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Evaluation of the enantioselectivity of glycogen-based dual chiral selector systems towards basic drugs in capillary electrophoresis

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ABSTRACT

Several chiral reagents including cyclodextrins (CDs) and derivatives, crown ethers, proteins, chiral surfactants and polymers have been involved in dual selector systems for enantioseparation of a series of chiral compounds by capillary electrophoresis (CE). In comparison to the chiral reagents abovementioned, there is no report concerning the use of polysaccharides in dual chiral CE system. In this paper we first investigate the enantioselectivity of polysaccharide-based dual selector systems towards some chiral drugs. During our recent work, glycogen belonging to the class of branched polysaccharides has been used as a novel chiral selector in CE. In this study, three glycogen-based dual chiral CE systems have been established for enantiomeric separations of several racemic basic drugs consisting of duloxetine, cetirizine, citalopram, sulconazole, laudanosine, amlodipine, propranolol, atenolol and nefopam. These three dual systems combined glycogen (neutral polysaccharide) with chondroitin sulfate A (CSA, ionic polysaccharide), β-CD and HP-β-CD, respectively. It was found that the dual system of glycogen/CSA exhibited good enantioselective properties toward the tested drugs. More importantly, compared to the single selector systems, synergistic effect was observed when glycogen was used with CSA for most of the analytes. This indicated the enhancement of enantioseparation observed for these analytes in glycogen/CSA system might be due to some favorable interaction effects between glycogen and CSA. Moreover, in order to evaluate the stereoselectivity of glycogen/CSA, the influences of buffer pH and selector concentration on enantioseparation of the studied drugs were also investigated.

1. Introduction

Over the past decade, capillary electrophoresis (CE) has also become a powerful technique for the separation of enantiomers [1–7]. In CE, various kinds of chiral selectors have been developed, including cyclodextrins (CDs) and derivatives, polysaccharides, crown ethers, antibiotics, proteins, chiral surfactants and polymers, etc. However, the utility of these reagents as a single chiral selector to resolve enantiomers is not always satisfactory. Consequently, use of more than one chiral reagent in CE to improve the enantioseparation has drawn increase attention in recent years [8–14]. Although some of the afore-mentioned selectors have been utilized in dual CE separation systems for chiral analysis of a variety of compounds, there is no report concerned with the use of polysaccharides in dual system. In this paper we first evaluate of the enantioselectivity of polysaccharide-based dual selector systems towards some chiral drugs. The systems in this study combined a neutral polysaccharide with an ionic polysaccharide and two neutral CDs. The first dual system was presented by Kuhn et al. [15] in 1992. A synergistic effect on the separation of tryptophan enantiomers was found for CD (α-CD) and crown ether (18-crown-6 tetracarboxylic acid) dissolved in the same buffer electrolyte. Subsequently, a dual CD system was developed by Lurie et al. [16] for the separation of cationic drugs of forensic interest (amphetamine, cathinone, cocaine, cathine, etc.). The electrolyte solution contained a neutral CD derivative (DM-β-CD) and a charged CD derivative (sulfobutyl ether-β-CD). The concentration ratio of the two chiral selectors seemed the most important factor to influence the resolution and the migration times. Similarly, dual systems of CDs implementing a cationic β-CD-NH_2 and a neutral CD (TM-β-CD or DM-β-CD) were studied by Lelievre et al. [17] for separation of arylpropionic acid enantiomers. To sum up, Fillet et al. [18] presented an overview on the use of mixtures of neutral and charged CDs for the enantioseparation of drugs in CE. In this review, a brief theoretical background and

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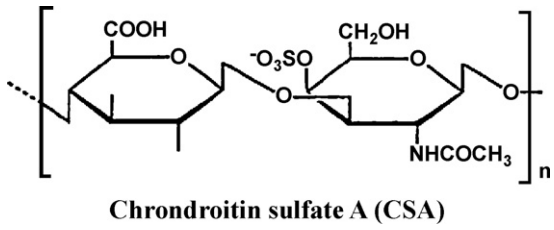

Fig. 1. Unit structure of chondroitin sulfate A (CSA) employed in this paper.

some typical examples of applications in the pharmaceutical field were described. Based on these reports as well as other references [19–25], in most cases combination of an electrically neutral selector and another ionic selector could probably improve selectivity and resolution in enantioseparation by CE.

