral substances.

Capillary electrophoresis (CE) with its various modes of operation (capillary zone electrophoresis, micellar electrokinetic chromatography, isotachophoresis, etc.) has proved to be a powerful and versatile separation technique. High plate numbers

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and speed of analysis in comparison with HPLC are easily achieved. Expanding the resolving power of CE towards stereoselective separations is possible by adding chiral modifiers to the background electrolyte, thus introducing a so-called chiral pseudo-stationary phase. Baseline resolution of chiral compounds has been demonstrated in the micellar electrokinetic chromatography mode, which consists of performing CE separations in electrolytes containing micelles. When using micelles composed of either achiral surfactants functionalized with chiral compounds [5] or chiral surfactants [6], an enantioselectivity factor is introduced and enantiomeric separation can be achieved. Alternatively, stereoselective complexing agents, *e.g.*, cyclodextrins, which have already been demonstrated to be most effective in chiral HPLC separations, have proved to be equally valuable in performing enantiomeric resolution by CE [7].

This paper gives an overview of the properties of linear oligosaccharides as potential chiral discriminators in CE. Oligosaccharides form a vast family of polymers, among which the following compounds were screened in order to obtain chiral separations of racemic 2-arylpropionic acid (2-APA) non-stereoidal anti-inflammatory drugs (NSAIDs): maltodextrin mixtures and corn syrups, pure maltooligosaccharides, linear non-α-(1–4)-linked glucose polymers and low-molecular-mass galactose–glucose–fructose copolymers and cyclodextrins.

Maltodextrins are defined by the US Food and Drug Administration as saccharide polymers consisting of D-glucose units linked primarily by α-(1–4) bonds and having a dextrose equivalent (*DE*, defined as percent reducing sugars calculated as glucose on a dry-substance basis) of <20. Corn syrups are maltooligosaccharide mixtures with *DE* $\geqslant 20$. These mixtures are prepared by partial acid and/or enzymatic hydrolysis of corn starch. Maltodextrins and corn syrups are available under different brand names as complex mixtures of maltooligosaccharides, obtained by various manufacturing procedures. During hydrolysis of starch, high-molecular-mass glucose polymers are converted into oligosaccharides of a lower degree of polymerization (*DP*, defined as the number of saccharide

TABLE I

DEXTROSE EQUIVALENT (*DE*) AND MALTOOLIGOSACCHARIDE COMPOSITION (RELATIVE AMOUNTS IN %, w/w, WITHIN THE LOWER *DP* RANGE) OF MALTODEXTRINS AND CORN SYRUPS USED

DP 1, *DP* 2 and *DP* 3 correspond to glucose, maltose and maltotriose, respectively.

Maltodextrin/corn syrup	*DE*	*DP* 1	*DP* 2	*DP* 3	DP > 3	DP > 2
Maltrin 200	20	2.3	7.9	9.6	80.2	
Maltrin 150	15	0.7	4.5	6.6	88.2	
Maltrin 100	10	0.5	2.7	4.3	92.5	
Maltrin 040	5	0.2	0.3	0.6	98.9	
Maldex 30	30	3	13	16	68	
Maldex 20	20	2.8	9.0	18.0	70.2	
Maldex 15	15	2.6	7.2	10.0	80.2	
Mylose HDE	60	38	37	7	18	
Mylose STD	38	14	12	11	63	
Mylose HM	44	7	50	14	29	
Glucidex 21	21	3	7			90
Glucidex 19	19	2	7			91
Glucidex 17	17	2	5			93
Glucidex 12	12	1	2			97
Glucidex 9	9	0.5	1.5			98
Glucidex 6	6	0.5	1			98.5
Glucidex 2	2	0.5	0.5			99
Lycasin 80/85	0	7	52.5			40

monomers within the oligo- or polysaccharide chain) and consequently the *DE* value rises. Therefore, maltodextrins and corn syrups of high *DE* are characterized by a lower average *DP*. The percentage of different oligosaccharides present in the maltodextrin mixtures and corn syrups depends on the hydrolysis procedure followed. As a consequence, the various brands of maltodextrins and corn syrups available display a wide range of compositions, as summarized in Table I.

Subfractionation of maltodextrins or corn syrups may be performed by either preparative gel permeation chromatography (GPC) or ultrafiltration. Some maltooligosaccharides may be purchased as pure compounds, *i.e.*, maltooligosaccharides up to maltoheptaose (*DP7*), the state of the art limit of preparative GPC.

Post-hydrolysis modifications may produce major changes in the properties of maltodextrins or corn syrups, as is the case with Lycasin corn syrup. Lycasin differs from conventional corn syrups in its extremely low *DE* value, which is obtained by subjecting corn syrup to hydrogenation, leaving a product devoid of reducing sugars.

Other oligo- and polysaccharides different from the linear α-(1–4)-linked glucose polymers are also vailable, such as linear raffinose, stachyose and dextrans, in addition to cyclodextrins, which are circular α-(1–4)-linked glucose oligomers.

In a first series of experiments, the enantiomeric resolving power of complex maltooligosaccharide mixtures was evaluated by their ability to obtain separate peaks for three racemic 2-arylpropionic acid non-steroidal anti-inflammatory drugs, flurbiprofen, ibuprofen and ketoprofen. Second, an attempt was made towards the identification of the chiral discriminating fraction using either corn syrup subfractions obtained through ultrafiltration or pure maltooligosaccharides. Moreover, a comparison was also made with various other oligosaccharides, different from the α-(1–4)-linked glucose polymers. Finally, the method was applied to racemic coumarinic anticoagulant drugs and to diastereoisomeric pairs of cephalosporin antibiotics.

