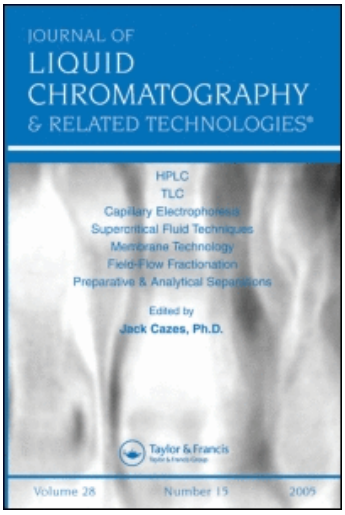

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CAPILLARY ELECTROPHORESIS OF INTACT HEPARIN AND IMPURITIES IN SHORT CAPILLARIES WITH HIGH CONCENTRATION PHOSPHATE BUFFERS USING LOW MOBILITY COUNTERIONS

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□ *Capillary electrophoresis (CE) of heparin, oversulfated chondroitin sulfate (OSCS), dermatan sulfate (DS), and related impurities were separated in 5 min using 1 M lithium phosphate buffer, pH 2.5 on an 8.5 cm × 25 μm i.d. capillary. The separation was optimized for overall speed, resolution between heparin, OSCS and DS, and in particular, the limit of detection (LOD) for OSCS. The LOD for OSCS was less than 0.03% of heparin. The migration time precision was always less than 0.5% for all solutes. The peak area precision was 1.9% and 2% for OSCS and DS at the 0.4% and 2% concentration levels, respectively. The response factors of OSCS and DS were found to be 3.69 and 6.40 times greater than heparin, respectively. Optimization experiments including buffer counterion and concentration, buffer pH, sample concentration, and injection size are reported. The buffer counterion impacted the speed and resolution of the separation beyond consideration of the effect of solution conductivity alone. A correlation between data generated by both CE and NMR for 29 separate lots of heparin known to be contaminated with OSCS was reported for the first time. The differences in OSCS measured by both techniques was not statistically significant.*

Keywords capillary electrophoresis, dermatan sulfate, heparin, heparin impurities, oversulfated chondroitin sulfate

INTRODUCTION

The heparin contamination problem of 2007–2008 was resolved in part through the use of a combination of Capillary Electrophoresis (CE) and Nuclear Magnetic Resonance spectroscopy (NMR). While it is not

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possible to design analytical methods to detect all possible contaminants and adulterants, the combination of spectroscopy and separation science provides powerful tools to protect the integrity of pharmaceutical products.

In a previous paper,[1] it was shown that 600 mM lithium phosphate buffer, pH 2.8, was effective in separating from heparin impurities such as oversulfated chondroitin sulfate (OSCS), dermatan sulfate (DS), and heparan sulfate (HS). The combination of high buffer concentration and low pH effectively reduced the electroosmotic flow (EOF) to near zero. This effect produced highly reproducible migration time precision. To reduce operating current to acceptable levels, 25 μm i.d. capillaries were employed.

Methodology reported in that first paper was immediately utilized to ensure the integrity of the heparin API tested. The method was validated and submitted to regulatory authorities. The next step was to fully understand and optimize the method.

Buffers and their counterions for CE have been studied for over 20 years. For example, it was shown that speed and resolution were better for sodium phosphate compared to potassium phosphate,[2] at least when applied to a series of dansyl amino acids. It was also shown that resolution improved as the buffer concentration was increased.[2] For a micellar electrokinetic chromatographic separation, lithium provided superior efficiency and resolution compared to sodium and potassium.[3] A counterion such as triethylamine reduced the electroosmotic flow, presumably through coverage of free silanol groups.[4] Despite these and other studies, it appeared that buffer design, particularly the buffer counterion, was not optimal for many separations.

A relatively high concentration of tris phosphate buffer had been used for the separation of human growth hormone.[5] With a variation of this buffer, a method was developed to separate heparin from its impurities in a manner that was similar to our method.[6] From these two approaches, the pathway to good heparin separations became clear: high concentration phosphate buffers with low mobility counterions produced superior separations.

Early on during our method development, we recognized that high concentration buffers function differently compared to their low concentration relatives. It was important to perform optimization studies at conditions that closely reflected the final conditions. Therefore, the method development stage became an iterative process. In this paper, we present data describing the optimization process for the separation of heparin from its impurities along with its validation information. Experimental details were carefully monitored, such as buffer pH, counterion, buffer concentration, injection size, capillary length, voltage, temperature, wash procedures, and sample concentration allowed for high speed, high

resolution separations to be performed with exceptional robustness and precision.

Over reliance on long capillaries to provide adequate resolution in CE occurs all too often. Since doubling separation time results in only a 41% improvement in resolution (due to diffusion), the capillary length should always be minimized. This can be accomplished by designing the buffer to optimize the resolution. Experimental data are reported here that allow the separation of heparin and its impurities to be performed on an 8.5 cm section of capillary in 5 min.

Both CE and NMR testing of unfractionated heparin have been required for use of heparin in drug products since March 2008. The regulations stipulated limit tests in which the limits were set to no OSCS detectable. A quantitative comparison, between the CE and NMR methods, of the %OSCS present in contaminated lots of heparin is reported here for the first time.

EXPERIMENTAL

Chemicals

Heparin Sodium USP active pharmaceutical ingredient (API) was supplied by Scientific Protein Laboratories, LLC. (Changzhou, China). USP Heparin Sodium System Suitability Reference Standard (Rockville, MD, USA). Heparin Sodium (USP grade), DS and HS were purchased from Sigma-Aldrich (St. Louis, MO, USA). OSCS, OS-Dermatan Sulfate (OSDS) and OS-Heparin were synthesized internally. Ortho-phosphoric acid was from Spectrum Chemicals (Gardena, CA, USA) or Sigma Chemical and Lithium phosphate, 99.99% was from Aldrich (Milwaukee, WI, USA). Buffers and sample solutions were prepared from Milli-Q water or Agilent CE water and were filtered through a 0.21 μm cellulose acetate filter prior to use. For NMR studies, deuterium oxide, 99.96% (Cambridge Isotope Laboratories, Andover, MA, USA) was used to prepare the heparin solutions.