Polysaccharides have been demonstrated to exhibit prominent enantioselective properties toward plenteous chiral compounds [26–34]. They have very low absorbance in the UV region, which is beneficial for high detection sensitivity. Additionally, varying structures and functional groups of polysaccharides provide a range of enantioselectivity in CE and many of them are water-soluble. Some examples that have been applied in chiral CE separation include chondroitin sulfates, dextrans, dextrins, aminoglycosides, carrageenans, heparin, etc. Chondroitin sulfates are naturally occurring charged polysaccharides employed successfully for CE enantiomer separation. They are linear mucopolysaccharides with N-acetylchondrosine as a repeating unit. The unit structure of chondroitin sulfate A (CSA) is shown in Fig. 1.

Recently, we have reported the use of glycogen as a novel polysaccharides selector for enantioseparation of some chiral compounds [35]. Glycogen is an electrically neutral and branched polysaccharide of high molecular weight playing a role in many tissues of the human and animal body as a storage carbohydrate. It has an average molecular weight of several million with α-(1,4)-linked glucose subunits and α-(1,6)-linked branching [36]. The unit of its structure is shown in Fig. 2. Additionally, glycogen possesses not only high solubility but also low viscosity in the water. With the lack of aromatic rings in the structure, it also exhibits very weak UV absorption. In this paper, we further investigated and evaluated the enantiorecognition capability three glycogen-based dual chiral selector systems towards several basic drugs. In the course of this work, synergistic effect was observed in the system of glycogen/CSA, while negative effects were in glycogen/β-CD and glycogen/HP-β-CD. Moreover, the influences of buffer pH and selector concentration on enantioseparations of the studied drugs in glycogen/CSA system were also investigated.

2. Experimental

2.1. Materials

Glycogen (≥90%) was purchased from Takeda Pure Chemicals, Ltd. (Osaka, Japan). CSA (sodium salt; molecular mass, 30,000–50,000) from whale cartilage was purchased from Seikagaku Kogyo (Tokyo, Japan). β-CD and HP-β-CD were purchased from Xi'an Deli Biology & Chemical Industry Co., Ltd. (Xi'an, China). Laudanosine (LAU), propranolol hydrochloride (PRO), and atenolol (ATE) were purchased from Sigma (St. Louis, MO, USA). Duloxetine hydrochloride (DUL), nefopam hydrochloride (NEF), sulconazole (SUL) and amlodipine besylate (AMB) were supplied by Jiangsu Institute for Food and Drug Control. Citalopram hydrobromide (CIT) and cetirizine hydrochloride (CET) were obtained from Chongqing Lummy Pharm Techn Co., Ltd. (Chongqing, China). All these drug samples were racemic mixtures.

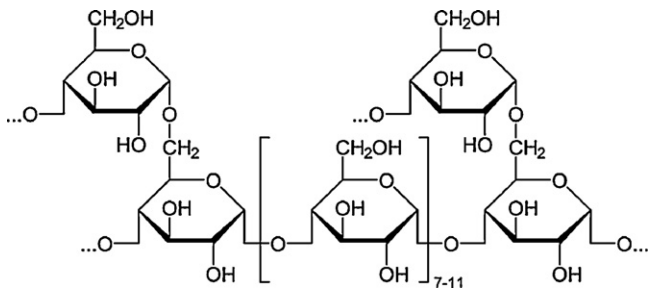

Fig. 2. Unit structure of glycogen used in this paper.

Methanol of HPLC grade, was purchased from Jiangsu Hanbon Sci. & Tech. Co., Ltd. (Nanjing, China). Sodium hydroxide, hydrochloric acid, phosphoric acid, and sodium dihydrogen phosphate, all of analytical grade, were purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Double distilled water was used throughout all the experiments.

