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# Determination of chitosan content with Schiff base method and HPLC

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ABSTRACT

Tremendous awareness of determination of chitosan content accurately is increasing, due to it has great significance to the quality control of chitosan. In this article, two kinds of chitosan-Schiff base derivatives (BCSB and PCSB) were synthesized by the different average degrees of deacetylation (DD) of chitosan with benzaldehyde or propanal, respectively. The total mass of Schiff base derivative product was dried and obtained without washing and loss. Then, a certain amount of the prepared Schiff base compound was taken to hydrolyze into glucosamine hydrochloride (GAH) in strong hydrochloric acidic environment, whose concentration was quantified by HPLC, and the mass of GAH contained in hydrolysis solution could be calculated. Subsequently, the total quality of GAH obtained by hydrolysis of all of the Schiff base product was calculated and obtained, and then the theoretical mass of chitosan could be deduced and calculated by further converse calculation. Finally, the chitosan content was obtained by combining the sample mass used in Schiff base reaction and the theoretical mass of chitosan. This method was accurate and convenient, providing a preeminent idea and method for the determination of chitosan content.



## 1. Introduction

Chitosan is the deacetylated derivative of chitin and it is the second most abundant copolymer present on earth [1–4]. Chitosan is a linear cationic amino-polysaccharide consisting of β-(1–4)-2-amino-D-glucose and β-(1–4)-2-acetamido-D-glucose units, mainly found from the cell walls of insect and the shells of crab, shrimp and other crustaceans [5–9]. The unique structure of chitosan is analogous to cellulose, in which the hydroxyl at C-2 has been replaced by amino or acetamido groups [1,10,11]. Chitosan, as a bountiful natural biopolymer, endowed with an uncommon blend of physicochemical properties and versatile biological activities [6,12,13]. Because of its merits of abundant availability, complete biodegradability, excellent biocompatibility, in conjunction with non-toxicity, chitosan has emerged with a wide array of applications and highly sophisticated and multidimensional functionalities. [14–16]. It has been confirmed that chitosan has a broad-spectrum antifungal and antimicrobial activities [17], antioxidant activities [18], obesity treatment [19], wound healing [20], immune-enhancing effects and drug delivery properties [15,21], and its application potential is multidimensional, such as agriculture [4], material science, medicine and pharmaceutical [22], biotechnology, food processing and nutrition [11], textile [23], cosmetics [24], environmental protection and etc. [14,25,26].

Furthermore, due to chitosan and its assortment of derivatives have diversified exciting properties, they have been regarded as multifunctional polymers applicable to numerous fields and has attracted substantial interest of researcher in recent decades [8,27]. Chitosan-Schiff base is one of the most important amino-functionalization reaction modification used to protect the amino groups of chitosan at the C-2 position [28], and it has been receiving great attention increasingly owing to its promising application in biological and pharmaceutical fields [29]. The newly formed imine groups (–N=CH–), which are introduced by the condensation reaction of aldehydes with amino groups of chitosan in Schiff base compounds, can enhance the biological activities of chitosan, such as anti-inflammatory, antioxidant, antiviral, antitumor and antibacterial activities [15,28,30,31]. In addition, the significant biological activities of a novel synthesized chitosan-Schiff base are strongly dependent on the nature properties of the substituent [26]. Recent years, a considerable amount of chitosan-Schiff bases have been synthesized widely and their biological activities and physicochemical properties were investigated [30,32–34].

With the wide range of applications of chitosan, accurate determination of chitosan content has a great significance to the stability and controllability of chitosan products quality, and it has been a meritorious topic that has been growing in importance for a variety of fields. To quantify and characterize chitosan, both direct quantitative methods and indirect

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analytical methods have been adopted [35–37]. The direct quantitative methods mainly include colorimetric detection, size exclusion chromatography and capillary zone electrophoresis, but these methods are too laborious, unstable, time consuming and not peculiar enough for chitosan's routine analysis in complex matrices [38]. Therefore, indirect quantitative methods, such as HPLC, spectrophotometry and ion exchange are more widely used at current period [39–41]. Generally speaking, chitosan is hydrolyzed into glucosamine salts in strong acidic environment whose concentration can be quantified by HPLC is the most commonly used indirect method to quantify chitosan [40–44]. Generous efforts have been made to enhance the hydrolysis of chitosan and as a result of the wide presence of amine groups, it is difficult to hydrolyze chitosan thoroughly under direct conditions [23,45,46]. Consequently, there is a strong incentive to modify C-2 amine groups to offer more effective hydrolysis of chitosan and quantitative method for chitosan content.

