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Maltooligosaccharides as Chiral Selectors for the Separation of Pharmaceuticals by Capillary Electrophoresis

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Complexation between the linear maltodextrin oligosaccharides and certain enantiomeric compounds of pharmaceutical interest in buffered solutions can lead to an analytically desirable chiral recognition. Different maltodextrins were assessed in their capacity to cause enantiomeric separations under various conditions of capillary electrophoresis. The mechanism of chiral recognition has been probed through electrophoretic mobility and selectivity measurements for different buffer solutions and organic solvent additives. A differential interaction of chiral solutes with the maltodextrin helical entities emerges as the basis of such enantioselectivity. This notion is further supported by ^{1}H- and ^{13}C-NMR experiments. Optimized separations of simendan, ibuprofen, warfarin, and ketoprofen enantiomers are demonstrated together with a chiral determination of ibuprofen in a blood serum sample at the therapeutic level.

Oligosaccharides, both linear and cyclic, are endowed with the capability of complexation to various classes of compounds.[1] The host–guest complexation is the most common interaction of this kind. Some of these complexations may play a role in the biological functions of polysaccharides and proteoglycans at the level of surface recognition in cell membranes. Additionally, chemically modified cyclodextrins (CDs), also displaying the host–guest behavior, have been used as model media for enzymatic reactions.[2] During the last decade, cyclodextrins (cyclomaltooligosaccharides) have found a very significant utilization in separation science. Besides various chromatographic applications, CDs are quite effective as chiral selectors in high-performance capillary electrophoresis (HPCE).[3-11]

In spite of the structural versatility of CDs, such as their variation in cavity size and the feasibility of preparing different derivatives, these cyclic "bucket-like" host molecules occasionally fail to achieve desirable separations of certain enantiomers. Recently, a search for suitable chiral selectors for acidic pharmaceuticals has led[12] to consideration of linear maltooligosaccharides (maltodextrins or amyloses). Both CDs and linear maltodextrins (MDs) consist of D-(+)-glucose units which are connected through Glu-(1–4)-α-D-Glu linkages. Hydrolysis of starch yields a mixture of amyloses which can further be hydrolyzed into shorter oligosaccharides. Linear MDs are characterized by their degrees of polymerization (DP), corresponding to the number of glucose residues in the molecule. The use of MDs with high DP in aqueous solutions is limited by their solubility, which decreases with molecular size. Isolation of the individual MD oligosaccharides from hydrolysis reaction mixtures is obviously a difficult task. The highest oligomer commercially available is maltoheptaose (DP = 7), while typical MDs are sold and used as complex oligomeric mixtures. A mixture is typically defined by its dextrose equivalent (DE) number. The higher the DE number, the higher is the extent of starch hydrolysis and, consequently, the shorter are the oligomeric chains present in a mixture.

In our study of MDs as chiral selectors, we initially followed the work of D'Hulst and Verbeke,[12] who screened a variety of commercial MD mixtures, corn syrups, and some individual oligomers for their capacity of chiral resolution with several acidic pharmaceuticals (such as non-steroid inflammatory drugs and coumarins). Using HPCE, they found differences in the chiral recognition properties among various commercial products. Chiral recognition with the aid of linear MDs has been a somewhat obscure albeit analytically useful type of host–guest complexation. Consequently, we decided to explore further and in more general terms the chiral recognition mechanisms with MDs. Both acidic and basic pharmaceuticals were included as model solutes. The studies presented here take into account the conformation (random coil/helix transitions) of MDs with respect to complexing molecules. Using simendan, ibuprofen, warfarin, and ketoprofen as model compounds, we demonstrate the effects of zwitterionic buffers and organic solvent modifiers on chiral separations. A series of ^{1}H- and ^{13}C-NMR spectroscopic complexation studies have been conducted for selected compounds to elucidate the chiral recognition mechanisms involved. Additionally, an MD-based chiral recognition system has been utilized here to measure ibuprofen enantiomers in blood serum spiked at the therapeutic level.

EXPERIMENTAL SECTION

Capillary Electrophoresis. Home-built CE instruments were used in all measurements. The high-voltage power supply (0–60 kV) was from Spellman High Voltage Electronics

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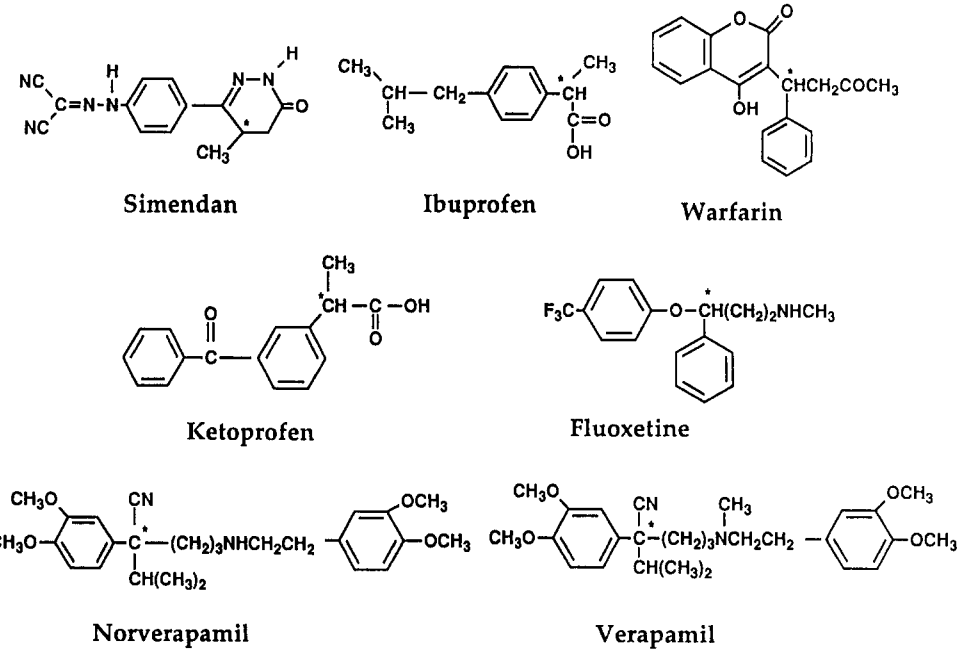

Figure 1. Chemical structures of the model solutes.