2.2. Instrumentation

Electrophoretic experiments were performed with an Agilent 3D capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany), which consisted of a sampling device, a power supply, a photodiode array UV detector (wavelength range from 190 to 600 nm) and a data processor. The whole system was driven by Agilent ChemStation software (Revision B.02.01) for system control, data collection, and analysis. It was equipped with a 50 cm (41.5 cm effective length) × 50 μm ID uncoated fused-silica capillary (Hebei Yongnian County Reafine Chromatography Ltd, Hebei, China). A new capillary was flushed for 10 min with 1 M HCl, 10 min with 1 M NaOH and 10 min with water, respectively. At the end of each day it was flushed successively with 0.1 M NaOH (10 min) and water (10 min). Between consecutive injections the capillary was rinsed with 0.1 M NaOH, water and running buffer for 3 min each. Sample injections were performed by pressure (50 mbar, 4 s). Enantioseparations were performed at a constant voltage in a range of 15–25 kV, and the temperature of capillary was controlled at 20 °C using an air-cooling system. The DAD was set at 230 nm (DUL and LAU), 210 nm (NEF and AMB), 220 nm (CET and SUL), 237 nm (CIT), 289 nm (PRO), 225 nm (ATE).

2.3. Preparation of BGE and sample solutions

Phosphate buffers were used as the buffers for CE. Buffer solution was a 40 mM phosphate buffer. The running background electrolytes containing glycogen and CSA were freshly prepared by dissolving glycogen (3%, w/v, if not stated otherwise) and CSA (2%, w/v, if not stated otherwise) in the buffer solutions having a specified pH, and then adjusting pH exactly to a desired value by adding a small volume of 10% (w/v) phosphoric acid or 1 M sodium hydroxide solution using a microsyringe. The pH values of these running buffers were adjusted in the range of 1.5–4.0, and then were checked before and after a run by a microglass electrode if needed. Each buffer solution was filtered through a 0.45 μm pore membrane filter before use.

The stock solution of each racemic sample was prepared at a concentration of 1 mg/ml in methanol. Sample solutions for CE were prepared by diluting one of the stock solutions with water to a concentration of 0.1 mg/ml.

2.4. Calculations

The resolution (Rs) and selectivity factor (α) of the enantiomers were calculated from $Rs = 2(t_2 - t_1)/(w_1 + w_2)$ and $\alpha = t_2/t_1$, where t_1 and t_2 are the migration times of the two enantiomers, and w_1 and w_1 are the widths of their peaks at the baseline.

