African Journal of Biotechnology Vol. 11(19), pp. 4311-4316, 6 march, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3516
ISSN 1684–5315 © 2012 Academic Journals

Review

Progress in structural analysis of glycosaminoglycans and their applications in biomaterials

Shuangli Xiong, Anlin Li and Dabin Hou

College of Life Science and Engineering, Southwest University of Science and Technology, 621010, Mianyang, Sichuan Province, China.

Accepted 24 January, 2012

The rising interest in the application of glycosaminoglycans (GAGs) is one of the main reasons for exploring different species and optimizing the extracting conditions of various GAGs in the last decade. Recent research data on GAGs have suggested that they have many new biological functions such as anti-atherogenesis, anticoagulation, prevention and cure of arthritis, morphogenesis and cell division. They are widely applied in functional food, clinical medicine, cosmetics and biomaterial. Especially, in the biomaterials industry, an increasing number of new applications have been found for GAGs such as tissue-engineering material, biodegradable film-forming materials and micro-encapsulating agents. However, these bioactivities and applications are dependent on their fine structure with different monosaccharide unit and substitute group patterns. This review provides recent information on GAGs preparation, determination and structural assay, as well as their potential novel or improved applications such as tissue engineering biomaterial.

Key words: Glycosaminoglycan, structure analysis, biomaterial.

INTRODUCTION

The glycosaminoglycan (GAG) containing hexosamine and uronic acid (or hexose) and other various substituent groups, are a group of structurally diverse polysaccharide polymers from animals and exist abundantly either natively or as part of proteolycans (PGs) in extracellular matrices and on cell surfaces. The GAGs chains consist of generally repeated disaccharide units that are negatively charged and assembled into long linear/ acidic chains with variable lengths and composition (hexosamine, uronic acid, sulfate group). Thus conveying a high polyanionic charge to these macromolecules (Raman et al., 2003). There are four major classes namely hyalu-ronic acid (HA), chondroitin/dermatan sulfate (CS/DS), heparin/heparan sulphates (HS) and keratin sulfate (KS) according to different kinds and quantities of monosaccharide component and substituent group in GAG molecular chain. The bioactivities and their applica-tions are quite distinct from each other. Some examples of GAGs uses in nature include heparin as an anti-coagulant, HA as a component in the synovial fluid lubricant in body joints and CS, which can be found in connective tissues, cartilage and tendons. Furthermore, the patterns and degree of sulfation, molecular mass, relative amounts of iduronic and glucuronic acid (GlcA) and hexosamine in the same kind of GAG considerably changes in quantity and position according to the species, age and/or tissues of origin and this plays an important role in the various bioactivities and applications (Volpi, 2004). For example, bovine tracheal cartilage is mainly sulfated in position four of GalNAc, which can protect high-dense lipoprotein against copper-induced oxidation (Albertini et al., 1999). CS from shark cartilage has a higher level of 2-sulfated GlcA residues as well as 6-sulfated N-acetylgalactosamine (GalNAc) which has anticancer function (Sim et al., 2007). GAGs reported to date have been used clinically for the treatment of chronic diseases such as degenerative arthritis, cirrhosis and chronic photo damage due to the emerging bioactivities such as antioxidant, anti-atherogenesis, anticoagulation, prevention and cure of arthritis, etc

*Corresponding author. E-mail: lxberry225@yahoo.com.cn.

Table 1. Structural characteristics and main preparation methods of GAGs.

GAG	Disaccharide unit	Modification state and rare moiety	Industrial preparation method and main source
Chondroitin sulfate (CS)	β-D-glucuronic acid (1→3)-linked to a 2-acetamido-2-deoxy-β-D-galactopyranose.	2-O-(IdoA), 4O-, 6O-, on NAc-Gal and rare 3-O on GlcA.	Extraction from cartilage such as pig laryngeal cartilage, chick keel cartilage, and bovine nasal cartilage, etc.
Hyaluronic acid(HA)	β-D-glucuronic acid (1→3)-linked to 2-acetamido-2-deoxy-β-D-glucopyranose.	Unsulfated	Extraction from crista galli, umbilical cord and pig skin; fermentation method
Heparin	α-L-iduronic acid (1→4)-linked to glucosamine.	N-sulfation and acetylation as well as 2-O-, 4O-, 6O- as well as 3-O- sulfation coupled with C5 of -COOH epimerization on GlcA and IdoA.	Extraction from intestinal mucosa of pig or bovine.
Heparin sulfate(HS)	α-L-iduronic acid (1→4)-linked to glucosamine or β-D-glucuronic acid (1→4)-linked to glucosamine.	N-sulfation and acetylation as well as 2-O-, 4O-, 6O- as well as 3-O- sulfation coupled with C5 of -COOH epimerization on GlcA and IdoA.	
Dermatan sulphates (DS)	α-L-iduronicacid (1→3)-linked to a 2-acetamido-β-deoxy-b-D-galactopyranose	2-O-(IdoA), 4O-, 6O- on NAc-Gal and rare 3-O on IdoA	
Keratin sulfate (KS)	β-D-galactose (1→4)-linked to 2-acetamido-2-deoxy-β-D-glucopyranose	Only 6O-sulfation, KS-II may be fucosylated	