(Plainview, NY). The applied voltages were 18–26 kV (positive or negative ground). For the on-column UV absorbance detection, Jasco UVIDEC-100-IV or UVIDEC-100-V (Tokyo, Japan) was set at the wavelength of 220 nm (0.005 AUFS). The on-column fluorescence detection of fluorescently tagged oligosaccharides[13] was accomplished with a Model 4112-50 helium/cadmium laser (Omnichrome, Chino, CA) as the excitation source operating at 442 nm. Emission was measured at 550 nm. Capillaries of 50 and 75 μm i.d. (180 μm o.d.) and various lengths were received from Polymicro Technologies (Phoenix, AZ). The separation capillaries were used uncoated or coated with a linear polyacrylamide.[14] Sample injection was performed in the hydrodynamic mode.

Materials. Zwitterionic buffers CHES (2-(*N*-cyclohexylamino)ethanesulfonic acid), TAPS (3-[*N*-[tris(hydroxymethyl)methyl]amino]propanesulfonic acid), Tricine (*N*-[tris(hydroxymethyl)methyl]glycine), MES (2-(*N*-morpholino)-ethanesulfonic acid), and Trizma base (Tris) were purchased from Sigma (St. Louis, MO). Sigma was also a source of pure maltooligosaccharides (triose, tetraose, pentaose, hexaose, and heptaose). Phosphoric acid (85%, HPLC grade), ammonium sulfate, and acetonitrile (HPLC grade) were received from Fisher Scientific (Fair Lawn, NJ). (*R*)-(−)-2-Butanol, (*S*)-(+)-ibuprofen, cyanoborohydride, and poly(vinylpyrrolidone) (average MW of 360 000) were obtained from Aldrich (Milwaukee, WI). (*R*)-(−)-Ibuprofen was from Research Biochemicals International (Natick, MA). Maltodextrins (Dextrin 20, Dextrin 15, and Dextrin 10, with DE 20, 15, and 10, respectively), were purchased from Fluka Chemie (Buchs, Switzerland). Maltrin M040 (DE 5) was a gift from Grain Processing Corp. (Muscatine, IA). Ethanol (absolute grade) was from McCormick (Weston, MO). Methanol and 2-propanol (Photrex) as well as *n*-hexane were from J. T. Baker (Phillipsburg, NJ). Potassium cyanide and boric acid were supplied by Mallinckrodt (Paris, KY). A fluorogenic reagent, CBQCA (3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde), was synthesized in our laboratory.[15]

Pharmaceuticals (racemic simendan, (*R*)-(−)-simendan, (*S*)-(+)-simendan, racemic ibuprofen, warfarin, ketoprofen, verapamil, norverapamil (desmethyl verapamil), and (*S*)-(+)-naproxen) were a gift from Orion-Farmos Pharmaceuticals (Espoo, Finland). Fluoxetine was donated by Eli Lilly Research Laboratories (Indianapolis, IN). The solutions of pharmaceuticals were prepared in methanol at concentrations of 100–200 μg/mL. The structures of these model solutes are shown in Figure 1. Maltodextrins Dextrin 10, 15, and 20 and Maltrin MO40 were tagged with the fluorogenic reagent CBQCA after reductive amination. This method is described elsewhere.[13]

Equations. Resolution (R_s) was calculated from $R_s = 2(t_{2migr} - t_{1migr})/(w_1 + w_2)$, where t_{1migr} and t_{2migr} are apparent migration times of enantiomers 1 and 2, respectively, and w_1 and w_2 are the corresponding widths at the peak base. Selectivities (α) were calculated using the equation $\alpha = (t_{2migr} - t_0)/t_{1migr} - t_0)$, where t_0 represents electroosmosis.

Serum Sample Preparation. Human serum was donated by a healthy volunteer and stored at −20 °C. A 0.5 mL aliquot of thawed serum was spiked with 4.25 μg (8.5 μg/mL) of the racemic ibuprofen, and 4.2 μg of (*S*)-(+)-naproxen was added as an internal standard. Additions were made in 25 μL of methanol. After the sample was mixed, 100 μL of 2 M HCl was added to acidify it. The ibuprofen enantiomers were extracted into 3.0 mL of *n*-hexane with 0.1% (v/v) 2-propanol by shaking the probe gently for 10 min. After centrifugation, 2.5 mL of the organic layer was evaporated to dryness under nitrogen flow. The residue was redissolved in 50 μL of methanol.

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NMR Spectroscopy. The ^1H- and ^{13}C-NMR spectra were recorded with a Bruker AM 500 MHz NMR spectrometer (Karlsruhe, Germany) in deuterium oxide. ^{13}C-NMR spectra were one-dimensional, proton decoupled recordings, referenced to TPS (trimethylsilyl)propanesulfonic acid) signal. The spectra were obtained after 256 scans at 25 °C. Deuterium oxide (D 99.9%) and deuteriomethanol (D 99.8%) were purchased from Cambridge Isotope Labs (Noburn, MA). TPS (sodium salt) was from E. Merck (Darmstadt, Germany).

In the complexation studies, maltoheptaose and Maltrin M040 (DE 5) were dissolved at concentration of 5% (w/w) (44 mM maltoheptaose) in deuterium oxide containing TPS (8 mM) and 10 mM Tris, which was added for pH adjustment and to imitate the conditions used in CE. The selected pharmaceuticals were added separately in 30 μL of deuteriomethanol, mixed for 15 min, and left equilibrating for an additional 30 min before the measurement. Concentrations in the samples were 4 mM for simendan (limited solubility) and 30 mM for ibuprofen.