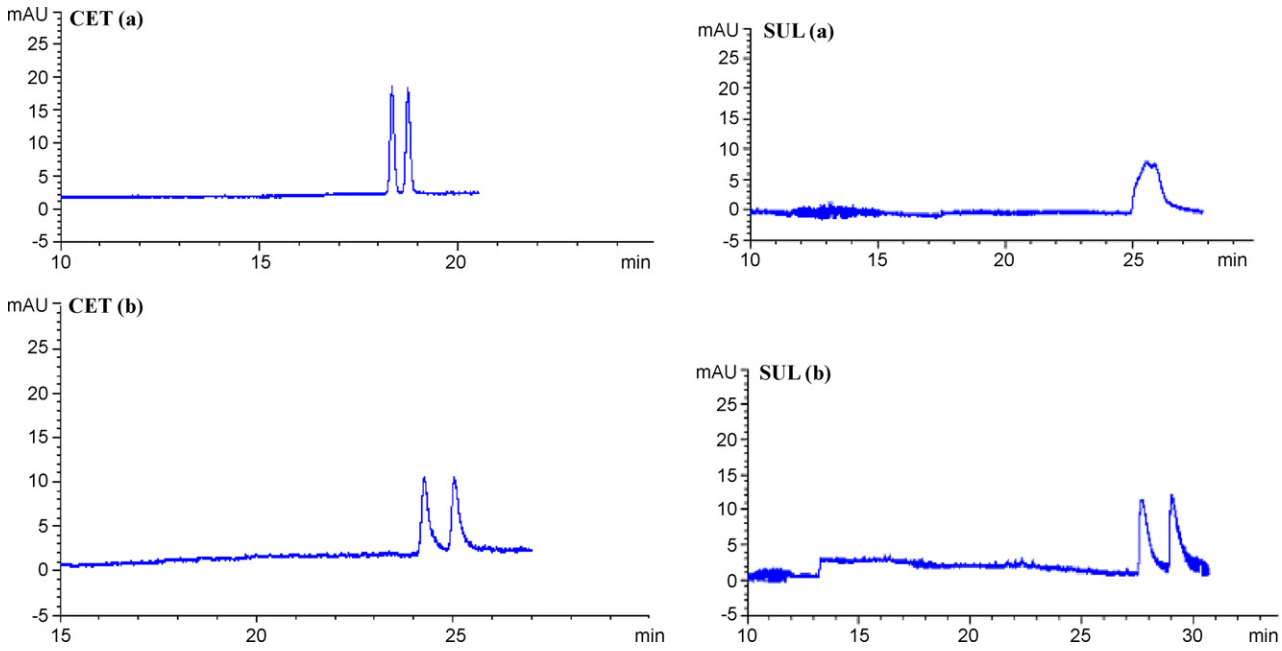

Fig. 3. Electropherograms of the chiral separations of CET and SUL in glycogen/CSA system. Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm ID; BGE, 40 mM phosphate buffer at pH 3.0; selector concentration, 3%glycogen alone for CET(a), 2%CSA alone for SUL(a), 3%glycogen + 2%CSA for CET(b) and SUL(b); applied voltage, 25 kV (CET) and 15 kV (SUL); capillary temperature, 20 °C.

3. Results and discussion

Several basic drugs were tested for the evaluation of enantioseparation capability of three glycogen-based dual chiral CE systems including glycogen/CSA, glycogen/β-CD and glycogen/HP-β-CD. Among them, glycogen/CSA showed good enantioselective properties towards most tested drugs. Table 1 shows the resolution (Rs) of these drugs obtained in glycogen/CSA system. As seen, this system allowed baseline separation of the enantiomers of DUL, CET, CIT and SUL. Furthermore, in glycogen/CSA synergistic effects were observed for the separations of DUL, CET, CIT, SUL, LAU, PRO and ATE, compared with the separation results obtained by the use of glycogen or CSA alone. These valuable results demonstrated that glycogen and CSA were complementary in respect of enantiorecognition. This also revealed the synergistic effects observed for these analytes in glycogen/CSA system might be due to some favorable interaction effects between glycogen and CSA. Polysaccharides have a number of stereogenic centers and functional groups, allowing them to have multiple interactions with chiral molecules. Electrostatic, dipole–dipole, π–π, hydrophobic interactions, hydrogen bonding and steric repulsion are assumed to be the interactions responsible for chiral recognition. In regard to neutral polysaccharides, two primary interactions are probably thought to be hydrogen bonding and hydrophobic interactions [28,29]. Since glycogen is a neutral polysaccharide, the hydrogen bonding existing between the amino and hydroxyl groups in basic analytes and the hydroxyl groups in glycogen is presumably expected to play a major role for the enantiorecognition. As regards charged polysaccharides, electrostatic interaction between the amino groups in basic analytes and the sulfate group in CSA can be mainly responsible for the chiral recognition [28,29]. In this study, the mixture of neutral and charged polysaccharides combines electrostatic interaction with hydrogen bonding and hydrophobic interactions. This induces or enhances the enantioseparation of these basic drugs when dual polysaccharide system is used. Examples of electropherograms (CET and SUL) are shown in Fig. 3. In addition, negative effect was observed in glycogen/CSA system for AMB. As shown in Table 1, glycogen (alone) allowed partial separation of the enantiomers of AMB, and CSA (alone) allowed baseline separation. However, glycogen/CSA system showed no enantioselectivity to AMB (see Fig. 4). In order to investigate this negative effect obtained, (−)-AMB was added in racemic AMB sample for enantioseparation using glycogen or CSA alone. As a result, it was observed that the order of the peaks of (−)-AMB and (+)-AMB was opposite (shown in Fig. 5). Thereby it can be concluded that the mixture of glycogen and CSA may counteract each enantioselectivity towards AMB.

