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Dermatan sulfate as useful chiral selector in capillary electrophoresis

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Abstract

Dermatan sulfate (DS), a complex, polydispersed, sulfated polysaccharide was investigated as a useful chiral selector in capillary electrophoresis for the enantioresolution of a variety of drugs. Analysis was carried out in a fused-silica capillary column of 48.5 cm length (40 cm to detector window)×50 μm I.D., and the separation buffer consisted of citric acid–Tris containing DS; the applied voltage was 15 kV and the detection wavelength was 220 nm. The effects of buffer pH, the dermatan concentration and run temperature on the enantioseparation and migration were examined. The method was applied to the enantioresolution of a representative set of twenty basic drugs. At all pH values used (3.0, 4.5 and 6.5) the addition of DS resulted in an increased migration time due to analyte–DS interaction. Using DS concentration of 2% (w/w), at pH 4.5, enantiomeric separations could be obtained for more than 50% of the examined drugs; resorcinic moiety was found to be a very favourable structural feature for obtaining high enantioresolution values.

Keywords: Chiral selectors; Enantiomeric separation; Dermatan sulfate; Drugs

1. Introduction

Capillary electrophoresis (CE), has become popular in various analytical fields owing to its high-resolving power, fast separation, minute sample loading and easy automation [1–5]. An interesting area of separation is that of chiral molecules: enantioresolution is an important issue in pharmaceutical and medical sciences because chirality can significantly affect biological activity.

Much work has been reported on the direct resolution of enantiomers of drugs by CE [3–5]. Most of the chiral selectors utilized in CE are water soluble and can be conveniently added to the running buffer; among these selectors, cyclodextrins and their derivatives have found a wide application in capillary zone electrophoresis (CZE) [4,5]. Electrokinetic chromatography (EKC) based on ionic polysaccharides constitutes another way to obtain enantioresolution; recently, linear maltodextrin oligosaccharides [6,7] and mucopolysaccharides as sodium heparin and chondroitin sulfate (A and C) [8–10] have been found to be effective chiral selectors in EKC mode.

This work was aimed at exploring the potential of dermatan sulfate (DS) as chiral selector in CE for the

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enantioseparation of racemic cationic drugs. DS is a complex, polydispersed, sulfated polysaccharide whose polysaccharide chains are mainly composed of a disaccharide unit: (1→4)–O–(α-idopyranosyluronic acid)–(1→3)–O–(2-acetamido-2-deoxy-β-D-galactopyranosyl-4-sulfate) (Fig. 1) [11].

DS unit structure differs from that of heparin and chondroitins for sites and sulfation degree and also for the presence of α-2-iduronic acid. The high anionic character, providing high aqueous solubility and considerable electrophoretic mobility, in conjunction with its inherent chirality, confers on DS interesting properties as potentially useful chiral selector to achieve enantiomeric separations by CE.

In the present study a series of racemic basic drugs, belonging to different therapeutic categories and representative of a wide structural variety, were analyzed by CE using DS as chiral selector. The influence of parameters such as buffer solution pH, DS concentration and run temperature on migration time and resolution was evaluated.

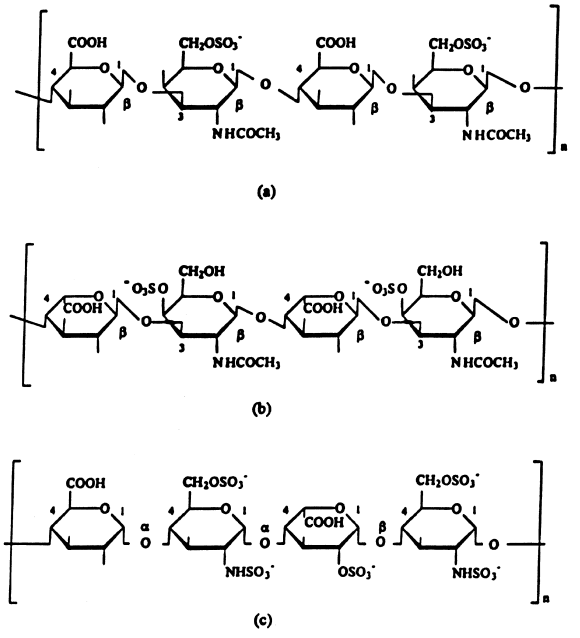

Fig. 1. Unit structure of anionic polysaccharides: (a) chondroitin sulfate C, (b) dermatan sulfate, (c) heparin.