RESULTS AND DISCUSSION

Complexation of the maltodextrin (MD) oligosaccharides with the optical isomers appears crucial to their successful separation. The conformational change from the flexible coil to a helix in the presence of complexing molecules and buffer salts is also believed to be essential in such selective interactions. The helical structure is hydrophobic inside, much like the cavity of cyclodextrins (CDs), but MDs represent a considerably more flexible entity than CDs, leading to less restriction toward a steric approach of an interacting solute. Similarities between CD and amylose complexation behaviors have been noted previously.[1] Amyloses bind small molecules, such as iodine, fatty acids,[16,17] linear and branched alcohols,[18] dimethyl sulfoxide,[19] and naphthyl and phenolic derivatives.[9,20] Crystalline complexes with amyloses are known as V-amyloses, in which adjacent turns of a helix are in contact with each other in the form of a collapsed helix.[21] In some circumstances, extended helical forms can be generated.[22] The general preferable properties of the molecules complexing with amylose include a hydrophobic core to which yet another nonpolar substituent is attached together with a more polar group.[1]

In our earlier studies[9] on resolving D- and L-monosaccharides (fluorescently labeled with a naphthyl moiety), we were successful in using both CDs and dextrins as the buffer additives. In a series of maltooligosaccharides, we noticed increased selectivity with increasing molecular weight and postulated that the helical structure (formed in a borate buffer) mimicking a CD cavity was responsible for chiral recognition. In the study by D'Hulst and Verbeke,[12] various commercial MD preparations were differently effective in resolving some acidic pharmaceuticals. Consequently, the effectiveness of MD types and their concentrations had to be related to the composition of these ill-defined MD mixtures and various solvating conditions. A series of electrophoretic and NMR measurements, described below, probe further the mechanisms of these selective interactions.

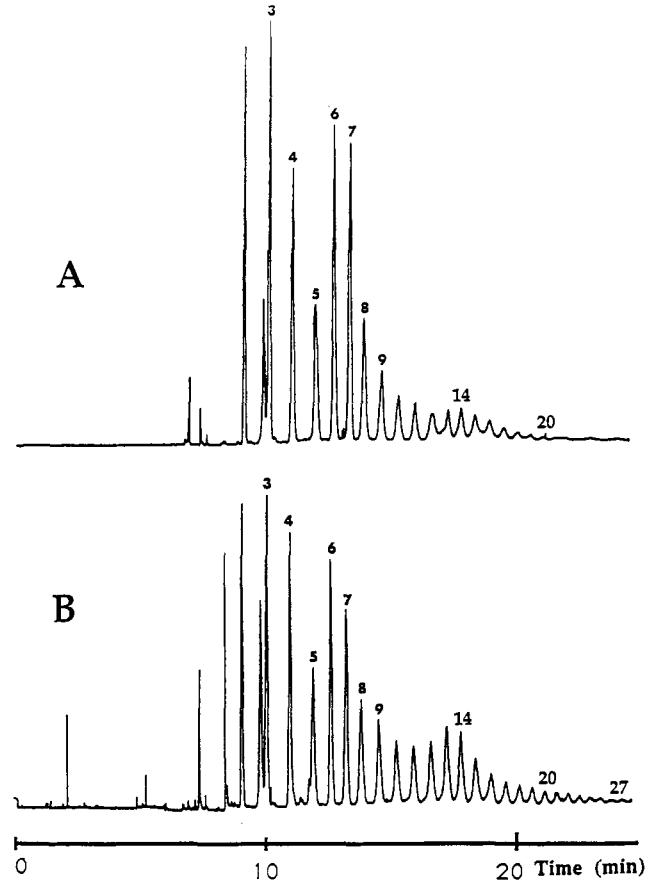

Figure 2. Oligosaccharide profiles from (A) Dextrin 10 (DE 10) and (B) Maltrin MO40 (DE 5) after reductive amination and fluorescent tagging with CBQCA. Detection, fluorescence, using He/Cd laser at 442 (excitation) and 550 (emission) nm wavelengths; separation capillary, linear polyacrylamide coated, 75 μm i.d. × 40 cm (25 cm effective length); run buffer, 0.1 M Tris/borate; pH 8; run voltage, 16 kV (positive ground). Peak numbers indicate the number of glucose residues in oligosaccharides.

Maltodextrin Type and Concentration. The maltodextrin mixtures were characterized by HPCE combined with the laser-induced fluorescence detection of their CBQCA derivatives. The HPCE profiles of Dextrin 10 and Maltrin MO40 revealed that the larger peaks range from three residues (triose) to seven residues (heptaose), but oligomers with a polymerization degree (DP) of as high as 27 were present as minor peaks in Maltrin MO40 (DE 5), as seen in Figure 2.

When the Dextrin 15 (DP = 8–16) and Dextrin 20 (DP = 8–13) mixtures were analyzed by HPCE (results not shown), higher oligomers were also visible but to a lesser extent than with Dextrin 10 and Maltrin M040. According to our experience, the higher oligomers are particularly effective as chiral selectors. Their quantities are not particularly small, as the later peaks are broader and the relative weights of the later-appearing components increase with increasing molecular size. (CBQCA labels a sugar molecule only at its reducing end.)