Up to now, the synthesis, characterization and biological activities of many chitosan-Schiff bases have been reported in a substantial amount of documents [31,47]. Nevertheless, the study of hydrolysis of chitosan-Schiff bases to be used for chitosan quantification with HPLC has not been investigated. In this paper, two kinds of chitosan-Schiff base derivatives (BCSB and PCSB) were synthesized by the condensation reaction of different average degrees of deacetylation (DD) of chitosan with benzaldehyde or propanal, respectively. The total mass of Schiff base derivative product was dried and obtained without washing and loss. Then, a certain amount of the prepared Schiff base compound was took to hydrolyze into glucosamine hydrochloride (GAH) in strong

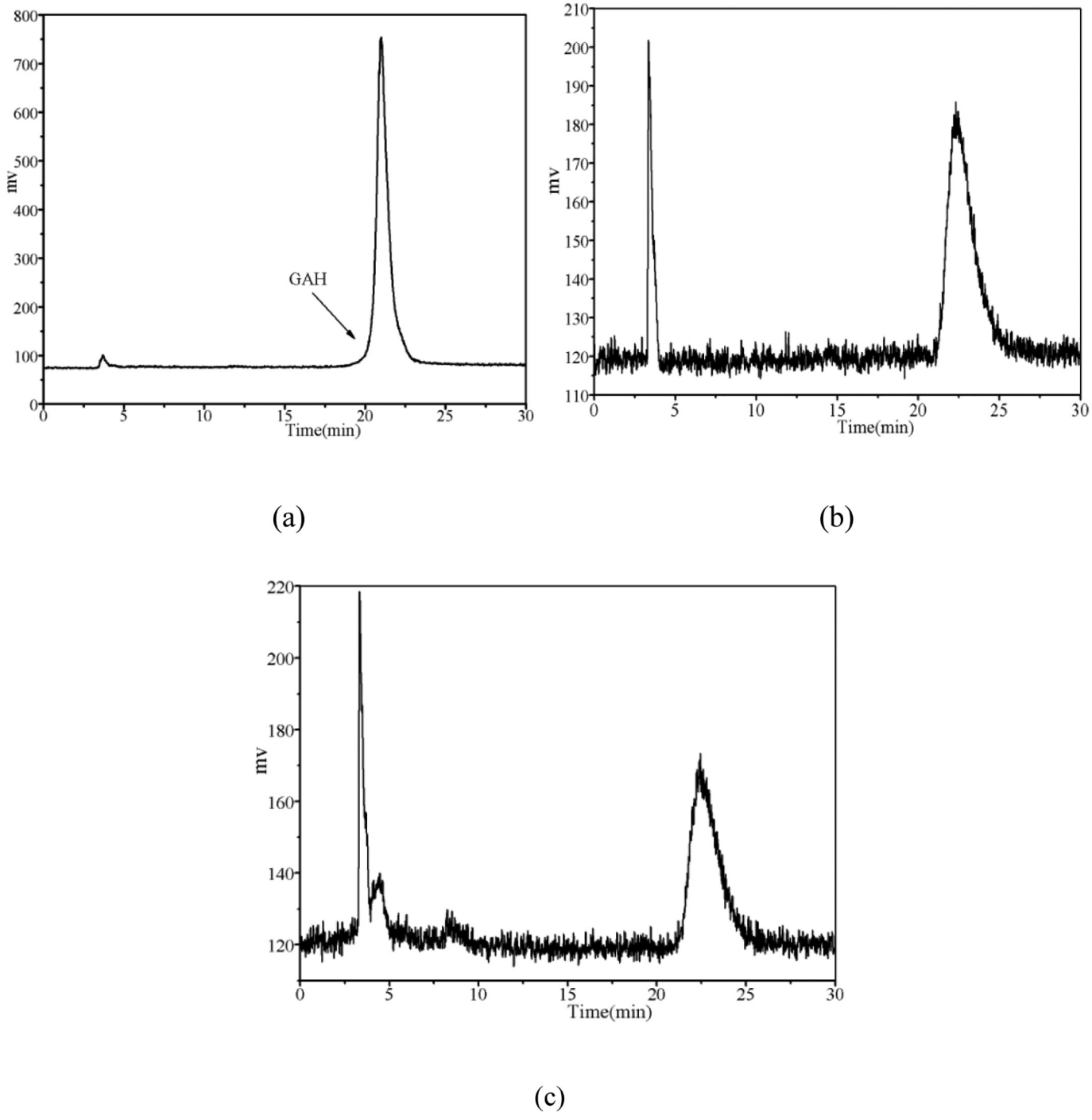


**Fig. 1.** HPLC chromatograms of GAH standard compound (a), hydrolysates of chitosan-Schiff base bearing benzaldehyde (BCSB) (b) and chitosan-Schiff base bearing propionaldehyde (PCSB) (c).