Instrumentation and Methods

CE

Agilent Technologies CE instruments (Waldbronn, Germany), equipped with a deuterium UV lamp and diode array detectors (190–600 nm), were used for all CE investigations. Instrument control was carried out using Agilent Chemstation software. Data analysis was performed using either the Agilent Chemstation or Dionex Corporation (Sunnyvale, CA, USA.) with Chromeleon software. Extended pathlength bare fused silica

capillaries were from Agilent Technologies and used as received. The detection wavelength was 195 nm with or without a reference wavelength (bandwidth 10 nm), reference 450 nm (bandwidth 80 nm). The capillary temperature was 20°C, unless otherwise noted.

Unless otherwise noted, the following protocol was used. Between each run, the capillary was flushed for 3 min with buffer. The samples were introduced into the cathodic end of the capillary by hydrodynamic injection for 10 s at 50 millibar; [500 millibar seconds (mbs)]. The capillary inlet was dipped with water prior to and after injection to minimize carryover. The separation was performed in the reverse polarity mode with a constant voltage of −14 kV. In between runs, the capillary was rinsed for 1 min each with 0.1 M sodium hydroxide and 600–1000 mM phosphoric acid.

Calibration curves were plotted for OSCS (1–15 mg/mL) and DS (0.5–8 mg/mL) relative to a fixed concentration of heparin (50 mg/mL). The slope of each line as determined by linear regression is the response factor of each impurity relative to heparin.

NMR

A Bruker-600 MHz Biospin III NMR Spectrometer (Billerica, MA, USA) equipped with a 5 mm dual carbon hydrogen (DCH) cryoprobe was used for all measurements. A 1-pulse experiment with ^{13}C decoupling to remove the ^{13}C satellites in the heparin spectrum was utilized.

Test solutions of heparin were prepared to concentrations of approximately 50 to 100 mg/mL in D_2O.The NMR spectra were acquired using a 90 degree pulse, a relaxation delay of 20 s, a 16 ppm spectral width, 32 k data points, and an acquisition time of 1.7 s. A 0.3 Hz line broadening was applied before Fourier transformation of the free induction decay (FID). Spectra were deconvoluted using Acorn NMR Inc.'s (Livermore, CA, USA) NUTS software.

In the deconvolution process, multiple peaks were used to obtain a best fit to the overall envelope of the methyl region of the spectrum (1.9 to 2.3 ppm). The individual deconvolution peaks were assigned to heparin, DS, or OSCS depending on their chemical shifts and the sum of their intensities was determined. For example, the amount of OSCS was determined from the sum of the intensities of deconvolution peaks that had shifts between 2.09–2.25 ppm, while the amount of DS was determined from the intensity of the single peak centered at 2.08 ppm and the amount of heparin was determined from the intensities of the deconvolution peaks between 1.98 and 2.07 ppm.

The percentages of DS and OSCS relative to heparin (H) were calculated using: $\%DS = 100 \times DS/(H/0.15)$ and $\%OSCS = 100 \times OSCS/(H/0.15)$, where 0.15 accounts for the fact that on average, only 15% of the heparin disaccharide units contain a methyl group compared to

100% for the disaccharide units of DS and OSCS,[7] and DS and OSCS refer to the integrals of the DS and OSCS peaks.

Buffer Preparation

Equimolar concentrations of lithium phosphate dibasic and phosphoric acid were prepared separately as follows. Lithium phosphate electrolyte (1 M) was prepared using 10.3 g (103.93 g/mole) of lithium phosphate dibasic in 100 mL with water. Phosphoric acid (1 M) was prepared by transferring 11.5 g (6.8 mL) of 85% phosphoric acid (98.0 g/mole) and diluting to volume with water. Using a pH meter, the pH was adjusted to 2.5 by titrating with an aliquot of the 1 M phosphoric acid solution.

Preparation of phosphate buffers with different counterions, concentrations, and pH values were performed using similar protocols.

RESULTS AND DISCUSSION

The Buffer Counterion

The selection of the buffer counterion has been underemphasized in CE. In most cases, the sodium salt is simply selected and, even if good results are obtained, this is usually not the best choice. Figure 1 shows a series of heparin separations using phosphate buffer with a series of buffer counterions, run at voltages that resulted in currents between 55–60 μA. The counterion concentration was held at 600 mM and the phosphate concentration was allowed to float. From these data, it was seen that the sodium salt was not the optimal choice. The sharpest peaks were found for bis-tris although the resolution was poor. The lithium salt provided the best resolution closely followed by tris buffer which provided sharper peaks. Ammonium ion was the poorest choice for buffer counterion.

Another way to prepare the buffer is to fix the phosphate concentration and allow the counterion concentration to float. Figure 2 shows data with 0.1% OSCS in heparin for a series of counterions, run in the constant voltage mode. For this means of buffer preparation, only lithium phosphate provided complete separation between OSCS and heparin. However, it was understood that higher concentrations of tris allowed for complete separation.[6] An additional disadvantage of tris was the higher UV absorbance of that buffer counterion. Based on these factors, lithium was selected as the buffer counterion.

