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Research Article

Layer-by-Layer Assembly of Polysaccharides and 6,10-Ionene for Separation of Nitrogen-Containing Pharmaceuticals and Their Enantiorecognition by Capillary Electrophoresis

Anna Ioutsi, Elena Shapovalova, Aleksandra Prokhorova, and Oleg Shpigun

Analytical Chemistry Division, Department of Chemistry, Lomonosov Moscow State University, Leninskie Gory 1/3, Moscow 119991, Russia

Correspondence should be addressed to Anna Ioutsi; freund-for-me@mail.ru

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Two silica capillaries modified layer-by-layer with 6,10-ionene and N-(3-sulfo-3-carboxy)-propionylchitosan (SCPC) and with 6,10-ionene and dextran sulfate (DS) were prepared and investigated. Dynamic coating of the capillary efficiently reduces the adsorption of the background electrolyte, sample matrix components, and analytes on its inner wall. Such coatings effect good reproducibility and sensitivity of determination. We demonstrate that separation of betablockers, calcium channel blockers, alpha-adrenergic agonists, H_1-blockers, and diuretics was the most efficient and rapid separation with a capillary modified with dextran sulfate. Tetrahydrozoline, carbinoxamine, and furacilin, which are commonly employed as treatments for allergic rhinitis, were identified in human urea. Their concentrations, independently verified by HPLC, were found to be 5.3 ± 0.8, 6.6 ± 0.5, and 0.9 ± 0.2 $\mu g\,mL^{-1}$, with LOD = 0.07, 0.03, 0.10 $\mu g\,mL^{-1}$, and LOQ = 1.0, 0.8, 0.6 $\mu g\,mL^{-1}$, respectively.

1. Introduction

Capillary electrophoresis (CE) is increasingly used for the determination of the qualitative and quantitative composition of pharmaceuticals. Medicines are often determined in biological fluids (blood plasma, urea) as part of investigation of their pharmacokinetics and mechanism of action in humans. Successful application of CE to the separation and identification of various compounds and their enantiomers owes to its high efficiency, the possibility to quickly and easily adjust separation conditions, and rapidity of separation. In the same time, a serious drawback of CE is low reproducibility of migration times and peak areas. However, this problem can be resolved through coating the capillary surface with modifiers. Modification of the capillary surface makes it possible to control the magnitude and direction of electroosmotic flow (EOF) and affords a decrease in adsorption of compounds and background electrolyte (BGE) on the inner capillary surface [1]. The modifiers can be classified into two groups, those that alter the EOF directions and the EOF suppressers, and their application can be done either by chemical modification of the inner surface of a capillary (e.g., with polyacrylamide [2, 3]) or by dynamic coating, with the latter option being more simple and convenient.

The cationic components of the BGE usually having a number of binding centers can efficiently interact with the capillary wall, thus charging the surface and affecting the EOF. To prevent that, various cationic polymers are used in the CE that physically adsorb to the capillary surface. They include ionenes [4, 5], quaternary ammonium bases, chitosan [6], and other natural cationic polymers [7, 8]. However, such coatings often are of low durability and thus compromise reproducibility. To enhance stability, bilayer or multilayer coatings can be introduced, for example, by means of successive application of two solutions: a polycationic one and then a polyanionic one [9, 10]. The coatings of this kind can improve separation of proteins, carbohydrates, pharmaceuticals, and their enantiomers by capillary electrophoresis. The first report of the technique of dynamic coating of the capillaries with multiple layers of polyelectrolytes including ionene is due to Katayama et al. The coating can be applied simply by flushing the capillary with a solution of positively