2. Experimental

2.1. Apparatus

The experiments were performed with a ^{3D}CE system (Hewlett-Packard, Palo Alto, CA, USA) equipped with a diode array detection system; data acquisition processing were done by HP Vectra 486/100 XMZ computer. All the electrophoretic separations were carried out using fused-silica capillaries of 48.5 cm length (40 cm to the detector)×50 μm I.D., purchased from Supelco (Milan, Italy) and operating at 15°C. The samples were introduced hydrodynamically for 10 s (injection pressure 5 kPa) and they were monitored by UV detection at 220 nm. The applied voltage was held constant at 15 kV.

2.2. Materials

Porcine DS and sodium heparin from mucosa and from skin were a kind gift from Opocrin (Corlo, Italy). The used polymers are characterized by molecular mass averages including: number-average (M_n), mass-average (M_w) and *z*-averaging (M_z). In particular M_z, is correlated to the polymer flex life and stiffness. DS number average (M_n=15 270), mass average (M_w=22 260), *z*-averaging (M_z=30 390), polydispersity ($D=M_w/M_n$=1.458) and sodium heparin number average (M_n=10 180), mass average (M_w=14 630), *z*-averaging (M_z=20 420) and polydispersity (D=1.438) were determined by calibration curve plotted with standards of glycosaminoglycans having known molecular mass, evaluated by means of analytical ultracentrifugation, according to Nieduszinski [12,13].

Tris buffer and citric acid were obtained from Carlo Erba (Milan, Italy); chlorpheniramine maleate, atenolol, metoprolol tartrate, pindolol, alprenolol, propranolol hydrochloride, acebutolol hydrochloride, salbutamol hemisulfate, isoproterenol hemisulfate, terbutaline hemisulfate, metaproterenol hemisulfate, chloroquine diphosphate, primaquine diphosphate and phenylalanine were purchased from Sigma (St. Louis, MO, USA).

Verapamil hydrochloride was from Fluka (Buchs, Switzerland), baclofen was from Ciba-Geigy (Origgio, Italy), dimethindene maleate was from Novartis

(Origgio, Italy), promethazine was from Pharmacia–Upjohn (Nerviano, Italy) and tryptophan was from Janssen (Geel, Belgium). Acetone, used as a tracer of the electroosmotic flow (EOF) was obtained from Carlo Erba.

Purified water from a TKA ROS 300 system was used to prepare buffers and standard solutions.

2.3. Procedure

Running buffer solutions were consisted of 25 m*M* citric acid solution adjusted to pH 3.0, pH 4.5 or pH 6.5 with Tris base: DS or heparin sulfate were then dissolved at the appropriate concentration in the buffer solution without changing in the pH values. The running buffer solutions and the analytes solutions (0.1 mg/ml in water) were filtered through 0.45 μm Millex-HV filter units (Millipore, Milford, MA, USA) prior to use. The capillary was conditioned prior to each run for 3 min with the separation electrolyte.

3. Results and discussion

DS is a polysaccharide belonging to the family of glycosaminoglycans with anticoagulant, profibrinolytic and antithrombotic properties. DS primarily catalyzes the inhibition of thrombin by heparin cofactor II (HC II) [11]. The structure of DS is largely represented by the repeating disaccharide sequences [IdoA-GalNAc4SO_3] where IdoA is α-L-iduronic acid and GalNAc4SO_3 is N-acetyl-β-galactosamine 4-O-sulfated, linked 1,3 and 1,4 respectively [11]. This structure is shown in Fig. 1. As shown, there is 0.5 sulfonic group per monosaccharide residue in DS, while there are 1.2 in heparin; moreover, the molecular mass of DS (≈22 000) is larger than that of heparin (≈14 600). This means that the electrophoretic mobility of DS is lower than that of heparin and this can affect both the migration and enantioseparation of basic racemic solutes.

Therefore, the conditions to develop a method for the enantioresolution of a set of representative basic drugs using DS as chiral selector were investigated.

3.1. Effect of pH on enantioresolution

The presence of the sulfate groups allowed these biopolymers to be used under acidic conditions: the effects of run buffer pH on effective mobility and resolution for the studied molecules are summarized in Table 1.

For each compound the migration time without DS is given at pH 3.0; all the analytes at the studied pH values migrated faster than the EOF. In the presence of DS a reduction in the effective mobility was observed for all the tested drugs (Table 1) and it is likely due to the strong ionic interaction between anionic DS and the cationic analytes.

