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Chiral analysis of basic drugs by oligosaccharide-mediated capillary electrophoresis

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Abstract

A low-dextrose-equivalent maltodextrin was investigated for application as an enantioselective electrolyte modifier in direct chiral capillary electrophoresis. A wide range of basic racemic drug substances belonging to different pharmacological groups (antiarrhythmic, anticholinergic, antifungal, antihistaminic, antidepressant and antipsychotic drugs) could be baseline resolved. The background electolyte cation and the anion were found to affect the enantioselectivity significantly. In addition, a definite effect of background electrolyte anion chirality was observed. The use of a single enantiomer background electrolyte could either enhance or completely abolish maltodextrin-mediated chiral separation and in one particular case (aminopromazine) was even sufficient to generate complete enantiomeric resolution.

Keywords: Buffer composition; Enantiomer separation; Drugs, basic; Maltodextrin

1. Introduction

Compared with the naturally derived substances, few chiral drugs obtained by de novo synthesis are single enantiomer compounds, yet the possible effects of chirality on activity or toxicity were, until recently, seldom addressed. However, nowadays chirality is receiving increasing attention in the medical world, and numerous publications have appeared [1–4]. Although a definite stereoselectivity of several synthetic chiral drugs has been acknowledged, many of them are still being marketed as racemic mixtures. "Astereognosis", as Ariëns [5] termed the neglect of the importance of the chirality issue of drugs in any process, whether it be in pharmacokinetics, pharmacodynamics, metabolism or toxicity, may therefore cause, e.g., erroneous interpretation of pharmacokinetic data or even complete misunderstanding of drug behaviour and should therefore be immunized against.

As regulatory offices now require stereochemical data for new candidate drugs, a high demand for stereoselective analytical methods is emerging. The most frequently applied techniques for the separation of enantiomers are undoubtedly high-performance liquid chromatography (HPLC) and gas chromatography (GC), for both preparative and analytical purposes. Stereoselectivity in chromatographic separations is basically obtained by applying the same principle, i.e., forming either permanent or transient diastereoisomeric derivatives or complexes, which present differences in physico-chemical characteristics.

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Derivatization of the analyte with a pure chiral compound allows enantioseparation with a conventional achiral column. However, if separation is then straightforward, the derivatization step is often cumbersome. Alternatively, chiral resolution may be obtained by incorporation of chiral modifiers in an HPLC mobile phase or immobilization on the stationary phase. Although much chiral HPLC research has focused on the development and the use of chiral stationary phases, the use of chiral columns also presents some more or less important drawbacks. Chiral columns are generally expensive. Also, because of a lack of general applicability, chiral column libraries have to be built. In addition, many chiral columns suffer from limited lifetime and efficiency. Hence the total cost for both method development and routine use may be high.

Since its introduction in 1981, capillary electrophoresis (CE) has proved to allow highly selective and efficient separations, so it has now become an established analytical separation technique, complementing and in some cases even replacing HPLC and GC techniques. The high selectivity which may be attained with CE has been shown to be especially useful for chiral separations. In addition, the easy switch between CE separation modes and mechanisms has further added to its widespread use in chiral applications. Several papers have been published reviewing the literature of chiral separations by CE [6–10] and a number of books on CE have appeared that mention numerous examples or have even devoted entire chapters to chiral separation methods [11–17]. Although several reports have described the successful use of chiral-coated capillaries, mimicking the principle of chiral HPLC or GC stationary phases, most applications have involved the addition of one or more chiral selectors to the background electrolyte. Since the only limitations are a sufficient solubility in the separation medium and an acceptable background absorption, the choice of chiral selectors, and therefore chiral separation mechanisms, is virtually infinite. Cyclodextrins, and their increasing number of analogues and derivatives, have been most frequently used as chiral electrolyte modifiers, and also chiral surfactants, producing chiral micellar electrolytes, or different combined systems. Biopolymers, such as glycosaminoglycans (heparin) and proteins, were also applicable as chiral discriminating additives, but the latter suffer from high background absorption. Although this may be considered an important drawback precluding their widespread use, the problem was circumvented by a simple but effective solution by putting the electrophoretic process itself to work [18].