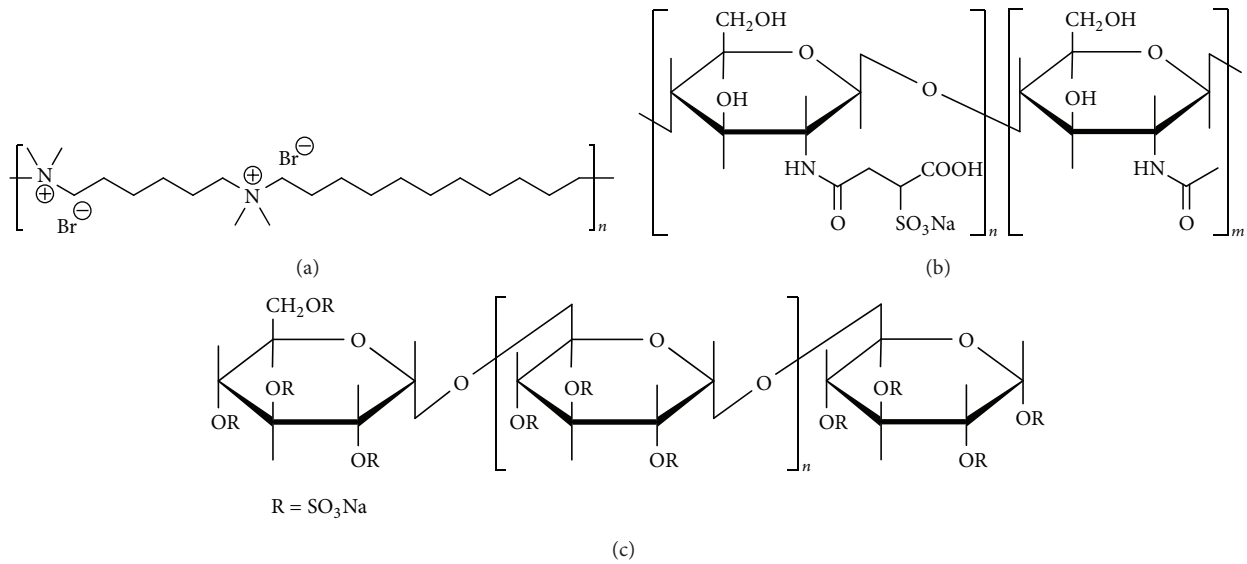

Figure 1: Structures of (a) 6,10-ionene, (b) N-(3-sulfo-3-carboxy)-propionylchitosan, and (c) dextran sulfate.

charged polybrene (hexadimethrine bromide) followed by a solution of negatively charged dextran sulfate (DS) polyelectrolyte [11]. With the coated capillaries, highly reproducible values of the EOF and the plateau height of free drug can be obtained [12].

In the 21st century, growing urbanization has made certain diseases very common, and some of them are even considered now as a new norm. Among them is allergic rhinitis, a disease highly prevalent in the metropolitan areas. While sounding rather innocuous, it can complicate the everyday lives of many persons, such as business people. Common therapies for the allergic rhinitis include α-adrenergic agonists, H_1-blockers, and antiseptics. The aim of the present study was to prepare and assess a capillary for rapid, efficient, and reproducible separation and determination of the above drugs in the biological fluids as a mean of monitoring the patient's condition. We introduce the new multiple layer capillary coatings consisting of polycationic 6,10-ionene and polyanionic N-(3-sulfo-3-carboxy)-propionylchitosan (SCPC) and dextran sulfate (DS) (Figure 1). Atenolol, pindolol, nadolol (β-blockers), tetrahydrozoline (α-adrenergic agonist), terbutaline (β-2-agonist), hydroxyzine, orphenadrine (M-cholinoreceptor blockers), doxylamine, carbinoxamine, and chlorpheniramine (H_1-blockers) were taken as model compounds (Figure 2).

2. Experimental

2.1. Chemicals and Materials. Pindolol, atenolol, nadolol, tetrahydrozoline, terbutaline, and hydroxyzine were purchased from Sigma-Aldrich (USA), and their samples were diluted with deionized water to a concentration of 100 μg/mL. Methyl ethyl ketone (3% water solution) obtained from the Chair of Analytical Chemistry (MSU, Russia) and deionized water itself were used as EOF markers. Sodium citrate, potassium phosphate trihydrate, dipotassium phosphate, and sodium hydroxide for background buffer preparation were purchased from Reachem (Moscow, Russia).

6,10-Ionene was obtained from the Division of Analytical Chemistry (MSU, Russia). N-(3-Sulfo-3-carboxy)-propionylchitosan (average molecular weight 5 kDa, degree of deacetylation 92%) was provided by Dr. V. P. Varlaamov. Dextran sulfate was purchased from Sigma-Aldrich (USA). A 37.8 cm × 50 μm i.d. fused silica capillary (effective length 29.3 cm) used in this study was obtained from Polymicro Technologies (Phoenix, USA).

2.2. Apparatus. The CE analyses were conducted with a Kapel-105M instrument (Lumex, Saint Petersburg, Russia) equipped with an autosampler and a photometric UV detector (λ = 235 and 270 nm). All CE operations were controlled by Elforan software. Migration time, separation efficiency, peak resolution, selectivity, and electrophoretic mobilities were calculated.

The capillaries were thermostated at 20°C using a liquid coolant, and the samples were injected at a pressure of 1000 mbar over 30 s. Electrophoresis was performed at an applied voltage of 8–15 kV.

All solutions were prepared in deionized water under pH control with the AV-74 ionometer and then degassed in an ultrasonic bath (Sapfir, Moscow). The samples were stored at 4°C.

The salts for preparation of the running buffer were diluted in deionized water at a concentration of 25 and 50 mM. Prior to the CE analyses, all buffers were filtered through the 0.22 μm Durapore PVDF membrane filters (Millipore, France). To prepare the background electrolytes, modifiers were weighed and dissolved in running buffers at concentrations of 0.1 and 1.5%.