At the studied pH values, the best results in terms of resolution were generally obtained at pH 4.5. Under more acidic conditions (pH 3.0) a general loss of resolution was observed although the EOF was slower than that at pH 4.5; nevertheless, the enantioresolution of pindolol, tetrahydrozoline and primaquine was improved. It is possible to assume that at this low pH value the carboxylic groups are prevalently undissociated and a cooperative effect of hydrogen bond interactions by the carboxylic groups with the ionic interactions by sulfate groups of DS may improve the enantioresolution of the cationic analytes.

For the racemic isoproterenol, terbutaline and metaproterenol very good enantioresolutions were obtained at pH 4.5 (Table 1, Fig. 2). These compounds exhibit some common characteristics such as a chiral center bearing a hydroxyl group and an aromatic ring, and a secondary amine located at position 2 from the chiral center. In addition, when the analytes possesses a resorcine moiety, very good resolution was observed. The presence of hydroxyl group on the aromatic ring seems to be favourable for the chiral recognition by DS; isoproterenol (catecholic system) was fully resolved (R_s=1.9), while salbutamol, that differs for the hydroxymethyl group at the position 3 on the aromatic ring, and for the ternary nitrogen in the side chain, was only partially resolved at any pH value. These results suggest that hydrogen-bonding interactions play an important role in the enantioselectivity of DS. In effect, β-blocker drugs (atenolol, metoprolol, alprenolol etc.), bearing a similar chiral side chain but

Table 1
Electrophoretic migration times (min), effective mobilities ($cm^2\ V^{-1}\ s^{-1}\cdot 10^{-5}$) and enantioresolution values for the tested compounds using DS as chiral selector

Compound	pH 3.0				pH 4.5			pH 6.5		
	t_m	t'_m	μ_e	R_s	t'_m	μ_e	R_s	t'_m	μ_e	R_s
Isoproterenol	9.0	23.0	0.7	0.8	21.0	−0.3	1.9	13.6	2.6	1.5
Terbutaline	9.5	22.0	1.2	3.3	21.0	−0.3	6.0	13.8	2.4	5.0
Metaproterenol	8.8	21.5	1.4	2.5	19.5	0.5	4.3	14.8	1.3	4.1
Salbutamol	7.1	15.0	5.8	0.9	13.2	38.5	0.9	9.7	8.8	0.8
Atenolol	9.6	21.3	1.5	–	16.1	2.9	–	12.0	4.8	–
Metoprolol	9.5	20.5	1.9	–	15.2	3.7	–	12.0	4.8	–
Alprenolol	8.5	10.0	13.0	–	10.7	9.5	–	11.2	6.0	–
Propranolol	8.3	22.3	1.0	–	16.7	2.4	–	12.3	4.3	–
Acebutolol	10.7	26.0	−0.3	–	18.8	0.9	0.7	13.4	2.8	–
Pindolol	8.7	23.0	0.7	1.0	13.8	5.1	–	12.2	4.4	0.7
Chlorpheniramine	6.2	/	/	–	22.0	−0.7	1.3	12.3	4.3	–
Baclofen	9.5	40.0	−3.2	0.7	48.6	−6.0	–	20.4	−2.7	–
Verapamil	10.8	/	/	–	/	/	–	13.4	2.8	–
Dimethindene	5.4	23.8	0.4	1.2	21.7	−0.5	1.0	11.0	5.8	–
Tetrahydrozoline	8.0	15.8	5	1.3	14.8	4.0	1.1	9.7	8.8	–
Phenylalanine	14.5	35.8	−2.6	–	31.5	−1.1	–	17.0	−0.6	–
Tryptophan	13.8	35.1	−2.4	–	35.2	−1.2	–	17.4	−0.8	–
Chloroquine	5.4	/	/	–	/	/	–	26.7	−5.2	1.5
Primaquine	5.6	26.8	−0.6	2.22	16.0	3.0	0.8	9.7	8.8	–
Promethazine	7.0	11.0	10.4	–	12.6	6.6	–	10.7	6.8	–

R_s: resolution.
t_m: migration time without DS.
t'_m: migration time in presence of DS (2%); it is relative to the first migrating enantiomer.
–: no resolution
/: migration time over 60 min.
Uncoated capillary of 48.5 cm (40 cm to the detector)×50 μm I.D., T=15°C. Voltage=15 kV. Hydrodynamic injection (10 s). UV detection at 220 nm.

lacking the appropriate aromatic substituents, were not fully enantioresolved.

Chloroquine, a very hydrophobic analyte, (log P=4.63, where P is *n*-octanol–water partition coefficient) [14], shows a high positive charge density and its low effective mobility (migration time over 60 min., at pH 3.0 and pH 4.5) can be ascribed to its strong ion interaction with DS. Under less acidic conditions (pH 6.5) the reduced positive charge of the protonated nitrogens (pK_a 8.8; 10.16) allows to obtain two well separated enantiomers in a relatively short time.

