

Preparation of Glucosamine Hydrochloride from Indigenous Shrimp Processing Waste

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Abstract

The present investigation described the effective preparation of glucosamine hydrochloride (GluHCl) from chitin which was extracted from indigenous shrimp shell. GluHCl has attracted much attention owing to its therapeutic activity in osteoarthritis and widely used dietary supplement. The key step involved was extraction of chitin from shrimp skeleton and then hydrolysis of chitin by concentrated hydrochloric acid. The reaction proceeds via break down of glycoside linkage. Structural analysis was carried out by melting point, TLC, FT-IR, elemental analysis and all the data were compared with that of standard GluHCl. The elemental (C, 32.75; H, 6.51; N, 6.20) analysis is good concord with the calculated value (C, 33.42; H, 6.54; N, 6.50). Absence of ν_{\max} at 1726 cm^{-1} indicates that GluHCl is a deacetylated product of chitin. The yields of the product mainly depend on reaction conditions. Maximum yield (63.5%) was obtained when chitin was hydrolyzed with concentrated HCl for 1.30 h.

Key words: Shrimp shell, Chitin, Acid hydrolysis, Glucosamine hydrochloride Osteoarthritis.

Introduction

Chitin is a natural polymer which is found in exoskeleton animals like shrimps. Chitin is the most second ubiquitous natural polysaccharide after cellulose on earth and is composed of β - (1 \rightarrow 4) linked 2-acetoamido-2-deoxy- β -D-glucose (Dutta *et al.*, 2004) (Fig.1). GluHCl is an important derivative of chitin.

Glucosamine (2-Amino-2-deoxy-D-glucose) (Fig. 2) is an amino monosaccharide precursor of the disaccharide unit of glycosaminoglycan which is the building block of proteoglycans, being the ground substance of articular cartilage (Fattahi *et al.*, 2008). Glucosamine exists as a component of proteoglycan in cartilage, skin, and connective tissue and recently has attracted much attention due to its therapeutic activity in osteoarthritis and been evaluated as a food supplement (Aiba *et al.*, 2005). Glucosamine is mostly found cartilage and provides an important function in cartilage resilience and joint lubrication. Human body synthesizes glucosamine by combining glucose with amino acid glutamine (McGrow-Hill, 1966). Glucosamine has been widely promulgated to relieve symptoms in osteoarthritis (Anderson *et al.*, 2005). Glucosamine products for arthritis are usually

formulated as the hydrochloride salt or glucosamine sulfate and are often combine with chondroitin sulfate (Abdel Fattah *et al.*, 2001). In the USA it is one of the most commonly used non-vitamin, non-mineral, natural products used by adults as complementary or alternative medicine and most popular dietary supplement (Dahmer and Schiller, 2008). The preparation of glucosamine hydrochloride from chitin is a simple hydrolysis reaction. During this reaction, chitin is deacetylated and depolymerized to glucosamine hydrochloride in the presence of hydrochloric acid. Enzymatic hydrolysis of chitin to prepare N-acetyl-D-glucosamine is reported in literature (Setgthbakset *et al.*, 2008). Acid hydrolysis of chitin and chitosan is also reported (Novikov, 2004). This process involves two acid-catalyzed hydrolysis reactions, i.e. that of glycosidic linkage (depolymerisation) and that of the N-acetyl linkage (deacetylation). Edward (1955) illustrated the most accepted reaction mechanism for the acid catalyzed hydrolysis of a glycosidic linkage. Stryer (1995) illustrated the reaction mechanism for acid hydrolysis of the N-acetyl linkage. It is also reported in literature that the exhaustive hydrolysis of chitin in the concentrated acid proceeds with the formation of acetic acid and D-glucosamine salt and the

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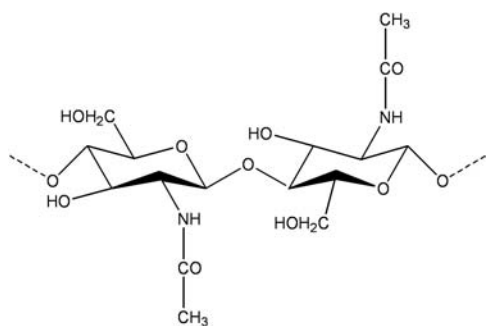


Fig. 1: Chemical structure of chitin.

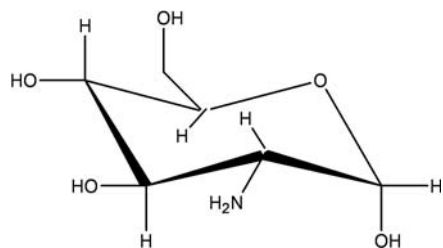


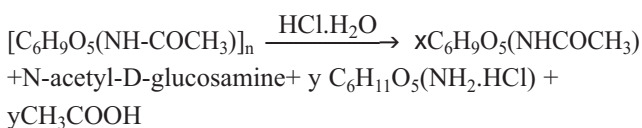
Fig. 2: Structure of glucosamine



Fig. 3: Needle shape crystal of prepared GluHCl

hydrolysis of chitosan results in the formation of D-glucosamine salt (Shabrukova *et al.*, 2002).

In weak acid chitin is hydrolyzed with the formation of N-acetyl-D-glucosamine and D-glucosamine salt (Shabrukova *et al.*, 2002).



Hackman (1962) investigated the effect of HCl and other strong mineral acids on the size of the chitin chain and reported that most of the degradation of the chitin chain occurred during the first few minutes, that the products formed were oligosaccharides. The main goal of this present work was to prepare GluHCl from fishery waste materials which are hazard and toxic for environment. Shrimps are in general sold headless and often peeled of the outer shells and tail. About 30-40% by weight, shrimp raw material is discarded as waste when processed shrimp is headless, shell on products (Begum *et al.*, 2006).