Dextrin 10 was used further in demonstrating the effect of its concentration on enantiomeric separation. Using a constant buffer composition (TAPS/Tris, with 4% ethanol addition) and uncoated capillaries, the Dextrin 10 concentra-

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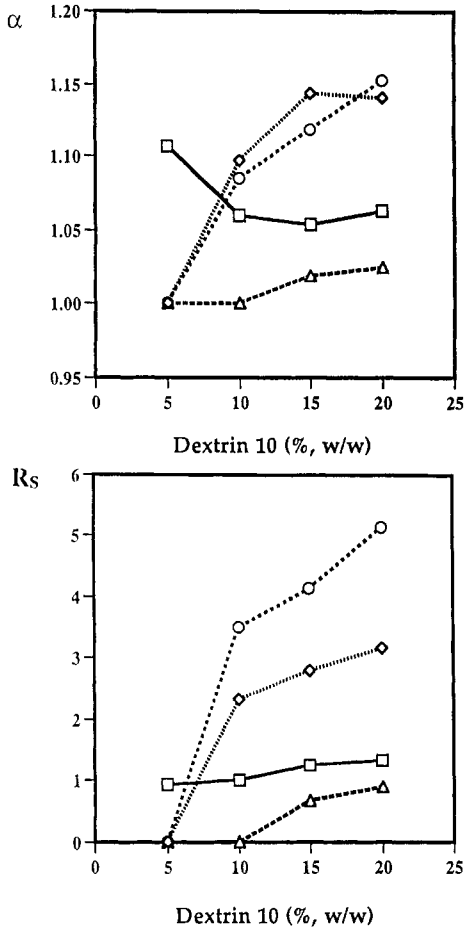

Figure 3. (A, top) Selectivity (α) and (B, bottom) resolution (R_s) of simendan (□), ibuprofen (◊), warfarin (○), and ketoprofen (△) enantiomers as a function of Dextrin 10 concentration. Buffer, 20 mM TAPS/6.5 mM Tris/4% (v/v) ethanol, pH 7.7; uncoated capillary, 50 μm i.d. × 60 cm (44 cm effective length); UV detection at 220 nm; run voltage, 26 kV.

tion was varied from 5 to 20% (w/w). The simendan enantiomers were partially separated already at 5% MD concentration, while ibuprofen and warfarin necessitated at least 10% concentration and ketoprofen isomers at least 15%. Figure 3 shows the selectivity (α-values) and resolution (R_s) plotted against concentration of Dextrin 10 in the buffer solution. Ibuprofen, warfarin, and (to a lesser degree) ketoprofen show increasing α-values and resolution with Dextrin 10 concentration. An anomalous situation is observed for the simendan enantiomers, which show more favorable α-values at lower concentration and a partial loss of selectivity at higher oligosaccharide concentrations. Differential behavior of the simendan enantiomers from the other test solutes might be explained through the presence of polar nitrogen groups in the molecule which may exhibit hydrogen-bonding abilities in the enantiospecific complexation with the maltooligosaccharides. It is presently unclear whether the sugar–sugar interactions[23] at higher concentration compete with the enantiomer complexation in this particular case. The effective mobilities (an average of three measurements) of the four acidic pharmaceuticals are plotted against Dextrin 10 concentrations in Figure 4. Once again, simendan appears here different from the other model solutes, which is likely due to

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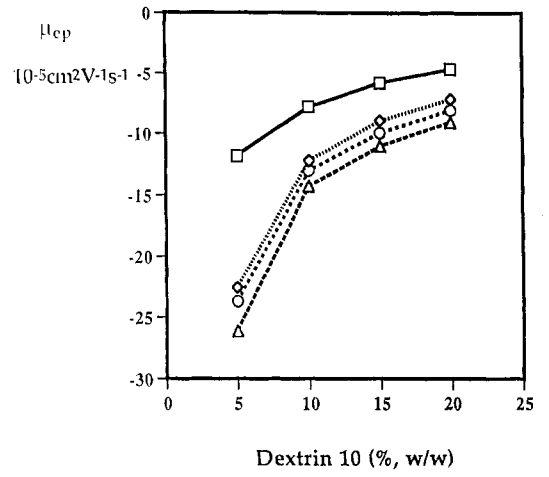

Figure 4. Electrophoretic mobilities (μ_{ep}) of simendan (□), ibuprofen (◊), warfarin (○), and ketoprofen (△) for the first eluted enantiomer. Analytical conditions as in Figure 3. (Results are an average over three runs.)

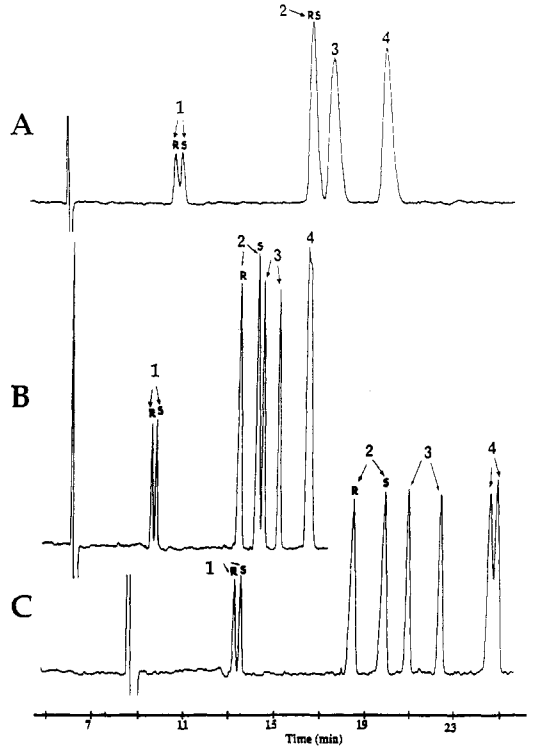

Figure 5. Electropherograms of the acidic model compounds with Dextrin 10 concentrations (w/w) (A) 5%; (B) 10%; (C) 15%. Solutes: peaks 1, R = (*R*)-(−)-simendan, S = (*S*)-(+)-simendan; peaks 2, R = (*R*)-(−)-ibuprofen, S = (*S*)-(+)-ibuprofen. Peaks 3, warfarin enantiomers. Peaks 4, ketoprofen enantiomers. Analytical conditions as in Figure 3.

the larger complexation constant or the changed complexation mechanism with the maltooligosaccharides. Since these model solutes exhibit different migration times, the example electropherograms for three different maltodextrin concentrations are demonstrated in Figure 5. The general enhancement of enantioseparation with the increase of MD concentration is clearly indicated. The role of electroosmosis is less important, as documented at 5% (Figure 5A) and 10% (Figure 5B). Dextrin concentrations had quite similar electroosmotic properties; the respective enantioseparations were considerably improved.