hydrochloric acidic environment, whose concentration was quantified by HPLC, and the mass of GAH contained in hydrolysis solution could be calculated. Subsequently, the total mass of GAH obtained by hydrolysis of all of the Schiff base product was calculated and obtained, and the theoretical mass of chitosan could also be deduced and calculated. Finally, the chitosan content was obtained by combining the sample quality used in Schiff base reaction and the theoretical mass of chitosan.

## 2. Materials and methods

### 2.1. Materials

Chitosan with different average DD values of 57.97% (average Mw = 600 kDa, viscosity 263 mPa·s), 69.40% (average Mw = 500 kDa, viscosity 221 mPa·s), 75.40% (average Mw = 370 kDa, viscosity 197 mPa·s), 83.90% (average Mw = 700 kDa, viscosity 400 mPa·s), 90.84% (average Mw = 300 kDa, viscosity 136 mPa·s), 94.73% (average Mw = 88 kDa, viscosity 50 mPa·s), 97.93% (average Mw = 100 kDa, viscosity 62 mPa·s), 98.91% (average Mw = 80 kDa, viscosity 49 mPa·s), 81.35% (average Mw = 11 kDa, viscosity 35 mPa·s), 88.98% (average Mw = 200 kDa, viscosity 107 mPa·s), 95.84% (average Mw = 150 kDa, viscosity 79 mPa·s) was purchased from Golden-Shell Pharmaceutical Co. Ltd., Zhejiang, China. The average DDs of chitosan have been detected by acid-base titration method in our preliminary experimental study [48]. Chitosan samples were dried to constant weight in a drying oven at 60 °C. Glucosamine hydrochloride (GAH) standard compound was supplied by the Sigma-Aldrich Chemical Corp, Shanghai, China. Absolute ethyl alcohol, chromatographic acetonitrile, acetic acid, hydrochloric acid, benzaldehyde and propyl aldehyde were supplied by Sinopharm Chemical Reagent Co. Ltd., Shanghai, China. Purified water was supplied by Wa-haha Group Co. Ltd., Shandong, China.

### 2.2. Preparation of chitosan-Schiff bases bearing propionaldehyde or benzaldehyde

The synthesis of chitosan-Schiff base was carried out according to the method reported in previous literatures [29,31,49]. Chitosan (1.0 g) was added to 50 mL 1% (*w*/*v*) of aqueous acetic acid solution and the suspension liquid was mixed with magnetic stirrer for over 4 h until it became a homogeneous, transparent and viscous solution. Absolute ethanol (50 mL) was added under continuous stirring for 2 h. Then, homogeneous mixed solution of ethanol (30 mL) and benzaldehyde (30 mmol) was added to the chitosan solution dropwise with constant stirring and reacted at 70 °C for 6 h. After reaction, all of the residual products were dried to obtain the solid of chitosan-Schiff base bearing benzaldehyde (BCSB) with a constant temperature drying oven. Two parallel experiments were conducted for each sample at the same time.

In parallel, the chitosan-Schiff base derivatives bearing propionaldehyde (PCSB) were obtained under the same reaction conditions and experimental operation.

### 2.3. Acid hydrolysis of chitosan-Schiff bases

The hydrolysis of chitosan-Schiff bases was carried out on the basis of the previous proposals with some modifications [23,38,46,48,50]. Chitosan-Schiff bases (40 mg), concentrated hydrochloric acid (3.0 mL) and distilled water (0.5 mL) were mixed together to carry out hydrolysis reaction for 1.5 h at 55 °C, 1.5 h at 75 °C, and 12 h at 100 °C in a heating magnetic stirrer. After reaction, the hydrochloric acid was removed using a rotary evaporator and the residual was transferred and diluted to a 25 mL volumetric flask with distilled water. Then, the acid hydrolysis solution of Chitosan-Schiff bases was obtained.

### 2.4. Determination of hydrolysis solution

Determination of GAH standard compound and hydrolysates of Chitosan-Schiff bases were performed on HPLC with an evaporative light-scattering detector (ELSD). GAH separation and detection was achieved with a mobile phase of acetonitrile–ultrapure water (80:20, *v*/v) on a Carbohydrate column (250 mm × 4.6 mm, 5 μm) at a flow rate of 1.0 mL·min$^{-1}$. The injection volume was 20 μL and the column temperature was 30 °C. ELSD was used at the atomization temperature of 30 °C, evaporation temperature of 60 °C and high purity nitrogen flow rate of 1.5 L·min$^{-1}$.

