第 11 卷第 2 期　　上 海 水 产 大 学 学 报　　Vol.11，No.2
2002 年 06 月　　JOURNAL OF SHANGHAI FISHERIES UNIVERSITY　　June，2002

文章编号：1004－7271(2002)02－0145－04

Preparation and characterization of glucosamine sulfate

ZHOU Pei-gen，YOU Yu-min，NI Ye，QI Xiao-yu，TU Shu-xia
(*College of Food Science and Technology，Shanghai Fisheries University，Shanghai* 200090，*China*)

Abstract：By using anion-exchange column chromatography，glucosamine sulfate was prepared from glucosamine hydrochloride that was produced by acidic hydrolysis of chitin and some properties of the product were studied. The contents of glucosamine，sulfate radical and chloridion in the product were 71.60%，18.25% and 0.038%，respectively. The R_f value of the glucosamine sulfate was 0.36 measured by paper chromatography with the solvent system of pyridine，ethyl acetate，water and glacial acetic acid (5∶5∶3∶1 in volume). While redissolved in water at 20℃，the specific rotation of glucosamine sulfate decreased with the time from 92.1° to about 56.7° and was，however，stable after 1.5 h at 56.7°. It exhibited the characteristic absorption bands at 3307.9，1415.6，1215.6，1181.2，1118.7，1093.7 and 1034.4 cm^{-1} in IR spectrum. The elemental analysis for the glucosamine sulfate showed that the contents of carbon，hydrogen，nitrogen and sulfur were 29.63，6.34，5.39 and 6.56 %，respectively.
Key words：glucosamine sulfate；an ion-exchange chromatography；paper chromatography；infrared spectrum；elemental analysis

氨基葡萄糖硫酸盐的制备及其性质

周培根，尤瑜敏，倪　晔，戚晓玉，涂书霞

(上海水产大学食品学院，上海　200090)

摘　要　以甲壳素的浓盐酸水解产物氨基葡萄糖盐酸盐为原料，通过阴离子交换层析法制备氨基葡萄糖硫酸盐，并对其的部分性质进行研究。采用本方法制备的氨基葡萄糖硫酸盐产物的氨基葡萄糖含量为 71.60%，硫酸根含量为 18.25%，氯离子含量为 0.038%。经纸层析鉴定，以吡啶、乙酸乙酯、水和冰醋酸按体积比 5∶5∶3∶1 组成的溶剂系统进行展层，氨基葡萄糖硫酸盐的 R_f 值为 0.36。将氨基葡萄糖硫酸盐产物的晶体重新溶解于 20℃水中时，它的比旋光度随时间的延长而下降，1.5h 后趋于稳定，从 92.1°降低至 56.7°。对它的红外光谱鉴定表明，在波数为 3303.9、1415.6、1215.6、1181.2、1118.7、1093.7 和 1034.4 cm^{-1} 处有特征吸收峰。通过对氨基葡萄糖硫酸盐产物的元素分析，碳、氢、氮和硫的含量分别为 29.63%、6.34%、5.39% 和 6.56%。
关键词　氨基葡萄糖硫酸盐；离子交换层析；纸层析；红外光谱；元素分析
中图分类号：TQ226.36　　**文献标识码**：A

Chitin is one of the richest natural resources on the earth and widely used in various industries due to its inherent features of non-toxicity，biodegradability and good biological compatibility. Being an important derivative of chitin，glucosamine sulfate has been proved to be efficacious for the therapy of rheumatic fever，arthritic and

Received date：2001-11-28
Brief introduction of the author：ZHOU Pei-gen (1941－)，Male，Professor of Biochemistry，Research area：utilization of marine biological resources. Tel. 021－65710058；E-mail：pgzhou @ shfu.edu.cn

arthrosic complaints. Many studies have shown that glucosamine sulfate exerts a protective effect against joint destruction and is selectively used by joint tissues, exerting a powerful healing effect on osteoarthritis which is the most common form of arthritis. In addition, glucosamine sulfate is virtually free of side effects and there are no known contra-indications[1,2].

Glucosamine sulfate is usually prepared from glucosamine hydrochloride reacted with an ethanolic solution of a tertiary base such as triethylamine, but too much organic solvents are used in the process, thus making its cost high and causing environment polluted.

Glucosamine sulfate was prepared from glucosamine hydrochloride as starting materials by ion-exchange chromatography and some properties were examined in this paper.

1 Materials and Methods

1.1 Glucosamine hydrochloride

Glucosamine hydrochloride was prepared from chitin hydrolyzed with concentrated hydrochloric acid and then purified. The purity of glucosamine hydrochloride obtained was 99.8%.