2.3. Layer-by-Layer Coating Protocol. Bare fused silica capillary was successively rinsed with deionized water, 1 M NaOH,

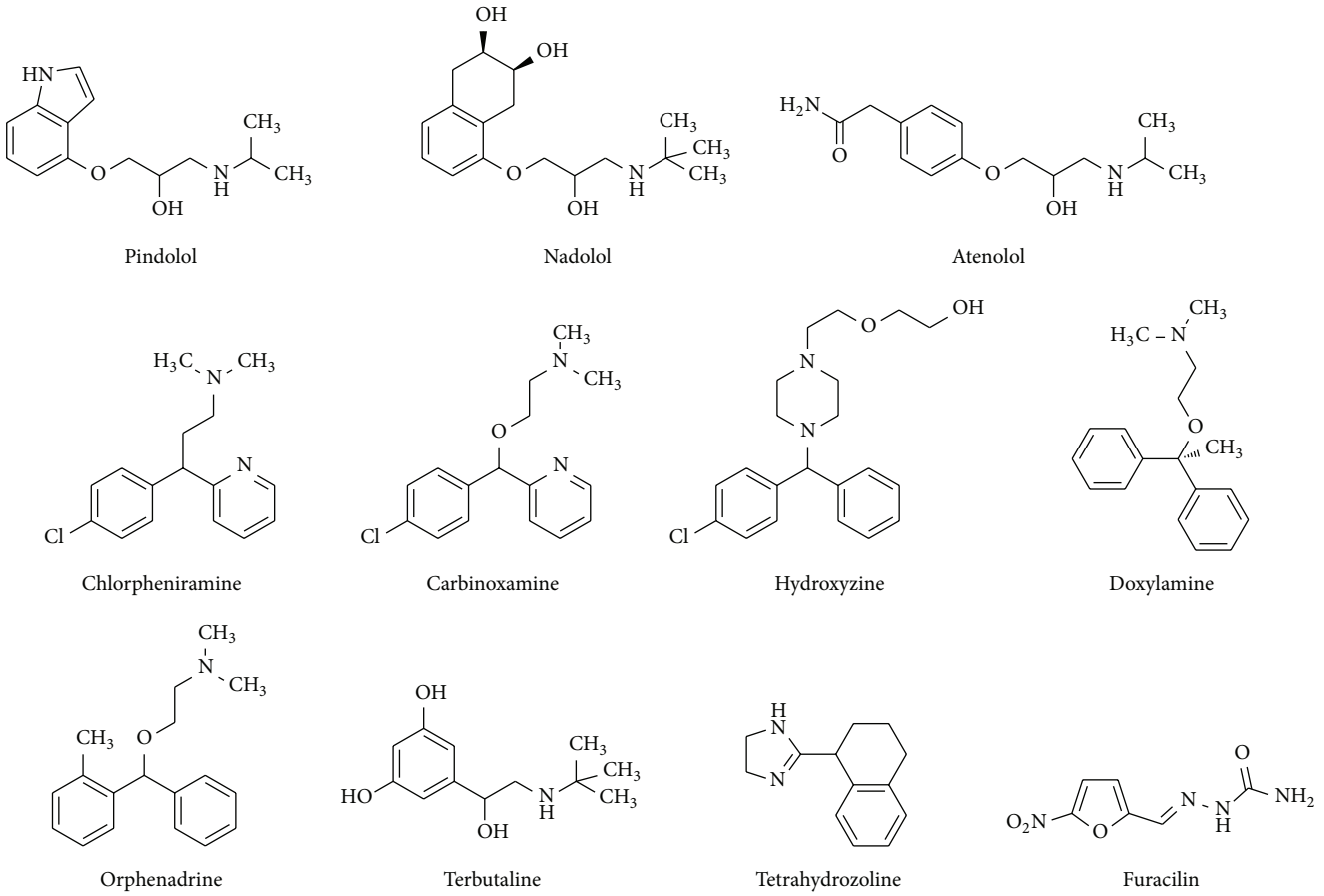

Figure 2: Structures of drugs studied.

and again with water for 10 minutes each. After preconditioning, the capillary was rinsed with a solution of 6,10-ionene (2 mg mL^{-1}) for 20 min at 1000 mbar. Then, the tips of the capillaries were kept overnight in deionized water. On the next day, the capillaries were rinsed with a polysaccharide solution (1 mg/mL) for 40 min and once again left overnight in water.

Before starting an experiment, a fresh capillary was rinsed with water for 10 min and with BGE (25 mM citric buffer at pH 6.5) for 10–30 min. Between any run, the capillary was rinsed with distilled water for 1 min and then with BGE for 2 min. BGE was refreshed every 4-5 analyses.

3. Results and Discussion

3.1. Development of the Method

3.1.1. Selection of the Modifiers. The separation of compounds by means of CE is strongly affected by adsorption on the bare fused silica capillaries, which is due to the electrostatic interactions and hydrogen bonding of the analytes with the silanol groups of the capillary surface. Adsorption results in deterioration of reproducibility and separation performance. Several approaches to overcome this problem have been explored; among them are various coating procedures [8, 11].