We have previously reported the use of maltodextrins as enantioselective electrolyte supplements to separate a wide range of acidic racemic drug and herbicidal substances by CE [19]. Maltodextrins are complex malto-oligo- and polysaccharide mixtures, i.e., mixtures of $\alpha(1\text{–}4)$-linked D-glucose oligo- and polymers obtained through a partial acid and/or enzymatic hydrolysis of corn starch. Depending on the manufacturing procedure and the extent of hydrolysis, the average degree of polymerization (DP) of the maltooligosaccharides obtained may differ and is indirectly measured through the dextrose equivalent (DE) value, defined as the percentage of reducing sugars calculated as glucose on a dry substance basis, with glucose given the value 100. We found that low-DE maltodextrins, i.e., maltodextrins produced by limited hydrolysis of starch and thus composed of maltooligosaccharides in the high-molecular-mass range, were most efficient in separating enantiomers.

We have investigated a low-DE maltodextrin for use in acidic separation conditions. Effects on the chiral selectivity towards a series of test mixtures of basic drugs by modifying the background electrolyte were also investigated. In addition, the question of a possible influence of background electrolyte chirality on the overall enantioselectivity was addressed.

2. Experimental

2.1. Chemicals

Racemic drug substances used were doxylamine (DOX) Merrell Dow, Edegem, Belgium), carbinoxamine (CAR), thiazinamium (TA)

(Pharmachemic, Antwerp, Belgium), orphenadrine (ORP) (Riker Laboratories, 3M, Loughborough, UK), bromodiphenhydramine (BDH) (Parke Davis, Zaventem, Belgium), oxyphencyclimine (OXY) (Pfizer, Brussels, Belgium), trihexyphenidyl (THP) (Lederle, Mont-Saint-Guibert, Belgium), dimethindene (DIM) (Zyma, Brussels, Belgium), pheniramine (Ph), thioridazine (TO) (Sandoz, Basle, Switzerland), chlorpheniramine (CPh), brompheniramine (BPh) (Schering-Plough, Brussels, Belgium), hydroxyzine (HYD), meclozine (MEC) (UCB Pharma, Brussels, Belgium), alimemazine (AL) (de Bournonville, Brussels, Belgium), aminopromazine (AM), oxomemazine (OX), promethazine (PM) (Specia, Paris, France), ethopropazine (ET) (Rhône-Poulenc, Brussels, Belgium), viloxacine (VIL) (ICI, Macclesfield, UK), mianserine (MIA) (Organon, Brussels, Belgium), fluoxetine (FLU) (Eli Lilly, Indianapolis, IN, USA), sulpiride (SP), sultopride (ST) (Synthelabo, Bagneux, France), verapamil (VER) (Knoll, Brussels, Belgium) and miconazol (MIC) (Janssen Pharmaceutica, Beerse, Belgium). Two achiral compounds used were diphenylpyraline (DPP) (Riker Laboratories) and promazine (PA) (Federa, Brussels, Belgium).

The Glucidex6 maltodextrin was kindly donated by Roquette (Lestrem, France). Other chemicals used were phosphoric, formic and oxalic acid, potassium hydroxide, diethylamine (DEA), triethylamine (TEA) and ammonia (UCB, Leuven, Belgium), acetic acid, tris (hydroxymethyl)aminomethane (Tris) and sodium hydroxide (Merck, Darmstadt, Germany), lithium hydroxide (Sigma, Deisenhofen, Germany), *rac*- and L-(+)-lactic acid (L), *rac*- and L-(−)-malic acid (M), L-(+)- and D-(−)-tartaric acid (T), citric acid (C), gluconic (GlcA) and glucuronic acid (GlcUA) (Fluka, Buchs, Switzerland) and HPLC-grade methanol (Carlo Erba, Milan, Italy). All compounds were used without further purification.

2.2. CE operation

A Waters Quanta 4000 CE apparatus with a fixed-wavelength (214 nm) UV detector was used for all experiments. A fused-silica capillary (49 cm × 50 μm I.D.) was used, unless mentioned otherwise. The system was operated at ambient temperature and a constant voltage (30 kV) using the normal polarity mode, with detection towards the cathodic end of the capillary. Capillaries were stored overnight filled with water. Each day operation was started by a vacuum purge with 0.5 *M* NaOH followed by water. The capillary was then subjected to an electroosmotic purge following a vacuum purge with the electrolyte. All runs were preceeded by a 3-min purge with the electrolyte used. Electrolyte solutions were freshly prepared in water obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA), filtered and degassed immediately prior to use. Samples were introduced by gravity-induced siphoning (Δh = 10 cm, injection time 30 s). Data were collected through Waters Maxima software.