In order to evaluate the stereoselectivity of dual system of glycogen/CSA towards the studied drugs, the effects of buffer pH and selector concentration on enantioseparation were also investigated.

Table 1
The resolution (Rs) of nine tested drugs obtained in glycogen/CSA system.

Chiral compounds	Glycogen/CSA system		
	Glycogen	CSA	Glycogen/CSA
DUL	1.84	–	3.93
CET	2.08	–	2.40
CIT	1.78	–	2.01
SUL	–	0.37	1.87
LAU	–	0.58	0.91
AMB	0.65	2.19	–
PRO	–	–	0.42
ATE	–	–	0.34

Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm ID; BGE, 40 mM phosphate buffer at pH 3.0; glycogen concentration, 3% (w/v); CSA concentration, 2% (w/v); capillary temperature, 20 °C; applied voltage, 25 kV (DUL, CET, CIT and AMB), 15 kV (SUL and LAU), 20 kV (PRO and ATE); –, not separated.

3.1. Glycogen/β-CD and glycogen/HP-β-CD systems

Five basic drugs consisting of DUL, NEF, CET, CIT and AMB were tested as model drugs for the evaluation of enantioselectivity of glycogen/β-CD and glycogen/HP-β-CD systems, and it was found that they showed poor enantioselectivity to the five drugs through optimization of the experimental conditions. As shown in Table 2, both of the systems had negative effects on the separations of these drug enantiomers under our experimental conditions. This

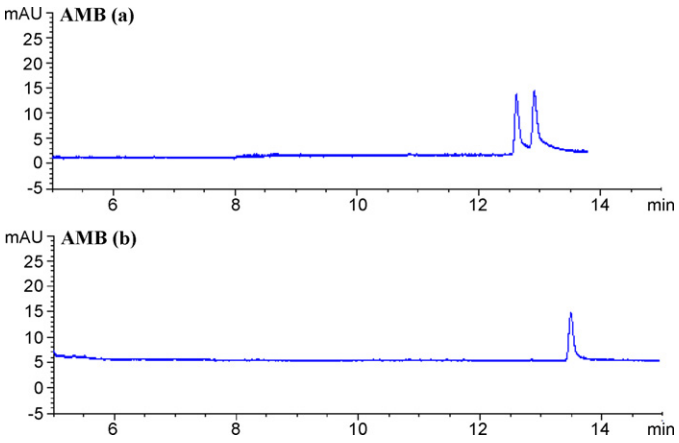

Fig. 4. Electropherograms of the chiral separation of AMB in glycogen/CSA system. Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm ID; BGE, 40 mM phosphate buffer at pH 3.0; selector concentration, 2%CSA alone (a), 3%glycogen + 2%CSA (b); applied voltage, 25 kV; capillary temperature, 20 °C.