Promethazine (pK_a=9.1) is a hydrophobic drug as well as chloroquine (almost the same measured log P) but migrated at each pH value in a short time without separation. The lack of a quinolinic nitrogen and tricyclic structure could explain the minor selector–selectand interactions in terms of reduced positive charge density and steric hindrance.

The antiarrythmic drug verapamil, possesses only a single nitrogen moiety and exhibits low effective mobility at acidic pH values (t_m>60 min). Increasing the pH to 6.5, the drug showed a t_m of 13.4 min but was not enantioresolved.

The antihistamine drugs chlorpheniramine and dimethindene were partially enantioresolved at pH 4.5. They posses structural analogies (alkylamine and pyridine moieties) but chlorpheniramine is one of the more lipophilic tested analytes and the pH value (4.5) was found to be critical for the enantioresolution.

For amino acid compounds such as baclofen, phenylalanine and tryptophan with hydrophilic properties (negative log P values), characteristic, slow

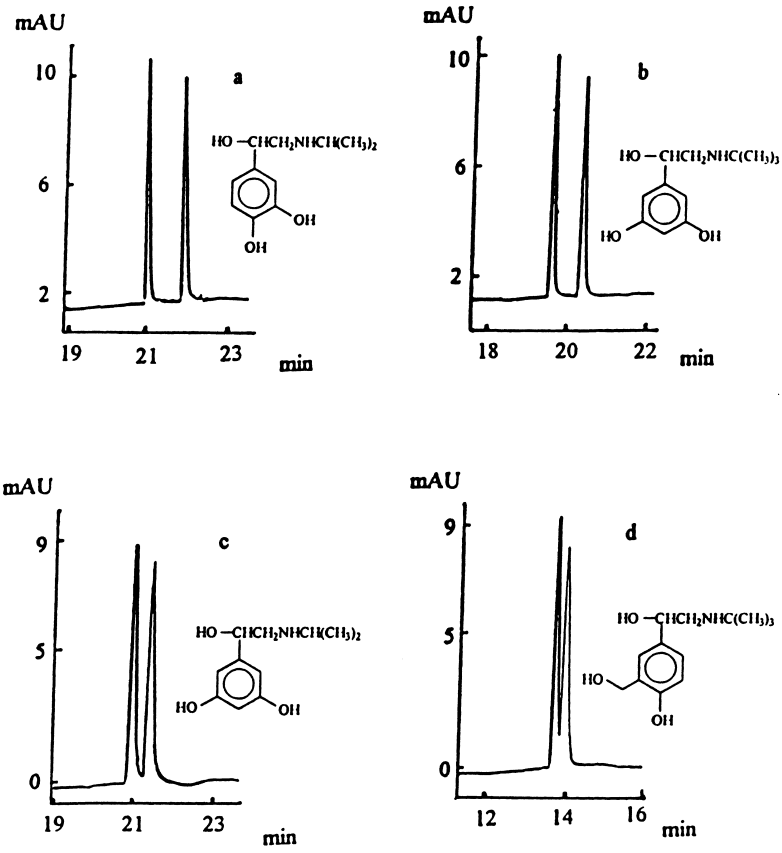

Fig. 2. Separations of enantiomers of terbutaline (a), metaproterenol (b), isoproterenol (c) and salbutamol (d) by EKC with DS. Conditions: buffer, 25 m*M* citric acid adjusted to pH 4.5 with Tris base containing 2% DS; fused-silica capillary 48.5 cm (40 cm effective length)×50 μm I.D.; applied voltage, 15 kV; detection 220 nm; temperature 15°C; injection time 10 s.

mobilities were observed. At pH 3.0, strong interactions between DS and amino acid compounds can be ascribed to electrostatic interactions and also to hydrogen-bonding due to the presence of the undissociated carboxylic groups on both selector and selectand analyte. In these pH conditions a partial enantioresolution was obtained for baclofen. At pH 6.5 a minor difference in the mobility with and without DS ($\Delta\mu_e \approx 0.5 \cdot 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$) was observed for the studied amino acids; this could be due to the fact that these compounds are in the zwitterionic form (baclofen: pK_a 3.9, 9.6; phenylalanine: pK_a 2.58, 9.24; tryptophan: pK_a 2.38, 9.39) and hence with a low charge; weak electrostatic interactions with the chiral selector could be responsible for the lack of enantioresolution.