Stock standard solutions of single compounds and test mixtures were prepared in methanol. Test mixture A contained racemic doxylamine, carbinoxamine, orphenadrine, bromodiphenhydramine, oxyphencyclimine, trihexyphenidyl and the achiral analogue diphenylpyraline, test mixture B consisted of dimethindene, hydroxyzine, meclozine, pheniramine, chlor- and brompheniramine, test mixture C contained eight phenothiazines (racemic alimemazine, aminopromazine, ethopropazine, oxomemazine, promethazine, thiazinamium, thioridazine and the achiral analogue promazine) and test mixture D was composed of viloxacine, mianserin, fluoxetine, sulpiride and sultopride. Concentrations in the stock standard solutions ranged from 0.75 to 1.5 mg/ml and were diluted 50–100-fold with Milli-Q water to give working standard solutions.

2.3. Calculations

For monitoring trends in enantioselectivity with varying electrolyte composition, the percentage chiral separation (%CS) factor was used as outlined previously [19]. Briefly, %CS is calculated according to

$$\%CS = [1 + (\Delta T - 2w)/w] \cdot 100$$

where ΔT is the time lapse between peak start and peak end of the first and second enantiomer peaks, respectively, and w is the mean peak width (measured at the peak base). %CS thus relates absolute chiral separation ($\Delta T - 2w$), i.e., the actual distance between or the overlap of enantiomeric peaks, to the peak width. For partially resolved or co-migrating peaks the corresponding %CS values will be smaller than 100 or 0, respectively. %CS values of 100 or higher are obtained for fully separated peaks.

3. Results

3.1. Selection of background electrolyte cation and pH

In a first series of experiments, the effect of varying the cation of a phosphate-based electrolyte on selectivity under non-chiral conditions was studied. Using 50 m*M* phosphoric acid solutions adjusted to pH 4 with either lithium, sodium or potassium hydroxide, ammonia, Tris, diethylamine or triethylamine, and four test mixtures (A, B, C and D, as described under Experimental), a definite dependence of selectivity on electrolyte cation was noted (results not shown). The selectivity and the migration times generally increased along the series $K < NH_4^+ < Na < Li < Tris < DEA < TEA$. For test mixtures A and B, baseline separation of all compounds was only observed with DEA and TEA. The phenothiazines were in no case completely resolved; however, TEA phosphate could clearly be distinguished as being considerably more selective. In the case of test mixture D, except for mianserin and viloxacine, which co-migrated, baseline separation was observed only with TEA, while the reverse was true for all other electrolytes. Owing to the overall superior selectivity, TEA phosphate was used in subsequent experiments.

TEA phosphate electrolytes covering the pH range 2.5–5.5 were compared with respect to selectivity towards the four test mixtures. As illustrated in Figs. 1 and 2, the overall selectivity increased with increase in pH and declined after reaching a maximum between pH 3 and 4. Migration times showed maxima in the same pH region. Although the selectivities were lower in all instances, analogous trends were observed for Tris–phosphate electrolytes.

3.2. Effect of background electrolyte anion species and chirality

Using TEA for pH adjustment to 3.25, electrolyte anion species were varied to investigate their effect on selectivity. When performing separations in acetate, formate or oxalate electrolytes, no improvement was observed in comparison with phosphate. At equal concentration,

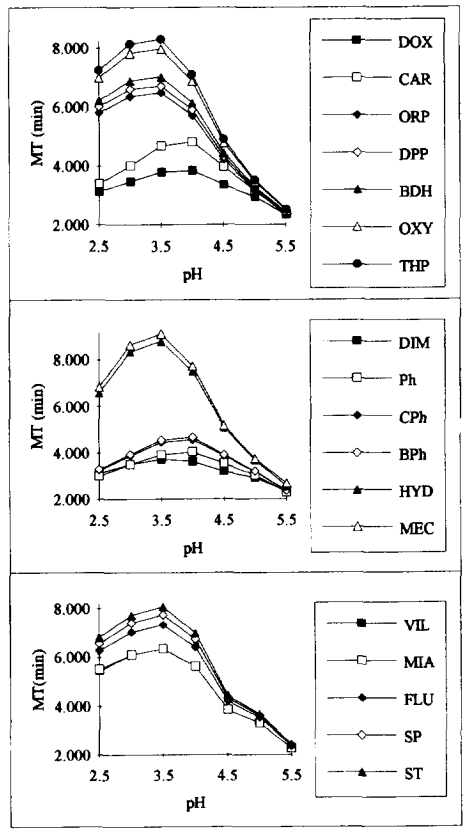

Fig. 1. Effect of electrolyte pH on migration times (MT) of solutes in test mixtures A, B and D. Electrolyte: 50 m*M* TEA phosphate.