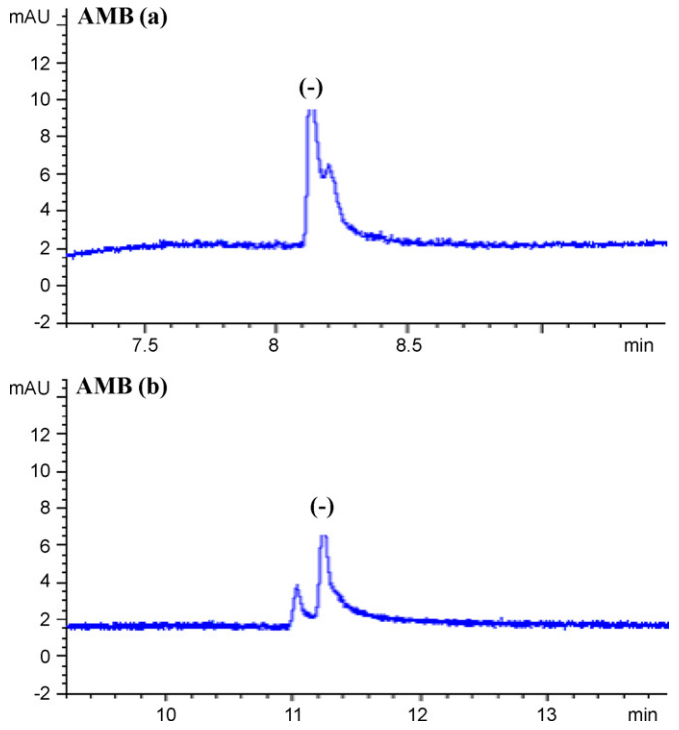

Fig. 5. Electropherograms of the chiral separation of AMB using glycogen or CSA alone. Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm ID; BGE, 40 mM phosphate buffer at pH 3.0; selector concentration, 3%glycogen alone (a), 2%CSA alone (b); (−)-AMB concentration, 0.15 mg/ml; (+)-AMB concentration, 0.05 mg/ml; applied voltage, 25 kV; capillary temperature, 20 °C.

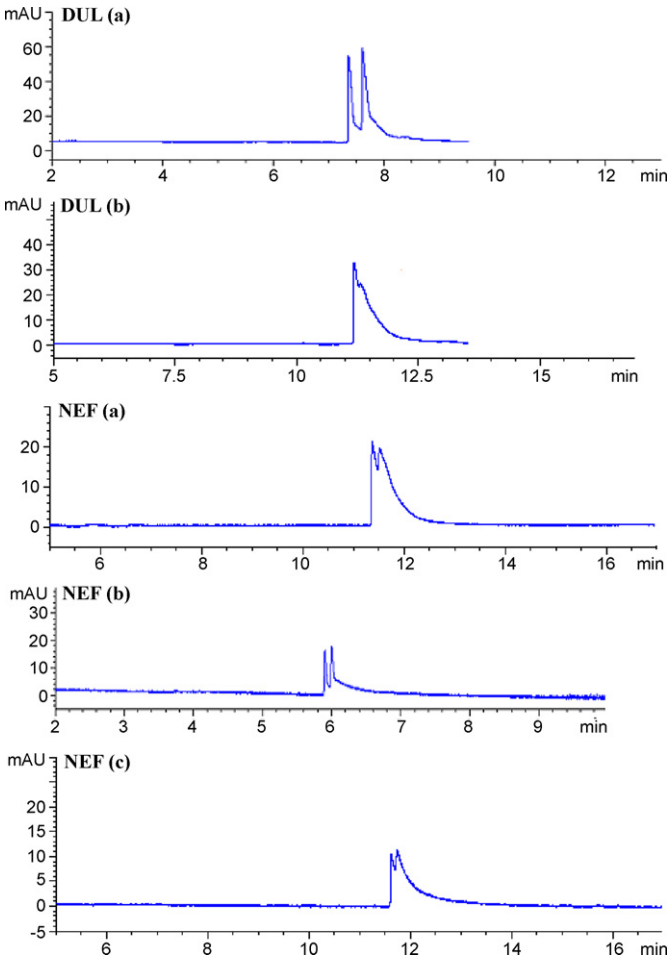

Fig. 6. Electropherograms of the chiral separations of DUL and NEF in glycogen/HP-β-CD system. Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm ID; BGE, 40 mM phosphate buffer at pH 3.0; selector concentration, 3%glycogen alone for DUL(a), 3%glycogen + 20 mM HP-β-CD for DUL(b), 20 mM HP-β-CD alone for NEF(a), 3%glycogen alone for NEF(b), 3%glycogen + 20 mM HP-β-CD for NEF(c); applied voltage, 25 kV; capillary temperature, 20 °C.

revealed that combination of two electrically neutral chiral selectors might probably bring negative effect in enantioseparation. Examples of electropherograms (DUL and NEF) in glycogen/HP-β-CD system are shown in Fig. 6.

3.2. Glycogen/CSA system

In chiral CE separation, buffer pH and chiral selector concentration are two important parameters in controlling and optimizing the separation. When ionizable chiral selectors and analytes are

Table 2
The resolution (Rs) of five tested drugs obtained in glycogen/β-CD and glycogen/HP-β-CD systems.