EXPERIMENTAL

Chemicals

Maltodextrins and corn syrups of different origin were used. The Maltrin, Maldex and Glucidex maltodextrin series were kind gifts from Grain Processing Corp. (Muscatine, IA, USA), Amylum (Aalst, Belgium) and Roquette (Lestrem, France) respectively; the Mylose and Lycasin corn syrups were gifts from Amylum and Roquette, respectively. D-(+)-Maltose, stachyose, dextran 1500 and dextran 6000 were purchased from Fluka (Buchs, Switzerland); raffinose, separate maltooligosaccharides (from maltotriose to maltoheptaose) and maltooligosaccharide mixture were obtained from Merck (Darmstadt, Germany). β-, Dimethyl-β- and hydroxypropyl-β-cyclodextrin were obtained from Sigma (Deisenhofen, Germany), Avebe (Veendam, Netherlands) and Janssen Drug Delivery Systems (Beerse, Belgium), respectively. 3-[(3-Cholamidopropyl)-dimethylammonio]-1-propanesulphonate (CHAPS), N,N-dimethyl-N-myristyl-3-ammonio-1-propanesulphonate (DMAP) and *n*-dodecyl-β-D-maltoside (DDM) surfactants were purchased from Fluka.

Pharmaceutical compounds included racemic flurbiprofen and ibuprofen (gifts from Boots, Nottingham, UK) and ketoprofen (gift from Rhône-Poulenc, Paris, France), the (*S*)-(+)-enantiomers of ibuprofen (gift from Profarma, Beerse, Belgium) and naproxen (gift from UCB, Brussels, Belgium), warfarin and 3-(α-acetonyl-*p*-chlorobenzyl)-4-hydroxycoumarin (Sigma), phenprocoumon and *p*-chlorophenprocoumon (gift from Roche, Basle, Switzerland), acenocoumarol (gifts from Ciba-Geigy, Basle, Switzerland) and the diastereoisomers of cefalexin and cefadroxyl (courtesy of Professor J. Hoogmartens, Laboratory of Pharmaceutical Chemistry, K.U. Leuven, Belgium).

Sodium dihydrogenphosphate and sodium hydroxide were obtained from Merck.

Capillary electrophoresis

Experiments were performed with a Waters Quanta 4000 CE system, with detection using a fixed-wavelength UV detector equipped with a zinc lamp and a 214-nm filter. The system was operated at a constant voltage (30 kV). Fused-silica capillaries of 50 and 75 μm I.D. were used with a capillary length ranging from 50 to 90 cm. Capillaries were stored overnight filled with water. Each day operation was started by purging with 0.5 *M* sodium hydroxide solution followed by water. When

changing electrolytes, the capillary was subjected to an electroosmotic purge following a vacuum purge with the new electrolyte. All runs were preceded by a 3-min purge with the electrolyte used. Samples were introduced by gravity-induced siphoning (ΔHeight · time = 100 cm s). Data were collected through Waters Maxima Software.

All sample solutions were prepared with water obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) or HPLC-grade acetonitrile (Carlo Erba, Milan, Italy). All CE running buffers were freshly prepared in Milli-Q-purified water, filtered and degassed immediately prior to use. The background electrolyte consisted of 10 m*M* sodium phosphate buffer (pH 7.0) unless stated otherwise.

Ultrafiltration

A 20% Mylose STD solution in water was subjected to three subsequent ultrafiltrations through membranes with decreasing molecular mass cut-offs (3000, 1000 and 500) using an Amicon Model 8050 ultrafiltration cell. After each step samples of the filtrate and retentate were taken and kept refrigerated until further use (within 1 day). To all samples sodium phosphate was added to give a final concentration of 10 m*M* and the pH was adjusted to 7. Ultrafiltration subfractions were either reconstituted with respect to the Mylose starting solution (filtrate fractions) or adjusted to obtain a multiple of the original corn syrup concentration (retentate fractions).

Calculations

As in chiral separations the peaks corresponding to the separate enantiomers are always geminate, the usual terms of resolution or efficiency often do not describe accurately the actual separation of the optical antipodes. Instead, a relative chiral separation (*RCS*) factor was introduced in order to compare the oligosaccharide-modified electrolytes with respect to their enantiomeric resolving power:

$$RCS = \frac{\Delta T - 2\bar{w}}{\overline{MT}} \cdot 100$$

where ΔT is the time between the start and end of the first and second enantiomeric peaks, respectively, $\bar{w}$ is the mean peak width and $\overline{MT}$ is the mean migration time. Baseline separation of the antipodes thus results in positive values of the *RCS* factor, whereas negative values are obtained for poorly resolved enantiomers. In addition, the *RCS* factor indicates the efficiency of the separation conditions studied: increasing *RCS* values represent an increase in efficiency, owing to a shorter analysis time and/or better peak separation. However, for partially resolved enantiomers with comparable peak overlap a decrease in migration time, which in fact represents a more efficient separation, is reflected by a more negative *RCS*.

RESULTS

Complex maltooligosaccharide mixtures as electrolyte additives for chiral separations

Influence of experimental conditions on chiral separation with a homogeneous series of maltooligosaccharide-modified electrolytes. Four maltodextrin mixtures of the Maltrin series having different *DE* values were compared with respect to their resolving power towards three racemic 2-APA NSAIDs (flurbiprofen, ibuprofen and ketoprofen) in three different capillaries (two 75 μm I.D. capillaries of length 50 and 90 cm, respectively, and a 50 μm I.D. × 60 cm length capillary. The results are reported in Table II.

Baseline resolution of ibuprofen enantiomers was achieved under almost all conditions (as indicated by the positive values of the *RCS* factor). The separation of the optical antipodes of flurbiprofen was more difficult to achieve, and ketoprofen enantiomers co-migrated under all conditions tested.

Increasing the maltodextrin contents of the electrolyte increased the migration times, as expected from the rise in viscosity, and improved the resolution. Modifying the electrolyte with maltodextrins of lower *DE* value also allowed an improved separation.