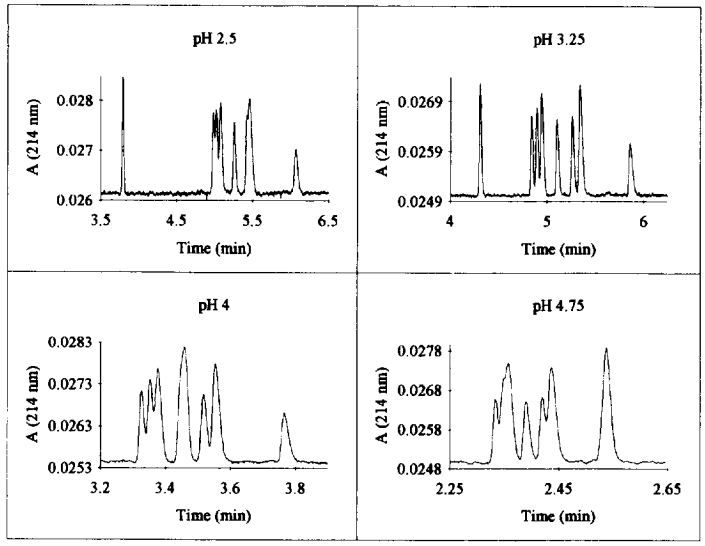

Fig. 2. Electrophoretic profiles of phenothiazine mixture C with varying electrolyte pH. Electrolyte: 50 m*M* TEA phosphate.

TEA *rac*-lactate was also considerably less effective in separating all peaks. However, the resolution increased with increase in concentration (Fig. 3), reaching moderately to slightly lower selectivities at 250 m*M* for test mixtures A, B and C, while the selectivity for test mixture D was significantly higher, as the mianserin and viloxacine peaks were fully resolved, compared with co-migration in phosphate. The same trend was observed when *rac*-malate- or citrate-based electrolytes were used. *rac*-Tartrate, on the other hand, resembled phosphate in that the higher overall selectivity was also accompanied by co-migrating viloxacine and mianserin peaks (Fig. 4) When using single enantiomer anions where possible, i.e., L-(+)-lactate, L-(−)-malate or L-(+)-tartrate, electrophoretic profiles could not be distinguished from the racemic acids, except for the electropherogram of test mixture C when L-(+)-tartrate was used. Whereas TEA gluconate and glucuronate gave rise to analogous electropherograms for test mixtures A, B and D, a slightly different pattern was obtained for mixture C (Fig. 5). The different results for the phenothiazine sample were found to be caused by the chiral resolution of aminopromazine. Stereoselectivity varied with concentration, as shown in Fig. 6, with a clear optimum for the

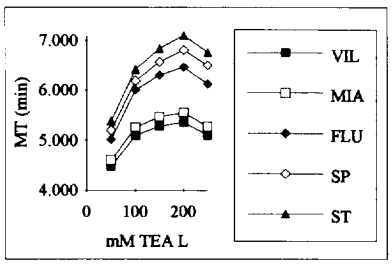

Fig. 3. Effect of electrolyte concentration on migration times (MT) of solutes in test mixture D. Electrolyte: TEA *rac*-lactate (pH 3.25).

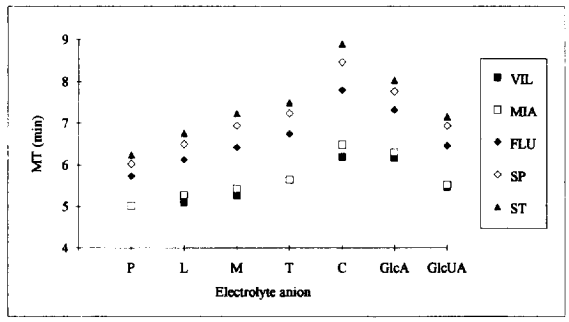

Fig. 4. Effect of electrolyte anion on migration times (MT) of test mixture D solutes. Electrolytes: TEA phosphate (P), *rac*-lactate (L), *rac*-malate (M), *rac*-tartrate (T), citrate (C), gluconate (GlcA) and glucuronate (GlcUA).