(Xiong et al., 2007). More recently, there have been a number of reports on the biomedical activities including tissue engineering, drug delivery, regenerative medicine and surgery (Strehin et al., 2010; Fajardo et al., 2010; Eun et al., 2007).

GAGs have become well known owing to their biomedical bioactivities and material characterization, the market of nutraceuticals containing various GAGs have dramatically increased, they are being developed as functional food, biochemical pharmaceutical, cosmetics and biomaterial. In order to better utilize various GAGs, more research is being focused on the preparation, functional characteristics, bioactivities and their applications in different areas. In this review, we mainly focused on the latest preparation, separation, determination and identification methods and applications of GAGs.

STRUCTURAL CHARACTERISTICS AND PREPARATION OF MAIN GAGS

As shown in Table 1, the disaccharide units of each GAGs are remarkably different, especially, the molecular components including kinds of hexosamine and uronic acid, position of sulfate group, which display a very large variety of structures. HA is the relatively simple polymeric species which has no sulfation pattern. CS can also be classified into some sub-groups such as CS-A (galactosamine can be sulfated on the C-4, mainly from pig laryngeal/ bovine nasal cartilage), CS-C (galactosamine can be sulfated on the C-6, mainly from pig laryngeal/ bovine nasal cartilage) and CS-E (galactosamine can be sulfated on the C-4 and C-6, originating from shark cartilage). Repeating disaccharide units in heparin and HS are either α-L-iduronic acid (1→4)-linked to glucosamine or β-D-glucuronic acid (1→4)-linked to glucosamine. The glucosamine is either N-sulfated or N-acetylated, and can be ester O-sulfated at the C-3 or C-6 position, while the uronic acid can be sulfated on C-2 position (Imberty et al., 2007). For example, HA is the sole GAG which is free from sulfate and protein. General boiling water extraction is suitable for it.

For the other GAGs, sodium salts such as NaCl and NaAc with different concentrations are the common extraction reagents. At present, GAGs are mainly extracted by enzymolysis of exogenous proteinase or sodium hydroxide for their attachment to a protein core and formation of GAG-protein/fat aggregates (Chen et al., 2011). Their usual and advanced separation and purification technologies are membrane separation, chromatographic separation (ion-exchange chromatography), hydrogen peroxide oxidation for decoloration and fractioned precipitation of alcohol. For promoting the bioactivities and applications, some of their chemical modifications are becoming hot topics in recent years.

For example, polysulfated CS derivatives are prepared because of their higher antiviral and anticoagulant capacity, heparin with low molecular weight is generally popular for its stronger antithrombotic effect, longer half-time, weaker hemorrhage side effect and higher bioavailability. The industrial production methods mainly include degradation of nitrous acid/hydrogen peroxide, β-elimination and enzymolysis.

COMPOSITION ASSAY OF GAG

The difference of GAGs are attributed to the component including hexuronic acid, hexosamine and sulfate pattern, thus their determination and identification are becoming the basis of understanding the first structure which further illustrates the structure-effect relationship of various GAG.