Impact of pH

The net charge on heparin and its impurities was generally independent of pH since sulfate was ionized at all conventional CE pHs. When the sodium salt was used as the counterion, separations were clearly

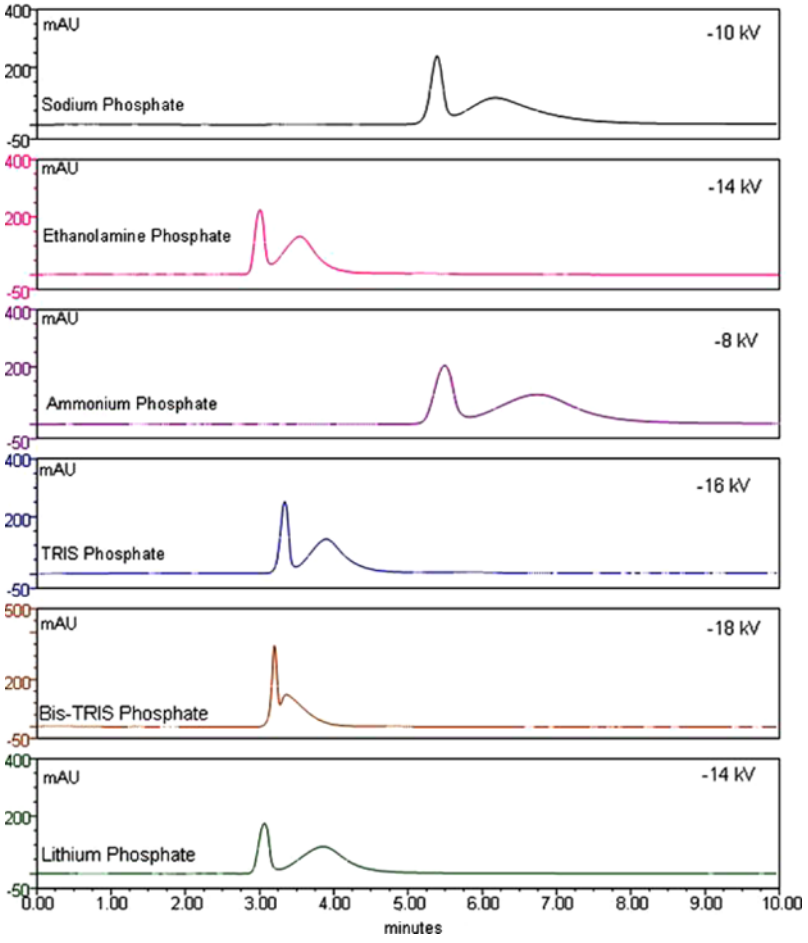

FIGURE 1 Impact of the buffer counterion on the heparin/OSCS separation. Buffer: 600 mM phosphate, pH 2.5, buffer counterion: 600 mM, capillary: 25 µm i.d., 31 cm total length, 21.5 cm effective length. Injection: 500 mbs, wavelength 195 nm. USP Heparin System Suitability Reference Standard: 50 mg/mL. (Figure available in color online.)

superior at lower pH[1]. As Figure 3 indicates, the impact of pH was less important when the lithium salt was employed. In any event, pH 2.5 was selected as additional bandbroadening can be seen at lower pHs.

Buffer Concentration and Capillary Length

Using identical conditions (buffer, sample, temperature, and field strength), a comparison of a heparin separation on the short end (8.5 cm) and the long end (24.5 cm) of the capillary is shown in Figure 4.

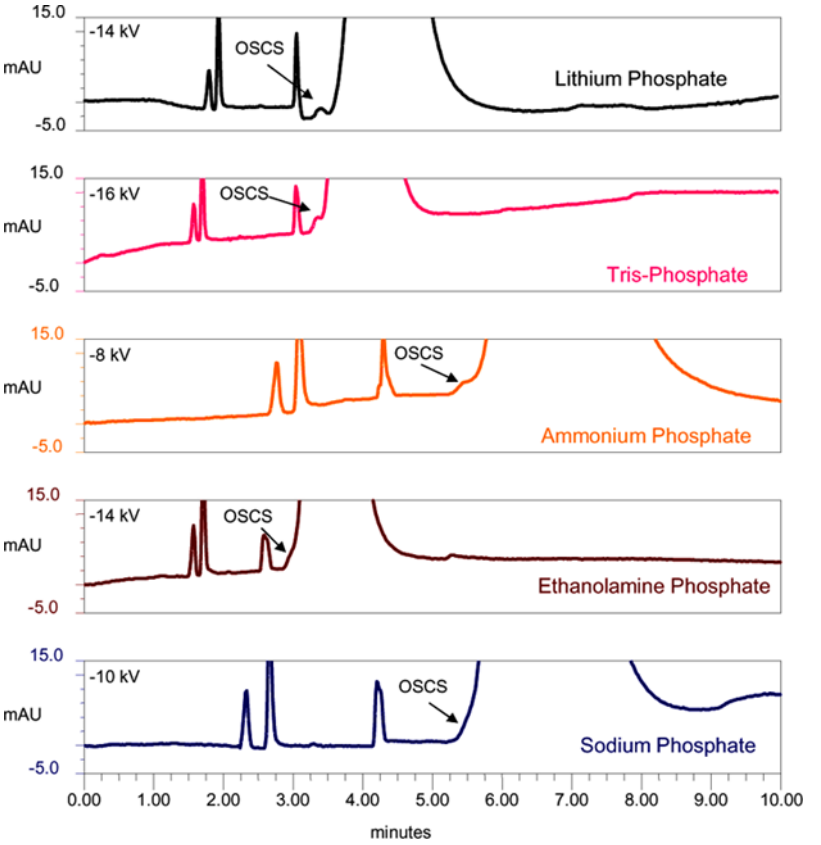

FIGURE 2 Effect of the buffer counterion on the heparin/OSCS separation and OSCS LOD. Buffer: 600 mM phosphate, pH 2.5, Capillary: 25 µm i.d., 31 cm total length, 21.5 cm effective length, Injection: 500 mbs, wavelength 195 nm. 0.1% OSCS in 50 mg/mL Heparin API. (Figure available in color online.)

With 600 mM lithium phosphate, pH 2.5 the separation was close to the baseline on the long end. For the short end, further adjustments to the buffer were required to obtain the baseline.