1.2 Ion-exchange chromatography

An ion-exchange chromatography column (2.6 x 24.8 cm) packed with anionic resin [201 × 7(717), Shanghai] was used for preparing glucosamine sulfate from the purified glucosamine hydrochloride. The column was conditioned with 1.0 M sodium sulfate solution and then washed several times with redistilled water to remove the residual sodium sulfate. 250 mL of 0.3 M glucosamine hydrochloride solution was applied onto the column at a flow rate of 30 mL / h. 3.5 mL fractions were collected with a fractional collector. The fractions containing both glucosamine and sulfate radicals were combined and concentrated under vacuum at 50℃, and then freeze-dried.

1.3 Glucosamine determination

The content of glucosamine in the glucosamine sulfate product was determined according to the modified Elson & Morgan method[3].

1.4 Sulfate radical determination

The content of sulfate ions in the product was assayed with the tubidimetric method[4].

1.5 Chloridion determination

The content of chloridion in the product was determined by the mercuric nitrate method[4].

1.6 Glucosamine sulfate detection by paper chromatography

1 ~ 2 μL of 10% (W/V) glucosamine sulfate solution was sampled on Whitman No.3 filter paper (36 x 5 cm). After ascending development with a solvent system consisting of pyridine-ethyl acetate-water-glacial acetic acid (5∶5∶3∶1 in volume), the paper was dried and detected by spraying with 0.1% ninhydrin reagent and then heating for about 10 min at 105℃ or by treating with Elson & Morgan color reagent, in which firstly it was sprayed with acetylacetone reagent and heated for 15 min at 80℃, then sprayed with p-dimethylaminobenzaldehyde solution and heated at 70℃ until the color spot was developed[5].

1.7 Optical rotation measurement

By dissolving 5.000g of glucosamine sulfate in 50 mL of redistilled water, the optical rotation of the solution was measured at various periods of time at 20℃. The specific rotation was calculated by $[\alpha]_D^t = \alpha/LC$, here t∶the temperature of the solution; D∶the sodium D line as the light source; L∶the length of the tube in decimeters; C∶the concentration of the solute in g/mL.

1.8 Infrared (IR) spectrum

The IR spectrum of glucosamine sulfate was surveyed on Perkin Elmer-1650 IR spectrophotometer.

1.9 Elemental analysis

The elemental analysis of glucosamine sulfate was conducted on CARLO-ERBA 1106 elemental analysis apparatus.

2 Results and Discussion

2.1 Elution of glucosamine sulfate from an anion-exchange column

Fig. 1 shows the elution profile of glucosamine sulfate from the anion-exchange chromatographic column. It indicated that the variation in contents of glucosamine and sulfate ion during the elution was very similar in the pattern. The fractions from 10 to 94 corresponding to the major peaks were collected and concentrated under vacuum at 50℃. After freeze-dried, the powder product was obtained with a yield of 98%.

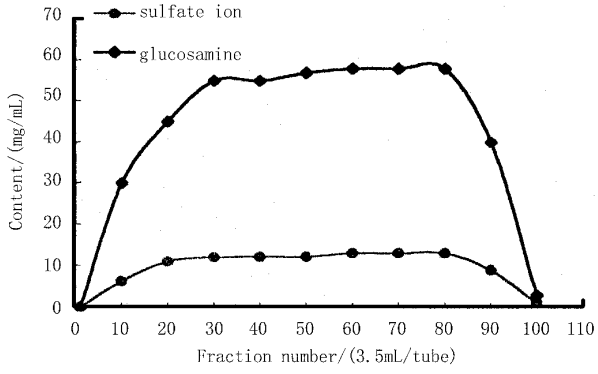

Fig.1 The elution profile of glucosamine sulfate from ion-exchange chromatographic column

The analytical results showed that the contents of glucosamine and sulfate ion in the product were 71.6 % and 18.25 %, respectively, and both of them were lower than their theoretical values which might be due to some of water molecules bound to glucosamine sulfate. The content of chloridion in the product was 0.038 %, mainly originated from the glucosamine hydrochloride residue not exchanged on the ion-exchange column.

2.2 Paper chromatography

The paper chromatogram (not shown here) of the glucosamine sulfate product exhibited one color spot with no tailing appearing on each chromatographic filter paper. The purple-red spot represented the strip treated with ninhydrin reagent. It implied that the compound contained amino groups. Similarly, the red-cherry spot stood for the strip treated with Elson & Morgan color reagent. It was suggested that the compound might have the structure of hexosamines. The R_f values for these two spots on the chromatogram were 0.36. Therefore, it can be concluded that the product was glucosamine sulfate with quite high purity.