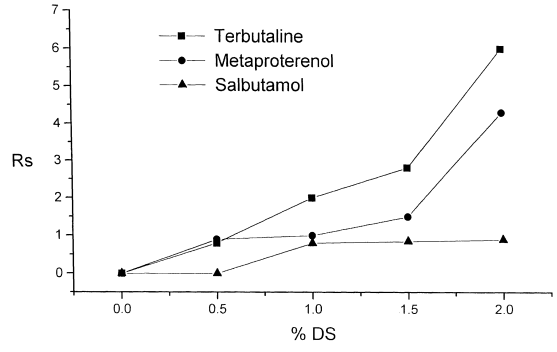

Fig. 3. Effects of the concentration of DS on the enantioseparation of terbutaline, metaproterenol and salbutamol. Buffer: 25 m*M* citric acid adjusted to pH 4.5 with Tris base, containing 0–2% DS. Other conditions as in Fig. 2.

Table 2
Effect of DS concentration on migration time (t_m, min) of three selected drugs and on the EOF ($cm^2\ V^{-1}\ s^{-1}\cdot 10^{-5}$)

Analyte	0% DS	0.5% DS	1% DS	1.5% DS	2% DS
Salbutamol	7.1	11.0	12.0	13.0	13.2
Metaproterenol	8.6	10.3	13.0	15.0	19.5
Terbutaline	8.2	10.0	12.8	16.4	21.0
EOF	8.6	21.5	15.4	14.4	10.5

Conditions: buffer, 25 m*M* citric acid adjusted to pH 4.5 with Tris base.
Uncoated capillary of 48.5 cm (40 cm to the detector)×50 μm I.D., T=15°C. Voltage=15 kV. Hydrodynamic injection (10 s). UV detection at 220 nm.

As a whole, an appropriate pH adjustment was found to improve the chiral recognition by DS and about 50% of the representative set of basic drugs was enantioresolved.

3.2. Effect of DS concentration

The effect of DS concentration on enantioresolution was investigated at pH 4.5 for selected, enantioresolved compounds. The resolution values of salbutamol, metaproterenol and terbutaline were evaluated over the 0–2.0% DS concentration range. As shown in Fig. 3, salbutamol reached maximum resolution at 1% concentration of DS, whereas metaproterenol and terbutaline exhibited a strong increase in resolution between 1.5 and 2%. The increase of the enantioresolution with the DS concentration was accompanied by an increase in the migration time. This effect was also due to the reduction of the EOF caused by the increased viscosity of the run buffer (Table 2). Thus, a concentration of 2% was chosen as the best compromise between good resolution and short analysis time. In fact, higher DS concentrations resulted in longer analyses with negligible gain in the enantioresolution.

All the electrophoretic runs were carried out at 15°C. Limited experiments on primaquine and chlorpheniramine, showed that higher temperatures increased the analyte mobilities yielding worse enantioresolution (Fig. 4). These effects were found to be comparable to those observed when heparin was used.

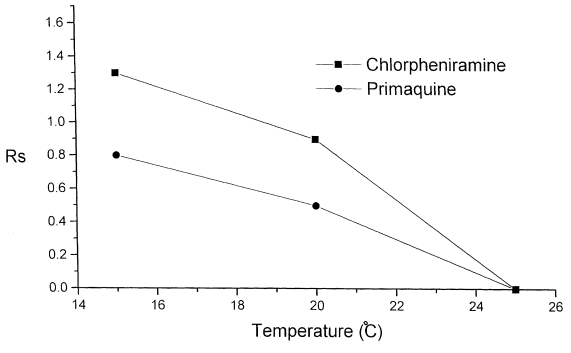

Fig. 4. Effects of the run temperature on the enantioresolution of chlorpheniramine and primaquine. Buffer: 25 m*M* citric acid adjusted to pH 4.5 with Tris base and containing 2% DS. Other conditions as in Fig. 2.

4. Conclusion

DS, an ionic polysaccharide, was found to be an effective chiral selector in CE, under acidic conditions (EKC) mode. CE with DS was successful for the enantioseparation of a number of basic racemic drugs, particularly for structures characterized by a phenolic moiety. The large R_s values obtained for various drugs makes the method useful for enantiomeric purity testings. Although the enantioseparation mechanism with mucopolysaccharides is not clear, the data obtained support the supposition that a combination of ionic, hydrogen-bonding and hydrophobic interactions is the basis of the chiral separation.

Studies are in progress to investigate the effect of the molecular mass and the sulfation degree on the enantioselectivity of glycosaminoglycans as chiral selectors in CE.

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