The effects of single maltodextrins in concentrations of 2–6% (w/w) were tested for simendan, ibuprofen, and warfarin with coated capillaries under the negligible electroosmotic flow. Maltoheptaose in a buffer of pH 6.7 separated the enantiomers of simendan and ibuprofen but not warfarin. The hint of separation for ibuprofen was first observed at the concentration of 3% (w/w). Severe peak-tailing, observed particularly with the later-migrating enantiomers (stronger binding), indicated slow equilibration of the binding process. Maltohexaose was effective in chiral recognition for simendan only, starting at the concentration of 2% (w/w) and showing only a partial enantiomer separation. This observation is consistent with the earlier observations on simendan at low Dextrin 10 concentrations. Maltopentaose, maltotetraose, and maltotriose (5% w/w concentration) lacked the enantioseparation ability for all solutes at pH 6.7 and 7.7 when coated capillaries were used. Furthermore, maltotetraose up to 15% (w/w) did not separate the enantiomers of ibuprofen and simendan. The success in chiral recognition with higher oligomers is probably due to the ability of pentaose and higher oligosaccharides to overcome an induced-fit conformation change depending on the size of a complexing molecule.[20] The oligomers with a DP higher than 7 thus seem most suitable for the separation process.

Solvation Effects. With uncoated capillaries, aminopropane- and aminoethanesulfonic acids (TAPS, CHES) and Tricine zwitterions had important effects on resolution, selectivity, and separation times for the enantiomers of acidic pharmaceuticals. The buffer systems Tricine/Tris (pH 7.4), TAPS/Tris (pH 7.8), and CHES/Tris (pH 8.5) were primarily used. With increasing hydrophobicity near the zwitterion's cationic group (e.g., cyclohexyl group in the CHES molecule), the electroosmotic velocity decreased and migration times increased, resulting in improved resolution of the enantiomeric pairs. The separated zone broadened, but the peak shapes remained symmetrical. Apparently, interactions of the wall-adsorbed zwitterions and the analytes were increased. The enantiomers of ketoprofen were separated with TAPS- and CHES- but not with Tricine/Tris buffers. The migration times with CHES buffer were prolonged to more than 1 h.

Decreasing the zwitterion concentration increased both the resolution and the selectivity (α), but also the migration times were increased due to a slower electroosmotic velocity. Limited availability of zwitterions for both the capillary wall and dynamic complexation with dextrins and analytes may influence certain complexation equilibria at the wall. The presence of the analytes and dextrins close to the capillary surface has obviously changed the magnitude of electroosmosis. The four-component test mixture of racemic compounds, simendan, ibuprofen, warfarin, and ketoprofen, listed in their migration order, was used in these experiments. The enantiomeric separation of warfarin (best resolution) and ketoprofen (worst resolution) using a series of dilutions with the TAPS/Tris buffers is shown in Figure 6. The resolution of ibuprofen was not increased in the same way as with the other solutes because of a pronounced band-broadening effect (asymmetrical fronting edge) observed with a decreasing ionic strength. This band-broadening has been assumed to be a sign of multiple complexation of the ibuprofen enantiomers with dextrins differing in their rate constants. Either association or dissociation of some of the complexes is slow, while the material competing for the available sites has been diminished. The ^{13}C-NMR studies, described below, at least partially support this assumption. Among the test solutes, the ibuprofen molecular structure featured the highest flexibility; therefore, also the least geometrical restrictions for interactions with the maltooligosaccharides were expected.

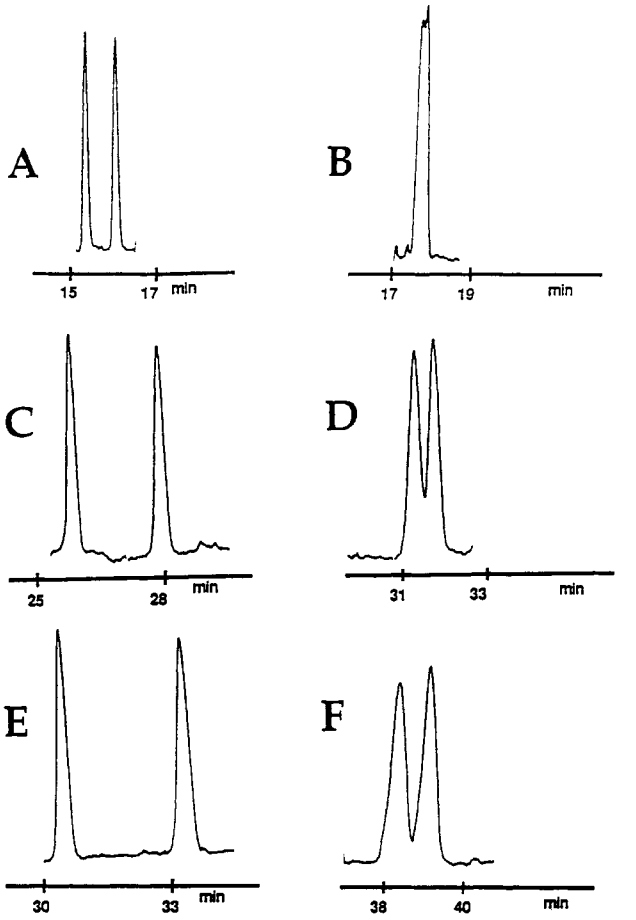

Figure 6. Effect of buffer concentration on the enantiomeric separation of warfarin (A, C, E) and ketoprofen (B, D, F). A, B: 120 mM TAPS/40 mM Tris. C, D: 40 mM TAPS/13 mM Tris. E, F: 20 mM TAPS/6.5 mM Tris. Uncoated capillary, 50 μm i.d. × 60 cm (44 cm effective length); additive, Dextrin 10, 20% (w/w), pH 7.7; UV detection at 220 nm; run voltage, 24 kV.