2.3 Variation in optical rotation

The variation in specific rotation for 10% glucosamine sulfate solution was illustrated in Fig.2. The specific rotation of the solution decreased with the time from nearly 92.1° to about 56.0° and became stable after 1.5 h at 56.7°. The optical depression indicated that there was a mutarotation of glucosamine sulfate occurred in aqueous solution. It was considered that the glucosamine sulfate prepared by this method existed in the form of α-isomer. When redissolved in water, the interconversion between α-isomer and β-isomer of

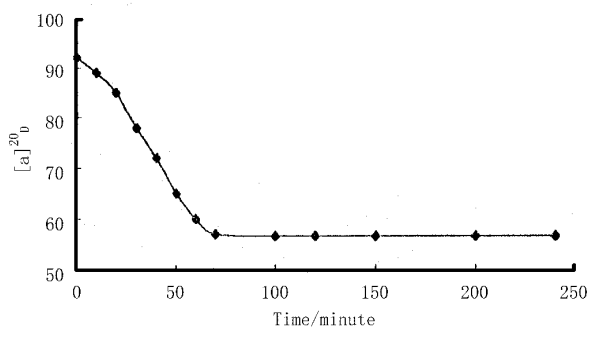

Fig.2 Variation of specific rotation with time for glucosmine sulfate solution (10%)

glucosamine sulfate existed in aqueous solution and eventually reached an identical equilibrium mixtures at the optical rotation of 56.7°.

2.4 Infrared spectrum of glucosamine sulfate

The IR spectrum of the glucosamine sulfate product was illustrated in Fig. 3. The characteristic absorption bands of primary amine group at 3303.9 cm^{-1}, primary alcohol group at 1415.6 cm^{-1} and pyranoid ring at 1093.7 cm^{-1} were found in the IR spectrum. The characteristic absorption bands of sulfate radical existed at 1034.4, 1215.6, 1181.2 and 1118.7 cm^{-1}.

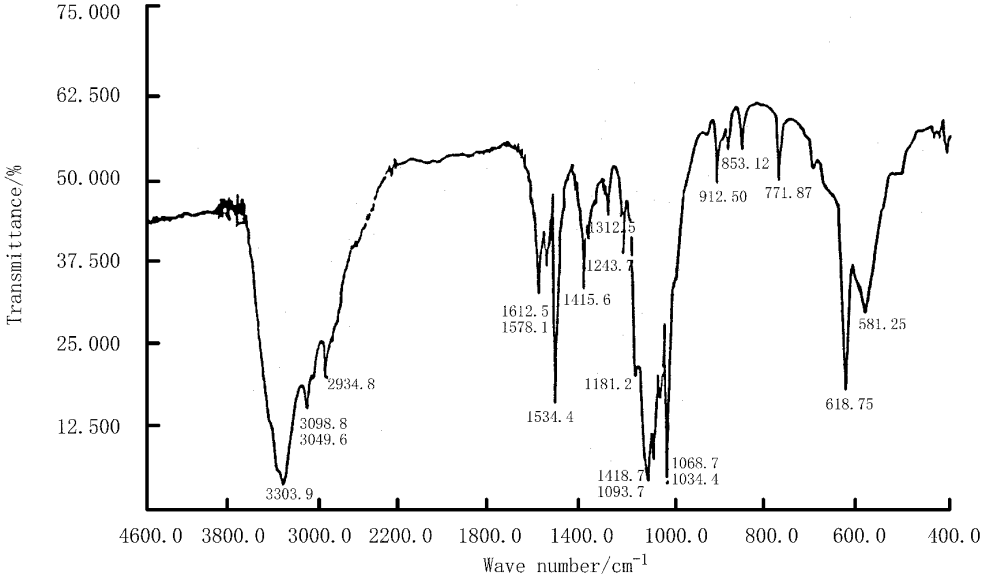

Fig. 3 Infrared spectrum of the glucosamine sulfate product

2.5 Elemental analysis

The results of elemental analysis indicated that the glucosamine sulfate product contained 29.63% of carbon, 6.34% of hydrogen, 5.39% of nitrogen and 6.56% of sulfur, respectively. The ratio of C:N:S was 12.0:1.9:1, being very close to the theoretical ratio of 12:2:1. However, it was found that the content of hydrogen in the product was little higher than its theoretical value, which was probably caused by the strongly hydroscopic property of glucosamine sulfate.

3 Conclusions

By the experimental results, it can be concluded that the preparation of glucosamine sulfate is feasible by means of the ion-exchange chromatography. The method has the merits of convenience and simplicity in the process and less environmental pollution.

References:

[1] Tapadinhas M J. Oral glucosamine sulfate in the management of arthrosis: Report on a Milti-Center Open Investigation in Portugal [J]. Pharmatherapeutica, 1982, 3(3): 157 – 168.

[2] Rovati L C. Clinical research in osteroarthritis: design and results of short-term and long-term trials with disease-modifying drugs [J]. Int J Tiss Reac. 1992, 14 (5): 243 – 251.

[3] Johnston J P. A modification of Elson and Morgan method for the estimation of glucosamine [J]. Analyst, 1951, 76 38.

[4] Horwitz W. Official methods of analysis of the association of official analytical chemists [M]. Washington: Association of Official Analytical Chemists, 1975, 623 – 625.

[5] Partridge S M. Fillter paper partition chromatography of sugars [J]. Biochem J, 1948, 42 (2), 238 – 248.