The simplest means to accomplish baseline resolution was to further increase the buffer concentration. Figure 5 illustrates the separation at 3 buffer concentrations: 0.6, 0.8, and 1.0 M. As the buffer concentration was increased, the resolution continued to improve. Even at the 1 M level, the current was only 75 µA.

The reduction of the capillary length by approximately two thirds only slightly decreased the resolution of OSCS and heparin but significantly improved the sensitivity and resolution between the heparin and DS peaks. Both heparin and DS appeared to be more polydisperse compared to OSCS; thus, these peaks broadened as a function of capillary length.

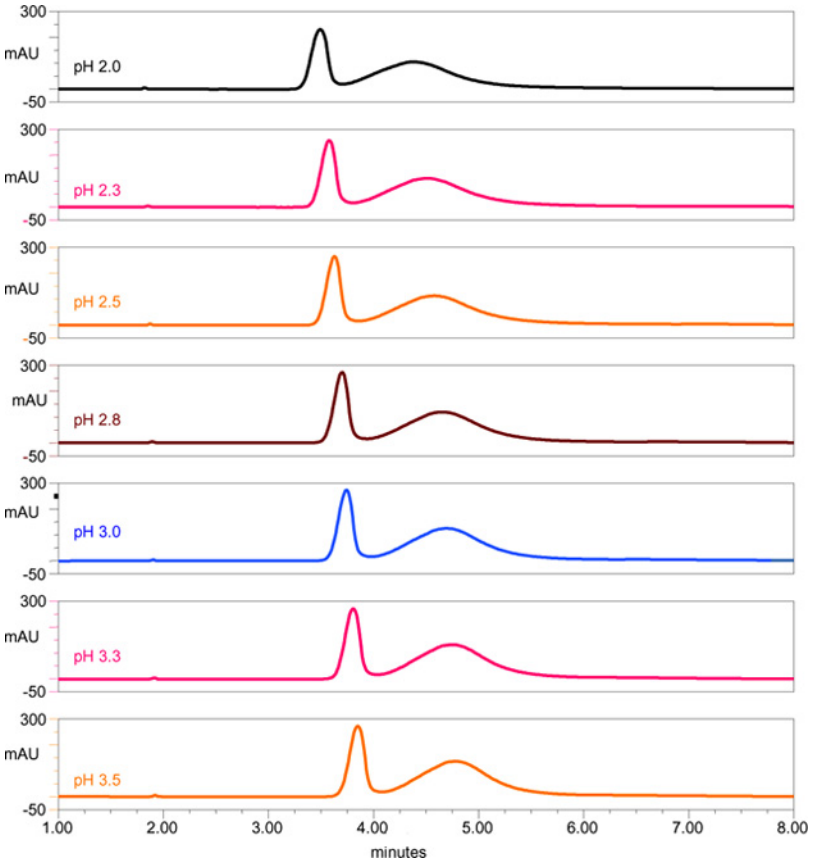

FIGURE 3 Impact of pH on the heparin OSCS separation. Buffer: 600 mM lithium phosphate, pH variable, capillary: 25 µm i.d., 31 cm total length, 21.5 cm effective length. Voltage: −14 kV, Injection: 500 mbs, wavelength 195 nm, Sample: USP Heparin System Suitability Reference Standard: 50 mg/mL. (Figure available in color online.)

Injection

The size of the injection was a compromise between the requisite limit of detection of the method and the amount of tolerable bandbroadening. When using a 1 M buffer, the amount of sample stacking should be substantial, but there were 2 factors that worked against stacking. First of all, heparin had an extremely high charge density due to the number of sulfate groups on the molecule. Secondly, the heparin concentration was very high (50 mg/mL) compared to usual CE practice.

An injection study using the short-end is shown in Figure 6. A small but tolerable amount of bandbroadening for OSCS was noticed when the injection size was increased from 250 to 500 millibar seconds (mbs). Further increases in the injection size resulted in more substantive

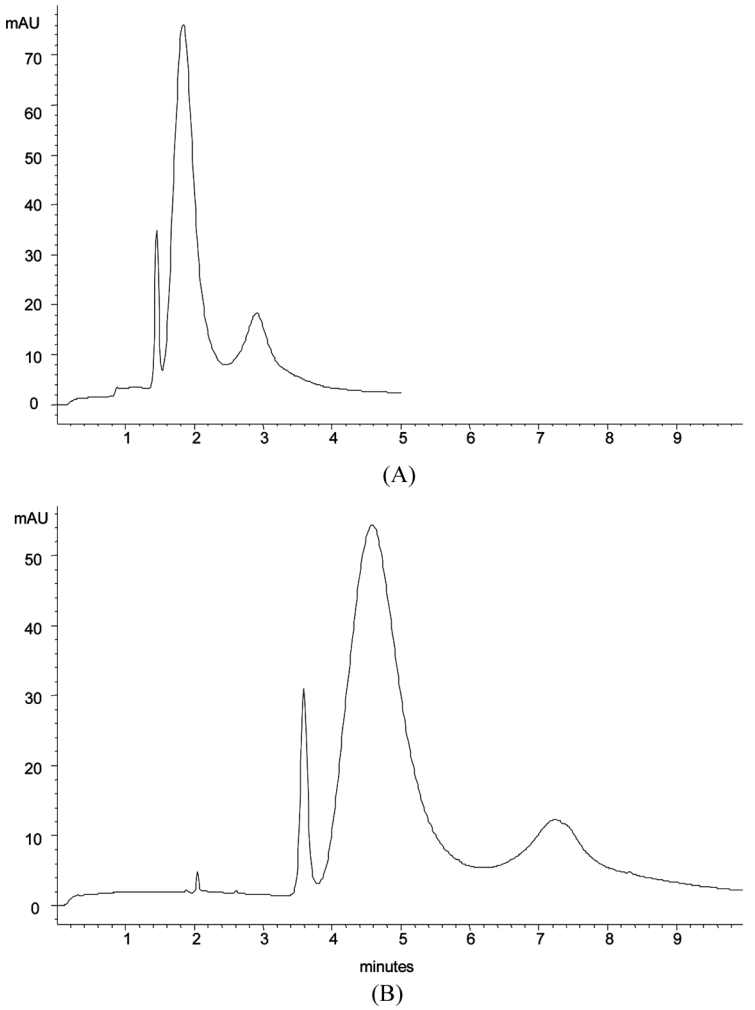

FIGURE 4 Comparison of the heparin/OSCS separation on the short-end (8.5 cm) and the long-end (24.5 cm). Buffer: 600 mM Lithium Phosphate, pH 2.5, Capillary: 25 μm i.d., 33 cm total length, (A) 8.5 cm and (B) 24.5 cm effective length. Voltage −14 kV, Injection: 500 mbs, wavelength 195 nm. Heparin API, 30 mg/mL.