Addition of acetonitrile to the electrolyte, generally known to decrease migration times and improve resolution [8], had the opposite effect, *i.e.*, an increased migration time without improved resolution. As it has been reported elsewhere that combining surfactants with circular oligosaccharides was beneficial to chiral separation [9], we added various surfactants to Maltrin-modified electrolytes. It was found that the resolution of flurbiprofen and ibu-

TABLE II

CAPILLARY ELECTROPHORETIC CHIRAL SEPARATION OF THE 2-APA NSAID COMPOUNDS FLURBIPROFEN (F), IBUPROFEN (I) AND KETOPROFEN (K) WITH MALTRIN-MODIFIED ELECTROLYTES

Migration time (*MT*) in minutes. Relative chiral separation (*RCS*) in arbitrary units.

Capillary	Maltrin		F		I		K[a]
	DE	Concentration (%)	*MT*	*RCS*	*MT*	*RCS*	*MT*
(a) 50 cm × 75 μm I.D.	15	10	9.61–9.96	−1.022	12.17–12.99	3.498	15.69
		7.5	7.68–7.90	−1.284	9.30–9.68	0.316	10.96
		5	6.93–7.11	−2.137	8.17–8.45	0.241	9.13
	10	5	6.84–7.04	−2.306	8.08–8.36	−1.095	9.22
	5	2.5	8.67–9.03	−1.695	10.66–11.27	2.462	12.43
(b) 90 cm × 75 μm I.D.	15	2.5	14.05–14.26	−0.212	15.35–15.60	0.582	15.77
		5	15.02–15.29	−0.367	16.81–17.22	1.117	17.85
		7.5	16.61–16.89	−0.359	18.81–19.32	1.259	20.40
		10	19.64–20.01	1.160	22.49–23.21	1.838	25.00
	10	5	12.75–12.94	−0.078	14.04–14.32	1.120	14.87
		7.5	16.34–16.63	0.485	18.37–18.93	1.877	>20
		10	17.86–18.16	0.333	20.02–20.67	2.162	22.46
(c) 60 cm × 50 μm I.D.	20	5			14.25–14.80	1.308	
		6.25			14.50–15.07	1.285	
		7.5			18.57–19.56	2.255	
	15	3.75			10.75–11.12	−0.823	
		5			12.04–12.56	1.301	
		6.25			14.06–14.79	2.288	
		7.5			17.65–18.84	4.166	
	10	2.5			9.13–9.36	−1.190	
		3.75			10.60–11.00	0.926	
		5			11.89–12.41	1.070	
		6.25			12.44–13.04	1.570	
	5	2.5			8.20–8.51	−0.477	
		3.75			9.45–9.82	0.415	
		5			10.00–10.52	2.047	

[a] No separation of enantiomers.

profen enantiomers disappeared completely on addition of the non-ionic surfactant DDM at a concentration well above the critical micellar concentration (cmc), the cmc of DDM in water being 0.16 m*M*. Increasing the maltodextrin concentration while decreasing the surfactant concentration restored the chiral separation with migration times slightly longer. Addition of increasing amounts of the amphoteric surfactants DMAP (cmc = 0.33 m*M*) initially resulted in an increased migration time and eventually in complete loss of enantioselectivity. Adding CHAPS (cmc = 8 m*M*), another amphoteric surfactant, had an even more pronounced deleterious effect on chiral resolution.

With a higher field strength the migration velocity increased [Table II, compare (a) and (b)]. Although higher plate numbers are expected to be achieved by increasing the field strength, this may cause a rise in temperature. Where heat dissipation is less efficient, as with shorter capillaries, separation is negatively affected. Consequently, decreasing the capillary diameter results in a higher efficiency as the increased surface to volume ratio favours convection. In a 50 μm I.D. × 60 cm capil-

lary chiral separation of ibuprofen was more easily obtained, *i.e.*, at lower Maltrin concentrations and with shorter migration times, as demonstrated by the results shown in Table II, part (c).

Influence of the composition of maltodextrin mixtures on chiral separation. Fig. 1. compares the chiral separation of the 2-APA NSAIDs in electrolytes modified with maltodextrins of the Maltrin (Fig. 1A), Maldex (Fig. 1B) and Glucidex (Fig. 1C) series, which display DP spectra slightly different from each other, and with Mylose corn syrups (Fig. 1D). Complete resolution of ibuprofen enantiomers was demonstrated for all Maldex and Mylose maltooligosaccharide mixtures studied and for the lower *DE* range of Glucidex, as indicated by the positive values of the *RCS* factor. The chiral separation of flurbiprofen was more limited whereas ketoprofen enantiomers did not separate at all.