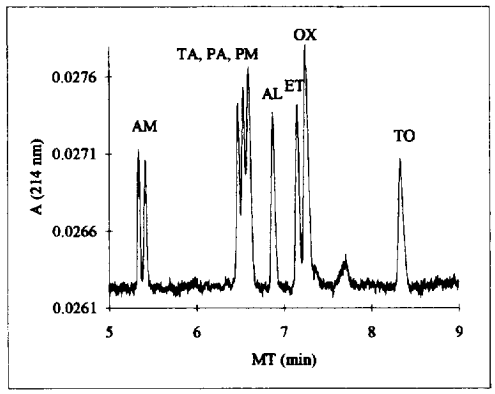

Fig. 5. Electropherogram representing the separation of phenothiazines (test mixture C) with chiral resolution of aminopromazine (AM). Electrolyte: 250 m*M* TEA gluconate (pH 3.25).

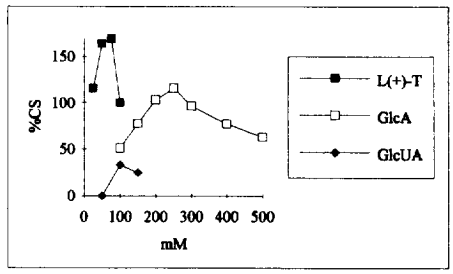

Fig. 6. Influence of chiral electrolyte anion concentration (m*M*) on enantioselectivity (%CS) towards aminopromazine.

three anions studied. Aminopromazine enantiomers were baseline resolved for TEA L-(+)-tartrate and gluconate with maxima at 50–75 and 250 m*M*, respectively.

The enantioselectivity towards aminopromazine was examined more closely by varying the anion chirality. As shown in Table 1, discrimination of aminopromazine isomers was greatly affected by the electrolyte chiral composition. Either tartrate enantiomer was effective in distinguishing between aminopromazine enantiomers, whereas enantioselectivity was lost when both L-(+)- and D-(−)-tartrate were present.

3.3. Effect of Glucidex6 as chiral electrolyte modifier

Glucidex6 added to phosphate-based electrolytes was found to permit the chiral separation of a wide range of racemic drug substances (Table 2). The enantiodiscriminative power of Glucidex6-modified chiral electrolytes was also significantly affected by the nature of the background electrolyte cation. Although selectivity was greatly enhanced in TEA-neutralized phosphate, an important increase in migration times, up to threefold in some cases, was noted. Eight racemic compounds out of twelve of the group of the antihistaminic drugs and some structurally related anticholinergics (mixtures A and B) were separated, and also five out of seven racemic phenothiazines. Except towards orphenadrine enantiomers, which were poorly separated, the selectivity displayed by Glucidex6 was generally very high. Glucidex6 was also highly efficient in discriminating between mianserin and fluoxetine enantiomers.

Table 1
Effect of electrolyte anion chirality on enantioselectivity (%CS) towards aminopromazine

Electrolyte	Concentration (m*M*)	%CS	MT1 (min)[a]	MT2 (min)[a]
TEA GlcA	250	90.9	5.012	5.078
Na GlcA	250	29.4	2.853	2.870
TEA L-(+)-T	75	187.3	5.413	5.628
TEA D-(−)-T	75	155.3	5.403	5.558
TEA *rac*-T	75	0.0	5.508	5.508
Na L-(+)-T	75	80.0	3.805	3.878
Na D-(−)-T	75	95.7	3.820	3.893
Na *rac*-T	75	0.0	3.867	3.867

[a] MT1 and MT2 are migration times of first- and second-migrating enantiomers, respectively.

Table 2
Chiral separation (%CS) and migration times of selected compounds in Glucidex6-modified electrolytes: effect of background electrolyte cation

Compound	Na P[a]		Tris P[a]		TEA P[a]	
	MT1/MT2 (min)[b]	%CS	MT1/MT2 (min)[b]	%CS	MT1/MT2 (min)[b]	%CS
MIC	5.552/5.638	92.9	7.667/7.827	111	ND[c]	ND
VER	5.383/5.448	72.2	6.632/6.722	81.8	16.50/17.14	259
ORP	4.583	0	5.827	0	12.00/12.10	43
THP	5.170/5.613	360	6.662/7.387	509	17.04/23.34	1159
OXY	5.257/5.362	122	6.822/6.965	125	18.12/19.34	391
BDH	6.050/6.227	136	8.157/8.432	138	ND	ND
CPh	3.335/3.347	18.9	4.035/4.055	30.8	6.172/6.237	69.6
BPh	3.562/3.602	63.2	4.352/4.408	81.0	6.993/7.153	167
HYD	6.773/6.967	141	8.738/9.008	154	ND	ND
MEC	7.452/7.690	143	9.685/10.03	144	ND	ND
AM	4.405/4.480	125	5.500/5.612	118	10.73/11.20	285
OX	4.597	0	5.757/5.797	39	11.60/11.78	84.8
ET	5.442	0	ND	ND	15.82/16.03	78.7
TA	5.430/5.493	82.6	ND	ND	16.71/17.34	147
AL	5.817/6.020	242	7.680/7.997	255	24.50/28.54	620
MIA	4.650/4.892	283	5.748/6.117	334	11.49/13.18	827
FLU	5.628/5.732	103	7.182/7.320	109	20.44/21.78	315