Poly (diallyldimethylammonium) chloride is a polycation most commonly used for multilayer coating of capillaries; it serves as a popular test case for studying the stability issues of the coatings. Other promising though less studied polycations are ionenes. Among them, polybrenes have been reported in the literature as capillary coatings. We believe that such compounds can contribute to the expansion of the range of applicable ionenes. In view of that, we employed 6,10-ionene for capillary modification.

Also, polysaccharides are widely used. Capillary coatings based on charged polysaccharides ensure higher reproducibility of migration times compared to the neutral coating techniques. Furthermore, such coatings are mostly devoid of solubility related problems. Negatively charged polysaccharides are used more universally than the neutral ones. In particular, Park et al. have synthesized highly sulfated cyclosophoraoses [13], the sulfated derivatives of chiral unbranched cyclic β-(1 → 2)-D-glucans, and successfully used them to separate five basic chiral drugs (arterenol, atenolol, isoproterenol, propranolol, and metoprolol). The seanionic derivatives enabled better resolution than the original cyclosophoraoses and were therefore recommended for separation of basic drugs. Nishi [14] has compared the performance of the two types of polysaccharides, the neutral dextran and dextrin and the charged chondroitin sulfate C and chondroitin sulfate A, in separation of some basic drugs. Again, anionic polysaccharides provided higher separation efficiency. The most interesting results were obtained with

heparin [15, 16], chondroitin sulfates (A, B, C) [17], colominic acid [18], dextran sulfate [19, 20], and SCPC [6].

Polycations form stable complexes with anionic polyelectrolyte polysaccharides. This behavior can be utilized in the creation of stable capillary coatings, which has been already demonstrated in several studies. Thus, stable and efficient capillary coating with polybrene and dextran sulfate has been obtained [12, 21]. Additional particular attractiveness of anionic polysaccharides is due to their enantiorecognition ability.

Previously, we have effected CE separation of the enantiomers of pindolol, atenolol, nadolol, fluoxetine, chlorcyclizine, hydroxyzine, orphenadrine, terbutaline, and tetrahydrozoline in presence of SCPC in BGE. For enantiomeric separation of fluoxetine, tetrahydrozoline, doxylamine, carbinoxamine, chlorpheniramine, and orphenadrine, we also used an additive of DS in BGE [22]. Comparison of the separation efficiency between original bare fused silica capillaries and modified capillaries would be of high interest.

In view of the foregoing, the present study reports two especially promising modifications of the fused silica capillaries: with N-(3-sulfo-3-carboxy)-propionylchitosan (SCPC) and with dextran sulfate (DS).

3.1.2. Verification of the Capillary Surface Modification. Two coated capillaries were prepared. After initial treatment of a fused silica capillary with 6,10-ionene, one was further coated with N-(3-sulfo-3-carboxy)-propionylchitosan (SCPC capillary) and the other with dextran sulfate (DS capillary). Capillary with four coating layers was also prepared. It will be discussed separately in another part.

Properties of the capillaries were examined after each act of coating by monitoring the EOF direction and value. In the fused silica capillaries, EOF is directed towards the cathode and, accordingly, transfers positive charge (normal polarity mode). After coating with 6,10-ionene, the peak due to the EOF marker was identified in the reversed polarity mode, which agrees with the expected inversion in surface charging (negative to positive). However, the reproducibility of the electroosmotic mobility was less than desired (s_r = 0.09, n = 3) and, moreover, the EOF strength itself was low ($\mu_{EOF} = -8.77 \times 10^{-5}$ cm^2·V^{-1}·s^{-1}). After the second modification step, that is, coating with polysaccharides, EOF was once again detected in the normal polarity mode. This observation confirms the adsorption of the polysaccharides on the surface modified with 6,10-ionene. The electroosmotic mobility value for the SCPC capillary and the DS one was $1.24 \cdot 10^{-4}$ and $2.00 \cdot 10^{-4}$ cm^2·V^{-1}·s^{-1}, respectively (s_r = 0.08 and 0.05; n = 3).

3.1.3. Selection of the Buffer pH and Composition. In this study, we used a buffer with pH 6.5. Each of the test compounds contains a nitrogen atom, which can be positively charged at pH 4–7. In our preliminary enantioseparation investigations, where we studied some test compounds on a fused silica capillary in presence of DS, the highest efficiency was obtained in the pH range of 6.5–6.8 while at pH above 6 or below 7 none of the compounds were identifiable.

We have tested the phosphate and the citrate buffer known for high buffer capacity in the pH range of interest. With the phosphate buffer, the model compounds migrated in less than 2 min and no separation was achieved even up to buffer concentration of 50 mM. This led us to select the 25 mM citrate buffer, with which we had good experience in the study of capillary modification with citrate-stabilized gold nanoparticles [20]. This buffer afforded resolution of the model compounds within 3–6 min with sufficient quality (R_s = 0.7–10.3). The concentration of 25 mM was found to provide the optimal trade-off between the resolution, which suffers from the increase in the electrophoretic mobility at lower buffer concentrations and the duration of the analyses. Note that apart from the decreased mobility and hence tedious analytical work, higher buffer concentrations can bring about the Joule heating issues.