The linearity equation of standard curves was Y = 1.177 X + 3.699, where X was the logarithm of GAH concentration, Y was the logarithm of peak area, and the correlation coefficient $R^2$ was 0.9998. The detector response for GAH showed linearity over the selected concentration range from 0.5 to 3.5 mg·mL$^{-1}$.

The content of chitosan was calculated based on the following equations:

$$m = \frac{(C \times V) \times m_2 \times \gamma}{m_1 \times 10^3} \quad (1)$$

$$w = \frac{m \times Mr_i}{Mr_1 \times m_3} \times 100 \quad (2)$$

where $m$ represented the total mass of GAH, $V$ was the volume of hydrolysis solution, $C$ was the concentration of GAH in hydrolysis solution, $m_1$ was the mass of chitosan-Schiff base used for acid hydrolysis, $m_2$ was

**Table 1**
Determination of chitosan content with Schiff base bearing benzaldehyde (BCSB).

| DD of chitosan/% | Mass of chitosan sample/g ($m_3$) | Mass of BCSB with complete substitution/g | Total actual mass of prepared BCSB/g ($m_2$) | Mass of GAH in hydrolysis solution/mg | Total mass of GAH/g ($m$) | Theoretical mass of chitosan/g | Content of chitosan/% ($w$) | Average value of $w$/% |
|---|---|---|---|---|---|---|---|---|
| 57.97 | 1.0024 | 1.2883 | 1.4103 | 38.3057 | 1.1975 | 0.9933 | 99.09 | 99.05 |
| | 1.0019 | 1.2876 | 1.4141 | 38.8107 | 1.1960 | 0.9920 | 99.01 | |
| 69.40 | 1.0017 | 1.3532 | 1.4706 | 37.7239 | 1.2238 | 0.9878 | 98.61 | 98.82 |
| | 1.0018 | 1.3533 | 1.4689 | 37.0955 | 1.2291 | 0.9921 | 99.03 | |
| 75.40 | 1.0013 | 1.3886 | 1.5001 | 37.3762 | 1.2490 | 0.9936 | 99.23 | 99.10 |
| | 1.0054 | 1.3943 | 1.5104 | 36.6272 | 1.2510 | 0.9951 | 98.98 | |
| 83.90 | 1.0007 | 1.4406 | 1.5634 | 36.2449 | 1.2782 | 0.9956 | 99.49 | 99.26 |
| | 1.0022 | 1.4427 | 1.5645 | 36.3811 | 1.2743 | 0.9926 | 99.04 | |
| 90.84 | 1.0038 | 1.4900 | 1.6157 | 36.8853 | 1.3018 | 0.9965 | 99.27 | 99.19 |
| | 1.0035 | 1.4895 | 1.6198 | 36.8107 | 1.2994 | 0.9946 | 99.11 | |
| 94.73 | 1.0012 | 1.5120 | 1.6448 | 35.3038 | 1.3131 | 0.9951 | 99.39 | 99.22 |
| | 1.0006 | 1.5111 | 1.6500 | 35.6676 | 1.3078 | 0.9911 | 99.05 | |
| 97.93 | 1.0024 | 1.5354 | 1.6784 | 35.8685 | 1.3247 | 0.9957 | 99.33 | 99.14 |
| | 1.0028 | 1.5360 | 1.6792 | 35.9069 | 1.3203 | 0.9924 | 98.96 | |
| 98.91 | 1.0056 | 1.5470 | 1.6752 | 35.5699 | 1.3307 | 0.9976 | 99.21 | 99.15 |
| | 1.0047 | 1.5456 | 1.6801 | 35.7429 | 1.3279 | 0.9956 | 99.09 | |