Determination and identification of hexuronic acid

During the past decades, high-performance liquid chromatography (HPLC), ion chromatography (IC) and gas chromatography (GC) were used to determine uronic acid (Kakit et al., 2006). These methods are not widespread because they easily give lower determination value due to the lability of uronic acids in aqueous acid which need complicate derivation process before column analysis. Thus, the most common assay method for the analysis of hexuronic acid is spectrophotometric method, which is based on the chromogenic formation between hexuronic acid produced by sulfuric acid hydrolysis of hexuronic acid and the color forming reagents including carbazole or meta-hydroxydiphenyl (Filisetti-Cozzi and Carpita, 1991). For determination and identification of hexuronic acid simultaneously, IC and GC methods are better than the above colourimetry. Owing to the fact that glucosidic bond which is consecutive to uronic acid is difficult to be hydrolyzed by acid and hydrolyzed uronic acid is easy to form lactone, uronic acid is determined and identified by GC analysis after reduction of carboxy group in uronic acid by 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride, aldoononitrile acetates deviation (treated by hydroxylamine hydrochloride, pyridine and acetic anhydride, respectively). It makes uronic acid easier to be hydrolyzed by acid and avoids lactone production and use of uronic acid standard (Kakit et al., 2006).

Determination and identification of hexosamine

Elson-Morgan method is very easy for assay of hexosamine. However, the hydrolysis at 100℃ and acetylation again usually demands intensive time and labor leads to incomplete hydrolysis and lower results. In recent years, more attentions have been paid to HPLC including amino-bound column, strong-anion exchange high-performance liquid chromatography (SAX-HPLC), reverse phase high-performance liquid chromatography (RP-HPLC) and so on. HPLC and pulsed amperometric detection (PAD) with a single isocratic analysis are adapted to isolate and assay simultaneously amino sugars as well as neutral saccharides (Cheng and Kaplan, 2003). In our laboratory, we used RP-HPLC procedures and ultraviolet and detection before automated precolumn derivation of ChS hydrolyzate with o-phthalaldehyde (OPA) to detect the content of GalNAc in CS from pig laryngeal cartilage. This method, similar to the above anion-exchange column chromatography, has higher resolution and reproducibility than Elson-Morgan method. Especially, it can also distinguish GalNAc from GlcNAc.

Determination and identification of sulfate group

Main methods used for the assay of sulfate group include gravimetric estimation as barium sulphate, turbidimetry and ion chromatography (Wang et al., 2004). In contrast to turbidimetry, the advantage of ion chromatography is simplicity, good reproducibility and sensitivity, and no other interference of impurities.

COLORIMETRY ASSAY AND IDENTIFICATION OF GAG

Considering the complexity and inaccuracy caused by hydrolysis or derivation procedure for determination of substituent groups, the direct determination methods such as colorimetry, chemical titrimetry and chromatography are rapidly developed. Owing to the polyanionic property of GAG, they can form soluble GAG-dye complex with cationic dye such as alcian blue, azure A, victoriapure blue BO or dimethymethyl blue, which alter the absorption spectra as compared to that of the dye alone (Liu and Qian, 2005). The merit of this method is rapid, simple and does not need strong acid or high temperature. However, it may be affected by the other

polyanionic macromolecules such as other carbohydrates, protein, DNA and RNA. At present, the emerging structure analysis or identification methods of GAG are spectrum analysis combined with enzymolysis technology (Ola et al., 2005). The GAGs are long chain molecules with higher molecular weight. Their complete analysis often needs hydrolysis before spectrum analysis. However, the occurrence of hexuronic acid, hexosamine and sulfate usually make them to inhibit partly the chemical hydrolysis such as hydrolysis, methylation analysis, smith degradation, periodate oxidation which are suitable for other general polysaccharide.

GAG lyases from bacterial sources but more recently of recombinant origin selectively degrade GAGs. For example, chondroitinase ABC will degrade virtually all chondroitin and DS, while leaving heparin (or HS, HA, KS) chains intact. Conversely, heparitinase enzymes will degrade nearly all forms of HS, but are unable to degrade CS, HA, DS and KS. Hyaluronidase will degrade HA but not others. More importantly, is that chondroitinase B only acts on the ChS-B, chondroitinase AC is for C4S and/or Ch6S and chondroitinase C for C6S. The generating oligosaccharides cotaining a $\Delta_{4,5}$-unsaturated uronic acid residue at the new non-reducing terminus exhibits an absorbance maximum at 232 nm, which permit the detection of the oligosaccharide products of the GAG lyases using ultraviolet spectroscopy. Thus, these specific GAG lyases, is followed by the diversified isolation techniques including size exclusion chromatography (SEC), SAX-HPLC, HPCE and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Ambrosius et al., 2008), which permit the identification of disaccharide characterization. Thus, analysis of unsaturated disaccharides is simple and rapid, avoiding any time-consuming derivatisation reactions. SAX-HPLC isolation and determination for unsaturated disaccharides after treatment with chondroitinase ABC is used to evaluate the content and biochemical properties (sulfation patterns) of ChS in shark cartilage powders and finished products containing shark cartilage powder. A linear gradient of 0 to 1.0 M NaCl (pH 3.5) for 41.55 min is used and the profile is monitored at 232 nm. The recovery ranges from 95.27 to 102.39% with precision from 2.27 to 3.95% (Xiong et al., 2007). Separation of milligram amounts of heparin oligosaccharides with sufficient purity ranging in degree of polymerization from four to 32 is achieved using continuous elution PAGE. The results of PAGE, strong anion exchange (SAX)–HPLC, electrospray ionization Fourier transform mass spectrometry (ESI-FTMS), and nuclear magnetic resonance (NMR) characterization of the gel-eluted oligosaccharides demonstrate that this method of separation does not introduce chemical artifacts and alter the oligosaccharide structure (Tatiana et al., 2010).