3.1.4. Selection of the Working Voltage. Trial separations of the test compounds were performed under the applied voltage of 8, 15, and 25 kV. Under 25 kV, the mobility of the test compounds was exceedingly high, which precluded any possible separation. Voltage reduction to 8 kV resulted in the increase in the separation time to 12 min. Yet, for some of the test compounds, complete separation has not occurred. Under the compromise 15 kV conditions, we achieved separation of the same compounds as under 8 kV but with considerable time saving. Thus, we were conducting subsequent experiments under 15 kV.

3.2. Analysis of the Pharmaceuticals. Initially, the model compounds were analyzed using a conventional fused silica capillary. The order of migration of the model compounds was as follows: tetrahydrozoline, pindolol, atenolol, terbutaline, nadolol, and finally hydroxyzine. Electrophoretic mobilities were rather high (up to $7.02 \cdot 10^{-4}$ cm^2·V^{-1}·s^{-1}). The mixture of tetrahydrozoline, pindolol, terbutaline, nadolol, and hydroxyzine was separated within 5 minutes (Figure 3). This figure shows the low height of peaks and bad peak resolution (not to baseline). Stability of the fused silica capillary was unsatisfactory and so was the reproducibility of the electroosmotic mobility and analyte mobility (s_r = 0.82 and 0.57, resp.; n = 3). Within three days, the efficiency of the capillary dropped by 30% and more. However, as we show below, the separation performance was improved through the use of the coated capillaries.

3.2.1. SCPC Capillary. The SCPC capillary was rinsed with BGE for 20 min for equilibration. The order of migration for the model compounds with the SCPC capillary was as follows: tetrahydrozoline, atenolol, pindolol, nadolol, terbutaline, and hydroxyzine. The migration rate depends on the value of the positive charge at the nitrogen atom, as well as the size and structure of the analytes. Higher charge and smaller molecular size result in higher mobility and, correspondingly, lower migration time of the substance. In addition, mobility can be influenced by the interaction of the analytes with SCPC at the inner capillary surface. Tetrahydrozoline, pindolol, nadolol, terbutaline, and hydroxyzine were separated

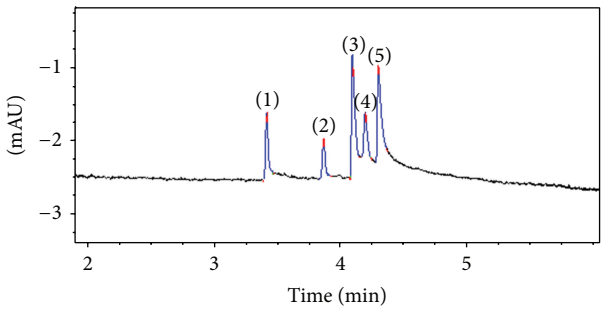

Figure 3: Separation of (1) tetrahydrozoline, (2) pindolol, (3) terbutaline, (4) nadolol, and (5) hydroxyzine by capillary zone electrophoresis with spectrophotometric detection (λ = 235 nm) on the fused silica capillary. CE conditions: $L_{tot/eff}$ 38.7/29.3 cm; BGE solution: 25 mM citric buffer at pH 6.5; voltage 15 kV, hydrodynamic injection for 30 s with 10 mbar.

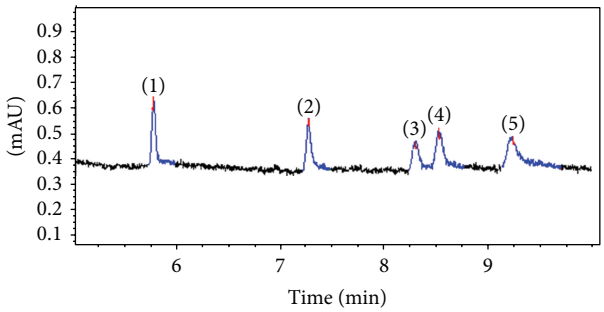

Figure 4: Separation of (1) tetrahydrozoline, (2) pindolol, (3) nadolol, (4) terbutaline, and (5) hydroxyzine on the SCPC capillary. Conditions as in Figure 3.

within 10 min with an efficiency of up to 125000 TP (Figure 4, Table 1).

The SCPC levels were varied in the range of 0 to 1.08%. The mobility of the compounds grew upon the increase in the SCPC concentration from 0.0 to 0.15% while beyond 0.15% it started to decrease back. This is likely due to the CS migration in the opposite direction with respect to the EOF. The highest efficiency for 4 of the 6 compounds studied (up to 170000) was reached at the upper limit of the tested SCPC concentrations range (Table 2).

3.2.2. DS Capillary. The capillary was rinsed with BGE for 10 min for equilibration. The migration order of model compounds altered again: pindolol and terbutaline were detected before atenolol and nadolol, respectively.