The organic solvent additives such as methanol, ethanol, (*R*)-(–)-2-butanol, and acetonitrile used at concentrations of 0.5–5% (v/v) were studied in Tricine/Tris (pH 7.4). Addition of organic solvents to CHES/Tris or TAPS/Tris buffer systems was found to be impractical due to excessive analysis times. On the basis of a reported complexation of alcohols[18] and other small molecules with amyloses, the added solvents were responsible for yet another set of competing equilibria in the buffer solution. Ethanol and acetonitrile had the most pronounced effect on the resolution of enantiomeric pairs but only a slight effect on selectivity. In general, the electroosmotic velocity decreased, peaks became broader, and the analysis times increased due to organic solvent additives.

The initially untreated capillaries were dynamically coated with poly(vinylpyrrolidone) (PVP) (average MW 360 000) during the separation of simendan enantiomers with Dextrin 20. Electroosmosis was negligible under such conditions. A base line separation due to reduced electroosmosis was achieved

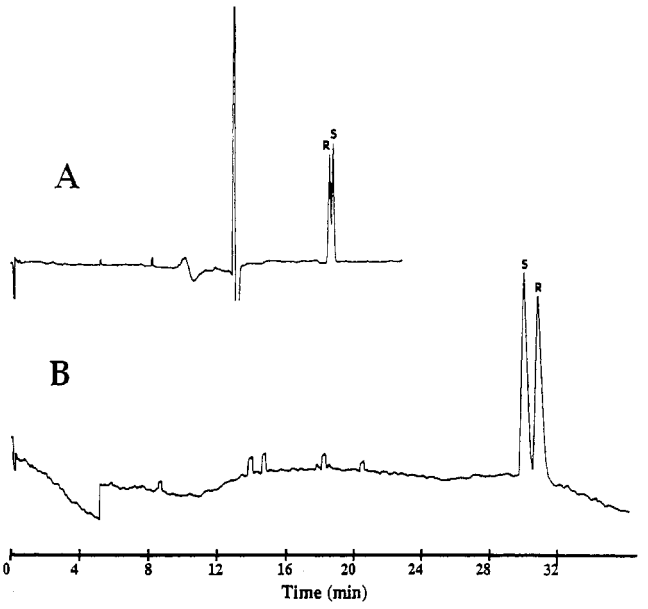

Figure 7. Separation of simendan enantiomers without polymer additive (A, buffer, 20 mM Tris/80 mM Tricine; Dextrin 20, 25% (w/w), *n*-propanol 2%, pH 7.4; voltage, +26 kV) and with PVP polymer additive (B, buffer, 10 mM Tris/40 mM Tricine pH 7.4; Dextrin 20, 12.5% (w/w), 1% (v/v) *n*-propanol, 0.1% (w/w) poly(vinylpyrrolidone) (MW 360 000); voltage, −26 kV. Other analytical conditions as in Figure 6. R = (*R*)-(−)-simendan, S = (*S*)-(+)-simendan.

for this analyte, which had previously not been separated under electroosmosis with a similar type of buffer system (Figure 7). The maltooligosaccharides were compatible in solution with PVP. A similar compatibility of two polymers and enhancement of enantioselectivity was observed earlier in this laboratory with poly(ethylene oxide) and cyclodextrins.[24]

Basic Pharmaceuticals. With uncoated capillaries, the basic solutes (fluoxetine, verapamil, and norverapamil) migrated under electroosmosis in relatively sharp but asymmetric tailing zones. To eliminate the electroosmotic flow, the polyacrylamide coated capillaries[14] were used to study such chiral separations. In the pharmaceuticals examined in the separation system, a position of the chiral center next to the aromatic ring was found necessary for chiral recognition. The enantiomers of basic pharmaceuticals fulfilling this requirement were easily separated, as demonstrated in Figure 8. In comparison, the same compounds could be separated in our previous study using trimethylated β-cyclodextrin containing buffers.[16] Single maltodextrins, from pentaose to heptaose (up to 6%, w/w), added in the same buffer system did not separate any of the three basic enantiomers (structures shown in Figure 1), suggesting that a beneficial chiral interaction must be due to the oligomeric chains longer than seven residues.

Serum Samples. In the analysis of racemic ibuprofen extracted from a spiked serum, an interference-free background was first ascertained through UV detection at 220 nm, using an uncoated capillary (Figure 9B). The levels of 8.5 μg/mL (4.25 μg/mL of each enantiomer) were within the concentration range reported after a single dose of 400 mg of racemic ibuprofen.[25] Maltrin MO40 (96% content of oligosaccharides longer than pentaose) was beneficial in 5% (w/w) concentration for the separation of equal amounts of enantiomers. Using (*S*)-(+)-naproxen as an internal standard, precision of peak–height ratios for each ibuprofen enantiomer and the internal standard was 1.0% (RSD, n = 5). The migration time stability was 1.3% (RSD, n = 5). (*R*)-(−)-Ibuprofen eluted as the first peak under electroosmotic conditions. When electroosmosis was suppressed with the use of a coated capillary, the elution order changed, showing

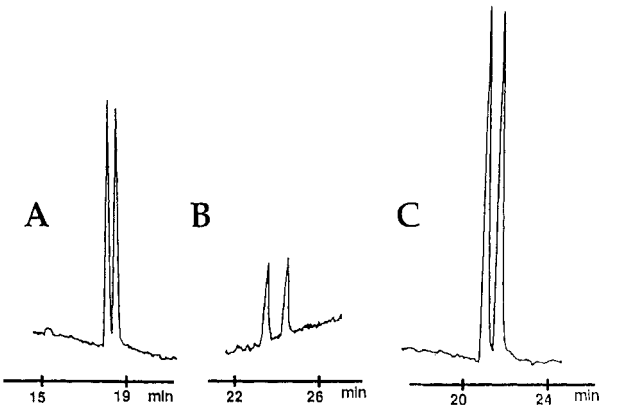

Figure 8. Separation of the basic drugs (A) verapamil, (B) norverapamil, and (C) fluoxetine with Dextrin 10, 20% (w/w) in 25 mM Tris/phosphoric acid buffer, pH 3.4. Coated capillary, 50 μm, i.d. × 60 cm (44 cm effective length); UV detection at 220 nm; run voltage, 22 kV (negative ground).