bandbroadening. The injection fill rate was calibrated using 50 mbar pressure and found to be 0.0258 cm/sec for a heparin sample at 30 mg/mL. The injection zone for a 500 mbs injection is 0.258 cm or 3% of the capillary volume. The same study was performed on the 24.5 cm long end (data not shown). Here 1000 mbs or 2% of the capillary could be injected before substantial bandbroadening of OSCS was noticed. Neither heparin nor DS showed any notable injection-related bandbroadening since these polydisperse materials produced naturally broad peaks.

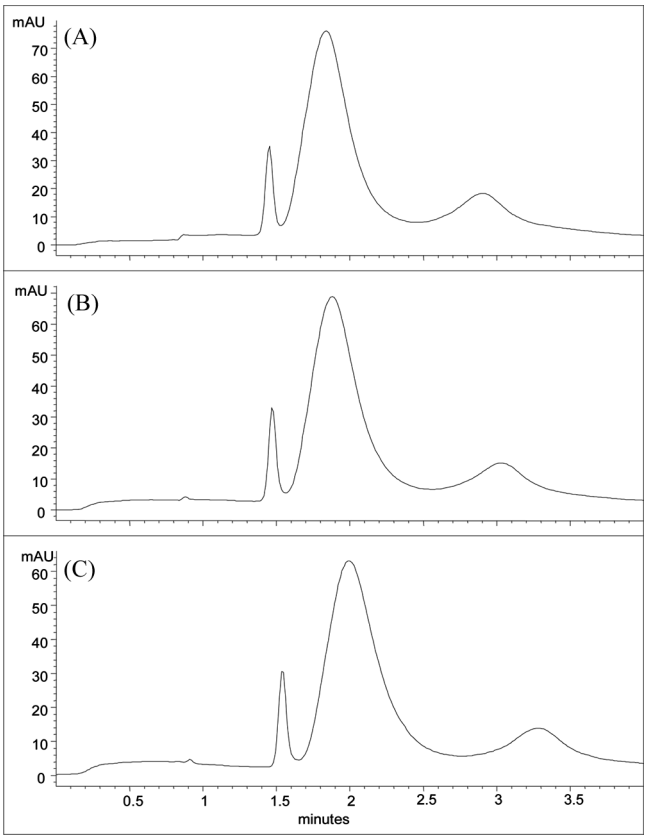

FIGURE 5 Effect of buffer concentration on the separation of heparin, OSCS, and DS. Buffer Concentration, (A) 600 mM, (B) 800 mM, and (C) 1 M Lithium Phosphate, pH 2.5, capillary: 25 µm i.d., 33 cm total length, 8.5, effective length. Voltage −14 kV, Injection: 500 mbs, wavelength 195 nm, Heparin API, 30 mg/mL.

Heparin Concentration

The LOD for OSCS and DS is based in part on the heparin concentration that is injected into the machine. For an impurity, per ICH guidelines, it is important that the LOD be less than 0.1%. A heparin concentration study on the 24.5 cm capillary is shown in Figure 7. A modest amount of bandbroadening for OSCS was seen at the 48 mg/mL level. At 100 mg/mL heparin, the amount of bandbroadening was substantial.

Capillary Temperature

The impact of capillary temperature was studied (data not shown) and the results were unremarkable. The migration time for OSCS was reduced by about 12 sec when the temperature was increased from 20 to 30°C. The