Migration times for this capillary were lower than those for the SCPC capillary but higher than those with a fused silica capillary. Tetrahydrozoline, pindolol, nadolol, terbutaline, and hydroxyzine were separated on a DS capillary within 7 min with higher efficiency (up to 200000 TP) and comparable or, sometimes, even higher selectivity (Figure 5, Table 3).

For an extended set of test compounds, the migration order was as follows: tetrahydrozoline, pindolol, atenolol,

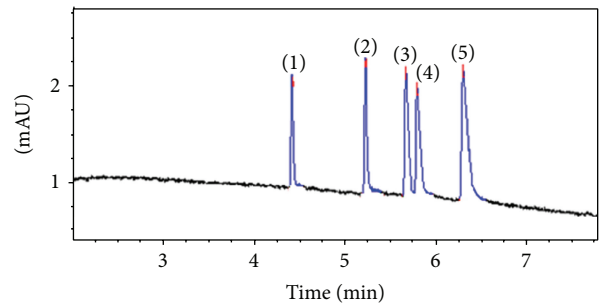

Figure 5: Separation of (1) tetrahydrozoline, (2) pindolol, (3) terbutaline, (4) nadolol, and (5) hydroxyzine on the DS capillary. Conditions as in Figure 3.

terbutaline, nadolol, orphenadrine, doxylamine, carbinoxamine, hydroxyzine, and chlorpheniramine.

The effect of the DS concentration in BGE on the mobility of compounds and on the efficiency of the capillary was investigated in the range of 0 to 1.5%. Similar to the SCPC case, the mobilities of the compounds showed initial increase up to SCPC concentration of 0.1% while beyond this level the trend was inverted. The effect of DS on the efficiency of the capillary was uneven over the reported range of compounds. However, addition of the DS to the citrate buffer always yielded higher efficiency compared to the neat buffer.

In general, the DS coating was found to be highly stable and efficient against analyte adsorption. It afforded better reproducibility and performance compared to the standard bare fused silica capillary.

3.2.3. Comparison of the Modified Capillaries and Their Advantages over Fused Silica Capillaries. As was shown above, modification of the capillary surface improved its chemical stability and reproducibility of the separation results compared to a fused silica capillary. Migration times of the model compounds became longer (with citrate running buffer as BGE), which may be explained by incomplete shielding of the cationic centers of 6,10-ionene by the sulfo groups of the polysaccharides. This implies lower integral negative charge on the inner capillary surface and consequential reduction in the EOF rate.

The changes in the order of migration of the model compounds may be due to the CH_2–OH functions in the SCPC. For example, terbutaline can develop stronger hydrogen-bonding interactions than nadolol as its hydroxy groups are attached to benzene rather than a cyclohexane ring. As a result, with SCPS, the migration time of terbutaline becomes longer than that of nadolol. The behavior of atenolol-pindolol pair, however, is likely to be governed by some different types of interactions or more subtle effects. Indeed, atenolol comes out earlier from the SCPC capillary (but not from the DS one) despite being more polar and capable of more efficient hydrogen bonding (an amide group versus a pyrrole ring in pindolol). Yet another advantage of the modified capillaries is higher separation efficiency, especially in the case of DS. We observe a 2-3-fold increase in separation efficiency peaking at 430500 TP. Furthermore, both capillaries provide improved

Table 1: Separation of tetrahydrozoline, pindolol, nadolol, terbutaline, and hydroxyzine on the SCPC and DScapillaries. Conditions as in Figure 3.

	Compound	t_m, min	α	R_s	N, TP
SCPC capillary	Tetrahydrozoline	5.78	1.26	10.7	125810
	Pindolol	7.27	1.14	7.3	102890
	Nadolol	8.30	1.02	1.4	90370
	Terbutaline	8.53	1.08	3.0	65640
	Hydroxyzine	9.24			28690
DS capillary	Tetrahydrozoline	4.42	1.24	7.4	197970
	Pindolol	5.22	1.08	3.2	166930
	Terbutaline	5.67	1.02	0.8	115310
	Nadolol	5.79	1.09	2.1	97310
	Hydroxyzine	6.30			52300

Table 2: Effect of SCPC concentration in BGE on the migration times of analytes and efficiency of SCPC capillary (λ = 235, 270 nm). Other conditions as in Figure 3.

Compound	SCPC concentration, %							
	0		0.10		0.15		1.08	
	t_m, min	N, TP	t_m, min	N, TP	t_m, min	N, TP	t_m, min	N, TP
Tetrahydrozoline	5.5	79800	3.1	98740	2.8	79970	3.5	141280
Athenolol	6.7	40370	3.5	34350	3.6	35280	3.8	60250
Pindolol	7.0	76260	3.6	136260	3.5	151600	3.9	57760
Nadolol	8.0	49450	3.7	80180	3.7	93880	4.0	170980
Terbutaline	8.2	66460	3.8	68690	3.8	70310	4.0	141230
Hydroxyzine	8.9	41700	3.8	81250	3.8	72510	4.2	8480

reproducibility of the compound mobilities with citrate buffer as BGE (s_r = 0.001–0.049 and 0.001–0.011 for the SCPC and the DS capillary, resp.; n = 3). Apparently, this is in connection with the stability of the capillary surface. Note that when the polysaccharides were used only as chiral selectors (CS) without surface modification, capillaries had to be replaced every 2-3 days [9, 16] while the modified capillaries retained their properties for at least 4 weeks (Table 3). In general, the DS capillary was found to be more promising for the purposes of separation and enantioseparation of various pharmaceuticals and for other related tasks.