Mass spectrometry (MS) has also been applied to the analysis of oligosaccharides obtained from various GAGs. It is comparably sensitive and has the particular advantage that individual GAG can be easily differentiated by the characteristic mass differences of their polymer repeat units. Fast-atom bombardment MS (FAB-MS), matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) MS, ESI-MS, matrix-assisted laser desorption/ionization MS (MALDI-MS) and tandem MS are suitable for determining the characterization of oligosaccharides. ESI-MS/MS techniques provide high mass accuracy, structural information and ability to quantify the fragments. They have become more attractive and prominent for the analysis of oligosaccharides (Nimptsch et al., 2009; Connie and Leary, 2010; Seoa et al., 2011). A mixture of GAG chains from a plasma proteoglycan bikunin was isolated and identified using native, continuous-elution PAGE alone or in combination with partial enzymatic depolymerization, and the resulting fractions were analyzed by ESI-FTMS (Tatiana et al., 2011).

However, HPCE, SAX-HPLC or MS usually gives no information about linkage positions and anomeric configurations. One- and two-dimensional NMR have been used to characterize their subtle structure.

APPLICATION OF GAG IN BIOMATERIALS

In many medicine system used in tissue engineering, surgery and drug delivery, there is need for materials which are both adhesive and *in situ* forming. These materials can be composed of synthetic, biological or a combination of such materials. Biological molecules such as GAGs have some advantages such as biodegradation, adhesiveness and bioactivities (stimulating cells to proliferate, migrate, differentiate and produce extracellular matrix) (Strehin et al., 2010). Recently, an increasing number of new applications of GAGs (mostly HA, CS and heparin) in biomaterial industry such as tissue-engineering material, biodegradable film-forming materials and micro-encapsulating agents were presented due to their physical-chemical (polyanionic, biodegradable and biologically compatible) and biological properties.

APPLICATION OF GAGS IN TISSUE ENGINEERING

Tissue engineering is a new and exciting technique which has the potential to create tissues and organs *de novo* (Chen et al., 2002). The scaffold acts as a physical support structure and insoluble regulator of cell activity. It has emerged as a promising alternative approach in the treatment of malfunctioning or lost organs. For facilitating cell adhesion, promoting cell growth and allowing the retention of differentiated cell functions and regeneration of tissue, the scaffold should be biocompatible, biodegradable, highly porous with a large surface/volume ratio, mechanically strong and malleable. Thus, various

scaffolds binding GAG alone or cross-linked with other natural/synthetic material have been developed quickly in recent years. Compared with placental decellular matrix scaffold, placental decellular matrix combined with cross-linked HA scaffolds also had a positive effect in terms of angiogenesis and adipogenesis although both of them macroscopically maintained their three-dimensional volume and supported mature adipocyte populations *in vivo* (in a subcutaneous athymic mouse model) (Flynn et al., 2009). The incorporation of HA with collagen can promote the strength of the collagen-based gels except for direct biological effect and inhibition of the cell-induced contraction. Collagen-HA scaffolds may offer robust, freely permeable 3-D matrices that enhance mammary stromal tissue development *in vitro* (Davidenko et al., 2010). 3D chitosan scaffolds simultaneously cross-linked with DS is beneficial for ECM production and enables statistical interpretation of the individual and synergistic effects of CS-C and DS (Chen et al., 2007).