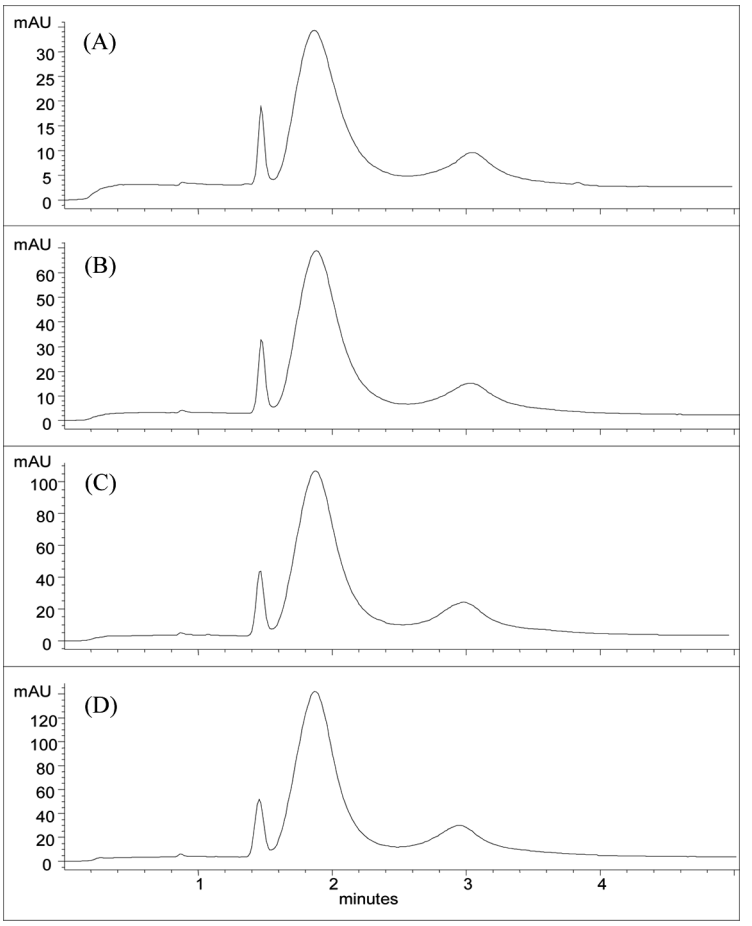

FIGURE 6 Effect of injection volume on the efficiency and resolution of OSCS. Buffer: 1 M Lithium Phosphate, pH 2.5, Capillary: 25 µm i.d., 33 cm total length, 8.5, effective length. Voltage 14 kV; Injection: (A) 250, (B) 500, (C) 750, and (D) 1000 mbs; wavelength 195 nm. Heparin API, 30 mg/mL.

resolution was best at the lower temperature so 20°C was adopted. Lower temperatures could work better but it might take too long for the instrument to achieve the target temperature, particularly during the summer months is less environmentally controlled areas.

Analytical Figures of Merit

The system was calibrated using OSCS ranging in concentrations from 0 to 0.5% in heparin (50 mg/mL). The blank was clear and it can be seen in Figure 8 that the LOD was less than 0.03%, S/N = 5.3. A correlation coefficient of 0.998 was obtained when plotting the peak area versus the concentration of these injections. The % relative standard deviation for

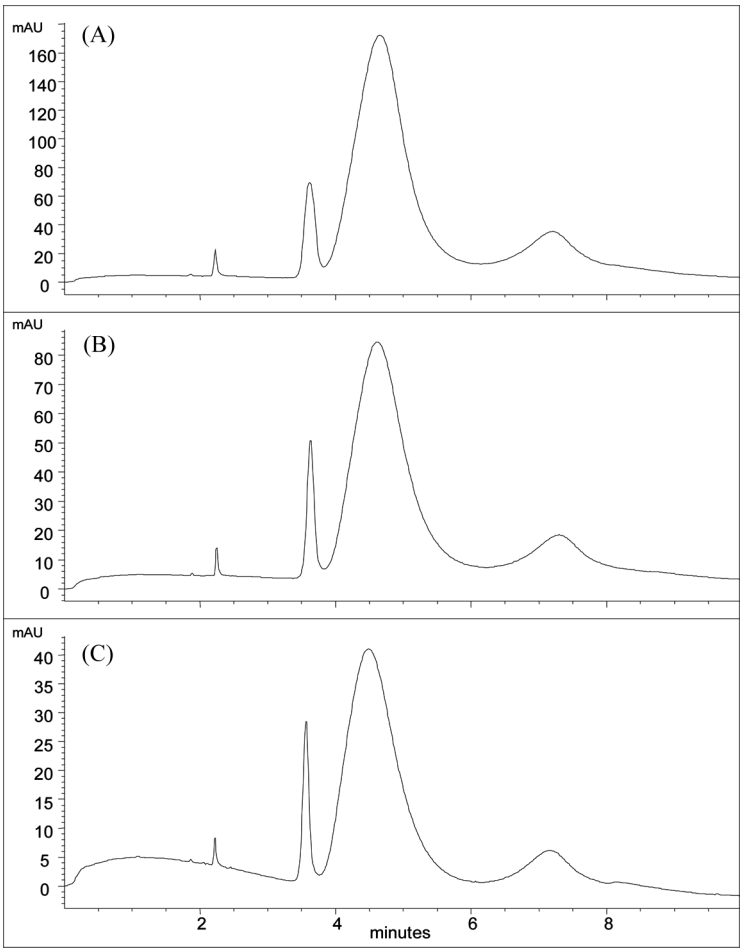

FIGURE 7 Effect of heparin concentration on the peak width of OSCS, Buffer: 1 M Lithium Phosphate, pH 2.5, Capillary: 25 μm i.d., 33 cm total length, 8.5, effective length. Voltage 14 kV, Injection: 500 mbs, wavelength 195 nm. Heparin Concentration: (A) 100 mg/mL, (B) 48 mg/mL, and (C) 24 mg/mL.

normalized peak areas obtained from 5 injections each of 5 levels of OSCS and DS over 3 days was determined. The results ranged from 1.9–7.8% for OSCS at the 0.4 and 0.04% levels and 2.0–9.8% for DS at 2.0 and 0.4% levels. The error of integration was 1.6 and 1.4% for OSCS and DS, respectively, at the highest level. The migration time variation was always less than 0.5% and usually much lower.

Selectivity for Additional Oversulfated Compounds

Since any polysulfated contaminate has the potential to cause the same type of clinical reaction that OSCS,[8] the selectivity of the method to other