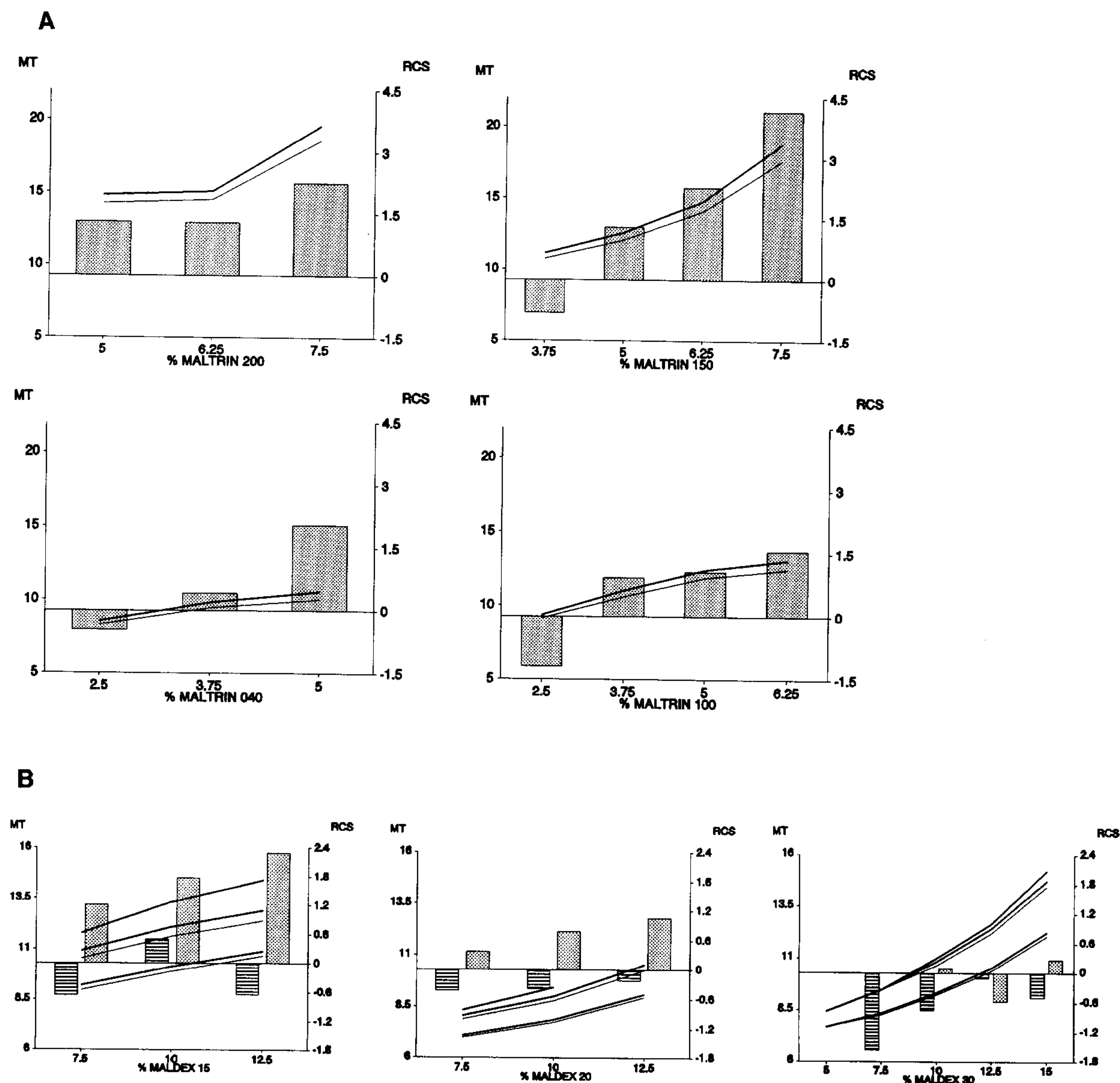


Fig. 1.

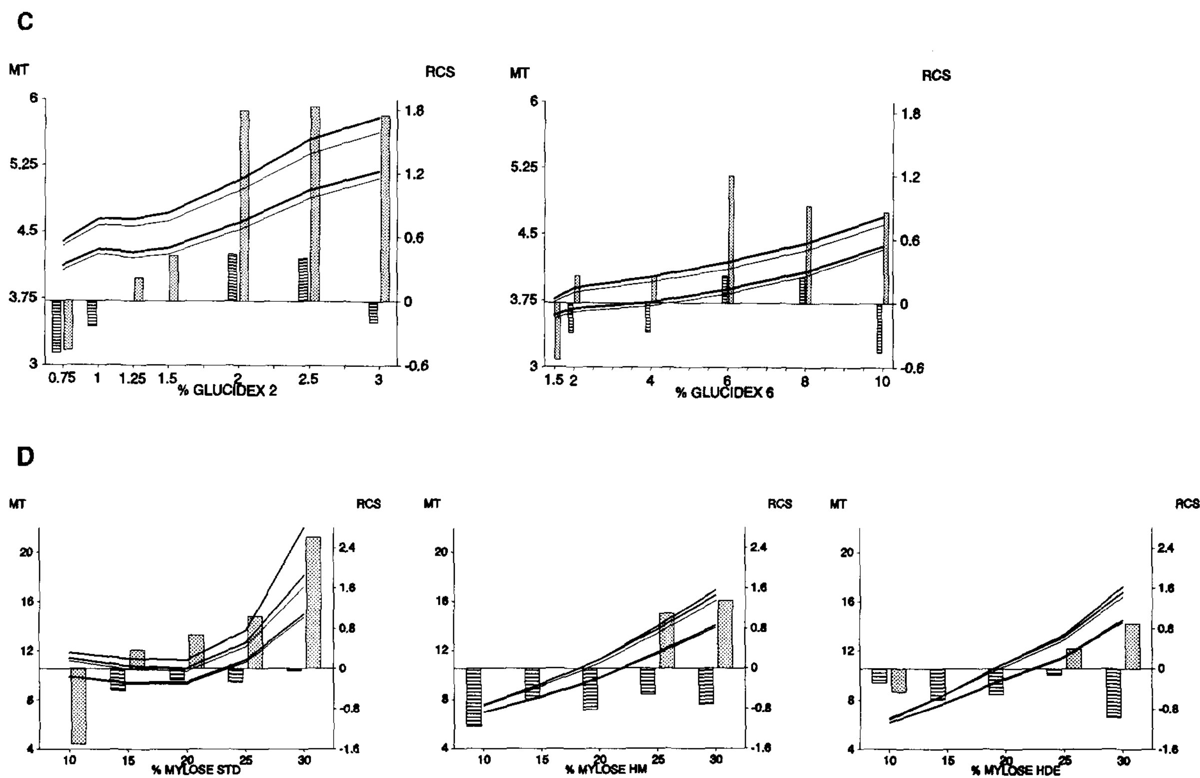


Fig. 1. Chiral separation of 2-APA NSAIDs: (*R/S*)-flurbiprofen (F), (*R/S*)-ketoprofen (K). Migration times of enantiomers (MT) in minutes (line graphs) and relative chiral separation (*RCS*) in arbitrary units (bar graphs) as a function of concentration in % of chiral modifier in background electrolyte. Dot-patterned bars, ibuprofen; stripe-patterned bars, flurbiprofen. Separation of ketoprofen enantiomers was not demonstrated in these experiments. (A) Modifiers, Maltrin series; sample, I. (B) Modifiers, Maldex series; sample, K (top line), I (middle pair of lines) and F (lower pair of lines). (C) Modifiers, Glucidex series; sample, I (upper pair of lines) and F (lower pair of lines). (D) Modifiers, Mylose series; sample, K (top line), I (middle pair of lines) and F (lower pair of lines).