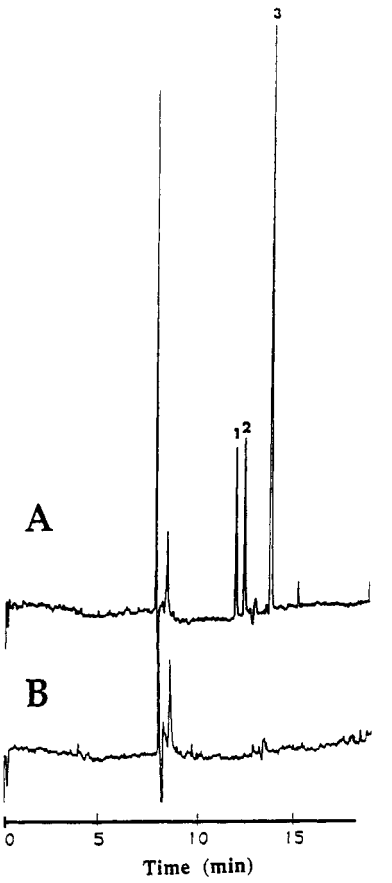

Figure 9. Electropherograms of (A) spiked serum sample (racemic ibuprofen, 8.5 μg/mL) and (B) blank serum after extraction with hexane/2-propanol. Separation buffer, Maltrin MO40 5% (w/w) in 30 mM TAPS/10 mM Tris, pH 7.8; run voltage, 24 kV. Other analytical conditions as in Figure 6. Peak 1, (*R*)-(−)-ibuprofen; peak 2, (*S*)-(+)-ibuprofen; peak 3, (*S*)-(+)-naproxen as an internal standard.

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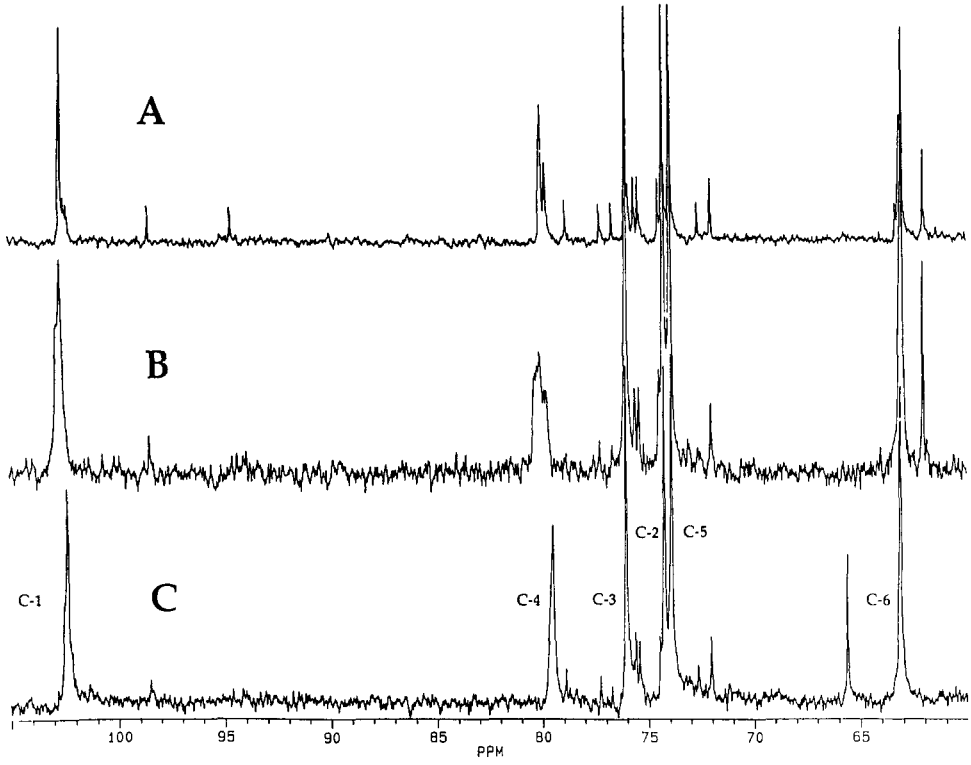

Figure 10. ^{13}C-NMR spectra for complexation of (A) racemic ibuprofen and maltoheptaose (DP = 7); (B) racemic ibuprofen and Maltrin MO40 (DP = 3–27); and (C) maltoheptaose (DP = 7) without solute. The solutions contained D_2O/Tris/TSP. ^{13}C-NMR signals (500 MHz) were proton decoupled and referenced to TSP proton signal (not shown).

(*R*)-(–)-ibuprofen binding more strongly with maltodextrins under the analytical conditions used. In comparison, the selective interaction was stronger for (*R*)-(–)-simendan than for (*S*)-(+)-simendan.