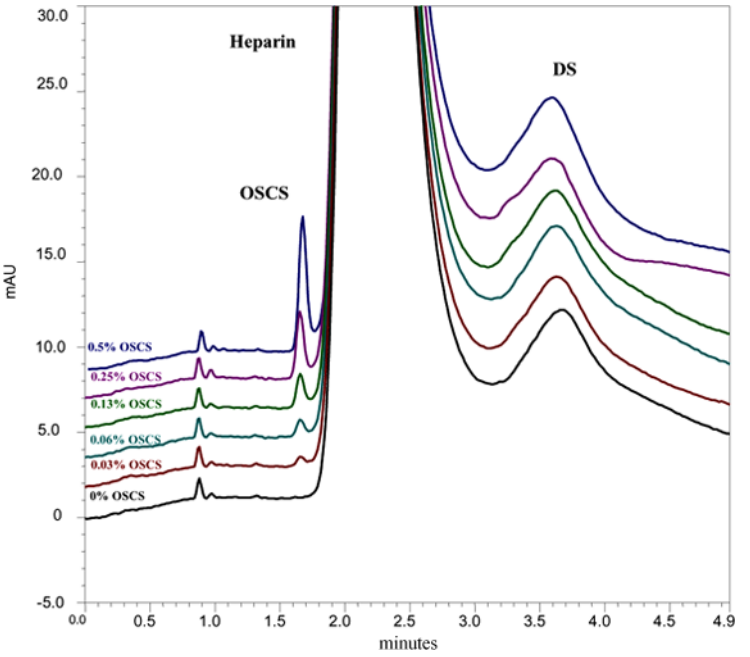

FIGURE 8 Linearity of OSCS in heparin near LOD, Buffer: 1 M LiPhosphate, pH 2.5, Capillary: 25 µm i.d., 31 cm total length, 8.5 cm effective length. Voltage: 14 kV, injection: 500 mbs, wavelength 195 nm. Heparin API: 50 mg/mL containing DS, spiked with OSCS. (Figure available in color online.)

oversulfated GAG's was evaluated. The short end analysis yields partial resolution of OSCS and OSDS (Figure 9). In addition, over sulfated heparin can be resolved from OSCS but not OSDS. Over sulfated heparan sulfate coeluted with the front of the heparin peak (not shown). A longer capillary could be used to improve the separation of OSCS and OSDS but not for the other aforementioned oversulfated solutes.

Correlation with NMR Spectroscopy

The 600 MHz ^{1}H spectrum of the methyl region of a contaminated heparin sample is shown in Figure 10. The spectral identification and deconvolution, as described in the experimental section is indicated on the figure.

CE and NMR are complementary techniques for the determination of impurities, notably OSCS in heparin samples. In order to perform a meaningful comparison, the %OSCS determined by both techniques needs to be calculated in an identical fashion. By NMR, both the %OSCS and %DS were calculated relative to the normalized peak area of the heparin signal.

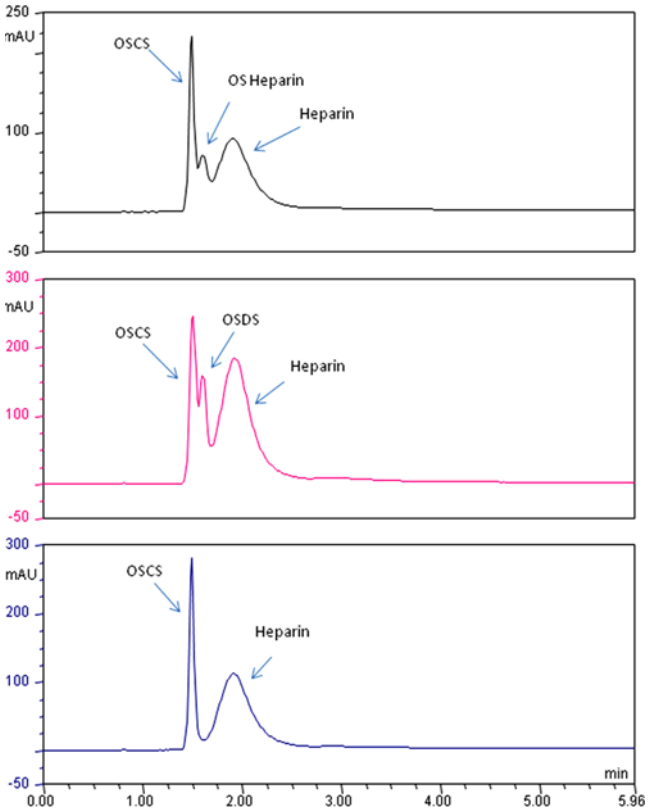

FIGURE 9 Separation of additional oversulfated compounds on the short end of the capillary. 1 M Lithium Phosphate, pH 2.5, Capillary: 25 µm i.d., 31 cm total length, 8.5 cm effective length. Voltage −14 kV, Injection: 500 mbs, wavelength 195 nm. USP Heparin System Suitability Reference Standard: 50 mg/mL spiked with 10% OSDS and OS Heparin. (Figure available in color online.)

By contrast, quantitation by CE was performed using a calibration curve consisting of external standards of varying concentration. Thus, the %OSCS was calculated based on the entire mass of the sample. For pharmaceutical grade material, the %OSCS calculated from the calibration curve or by normalization should be similar since extraneous impurities were quite low. This hypothesis was tested for a few samples known to be contaminated with OSCS.

The relative UV absorbances of OSCS and DS were found to be 3.69 and 6.40 times greater than heparin, respectively. The OSCS content of 4 heparin samples was calculated using both normalization and external standardization. The results in %OSCS were 17.0 and 16.8; 8.3 and 8.4; 5.1 and 5.4; and 1.9 and 2.3 for each technique, respectively. As expected, the results were quite similar for each technique. The advantage of the