As shown already for the Maltrin series, the chiral separation of ibuprofen enantiomers also improved for Maldex and Glucidex maltodextrins with increasing concentrations or decreasing *DE* value. Resolution was achieved in a shorter time with maltodextrins of the Maldex series as compared with Maltrin, even at higher concentrations. The analysis times with electrolytes containing low-*DE* Glucidex (*DE* 2 and 6) were found to be even lower. Glucidex 2 and 6 also allowed baseline separation of flurbiprofen enantiomers, which was not possible with the Maldex products. The longer analysis times in the presence of Maltrin as compared with Maldex of identical *DE* value and at the same concentration could possibly by ascribed to the higher contents of high-molecular-mass malto-oligosaccharides producing a higher viscosity. Apparently the presence of oligosaccharides with a higher *DP* value favours chiral separation.

From the *RCS versus* concentration course of the experiments with Glucidex 2 and 6 (Fig. 1C), an optimum in the maltodextrin concentration for chiral separation was observed. This phenomenon was not observed with the Maltrin- or Maldex-modified electrolytes. Indeed, owing to their lower transparency as compared with the electrolytes containing high concentrations of Glucidex 2 or 6, the concentrations of Maltrin and Maldex maltodextrins could not be increased so as to determine a possible decline in chiral resolution and/or efficiency in the presence of higher percentages of electrolyte modifier.

As shown in Table III, Glucidex maltodextrins in the higher *DE* range were not as efficient as electro-

TABLE III

CAPILLARY ELECTROPHORETIC CHIRAL SEPARATION OF THE 2-APA NSAID COMPOUNDS FLURBIPROFEN (F) AND IBUPROFEN (I) WITH GLUCIDEX-MODIFIED ELECTROLYTES

Capillary 60 cm × 50 μm I.D. Migration time (*MT*) in minutes. Relative chiral separation (*RCS*) in arbitrary units.

Glucidex		F		I	
DE	Concentration (%)	*MT*	*RCS*	*MT*	*RCS*
2	2	4.52–4.60	0.439	4.96–5.08	1.793
6	4	3.69–3.73	−0.270	3.96–4.02	0.251
9	5			10.48–10.96	
12	5			5.78–5.90	
17	5			8.61–8.87	
19	5			5.82	
21	5			5.37	

lyte modifiers; prolonged analysis times and loss of enantioselectivity were demonstrated as the *DE* value was increased from 2 to 21.

Notwithstanding their high *DE* values, reflecting comparatively large amounts of low-*DP* oligosaccharides, Mylose corn syrups also allowed chiral separation of ibuprofen and, to a variable extent, of flurbiprofen (Fig. 1D). As expected from the *DP* values (Table I) and the observations relating to the maltodextrins described above, Mylose STD performed best. The enantiomeric resolution of flurbiprofen improved on increasing the Mylose concentration but no baseline separation could be obtained. Chiral separation of ibuprofen was achieved in most instances. With Lycasin 80/85 hydrogenated corn syrup-based electrolytes a complete absence of enantiomeric separation for all three 2-APAs was demonstrated.

Typical electropherograms of the simultaneous chiral separation of ibuprofen and flurbiprofen using maltodextrin- or corn syrup-modified electrolytes are shown in Fig. 2A and B.

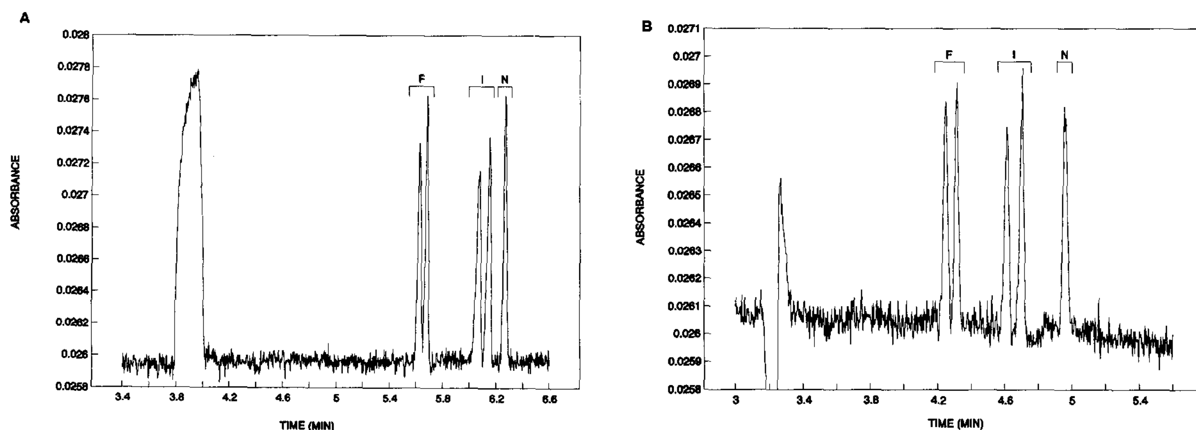


Fig. 2. Electropherograms representing chiral separation of 2-APA NSAIDs: (*R*/*S*)-flurbiprofen (F), (*R*/*S*)- and *S*-(+)-ibuprofen (I), (*S*)-(+)-naproxen (N). (A) Electrolyte: 10% Mylose STD–10 m*M* sodium phosphate (pH 7.07). Migration times (min): (*R*/*S*)-F, 5.63–5.68; (*R*/*S*)-I, 6.08 (*S*)–6.15 (*R*); (*S*)-(+)-N, 6.27. (B) Electrolyte: 1.5% Glucidex 2–10 m*M* sodium phosphate (pH 7.05). Migration times (min): (*R*/*S*)-F, 4.25–4.32; (*R*/*S*)-I, 4.62 (*S*)–4.71 (*R*); (*S*)-(+)-N, 4.96.