In order to take advantage of the distinct control in the scaffold's mechanical properties of synthetic biomaterials and bioactivities of GAGs, developing a biomaterial with both biological and synthetic components with tissue adhesive properties is of significant interest. A chondroitin sulfate–polyethylene glycol (CS–PEG) adhesive hydrogel is composed of CS, succinimidyl succinate and polyethylene glycol amine PEG–$(NH2)_6$. Its adhesive strength with cartilage tissue was shown to be 10 times higher than that of fibrin glue. Cells encapsulated or in direct contact with it also remained viable and metabolically active. Furthermore, CS-PEG material produced minimal inflammatory response when implanted subcutaneously in a rat model and enzymatic degradation was demonstrated *in vitro* (Sachlos and Czernuszka, 2003). The exploration on vascular endothelial growth factor (VEGF)-mediated angiogenesis in porous polycaprolactone (PCL) scaffolds cross-linked heparin showed that modification of the scaffold surface with heparin is a convenient and potent approach to deliver VEGF. So, the heparin immobilization technique can be applied to make any biomaterial more conducive for angiogenesis (Singh et al., 2011). HA is known to be present at high levels in viscous tissues and fluids such as the vitreous humour, umbilical cord and synovial fluid. It is a natural non-toxic mucoadhesive polysaccharide that is negatively charged and biodegradable (Oyarzun-Ampuero et al., 2011). Several studies have tested the efficacy of nanosystems based on HA using various applications, polyarginine, cancer and asthma.

CONCLUSION

The remarkable physical-chemical, therapeutic and biological importance of GAGs ensures a continued interest in advancing new technologies for their extraction, separation and purification, identification and applications in functional food, biochemical pharmaceutical, cosmetics and biomaterial.

ACKNOWLEDGEMENTS

This work was supported by Doctor Foundation of Southwest University of Science and Technology (grant no. 06zx7122) and Mianyang Shi Science and Technology Board (grant no. 10Y004-1).

REFERENCES

Albertini R, De Luca G, Passi A, Moratti R, Abuja PM (1999). Chondroitin-4-sulfate Protects High-Density Lipoprotein against Copper- Dependent Oxidation. Arch. Biochem. Biophys. 365(1): 143-149.

Ambrosius M, Kleesiek K, Götting C (2008). Quantitative determination of the glycosaminoglycan Δ-disaccharide composition of serum, platelets and granulocytes by reversed-phase high-performance liquid chromatography. J. Chromatogr. B. 1201(1): 54-60.

Chen GP, Ushida T, Tateishi T (2002). Scaffold Design for Tissue Engineering. Macromol. Biosci. 2: 67-77.

Chen SG, Xue CH, Yin LA, Tang QJ, Yu GL, Chai WG (2011). Comparison of structures and anticoagulant activities of fucosylated chondroitin sulfates from different sea cucumbers. Carbohydr. Polym. 83(2): 688-696.

Chen YL, Lee HP, Chan HY, Sung LY, Chen HC, Hu YC (2007). Composite chondroitin-6-sulfate/dermatan sulfate/chitosan scaffolds for cartilage tissue engineering. Biomaterials, 28(14): 2294-2305.

Cheng XH, Kaplan LA (2003). Simultaneous Analyses of Neutral Carbohydrates and Amino Sugars in Freshwaters with HPLC-PAD. J. Chromatogr. Sci. 41(8): 1-5.

Connie HJ, Leary JA (2010). A competitive binding study of chemokine, sulfated receptor, and glycosaminoglycan interactions by nano-electrospray ionization mass spectrometry. Anal. Biochem. 407(1): 134-140.

Davidenko N, Campbell JJ, Thian ES, Watson CJ, Cameron RE (2010). Collagen-hyaluronic acid scaffolds for adipose tissue engineering. Acta Biomater. 6(10): 3957-3968.

Eun SL, Keun-Hong P, Dongmin K, In SP, Hyo YM, Don HL, Sungwon K, Jong HK, Kun N (2007). Protein complexed with chondroitin sulfate in poly (lactide-co-glycolide) microspheres. Biomaterials, 28(17): 2754-2762.

Fajardo AR, Piai JF, Rubira A F, Muniz EC (2010). Time- and pH-dependent self-rearrangement of a swollen polymer network based on polyelectrolytes complexes of chitosan/chondroitin sulfate. Carbohydr. Polym. 80(3): 934-943.

Filisetti-Cozzi TM, Carpita NC (1991). Measurement of uronic acids without interference from neutral sugars. Anal. Biochem. 197(1): 157-162.

