Carbohydrate Polymers 141 (2016) 197–203

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Development of a rapid method for simultaneous separation of hyaluronic acid, chondroitin sulfate, dermatan sulfate and heparin by capillary electrophoresis

Ting Zhao [a], Xinlei Song [a], Xiaoqing Tan [a], Linghua Xu [a], Mingxiu Yu [a], Siyi Wang [a], Xiumei Liu [a,*], Fengshan Wang [a,b,**]

[a] School of Pharmaceutical Sciences, Shandong University, Jinan 250012, China
[b] Shandong Provincial Key Laboratory of Carbohydrate Chemistry and Glycobiology, Jinan 250012, China

ARTICLE INFO

Article history:
Received 16 September 2015
Received in revised form 5 January 2016
Accepted 9 January 2016
Available online 12 January 2016

Keywords:
Simultaneous separation
Glycosaminoglycans
Plasma
Capillary electrophoresis

ABSTRACT

This study reports the use of diethylenetriamine as background electrolyte for the simultaneous separation of hyaluronan acid, chondroitin sulfate, dermatan sulfate and heparin. The analytes were baseline separated by using an uncoated fused silica capillary at 37 °C with a run time of 23 min. The migration order, with hyaluronan acid at first and heparin at last, was related to the sulfation degree. The effect of salt concentration on resolution and migration order was also investigated. The developed method was applied to the simultaneous determination of hyaluronan acid and chondroitin sulfate in mouse plasma. Interferences in plasma were removed by protein precipitation and glycosaminoglycans were further purified by ethanol precipitation. The method was validated over the concentration range from 50 to 600 μg/mL for hyaluronan acid and 500 to 6000 μg/mL for chondroitin sulfate in mouse plasma. Results from assay validations showed that the method was selective and robust.

1. Introduction

Glycosaminoglycans (GAGs) are a family of polydisperse, negatively charged complex mixtures of linear polysaccharides that are involved in many biological processes, for example, enzyme regulation and cellular adhesion, growth, migration, or differentiation (Capila & Linhardt, 2002; Kreuger, Spillmann, Li, & Lindahl, 2006; Lyon & Gallagher, 1998; Selleck, 2000; Turnbull, Powell, & Guimond, 2001). Since GAGs are isolated from animal sources and have similar structures among different GAGs, it is difficult to obtain one kind of absolutely pure GAGs, for example, the most commonly used GAGs, heparin contains low levels (normally less than 1%) of several containments (e.g. heparan sulfate or chondroitin sulfate (CS) A, B or C) that are not related to adverse health effects (Keire et al., 2010). In order to control the quality of GAGs, it is very important to separate different intact GAGs simultaneously.

Recently, many studies pursuing the separation of GAGs have been reported, most of them are about the separation of GAGs after enzymatic degradation (Bendazzoli et al., 2010; Ucakturk et al., 2014; Volpi, Galeotti, Yang, & Linhardt, 2014; Volpi, Maccari, & Linhardt, 2009). Enzymatic degradation is time-consuming and expensive. There are also reports about the separation of intact GAGs. Previously reported separation methods of intact GAGs include: cellulose acetate membrane electrophoresis (Fateen, Ibrahim, Gouda, & Youssef, 2014; Nakano & Ozimek, 2014), polyacrylamide gel electrophoresis (Laremore, Ly, Solakyildirim, Zagorevski, & Linhardt, 2010), agarose gel electrophoresis (Breier, Ce, & Coelho, 2014), strong anion exchange high performance liquid chromatography (SAX-HPLC) (Hashii et al., 2010; Keire et al., 2010; Trehy, Reepmeyer, Kolinski, Westenberger, & Buhse, 2009) and capillary electrophoresis (CE) (Loegel, Trombley, Taylor, & Danielson, 2012; Wielgos, Havel, Ivanova, & Weinberger, 2009b). However, cellulose acetate membrane electrophoresis, polyacrylamide gel electrophoresis and polyacrylamide gel electrophoresis are laborious and time consuming, and ion-exchange HPLC requires expensive column. In contrast, CE is an optional technique which has high throughput and requires extremely small amount of sample. The most commonly used type of CE is capillary zone electrophoresis (CZE), which can separate analytes based on the difference in their charge: molecule weight ratio. Most of the