TABLE IV

CAPILLARY ELECTROPHORETIC CHIRAL SEPARATION OF THE 2-APA NSAID COMPOUNDS FLURBIPROFEN (F), IBUPROFEN (I) AND KETOPROFEN (K) IN ELECTROLYTES MODIFIED WITH PURE MALTOOLIGOSACCHARIDES

DP 2 is maltose, *DP* 3 is maltotriose, *DP* 4 is maltotetraose, etc. Capillary 60 cm × 50 μm I.D. Migration time (*MT*) in minutes. Relative chiral separation (*RCS*) in arbitrary units.

Maltooligosaccharide		F		I		K	
DP	Concentration (%)	*MT*	*RCS*	*MT*	*RCS*	*MT*	*RCS*
2	10	4.76		4.80		4.68	
3	5	5.30		5.38		5.31	
4	5	4.88–4.91	−1.839	5.01–5.10	0.396	5.59–5.62	−1.070
5	5	5.71–5.74	−0.699	5.68–5.76	−2.448	6.33	
6	5	5.76–5.82	−1.036	5.49–5.62	0.360	6.82–6.87	−0.730
7	6.25			5.52–5.60	−0.899		

Separate maltooligosaccharides as chiral electrolyte modifiers

Separate maltooligosaccharides of *DP* 2–7 were screened as electrolyte additives for chiral separation. The results, summarized in Table IV, were obtained after simultaneous injection of all three racemic 2-APAs unless co-migration was observed, in which event separate runs were performed. None of

TABLE V

CAPILLARY ELECTROPHORETIC CHIRAL SEPARATION OF THE 2-APA NSAID COMPOUNDS FLURBIPROFEN (F) AND IBUPROFEN (I) WITH ELECTROLYTES MODIFIED WITH MYLOSE ULTRAFILTRATION SUBFRACTIONS

Mylose and subfractions are referred to as S (Mylose starting solution), R (retentate) and F (filtrate), and characterized by the molecular mass cut-off of the filter (MCO) and the concentration relative to the starting solution (conc. ratio). Capillary 60 cm × 50 μm I.D. Migration time (*MT*) in minutes. Relative chiral separation (*RCS*) in arbitrary units.

Material	Mylose		F		I	
	MCO	Conc. ratio	*MT*	*RCS*	*MT*	*RCS*
S		1.00	8.43–8.53	0.236	9.30–9.48	0.745
		0.67	6.81–6.89	0.146	7.45–7.57	0.399
		0.50	5.63–5.68	−0.177	6.08–6.15	−0.164
R	3000	4.00	9.54–9.64	0.104	10.39–10.63	1.332
		2.00	5.55–5.61	0	6.04–6.15	0.656
		1.00	4.42–4.47	−0.225	4.76–4.82	0
		0.67	4.12–4.16	−0.242	4.38–4.42	−0.455
		0.50	3.97–4.00	−0.753	4.18–4.21	−0.715
F	3000	1.00	7.08–7.14	−0.422	7.67–7.76	0
		0.67	5.78–5.82	−0.517	6.14–6.19	−0.811
		0.50	5.21–5.24	−0.766	5.47–5.50	−0.729
R	1000	2.00	6.32–6.38	−0.157	6.80–6.90	0.438
		1.00	4.79–4.82	−0.624	5.07–5.11	−0.589
		0.67	4.46–4.48	−1.119	4.66–4.69	0
F	1000	1.00	5.80		5.95	
R	500	4.00	6.46		6.70	
F	500	1.00	4.97		5.03	

the maltooligosaccharides used was able to resolve completely and simultaneously ibuprofen, flurbiprofen and ketoprofen into their enantiomers. In the presence of maltose or maltotriose, no enantiomeric separation was observed and ketoprofen and flurbiprofen co-migrated. Maltotetraose and -hexaose allowed the complete resolution of ibuprofen, whereas maltopentaose and -heptaose did not. In addition, a partial separation of ketoprofen enantiomers was observed with maltotetraose and -hexaose.

Maltodextrin ultrafiltration subfractions

In order to analyse further the influence of the composition of maltodextrin mixtures on chiral resolution, a 20% Mylose solution was submitted to differential ultrafiltration, yielding subfractions composed of maltooligosaccharides with decreasing *DP*. The retentate and filtrate from each fractionation step were tested at various concentrations to obtain the chiral separation of simultaneously injected racemic flurbiprofen and ibuprofen. The results are given in Table V. The retentate of the first ultrafiltration step, *i.e.*, a Mylose solution from which oligosaccharides of molecular mass $\leqslant 3000$ had been removed and of a comparable concentration with respect to the higher *DP* fractions, showed a slight decrease in stereoselectivity and a substantial decrease in migration times. The corresponding filtrate, composed of maltooligosaccharides of molecular mass $\leqslant 3000$, *i.e.*, with $DP < 19$, again showed a moderate decrease in both stereoselectivity and migration times. The ultrafiltrate subfraction with molecular mass cut-offs between 1000 and 3000, corresponding to a maltooligosaccharide mixture with *DP* values ranging from 6 to 18, performed slightly less than the preceding fraction. Fractions consisting of maltooligosaccharides of molecular mass $\leqslant 1000$ completely lost enantioselectivity. The latter result was confirmed by using a commercially available *DP* 2–6 standard maltooligosaccharide mixture; this modifier was unable to resolve the ibuprofen enantiomers within the concentration range tested, *i.e.*, 2.5–12.5%.

Maltooligosaccharide-enriched maltodextrins

The importance of the quantitative composition of maltooligosaccharide mixtures with respect to enantioselectivity was also briefly investigated by selective enrichment of a maltodextrin mixture with separate maltooligosaccharides. Samples containing racemic flurbiprofen, ibuprofen and ketoprofen were run with electrolytes modified with 5% of Maltrin 150 and 2.5% of a pure maltooligosaccharide, either maltotriose, -tetraose, -pentaose or -hexaose. No influence on the chiral separation of any of the three 2-APAs was observed on addition of maltotriose (both migration times and *RCS* values were comparable), whereas the addition of *DP* 4–6 maltooligosaccharides to a maltodextrin-based electrolyte completely abolished chiral separation.