Chiral compounds	Glycogen/β-CD system[a]			Glycogen/HP-β-CD system[b]		
	Glycogen	β-CD	Glycogen/β-CD	Glycogen	HP-β-CD	Glycogen/HP-β-CD
DUL	1.84	–	–	1.84	–	0.42
NEF	1.64	–	–	1.64	0.50	–
CET	2.08	–	–	2.08	–	–
CIT	1.78	–	–	1.78	–	–
AMB	0.65	–	–	0.65	2.33	1.97

Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm ID; BGE, 40 mM phosphate buffer at pH 3.0; applied voltage, 25 kV; capillary temperature, 20 °C; –, not separated.

[a] Glycogen concentration, 3% (w/v); β-CD concentration, 10 mM.

[b] Glycogen concentration, 3% (w/v); HP-β-CD concentration, 20 mM.

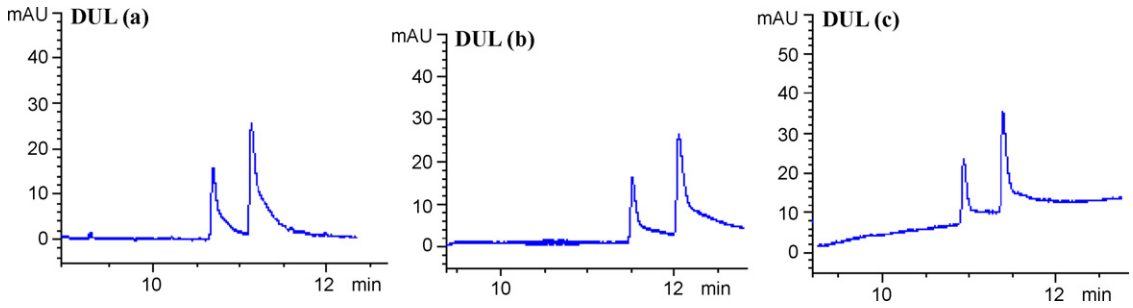

Fig. 7. Electropherograms of the chiral separation of DUL at different pH in glycogen/CSA system. Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm ID; BGE, 40 mM phosphate buffer containing glycogen (3%, w/v) and CSA (2%, w/v); buffer pH, 2.5 (a), 3.0 (b), 3.5 (c); applied voltage, 25 kV; capillary temperature, 20 °C.

Table 3
Effect of glycogen concentration on the selectivity factor (α) and resolution (Rs) of basic drugs in glycogen/CSA system.

Chiral drugs	Concentrations of glycogen and CSA (w/v)							
	1%glycogen + 2%CSA		2%glycogen + 2%CSA		3%glycogen + 2%CSA		4%glycogen + 2%CSA	
	α	Rs	α	Rs	α	Rs	α	Rs
DUL	1.03	2.01	1.03	2.51	1.04	3.93	1.04	3.32
CET	1.02	1.42	1.02	1.98	1.03	2.40	1.04	2.12
CIT	1.01	0.56	1.01	1.87	1.02	2.01	1.03	1.80

Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm ID; BGE, 40 mM phosphate buffer at pH 3.0; selector concentration, as shown in this table; applied voltage, 25 kV; capillary temperature, 20 °C.

involved, the pH of the running buffer can change the charge of both analytes and chiral selectors, which will in turn influence many aspects of the enantioseparation, such as the interactions between the selectors and analytes as well as the electrophoretic mobilities of analytes and the selectors. The chiral selector concentration is another important experimental parameter in CE enantioseparation. At an extremely low concentration, the amount of selector is not sufficient to form complexes with enantiomers and, therefore, the enantiomers cannot be separated. On the other hand, when the concentration of selector is too high, both enantiomers mostly complexed will have very similar mobility, and not be separated also in this case [9,10].

Accordingly, in glycogen/CSA system we investigated the effects of buffer pH and selector concentration on the enantiomeric separations of three basic drugs (DUL, CET and CIT), which gave baseline enantioseparations and were taken as model compounds.