* Corresponding author.
** Corresponding author at: School of Pharmaceutical Sciences, Shandong University, Jinan 250012, Shandong, PR China. Tel.: +86 531 88382013; fax: +86 531 88382548.
E-mail addresses: liuxium@sdu.edu.cn (X. Liu), fswang@sdu.edu.cn (F. Wang).

http://dx.doi.org/10.1016/j.carbpol.2016.01.016
0144-8617/

Table 1
The comparison of the resolution efficiency of different kind background electrolytes.

BGE(background electrolyte)	Capillary i.d. (μm)	Analytes	Reference
50 mM phosphate (pH 3.0)	50	CS, CS-B, HA;CS, KS (keratan sulfate), HA;HS, CS, HA	Malavaki et al. (2008)
600 mM phosphate (pH 3.5)	25	Heparin, OSCS, HS, DS	Wielgos et al. (2009a,b)
36 mM phosphate (pH 3.5)	50	OSCS, heparin, chondroitins	Keire et al. (2010)
850 mM Tris phosphate (pH 3.0)	25	Heparin, OSCS(oversulfated CS), DS	Somsen et al. (2009)
600 mM Tris phosphate (pH 3.0)	50	HA, CS, heparin, OSCS	Liu et al. (2012)
50 mM EDA, 18.7 mM phosphate (pH 4.5)	50	DS, heparin, CS, OSCS, DS, heparin, HA, OSCS	Loegel et al. (2012)

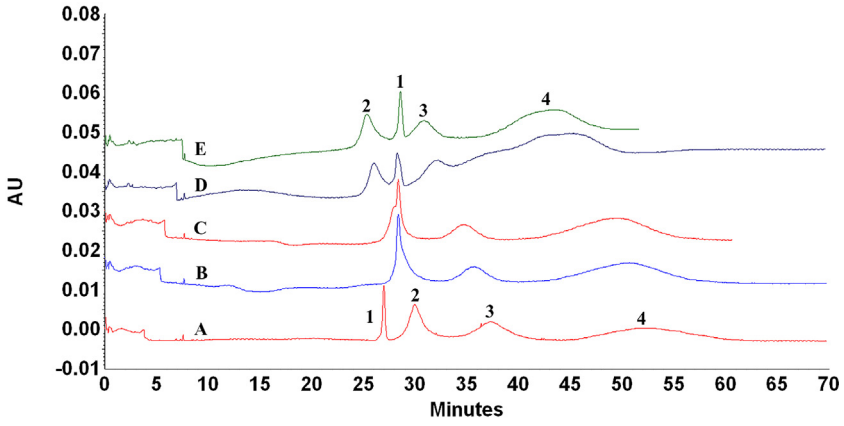

Fig. 1. Impact of NaH_2PO_4 concentration on the migration time and resolution of HA, CS, DS and heparin. Fused silica capillary: total length 60.2 cm, effective length 50 cm; Capillary i.d. 75 μm. Capillary temperature: 37 °C; Sample temperature: 4 °C; Injection: hydrodynamically at 0.5 psi×10 s; Detection wavelength: 200 nm. Standard sample mixture: 0.25 mg/mL HA, 1.08 mg/mL CS, 0.82 mg/mL DS, 10 mg/mL heparin. Voltage: -10 kV. Peaks identification: (1) HA; (2) CS; (3) DS; (4) heparin. BGE (pH 5.0, 80 mM diethylenetriamine phosphate buffer): (A) 0 mM NaH_2PO_4; (B) 40 mM NaH_2PO_4; (C) 80 mM NaH_2PO_4; (D) 120 mM NaH_2PO_4; (E) 140 mM NaH_2PO_4.