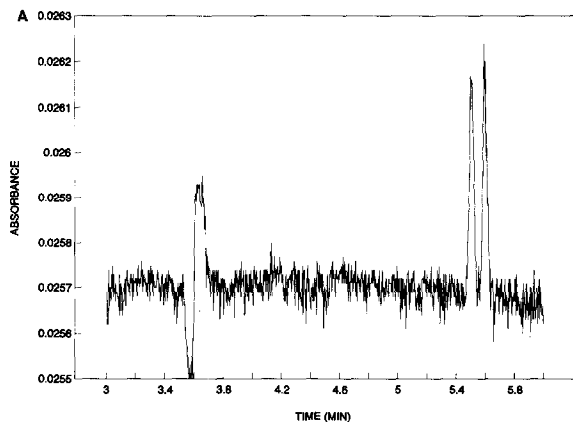
Oligosaccharides different from linear α-(1–4)-D-glucose polymers

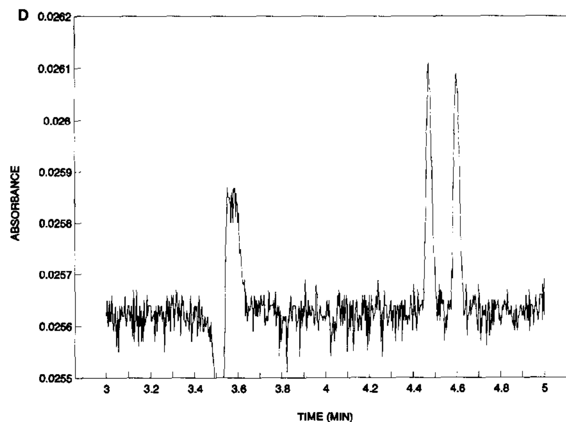
Several other linear non-α-(1–4)-linked glucose polymers and low-molecular-mass galactose–glucose–fructose copolymers were also studied. The electrolytes contained either stachyose (7.7%), raffinose (10%), dextran 1500 (8.2%) or dextran 600 (10%). At the concentrations used none of these oligosaccharides showed any chiral discrimination towards flurbiprofen, ibuprofen or ketoprofen.

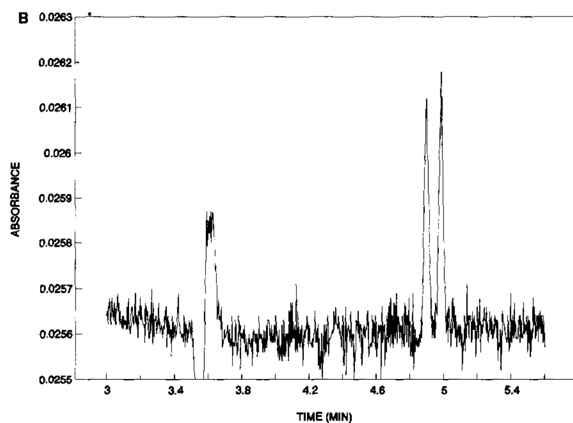
The circular α-(1–4)-linked glucose oligomeric cyclodextrins which have been reported to be effective electrolyte additives for chiral CE were also included. β-Cyclodextrin, dimethyl-β-cyclodextrin or hydroxypropyl-β-cyclodextrin were added to the background electrolyte within the concentration ranges 1.35–1.85%, 7.5–20% and 25–50%, respectively. None of the three 2-APAs tested was resolved using either of the cyclodextrin-modified electrolytes. Also, addition of 5–30% acetonitrile to the cyclodextrin-based electrolyte did not lead to chiral separation.

Applications

In order to assess the applicability of maltodextrins as chiral discriminators towards various other racemic compounds using comparable conditions with respect to pH, maltodextrin concentration and migration of the compounds tested relative to the electroosmotic flow, three coumarinic anticoagulant drugs were used. Two racemic analogues commonly used as their internal standards in HPLC were also included. Chiral separation was demonstrated for warfarin and phenprocoumon and for their HPLC internal standards, but not for acenocoumarol (Fig. 3A–D). Diastereoisomers could also







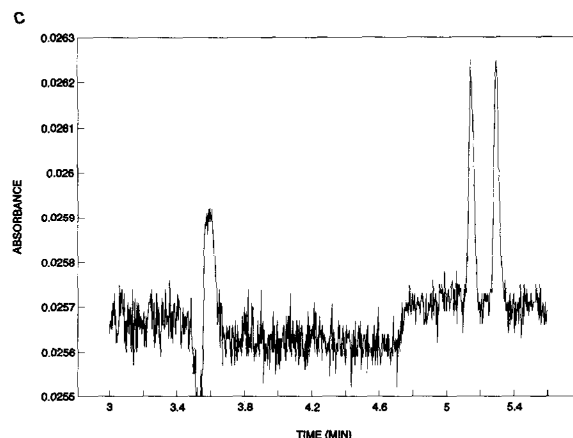


Fig. 3. Electropherograms representing chiral separation of coumarinic anticoagulants. Electrolyte: 2.5% Glucidex 2–10 m*M* sodium phosphate (pH 7.1). (A) (*R*/*S*)-Warfarin. Migration times: 5.50–5.59 min. (*R*/*S*)-3-(α-Acetonyl-*p*-chlorobenzyl)-4-hydroxycoumarin. Migration times: 4.90–4.99 min. (C) (*R*/*S*)-Phenoprocoumon. Migration times: 5.15–5.30 min. (D) (*R*/*S*)-*p*-Chlorophenprocoumon. Migration times: 4.47–4.60 min.

be separated, as demonstrated with two cefalosporin antibiotics, cefalexin and cefadroxyl (Fig. 4A and B).

DISCUSSION

Using 2-APA NSAIDs as test compounds, direct chiral separation by CE was demonstrated with electrolytes modified by complex maltooligosaccharide mixtures. Although the performance of the maltodextrins and corn syrups tested displayed large variations, the chiral separations generally improved on increasing the concentration or decreasing the *DE* value, demonstrating the importance of both the qualitative and quantitative composition of the maltooligosaccharide mixtures.