[a] Electrolytes composed of 10% Glucidex6 and 50 m*M* phosphate (P) (pH 3.25).
[b] See footnote to Table 1.
[c] ND = not done.

3.4. Combined effects of Glucidex6 chiral electrolyte modifier and background electrolyte chirality

The effect of additional chirality provided by the background electrolyte was investigated by using chiral background anions. As shown in Table 3, dimethindene could only be resolved in a glucuronate-based electrolyte, whereas in other instances the enantioselectivity was relatively indifferent to the nature of the background anion. For instance, the separation of the enantiomers of verapamil, oxomemazine and brompheniramine was only slightly affected by the anion species and no effect of anion chirality could be discerned. For fluoxetine, however, single-enantiomer background electrolytes consistently produced higher selectivities. Tartrate racemate produced a significantly better separation of trihexyphenidyl or alimemazine than the pure isomers, but this effect was not reproduced with the other anions tested. The greatest difference was noted for aminopromazine: whereas Glucidex6 allowed separation in the presence of D-(−)-tartrate, a complete loss of enantioselectivity was observed when its antipode was present, while the racemate produced an intermediate result.

The effect of Glucidex6 concentration on separation was studied with L-(+)-tartrate as chiral background electrolyte. The separation improved with increasing concentration in all instances, except for aminopromazine, for which enantiomers showed co-migration from 7.5% Glucidex6 upwards (Fig. 7). The effect of increasing Glucidex6 concentration on separation, both chiral and non-chiral, is also illustrated in Fig. 8.

4. Discussion

The applicability of a low-DE maltodextrin as an enantiodiscriminative electrolyte supplement for direct chiral CE of basic drugs was investi-

Table 3
Chiral separation (%CS) of selected compounds in Glucidex6-modified electrolytes: effect of background electrolyte anion[a]

Compound	P	*rac*-L	L-(+)-L	*rac*-M	L-(−)-M	L-(+)-T	D-(−)-T	*rac*-T	GlcUA
VER	259	217	202	253	291	317	317	294	292
ORP	43.4	33.8	33.9	42.9	42.5	63.8	54.2	52.1	40.5
THP	1159	780	830	1044	ND[b]	1458	1274	2573	1011
OXY	391	359	448	622	899	588	454	489	453
DIM	0	0	0	0	0	0	0	0	51.2
CPh	69.6	105	89.6	69.2	92.6	84.7	107.4	84.1	81.5
BPh	167	179	179	166	169	170	201	188	203
AM	285	205	198	275	323	0	541	336	347
OX	84.8	91.6	110	112	108	108	115	122	129
TA	147	ND	ND	126	142	214	240	211	304
AL	620	700	762	897	1084	ND	530	1008	ND
MIA	827	719	732	730	750	914	899	976	891
FLU	315	349	400	436	486	463	560	403	492

[a] Electrolytes composed of 10% Glucidex6 and 50 m*M* phosphate (P); 250 m*M* *rac*- or L-(+)-lactate (L); 150 m*M* *rac*- or L-(−)-malate (M); 75 m*M* L-(+)-, D-(−)- or *rac*-tartrate (T) or 100 m*M* glucuronate adjusted to pH 3.25 with TEA.
[b] ND = not done.

gated with test mixtures of racemic drug substances.