For the successful combination of 6,10-ionene and DS, we have further investigated the effect of repeated multilayer capillary coatings. A capillary modified with four alternating coating layers, two of 6,10-ionene and two of DS, was prepared. This modification was found to increase the electrophoretic mobility of the model compounds. Tetrahydrozoline, pindolol, doxylamine, chlorpheniramine, orphenadrine, nadolol, and hydroxyzine were separated within 7 min (U = 8 kV) with an efficiency of up to 225000 TP.

3.3. Ability of Modified Capillaries for Enantiorecognition. As was expected, in case of SCPC capillary with chiral selector- (CS-) free BGE, no enantioseparation was observed (as for other such capillaries). Enantiomers of atenolol and terbutaline (R_s = 0.6 and 0.8, resp.) were separated with BGE containing 0.1% SCPC. However, the capillary properties were found to change over time, which ultimately made enantioseparation impossible.

Enantiomers of tetrahydrozoline and atenolol were separated on a DS capillary at a rather low DS concentration of 0.1% within 3 min (R_s = 1.2 and 0.8 and s_r = 0.03 and 0.05, resp.; n = 3). Enantiomers of terbutaline (R_s = 1.0, s_r = 0.013; n = 3), orphenadrine (R_s = 1.0, s_r = 0.001; n = 3), doxylamine (R_s = 0.8, s_r = 0.001; n = 3), and chlorpheniramine (R_s = 0.8, s_r = 0.001; n = 3) were separated at 1.5% of DS in BGE within 4 min.

A capillary double modified with 6,10-ionene and DS (four coating layers) provided better resolution of the enantiomers of the model compounds and made it possible to conduct the analyses at lower DS concentrations in BGE. For instance, enantiomers of tetrahydrozoline (R_s = 1.4), doxylamine (R_s = 1.0), carbinoxamine (R_s = 0.9), and hydroxyzine (R_s = 0.8) were separated with 1.08% DS in BGE (Figure 6). Note that with a capillary with two coating layers, we did not observe separation of the latter two compounds even at 1.5% DS in BGE; similarly, the enantiomers of hydroxyzine were not separated in which a bare fused silica capillary was used and DS was added in BGE [23]. So, it is another advantage of coated capillaries over the bare fused silica capillaries.

However, it has to be said that the coated capillaries are far from perfect for the purposes of enantioseparation. The major problems include the less than preparative scale of the method and insufficient peak resolution. The latter drawback is likely due to a combination of two mechanisms: the HPLC-like mechanism on the capillary surface (its contribution is low since the amount of CS on the surface is rather small) and

Table 3: Comparison stability of the coatings in terms of electroosmotic mobility for the coated capillaries.

SCPC capillary						
EO mobility $\cdot 10^4$, $cm^2 \cdot V^{-1} \cdot s^{-1}$	RSD $n = 3$	EO mobility $\cdot 10^4$, $cm^2 \cdot V^{-1} \cdot s^{-1}$	RSD $n = 3$	EO mobility $\cdot 10^4$, $cm^2 \cdot V^{-1} \cdot s^{-1}$	RSD $n = 3$	Variation of EO mobility, RSD
Before treatment		After treatment (2 weeks)		After treatment (1 month)		Over time of life
1.24	0.08	1.20	0.04	1.15	0.06	0.07
DS capillary						
EO mobility $\cdot 10^4$, $cm^2 \cdot V^{-1} \cdot s^{-1}$	RSD $n = 3$	EO mobility $\cdot 10^4$, $cm^2 \cdot V^{-1} \cdot s^{-1}$	RSD $n = 3$	EO mobility $\cdot 10^4$, $cm^2 \cdot V^{-1} \cdot s^{-1}$	RSD $n = 3$	Variation of EO mobility, RSD
Before treatment		After treatment (1 week)		After treatment (5 months)		Over time of life
2.00	0.05	2.00	0.03	1.98	0.04	0.01

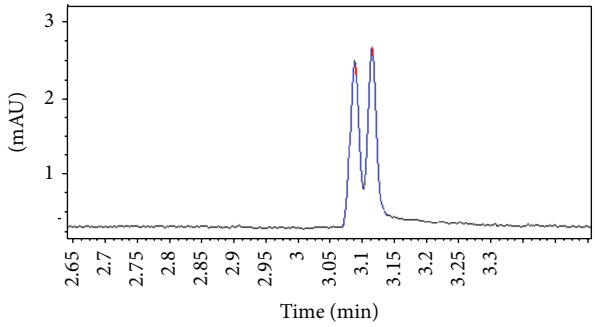

Figure 6: Separation of enantiomers of tetrahydrozoline in the capillary with four coating layers (6,10-ionene and DS). CE conditions: $L_{tot/eff}$ 38.7/29.3 cm; BGE solution: 1.08% DS in 25 mM citric buffer at pH 6.5; voltage 15 kV and hydrodynamic injection for 30 s with 10 mbar, λ = 235 nm.