NMR Measurements. There is ample documentation of the usefulness of ^{1}H- and ^{13}C-NMR techniques in elucidating the nature of complexation between complex carbohydrates and smaller molecules, as well as in some other aspects of the advanced structures. Jane et al.,[26] investigating complexation of amyloses with dimethylsulfoxide and α-naphthol by ^{13}C-NMR, found characteristic downfield shifts on C-1 and C-4. In their interpretation, the magnitude of the shift change ratio was found to reflect compactness of the polymer helices. Similar downfield shifts of ^{13}C-NMR signals were also observed during complexation of 3–0-methyl-α-(1–4)-mannan with acyl-CoA[27] and triiodine complexes with maltodextrins of DP = 1–20.[28] In linear maltooligosaccharides, C-1 and C-4 participate in the glycosidic bond. For the proposed process of helix formation, the glycosidic bonds bend into an energetically favored conformation, in which the adjacent turns of the helix are in contact with each other. A helical arrangement of the short-chain maltodextrins during complexation with derivatized monosaccharides in a borate buffer was also suggested by the ^{1}H-NMR and fluorescence measurements in our laboratory.[9]

In agreement with the earlier studies,[26] our ^{13}C-NMR measurements registered identical spectra for maltoheptaose and Maltrin MO40 (DE 5) in the absence of chiral molecules. After ibuprofen was added, large downfield shifts were observed for both maltoheptaose and Maltrin MO40. Line broadening and peak splitting were pronounced for Maltrin MO40 (DP = 3–27) in the presence of the pharmaceutical, while for the identical experiment with maltoheptaose, the peak at about 80 ppm was split (Figure 10). A similar splitting behavior was seen with the racemic ibuprofen and the two pure enantiomers (measured individually). One can assume that the ratios of chemical shift changes correspond to a compact helical form (0.6–0.7) and the linear, extended form (1.7) of maltodextrin.[26] The two forms are at equilibrium, which is slow at the time scale of NMR measurements. The spectra for maltotriose (DP = 3) and maltotriose plus ibuprofen were also registered for the sake of comparison. In this case, the chain bending is expected to be minimal, yielding the chemical shifts for C-1 and C-4 as useful comparative values. Furthermore, if interactions of ibuprofen or buffer molecules with maltotriose were to occur, they should be considerably less pronounced with the oligosaccharides smaller than maltopentaose. (Stability constants of molecules complexing with DP = 6–12 were reported[28] relatively unchanged.) In Table 1, we show the chemical shifts for C-1 and C-4 and the characteristic ratios of shift changes, $\Delta\delta$(C-1)/$\Delta\delta$(C-4), for the solutes complexing with various maltooligosaccharides.

The observed shift change ratios (0.6–0.7) for ibuprofen complexation with DP = 7 and DP = 3–27 maltodextrins seem to support a notion of a relatively tight helix form. The broadened signal bands with DP = 3–27 also suggest multiple complexations with varying equilibrium constants, which is in agreement with the above-mentioned electrophoretic experiments, showing asymmetrical peaks. In our ^{1}H-NMR experiments with the same solutions, we observed no significant

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Table 1. Chemical Shifts and Ratios of Shift Changes C-1 and C-4 Registered with Proton Decoupled ^{13}C-NMR Spectroscopy

complexation system	δ(C-1) ppm	δ(C-4) ppm	Δδ(C-1)/Δδ(C-4)
DP 3	3	102.167	79.538
(+)-ibuprofen/DP 3	102.378	79.636	2.153
(±)-ibuprofen/DP 7	102.550	80.013	0.806
		79.780	1.583
(+)-ibuprofen/DP 7	102.444	79.935	0.688
		79.700	1.685
(−)-ibuprofen/DP 7	102.440	79.923	0.709
		79.696	1.728
(±)-ibuprofen/DP 3–27	102.612	80.070[a]	0.836
(+)-ibuprofen/DP 3–27	102.485	79.988[a]	0.707
(−)-ibuprofen/DP 3–27	102.499	80.055[a]	0.642
(−)-simendan/DP 7	102.295	79.673	1.422
(±)-simendan/DP 3–27	102.538	79.839	1.233

[a] Broad signal band.

shift changes but a clear broadening of all proton signals. The signal broadening was also more extensive for (*R*)-(−)-ibuprofen compared to (*S*)-(+)-ibuprofen, supporting the previously observed electrophoretic migration behavior that suggested a stronger complexation of the *R*-isomer.

For simendan, both ^{1}H- and ^{13}C-NMR spectra suggested interaction with a relatively extended form of the dextrin polymer. All proton signals were broadened, but the ratios of carbon shift changes were 1.2–1.4, showing little support for bending at the glycosidic bond.[26] In this respect, a higher separation selectivity at lower concentrations of Dextrin 10 observed during capillary electrophoresis could be explained by a higher stability of complex with the extended chains. Complexation at higher Dextrin concentrations might involve less beneficial side reactions for the chiral recognition of simendan, such as 1:2 complexation, which would be negligible at lower Dextrin concentrations.

The helix formation of linear maltodextrins in the presence of various complexing compounds is extensively documented, but the exact mechanisms of complexation remain uncertain. Most likely, there are different mechanisms, depending on a momentary structure of the guest molecule. Electrostatic dipole–dipole[16] and CH-π interactions as well as multiple hydrogen bonding are obviously important parameters in the binding processes.[20] There has been evidence for some complexing molecules located inside the helix cavity in several studies.[17,26] However, partial inclusion[29] and complexation between helical structures[30] have been under consideration as well. Additionally, complexation and a chirally specific complexation may sometimes be two separate processes. Our evidence for the importance of the location of a chiral center between the aromatic moiety and the negative or positive charge in the chiral recognition is consistent with the observations of the stabilizing effects of a negative charge in complexation.[18]

Through a combination of electrophoretic and NMR measurements, we have gained some valuable insights into how various chiral molecules interact with linear oligosaccharides. Similar techniques can potentially be utilized in probing additional interactions with various carbohydrate molecules. Optimization of the analytical separations of acidic pharmaceuticals have been a practically important highlight of our investigation.

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