Using first non-maltodextrin-modified electrolytes, it was found that the electrolyte anion concentrations greatly affected the separation. However, upon varying the electrolyte anion species, the selectivities and electrophoretic profiles were roughly comparable, provided that the concentration of maximum selectivity was used. A remarkable difference was noted, however, when a phenothiazine mixture was run in tartrate-based electrolytes. It was found that the sole presence of a chiral anion was sufficient for complete resolution of aminopromazine enantiomers. Both L-(+)- and D-(−)-tartrate were effective to a comparable extent in distinguishing between aminopromazine enantiomers, through a mechanism probably involving diasteromeric ion-pair formation. In this case, the use of *rac*-tartrate should lead to an absence of enantioselectivity. Assuming identical ion-pair formation constants for one aminopromazine enantiomer with L-(+)-tartrate, and its antipode with D-(−)-tartrate, in the presence of racemic mixtures of L-(+)- and D-(−)-tartrate diastereomeric ion pairs will form, which are mirror images of each other in a net achiral environment. This was indeed observed: enantioselectivity was lost completely when *rac*-tartrate was used as the electrolyte. Although the use of single-enantiomer anions as electrolytes for chiral separations of

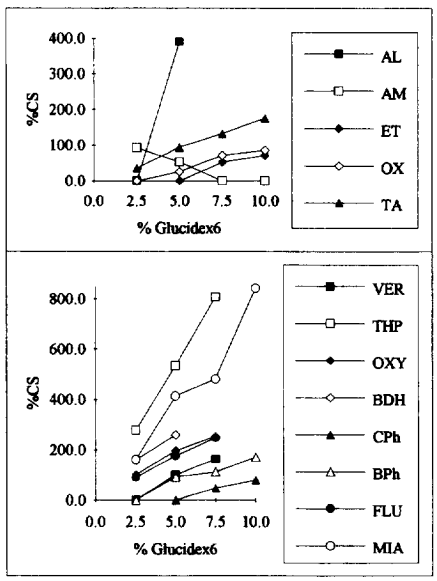

Fig. 7. Effect of Glucidex6 concentration on chiral separation (%CS). Background electrolyte: 50 m*M* TEA L-(+)-tartrate (pH 3.25).

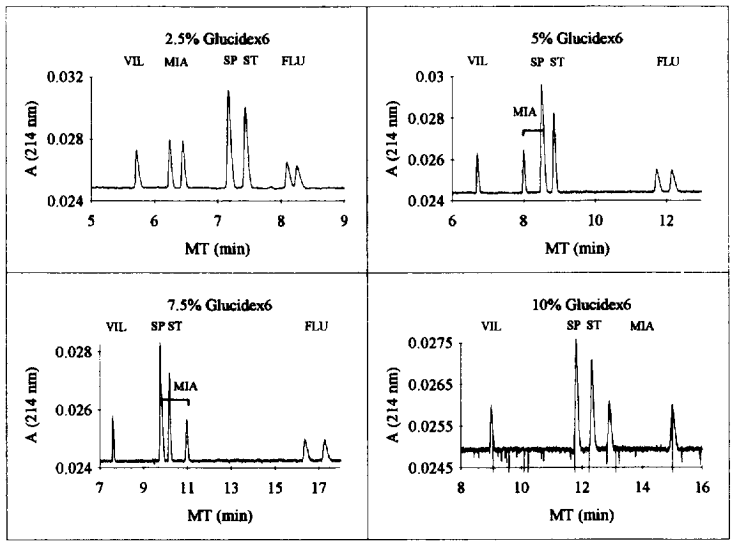

Fig. 8. Electropherograms representing the chiral separation of fluoxetine (FLU) and mianserin (MIA) at different Glucidex6 concentrations. Background electrolyte: 50 m*M* L-(+)-tartrate (pH 3.25). Other compounds: viloxacine (VIL), sulpiride (SP) and sultopride (ST).

drugs by CE has not received much attention, the principle has been put to use extensively to resolve amino acid enantiomers in both electrophoretic and chromatographic techniques. Also, preparative chiral separations by precipitation have used the same principle and tartaric acid has even been used frequently in this respect. The stereoselective separation of aminopromazine, either complete or partial, was also obtained with TEA gluconate and glucuronate, respectively. As observed for most chiral selectors, enantioselectivity varied with concentration, showing a distinct maximum in all cases.

We have previously shown that maltodextrins are extremely efficient in discriminating between enantiomers of a wide range of acidic compounds. As demonstrated here, Glucidex6 maltodextrin is equally applicable under acidic conditions for the chiral separation of a series of basic compounds. As expected and in analogy with non-chiral conditions (as discussed below), greatly enhanced selectivity was obtained with TEA as the background electrolyte cation. Although replacing sodium with TEA was a prerequisite for resolution to occur in some cases, sufficient selectivity was achieved for most compounds in sodium hydroxide-neutralized electrolytes. These may in fact be preferred because of the considerably shorter migration times.