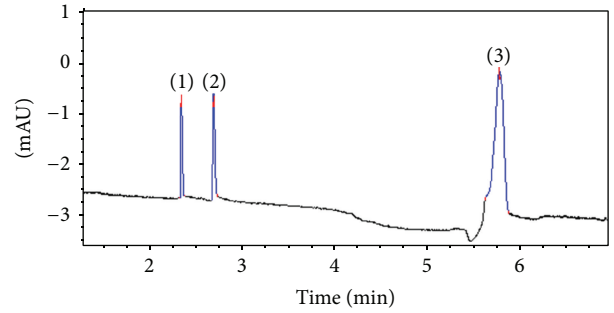

Figure 7: Separation of (1) tetrahydrozoline, (2) carbinoxamine, (3) and furacilin in human urea by capillary zone electrophoresis with spectrophotometric detection (λ = 235 nm) on the DS capillary. CE conditions: $L_{tot/eff}$ 38.7/29.3 cm; BGE solution: 25 mM citric buffer at pH 6.5; voltage 15 kV and hydrodynamic injection for 30 s with 10 mbar.

the CE mechanism in the bulk of the solution that involves the CS additives to the BGE. In some cases, the order of migration differs for these two concurrent mechanisms, and their interplay consequently results in lower peak resolution compared to the CE-only process in the pristine fused silica capillaries.

3.4. Application of the DS Capillary to the Analysis of Human Urea. The capillary modified with 6,10-ionene and dextran sulfate was used for separation of drugs for the treatment of the allergic rhinitis. Tetrahydrozoline (alpha-adrenergic agonist), carbinoxamine (H_1-blocker), and furacilin (antiseptic) (Figure 1) were separated within 6 min with an efficiency of up to 78000 TP (Figure 7). The applied voltage was 15 kV and the detection wavelength was 235 nm. The quantitative accuracy was independently verified by HPLC.

Sample preparation included tenfold dilution of the test sample with BGE and centrifugation for 5 min (10000 r.p.m.). Identification was carried out by means of the standard addition method. The results and the metrological parameters are presented in Table 4.

The control HPLC identification was performed using a Shimadzu HPLC system equipped with an SPD-M20A photodiode array detector and an LC-20AB binary pump. We used commercially available Mightysil C-18 as the stationary phase and 50/50 mixture of acetonitrile and 0.1% water solution of the phosphoric acid as an eluent. As shown in Table 4, there is a good agreement between the HPLC and the CE results.

4. Conclusions

The present study has demonstrated that 6,10-ionene and dextran sulfate can be recommended for modifying the CE capillaries by forming multilayer coatings on the inner surface. In addition, dextran sulfate can be used as a chiral selector as well. For the capillaries thus coated, we report the optimal conditions for identification and separation of tetrahydrozoline (alpha-adrenergic agonist), carbinoxamine (H_1-blocker), and furacilin in human urea. The DS-modified capillaries are also suitable for separation of other nitrogen-containing compounds and their enantiomers.

Table 4: CE and HPLC determination of tetrahydrozoline, carbinoxamine, and furacilin in human urea. CE conditions as in Figure 6 and, for sample preparation, see Results and Discussion.

	Contention, $\mu g\,mL^{-1}$		$c_{,min}$, $\mu g\,mL^{-1}$	linearity	S_r ($n = 3$)	Regression equation	R^2
CE	Tetrahydrozoline	5.3 ± 0.8	1.0	2-140	0.07	$y = (0.167 \pm 0.004)\,x$	0.996
	Carbinoxamine	6.6 ± 0.5	0.8	2-140	0.03	$y = (0.256 \pm 0.008)\,x$	0.997
	Furacilin	0.9 ± 0.2	0.6	2-140	0.10	$y = (1.696 \pm 0.615)\,x + (12.473 \pm 0.416)$	0.997
HPLC	Tetrahydrozoline	4.9 ± 1.7	0.5	2-100	0.08	$y = (20.6 \pm 0.9)\,x$	0.999
	Carbinoxamine	6.2 ± 0.4	0.5	2-100	0.05	$y = (7.8 \pm 0.2)\,x$	0.999
	Furacilin	0.6 ± 0.1	0.5	2-100	0.08	$y = (28.5 \pm 0.6)\,x + (1.9 \pm 0.3)$	0.999

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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