Materials and Methods

Materials

Shrimp shell materials were collected from Khulna, Bangladesh. D-(+) Glucosamine hydrochloride was purchased from KANTO CHEMICAL CO. INC. Japan as a standard. All the chemicals purchased from Merck were analytical grade.

Preparation of Chitin

Shrimp shells were scraped free of loose tissue, washed with cold water and dried in sun for 2 days. Chitin was extracted from shrimp shell following standard procedure (Hossain *et al.*, 2005; Rodde *et al.*, 2008). The process mainly involved the following steps: Demineralizations of Shells. In this step, the shells were suspended in 4% HCl at room temperature in the ratio of 1:14(w/v). After 36 hours, the shells were quite squashy and were rinsed with water to remove acid and calcium chloride. Deproteinization of Shells. The demineralized shells were then treated with 5% NaOH at 90°C for 24 hours with a solvent to solid ratio of 12:1(v/w). The residue was then collected and washed to neutrality in running tap water. Then it was dried in sun. Yield of chitin was 18.23%.

Preparation of Glucosamine hydrochloride

Chitin was treated with different ratios and concentrations of hydrochloric acid (Table I). The following major steps were under taken to obtain GluHCl from chitin. (i) grinding the chitin to fine particle; (ii) hydrolysis the chitin with HCl at 90°C or room temperature; (iii) dissolving the black precipitate in water and decolorizing with activated charcoal; (iv) filtering the solution and discarding the solids; (v) evaporating the solution to recover GluHCl crystal at 45°C; (vi)

washing the crystals with ethanol; (vii) drying the crystals at 50°C in an oven.

well agreement with the reported data (Pavia *et al.*, 2001; Silverstien *et al.*, 1991). A broad strong stretching band in

Table I: Optimizing the reaction conditions

Entry	Amount of Chitin (g)	Amount of HCl (mL)	Conc. of HCl (%)	Reaction time (h)	Reaction temp.(°C)	Yield (%)
1	3	60	37	1	90	52.12
2	3	60	37	1.30	90	63.45
3	3	60	37	2	90	55.36
4	3	60	37	24	Room temp	51.23
5	3	60	37	48	Room temp	60.32

Results and Discussion

The purity and quality of the obtained GluHCl is demonstrated by its melting point, IR spectra, TLC method which are similar with the results of that standard GluHCl. The melting point of the prepared GluHCl was 192-194 °C which is good agreement with 191-192 °C for standard GluHCl. The spectral data FT-IR spectrometry of chitin and prepared GluHCl are given bellow;

Chitin FT-IR (KBr): 3849.7, 3421.5, 2935.5, 2360.7, 2343.4, 1726.5, 1627.8, 1558.4, 1542.9, 1488.9, 1475.4, 1404.1, 1384.8, 1330.8, 1261.4, 1195.8, 1112.4, 896.8, 619.1, 603.7, 513.0, 453.2, 408.9 cm^{-1} .

Prepared GluHCl FT-IR (KBr):3600, 2788.9, 2613.9, 2472.6, 1946.0, 1888.2, 1831.3, 1801.4, 1616.2, 1585.4, 1533.2, 1456.2, 1421.4, 1394.4, 1363.6, 1332.7, 1317.3, 1217.3, 1249.8, 1184.2, 1137.9, 1002.9, 912.3, 887.2, 854.4, 773.4, 698.2, 592.1, 543.9, 507.2, 434.0, 408.9 cm^{-1} .

Standard GluHCl FT-IR (KBr):3600, 2842.9, 2570.9, 2538.1, 2432.1, 1944.1, 1830.3, 1801.4, 1616.2, 1583.4, 1535.2, 1421.4, 1394.4, 1363.6, 1332.7, 1317.3, 1217.0, 1249.8, 1184.2, 1002.9, 973.3, 887.2, 854.4, 773.4, 698.2, 592.1, 545.9, 507.2, 434.0, 407.0 cm^{-1} .

The finger print of the FT-IR spectra of prepared and standard GluHCl does not show any excess peaks. The FT-IR spectra of prepared GluHCl showed a deacetylation because the wave no. at about 1726 cm^{-1} for C=O, which exist in IR spectrum of chitin, has disappeared. The absorption bands at 1616.2 and 1585-1535 cm^{-1} are due to the NH_3^+ in GluHCl for asymmetrical and symmetrical NH_3^+ bending which is

the region 3400-2500 cm^{-1} also proved the formation of NH_3^+ in GluHCl as in reported data (Pavia *et al.*, 2001; Silverstien *et al.*, 1991). TLC plate was prepared manually, eluting solvents were butanol-acetic acid-water (3:1:1) and ninhydrin was used as a locating agent. The R_f value of spots was 0.46 for the both of prepared and standard GluHCl. TLC did not show any other spots for impurities. The needle shape crystals of prepared GluHCl (Fig. 3) also prove its high purity. The yield is high when the chitin was hydrolyzed with 37% HCl at 90°C for 1.30h. The yield decreased when the chitin was hydrolyzed for 2h. The explanation is that some of the GluHCl may decompose to fragments. Anal. Calcd for $\text{C}_6\text{H}_{11}\text{O}_5(\text{NH}_2\cdot\text{HCl})$: C, 33.41; H, 6.54; N, 6.50 Found: C, 32.75; H, 6.51; N, 6.20. The existence of nitrogen and chlorine was also confirmed by Lassaigne test.

Conclusion

Based on the experimental results, it is concluded that the preparation of GluHCl by acid hydrolysis method is effective. The method is convenient and simple in procedure. The preparation of GluHCl from shrimp shell via chitin would successfully minimize the environmental pollution by the shrimp shell processing.

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