3.2.1. *Effect of buffer pH in glycogen/CSA system*

The effect of buffer pH on enantioseparation of the studied drugs was investigated over the range of 1.5–4.0 using 40 mM phosphate buffer supplemented with 3% glycogen and 2% CSA. The five tested pH values were controlled at 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0. It was found that when the pH was under 2.5 or over 3.5, heavy noise and instable baseline were observed. Satisfactory enantioseparations of DUL, CET and CIT were achieved when pH was 2.5–3.5, and during the pH range of 2.5–3.5 the Rs of these three drugs did not vary obviously. This trend indicated that the pH range of 2.5–3.5 was optimal for the enantioseparation of the studied drugs. Electropherograms of chiral separation of DUL at pH 2.5, 3.0 and 3.5 are shown in Fig. 7.

3.2.2. *Effect of selector concentration in glycogen/CSA system*

In order to find the optimum concentrations of glycogen and CSA, we varied the concentration of one while keeping the other concentration fixed. The concentration of glycogen was fixed at 3%, when CSA concentration was increased from 1% to 4%. Since CSA was an ionic polysaccharide, it could be found that when CSA concentration was over 3.0%, high electric current and heavy noise were observed. Satisfactory enantioseparation was achieved when the concentration of CSA was at 2%. Thus, we kept CSA concentration at 2% and investigated the effect of glycogen concentration (1–4%) on the enantioseparations of DUL, CET and CIT using 40 mM phosphate buffer (pH = 3.0). As shown in Table 3, α and Rs of the three drugs increased in step with glycogen concentration ascending from 1% to 3%. When glycogen concentration reached 4%, apparent peak broadening was observed and peak shape of the analytes got worse. Consequently, the additive glycogen concentration of 3% was proved to be optimum for the enantioseparations of these drug enantiomers in glycogen/CSA system. Electropherograms of chiral separation of DUL at different selector concentrations are shown in Fig. 8.

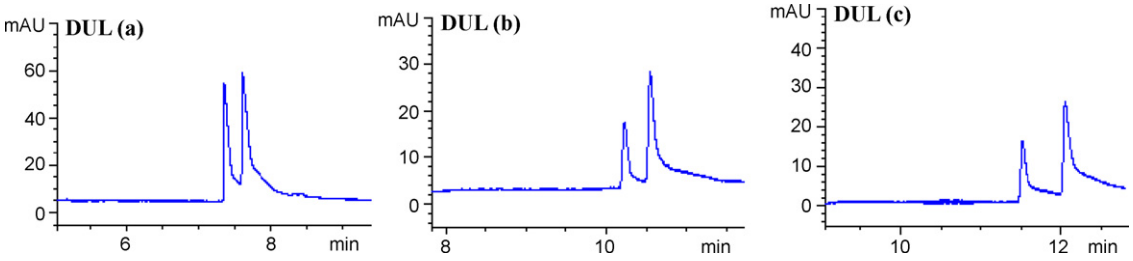

Fig. 8. Electropherograms of the chiral separation of DUL at different selector concentrations in glycogen/CSA system. Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm ID; BGE, 40 mM phosphate buffer at pH 3.0; selector concentration, 3%glycogen alone (a), 2%glycogen + 2%CSA (b), 3%glycogen + 2%CSA (c); applied voltage, 25 kV; capillary temperature, 20 °C.

4. Conclusions

In this paper, we investigated and evaluated the enantioselectivity of three glycogen-based dual selector systems towards several racemic basic drugs. Among the three systems, glycogen/CSA brought baseline separation of the enantiomers of duloxetine, cetirizine, citalopram and sulconazole. Furthermore, this system caused synergistic effect for chiral separation of most of the studied compounds. This illustrated the enhancement of enantioseparation observed for these compounds in glycogen/CSA system might be due to some favorable interaction effects between glycogen and CSA. We also studied the effects of buffer pH and selector concentration on the enantioseparations of the model drugs in glycogen/CSA system. Further studies are being carried out in order to verify the usefulness of glycogen and the synergistic effect on the separation mechanism in CE.

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