Flynn L, Prestwich GD, Semple JL, Woodhouse KA (2009). Adipose tissue engineering *in vivo* with adipose-derived stem cells on naturally derived scaffolds. J. Biomed. Mater. Res. Part A. 89(4): 929-941.

Imberty A, Lortat-Jacob H, Pérez S (2007). Structural view of glycosaminoglycan-protein interactions. Carbohydr .Res. 342(3-4): 430-439.

Kakit H, Kamishima H, Inouye K (2006). Uronic acid determination by high performance liquid chromatography with postcolumn fluorescence derivatization. J. Chromatogr. A 1129(2): 296-299.

Liu Y, Qian H (2005). Spectrophotometric quantitative determination of glycosaminoglycans in sea cucumber tissues with 1, 9-dimethylmethylene blue. Chin. J. Anal. Lab. 24(6): 59-61.

Nimptsch A, Schibur S, Schnabelrauch M, Fuchs B, Huster D, Schiller J (2009). Characterization of the quantitative relationship between signal-to-noise (S/N) ratio and sample amount on-target by MALDI-TOF MS: Determination of chondroitin sulfate subsequent to enzymatic digestion. Analytica. Chimica Acta. 635(2): 175-182.

Ola SM, Myers RA, Castleton DL, Leary JA (2005). Analysis of hyaluronan content in chondroitin sulfate preparations by using selective enzymatic digestion and electrospray ionization mass spectrometry. Anal. Biochem. 344(2): 232-239.
Oyarzun-Ampuero FA, Goycoolea FM, Torres D, Alonso MJ (2011). A new drug nanocarrier consisting of polyarginine and hyaluronic acid. Eur. J. Pharm. Biopharm. 79(1): 54-57.
Raman R, Myette JR, Shriver Z, Pojasek K, Venkataraman G, Sasisekharan R (2003). The heparin/heparan sulfate 2-o-sulfatase from flavobacterium heparinum. A structural and biochemical study of the enzyme active site and saccharide substrate specificity. J. Biol. Chem. 278(14): 12167-12174.
Sachlos E, Czernuszka JT (2003). Making Tissue Engineering Scaffolds Work. Review: The application of solid freeform fabrication technology to the production of tissue engineering scaffolds. Eur. Cells. Mater. 5(13): 29-40.
Seoa Y, Schenauer MR, Leary JA (2011). Biologically relevant metal-cation binding induces conformational changes in heparin oligosaccharides as measured by ion mobility mass spectrometry. Int. J. Mass Spectrom. 303(2-3): 191-198.
Sim JS, Im AR, Cho SM, Jang HJ, Jo JH, Kim YS (2007). Evaluation of chondroitin sulfate in shark cartilage powder as a dietary supplement: raw materials and finished products. Food Chem. 101(2): 532-539.
Singh S, Wu BM, Dunn JC (2011). The enhancement of VEGF-mediated angiogenesis by polycaprolactone scaffolds with surface cross-linked heparin. Biomaterials, 32(8): 2059-2069.
Strehin I, Nahas Z, Arora K, Nguyen T, Elisseeff J (2010). A versatile pH sensitive chondroitin sulfate-PEG tissue adhesive and hydrogel. Biomaterials, 31(10): 2788-2797.
Tatiana LN, Leach FE, Amster IJ, Linhardt RJ (2011). Electrospray ionization Fourier transform mass spectrometric analysis of intact bikunin glycosaminoglycan from normal human plasma. Int. J. Mass Spectrom. 305(2-3): 109-115.
Tatiana LN, Ly M, Solakyildirim K, Zagorevski DV, Linhardt RJ (2010). High-resolution preparative separation of glycosaminoglycan oligosaccharides by polyacrylamide gel electrophoresis. Anal. Biochem. 401(2): 236-241.
Volpi N (2004). Disaccharide mapping of chondroitin sulfate of different origins by high-performance capillary electrophoresis and high performance liquid chromatography. Carbohydr. Polym. 55(3): 273-281.
Wang SH, Raptis E, Yeh J (2004). Ion chromatography for the determination of sulfate in STEALTH liposomes. J. Chromatogr. A 1039: 51-58.
Xiong SL, Li AL, Jin, ZY, Chen M (2007). Effects of Oral Chondroitin Sulfate on Lipid and Antioxidant Metabolism in Rats Fed a High-Fat Diet. J. Food. Biochem. 31(3): 356-369.