In an attempt to identify the maltodextrin fractions necessary and/or sufficient for chiral separation to occur, separate maltooligosaccharides of *DP* 3–7 were studied. Maltotetraose and-hexaose showed chiral separations of all three 2-APAs tested, although to variable extents. Odd-numbered oligosaccharides performed less well under the same conditions. Additional information from experiments with maltooligosaccharides of higher *DP* is clearly needed, but so far pure separate maltooligosaccharides have been produced by preparative gel

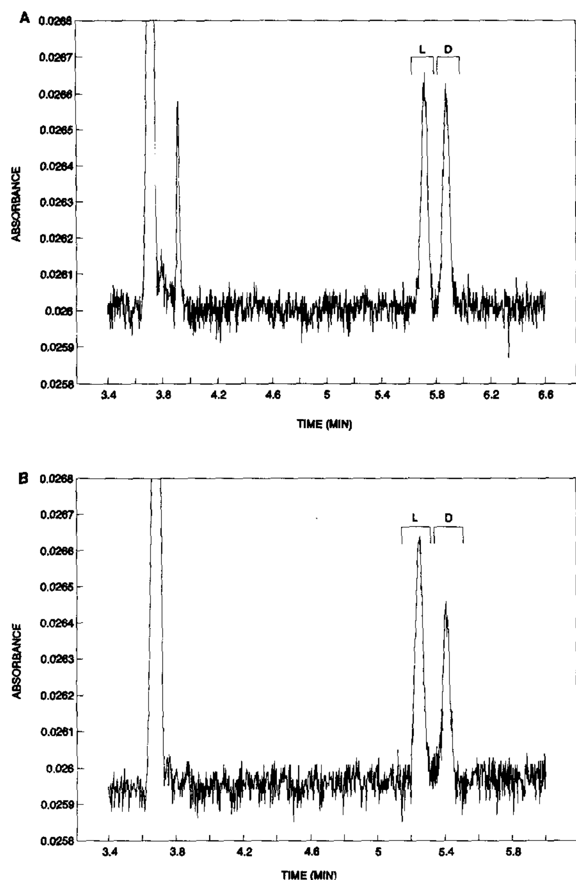


Fig. 4. Electropherograms representing the separation of the diastereoisomers of cephalosporin antibiotics. Electrolyte: 1% Glucidex 2–10 m*M* sodium phosphate (pH 7.5). (A) D/L-Cephalexin. Migration times: 5.72(L)–5.88(D) min. (B) D/L-Cephadroxyl. Migration times: 5.25(L)–5.41(D) min.

permeation chromatography, the upper limit of which is set at *DP* 7. Also, in order to link the chiral resolving capacity to a specific component or subfraction, a knowledge of the entire DP spectrum of the given maltooligosaccharide mixtures would be required. However, the analytical methodology available to date is limited to the identification and determination of the lower *DP* range, *i.e.*, up to *DP* 43 [10].

The importance of the qualitative composition of the maltooligosaccharide mixtures was revealed by comparing the various maltodextrins used, *i.e.*, maltodextrin mixtures and corn syrups of different origins and *DE* values. Circumstantial evidence was also provided by the experiments with separate maltooligosaccharides. Further conformation was obtained by the differential ultrafiltration experiments. As the maltodextrins and corn syrups are complex systems, one could speculate whether a single component might be responsible for the observed chiral separation. As pure oligosaccharides with $DP > 7$ are not available, a different approach elucidating this question was attempted by using several corn syrup ultrafiltration subfractions. It was found that maltooligosaccharide mixtures in the lower *DP* range were ineffective in separating 2-APA enantiomers, whereas high-*DP* corn syrup subfractions allowed chiral separation, although higher concentrations were needed as compared with the original solution. Apparently, the presence of high-*DP* subfractions was not the only factor determining chiral separation. From the results described above one may conclude that particular high-molecular-mass components were necessary but not sufficient for enantioselective separations to occur. The matrix seemed to be equally, if not more, important, as was illustrated most clearly by the chiral separation of ketoprofen. The enantiomers of this racemate were separated, although not to the baseline, with either maltotetraose or -hexaose, whereas none of the maltooligosaccharide mixtures used proved to be capable of resolving ketoprofen enantiomers.

The importance of the quantitative composition, on the other hand, could be demonstrated by selective enrichment of a maltodextrin mixture with separate maltooligosaccharides of low *DP*. Paradoxically, this resulted in a negative effect on enantiomeric separation.

Various other oligo- and polysaccharides did not show any enantioselectivity towards 2-APAs, including circular α-(1–4)- and linear β-(1–6)-linked D-glucose oligomers and polymers and saccharide copolymers, suggesting the key role of chains of D-glucose units linked through α-(1–4) bonds. It may be be speculated that in analogy with amylose some α-(1–4)-linked glucose oligomers and polymers display a balanced hydrophilic–hydrophobic surface, resulting from the helical conformation, and confer the steric environment necessary for chiral interactions to occur.

Using comparable running conditions, other enantiomeric (coumarinic anticoagulant drugs) and

diastereoisomeric (cefalosporin antibiotics) pairs of compounds were easily resolved. Hence the applicability of maltooligosaccharides to perform chiral separations is not restricted to the 2-APA compounds. So far and under the given experimental conditions, only acidic racemates were found to be effectively separated. However, no premature conclusions should be drawn about the nature of racemic compounds that could be resolved through this approach.

In conclusion, linear α-(1–4)-glucose polymers were demonstrated to be effective chiral discriminators in capillary electrophoresis. It was shown that an as yet undefined combination of variables relating to the maltodextrins is involved in their performance in the stereoselective separation by CE. The qualitative and quantitative composition of the maltodextrin mixtures, the importance of the viscosity of the electrolyte and the physico-chemical interactions involved in chiral separations using oligosaccharides need further examination.

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