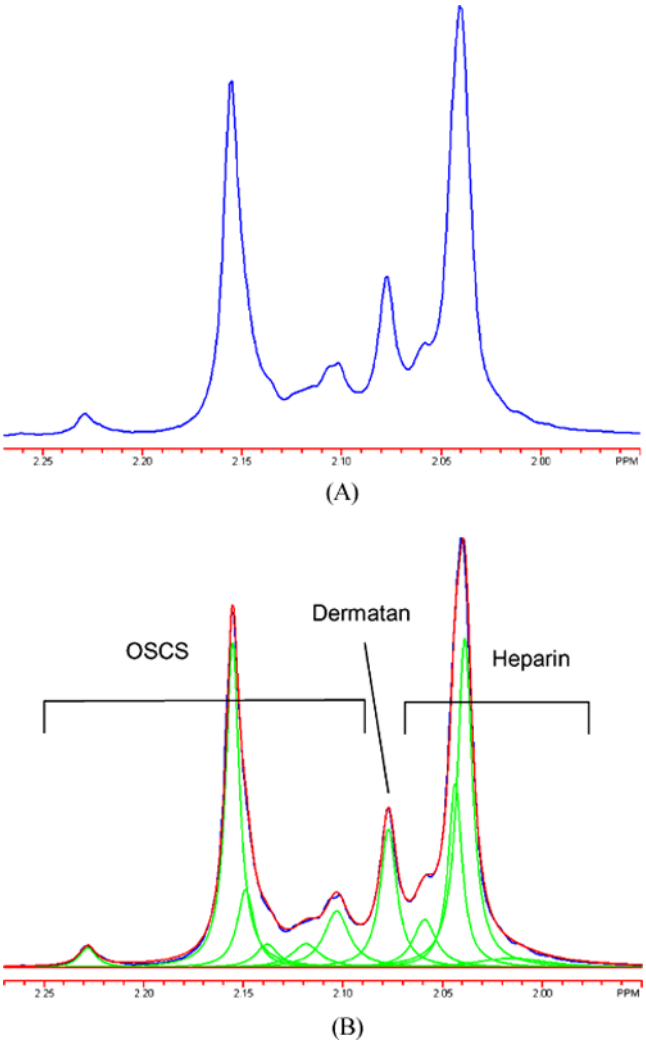

FIGURE 10 (A) 600 ^{1}H MHz NMR spectrum of the methyl region of heparin. (B) The deconvolution of the methyl region where the green (light) curves show individual peaks used in the fitting process while the red (dark) curve shows the overall fit, and the peaks associated with OSCS, Heparin, and Dermatan are indicated. (Figure available in color online.)

normalization approach was that impurity standards were not needed for quantitative analysis.

The data for 29 Heparin API samples known to be contaminated with OSCS and 1 negative control are shown in Table 1. With perhaps 1 exception (sample 19), the data provided similar results for OSCS using both CE and NMR. A paired t-test [P(T<=t) two-tail = 0.85394] demonstrated that both data sets were not significantly different from each other.

TABLE 1 OSCS Determined by NMR and CE (%)

	OSCS (%)	
Sample No.	NMR	CE
1	4.2	4.1
2	5.0	5.4
3	6.7	4.9
4	13.4	13.3
5	18.9	14.7
6	4.0	4.0
7	13.4	15.8
8	20.5	24.6
9	14.5	14.8
10	6.3	6.8
11	9.3	12.3
12	17.3	18.5
13	32.0	28.7
14	15.1	13.3
15	20.5	17.6
16	21.6	19.2
17	3.1	2.9
18	12.8	10.3
19	6.1	2.1
20	1.4	0.4
21	12.0	9.2
22	3.2	1.9
23	ND	ND
24	3.3	1.2
25	3.3	2.7
26	17.6	16.6
27	10.0	8.8
28	6.1	5.4
29	4.4	4.0
30	3.4	1.9

CONCLUSION

Data have been presented that demonstrate reproducible high speed, high resolution separations of heparin, and important impurities in only 5 min. Sample preparation involved simple dissolution of heparin with water followed by filtration. The good correlation of the %OSCS measured by this method with NMR establishes a continuity of the historical results. The method has undergone testing in multiple laboratories around the world and these results will be reported in a future paper.

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REFERENCES

1. Wielgos, T.; Havel, K.; Ivanova, N.; Weinberger, R. Determination of Impurities in Heparin by Capillary Electrophoresis Using High Molarity Phosphate Buffers. *J. Pharm. Biomed. Anal.* **2009**, *49*, 319–326.
2. Issaq, H. J.; Atamna, I. Z.; Metral, C. J.; Muschik, G. M. Factors that Influence Mobility, Resolution and Selectivity in Capillary Zone Electrophoresis. I. Sodium Phosphate vs. Potassium Phosphate. *J. Liq. Chromatogr.* **1990**, *13*, 1247–1259.
3. Ahuja, E. S.; Foley, J. P. Influence of Dodecyl Sulfate Counterion on Efficiency, Selectivity, Retention, Elution Range, and Resolution in Micellar Electrokinetic Capillary Chromatography. *Anal. Chem.* **1995**, *67*, 2315–2324.
4. Scapol, L.; Marchi, E.; Viscomi, G. C. Capillary Electrophoresis of Heparin and Dermatan Sulfate Unsaturated Disaccharides with Triethylamine and Acetonitrile as Electrolyte Additives. *J. Chromatogr. A* **1996**, *735*, 367–374.
5. Catai, J. R.; Torano, J. S.; Jongen, P. M.; de Jong, G. J.; Somsen, G. W. Analysis of Recombinant Human Growth Hormone by Capillary Electrophoresis with Bilayer-Coated Capillaries Using UV and MS Setection. *J. Chromatogr. B* **2007**, *852*, 160–166.
6. Somsen, G. W; Tak, Y. H.; Torano, J. S.; Jongen, P. M.; de Jong, G. J. Determination of Oversulfated Chondroitin Sulfate and Dermatan Sulfate Impurities in Heparin by Capillary Electrophoresis. *J. Chromatogr. A* **2009**, *1216*, 4107–4112.
7. Guerrini, M.; Bisio, A.; Torri, G. Combined ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectroscopy for Characterization of Heparin Preparations. *Thromb. Haemost.* **2001**, *27*, 473–482.
8. Kishimoto, T. K.; Viswanathan, K.; Ganguly, T.; Elankumaran, S.; Smith, S.; Pelzer, K.; Lansing, J. C.; Sriranganathan, N.; Zhao, G.; Galcheva-Gargova, Z.; Al-Hakim, A.; Bailey, G. S.; Fraser, B.; Roy, S.; Rogers-Cotrone, T.; Buhse, L.; Whary, M.; Fox, M.; Nasr, G. J.; Dal Pan, Z.; Shriver, R. S.; Langer, G.; Venkataraman, K. F.; Austen, J.; Woodcock, J.; Sasisekharan, R. Contaminated Heparin Associated with Adverse Clinical Events and Activation of the Contact System. *N. Engl. J. Med.* **2008**, *358*, 2457–2467.