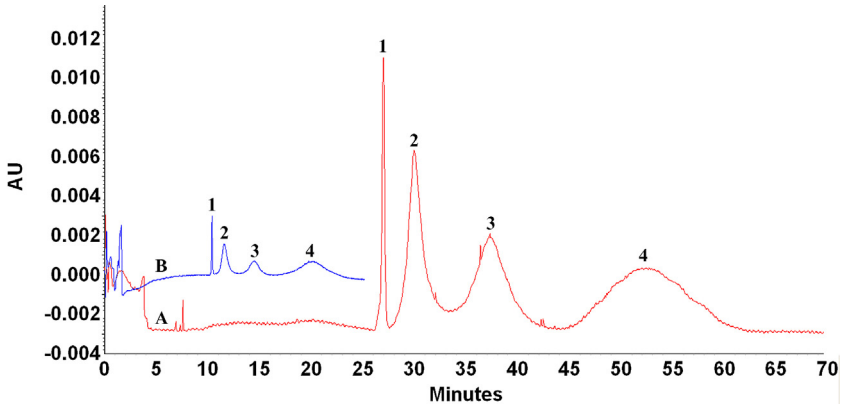

Fig. 2. Impact of capillary i.d. and voltage on the analysis of HA, CS, DS and heparin. Standard sample mixture: 0.25 mg/mL HA, 1.08 mg/mL CS, 0.82 mg/mL DS, 10 mg/mL heparin. Peaks identification: (1) HA; (2) CS; (3) DS; (4) heparin. BGE: 80 mM diethylenetriamine phosphate buffer (pH 5.0). (A) 75 μm i.d., Voltage: -10 kV. (B) 50 μm i.d., Voltage: -24 kV; Other conditions as in Fig. 1.

intact GAGs separations were achieved with low pH phosphate (Keire et al., 2010; Malavaki et al., 2008; Wielgos et al., 2009b) or Tris-phosphate buffer systems (Liu et al., 2012; Somsen, Tak, Torano, Jongen, & de Jong, 2009). Malavaki et al. (2008) developed a CE method for the quality control of CS in raw materials and formulations. By employing the established method, CS, hyaluronan (HA) and dermatan sulfate (DS), CS, HA and keratin sulfate (KS) and CS, HA and HS could be separated in three separate runs, but the resolutions were not satisfactory. With an increase in the number of adverse reactions associated with the administration of heparin, the study was focused on the separation of heparin and its impurities (Keire et al., 2010; Malavaki et al., 2008; Somsen et al., 2009; Wielgos, Havel, Ivanova, & Weinberger, 2009a), but none of the above studies could separate CS, DS and heparin in one run. Later, polyamines were investigated for further separation of GAGs (Loegel et al., 2012), in which many kinds of polyamines were investigated and finally found that ethylenediamine (EDA)-phosphate buffer system could give satisfactory resolution: DS, heparin, CS and over sulfated chondroitin sulfate (OSCS) could be separated simultaneously in one run and DS, heparin, HA and OSCS could be separated simultaneously in another run, but HA, CS, DS and heparin could not be separated in one run in the study. The comparison of the resolution efficiency of different kind background electrolytes (BGE) is shown in Table 1.

GAGs have been found in biological fluids such as blood plasma and urine (Sakai et al., 2002). In blood plasma, GAGs interact with biologically important proteins (Gotti, Parma, Spelta, & Liverani, 2013), cells (Dikov, Reich, Dworkin, Thomas, & Miller, 1998), and vascular endothelium (Kuschert et al., 1999). Since GAGs constitute the cellular environment and play many vital roles in physical process, the change of *in vivo* concentration of plasma GAGs levels can help initial diagnosis and be a measure for responsiveness to