Glucidex6 concentration obviously also affected chiral separation: except for aminopromazine, the resolution of enantiomers increased significantly, as did the migration times. This is to be ascribed primarily to the decreasing charge density of complexed analytes and the increasing viscosity of the separation medium. In Fig. 8 the enantiodiscriminating complexation is clearly illustrated. On comparing mianserin and fluoxetine enantiomers, which were separated, with the unresolved sulpiride, sultopride and viloxacine enantiomers, a relative increase in migration time is clearly distinguished. The extent of migration order changes becomes more pronounced as the Glucidex6 concentration increases, which may be regarded as indicative of increasing complexation of mianserin and fluoxetine and consequently lower apparent mobilities.

In general, a relatively limited influence, compared with the importance of the cationic species, of the nature of the background electrolyte anion on Glucidex6-mediated chiral sepa-

ration was observed. Only in the case of dimethindene was anion selection critical, as separation was not observed in any background electrolyte except the glucuronate. The incorporation of an additional element of chirality in Glucidex6-modified electrolytes through the use of a chiral electrolyte anion produced very dissimilar effects on stereoselectivity towards the compounds studied. The greatest effects were noted for trihexyphenidyl and aminopromazine. In the former case the use of *rac*-tartrate was definitely superior to the use of the single enantiomers, suggesting a cooperative effect of diastereomeric ion-pair formation and maltodextrin complexation in enantiodiscrimination.

The resolution of aminopromazine enantiomers presents itself as a particular case: being the only compound resolved by mere addition of a chiral background electrolyte, it was highly dependent on tartrate chirality for Glucidex6-mediated enantioseparation. Switching from D-(−)- to racemic to L-(+)-tartrate caused the selectivities to drop from very high to zero. The operating enantioselective mechanisms in the mixed electrolytes clearly showed significant interference and, depending on the relative concentrations used, complete incompatibility. A possible explanation might be extremely strong complex formation of the aminopromazine–L-(+)-tartrate diastereomeric ion pairs, preventing any equilibrium differences from appearing. This may be supported by an increase in migration times relative to the other phenothiazines, indicating a lower apparent mobility and therefore most likely stronger complexes with the maltodextrins.

Experiments set up to investigate the role of the background electrolyte cation under non-chiral conditions confirmed previous observations made by several workers, i.e., a significantly improved selectivity with TEA as neutralizing base. This may easily be explained by a decrease in the electroosmotic flow (EOF). According to the resolution equation given by Jorgenson and Lukacs [20], maximum resolution of cationic species is achieved when the EOF is completely suppressed. In the pH range 3–4, a small but definite dissociation of the silanol groups of the capillary wall occurs, generating an EOF. TEA is assumed to shield the capillary wall and thus reduce the EOF. This was confirmed by the increase in migration times on comparison with, e.g., sodium phosphate electrolyte. In addition, capillary wall interactions of the basic analytes are also expected to be significantly reduced in the presence of TEA, preventing adsorption (a phenomenon known to decrease selectivity) from taking place and leading to a further enhanced separation. The decreased peak tailing which was expected as a result was not observed. This could be ascribed, however, to the extremely fast separations with the compounds and conditions used, so that no peak asymmetry was seen.

The observed maximum in selectivity and migration times with changing pH can be explained by opposing effects of EOF and analyte protonation. At pH 2.5 the basic compounds are maximally protonated and thus acquire maximum mobility. According to the resolution equation, maximum selectivity should be observed, because of the more reduced EOF mobility, but this was obviously not the case. The high mobilities could possibly account for this observation, so that insufficient time may have been allowed for maximum separation to occur. With increase in pH to 3–4, the decreasing mobility is due to diminishing charge density, while a beneficial effect of reduced EOF by the use of TEA is still present. With a further increase in pH, the EOF effect predominates.

In conclusion, maltodextrin-supplemented electrolytes were shown to generate extremely high enantioselectivities for a wide range of basic racemic compounds. The resolving power may be further enhanced by selecting a suitable electrolyte cation and anion. In addition, the possibility of using or taking into account the background electrolyte as an additional source of chirality should be recognized. In the context of the possible use of the proposed method as a chiral analysis tool in pharmacokinetic studies, diphenylpyraline and promazine, achiral analogues of the antihistamines and the phenothiazines, respectively, were included in the test

mixtures. Both were well separated from all chiral compounds tested and should thus be useful as internal standards.

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