**Table 2**
Determination of chitosan content with Schiff base bearing propionaldehyde (PCSB).

| DD of chitosan/% | Mass of chitosan sample/g ($m_3$) | Mass of PCSB with complete substitution/g | Total actual mass of prepared PCSB/g ($m_2$) | Mass of GAH in hydrolysis solution/mg | Total mass of GAH/g ($m$) | Theoretical mass of chitosan/g | Content of chitosan/% ($w$) | Average value of $w$/% |
|---|---|---|---|---|---|---|---|---|
| 57.97 | 1.0035 | 1.1336 | 1.2462 | 42.9115 | 1.1942 | 0.9906 | 98.71 | 98.86 |
| | 1.0056 | 1.1360 | 1.2398 | 42.7092 | 1.2004 | 0.9956 | 99.01 | |
| 69.40 | 1.0062 | 1.1667 | 1.2869 | 42.6136 | 1.2370 | 0.9984 | 99.23 | 99.16 |
| | 1.0024 | 1.1623 | 1.2788 | 42.9820 | 1.2306 | 0.9933 | 99.09 | |
| 75.40 | 1.0086 | 1.1859 | 1.2897 | 44.0185 | 1.2616 | 1.0036 | 99.50 | 99.24 |
| | 1.0087 | 1.1861 | 1.2900 | 43.0302 | 1.2552 | 0.9985 | 98.99 | |
| 83.90 | 1.0041 | 1.2047 | 1.3045 | 43.4632 | 1.2789 | 0.9962 | 99.21 | 99.12 |
| | 1.0032 | 1.2036 | 1.3123 | 43.5198 | 1.2754 | 0.9935 | 99.03 | |
| 90.84 | 1.0040 | 1.2250 | 1.3208 | 43.7965 | 1.2983 | 0.9938 | 98.98 | 99.08 |
| | 1.0051 | 1.2263 | 1.3300 | 44.2775 | 1.3022 | 0.9968 | 99.17 | |
| 94.73 | 1.0060 | 1.2393 | 1.3544 | 42.7201 | 1.3150 | 0.9965 | 99.06 | 99.08 |
| | 1.0044 | 1.2373 | 1.3501 | 43.8862 | 1.3134 | 0.9954 | 99.10 | |
| 97.93 | 1.0035 | 1.2460 | 1.3775 | 43.2689 | 1.3278 | 0.9980 | 99.45 | 99.24 |
| | 1.0080 | 1.2516 | 1.3742 | 42.5285 | 1.3282 | 0.9983 | 99.04 | |
| 98.91 | 1.0084 | 1.2552 | 1.3873 | 42.6093 | 1.3333 | 0.9996 | 99.13 | 99.08 |
| | 1.0063 | 1.2526 | 1.3811 | 43.5195 | 1.3291 | 0.9964 | 99.02 | |

the total actual mass of the prepared chitosan-Schiff base, $\gamma$ ($\gamma = 0.9$) was impurity coefficient, $w$ represented content of chitosan, $m_3$ was the mass of chitosan sample used for Schiff base reaction, $A(\%) = \left(\frac{C_o - C_e}{C_o}\right) \times 100\% Mr_1$ was the molar mass of GAH, and that $Mr_i$ was the molar mass of chitosan with different average deacetylation degree (DD) values and it was calculated in terms of Eq. (3):

$$Mr_i = 161.2 \times DD + 203.2 \times (100 - DD) \quad (3)$$

where DD was the degree of deacetylation of chitosan, 161.2 was the molar mass of glucosamine (GlcN) and 203.2 was the molar mass of acetylglucosamine (GlcNAc).

### 2.5. Method validation

Hydrolysis solution of chitosan-Schiff bases bearing benzaldehyde (BCSB) or propionaldehyde (PCSB) was quantified by HPLC method, which was validated for routine laboratory analysis, including the construction of linearity equation of GAH standard compound, accuracy, precision (repeatability and reproducibility) and solution stability [35,36,38,50]. To demonstrate method accuracy, a known concentration of GAH standard compound was spiked with Schiff bases prior to hydrolysis and analysis following the method in the previous sections. Hydrolysates were determined and evaluated to obtain the recovery of GAH. For method repeatability, 2.5 mg·mL$^{-1}$ GAH standard solution was prepared and a total of five replicate determinations of the sample were analyzed. Five replicate GAH samples were prepared and analyzed to test reproducibility. To evaluate solution stability of HPLC method, the GAH standard solution was determined at 0 h, 2 h, 8 h, 12 h and 24 h respectively.

For further validation of the chitosan-Schiff base method, three random chitosan samples (average DD values were 81.35%, 88.98% and 95.84%; purity of chitosan was 98.95%, 99.13% and 99.08%) were selected and detected. Content of chitosan could be determined and calculated respectively, without loss of chitosan-Schiff bases and tedious purification procedure. Three replicates were conducted for each sample.

## 3. Results and discussion

### 3.1. HPLC chromatograms of chitosan-Schiff base hydrolysates

GAH standard solution and acid hydrolysis solution of chitosan-Schiff bases were determined and achieved on a Carbohydrate column, and the retention time of GAH standard compound was about 22.38 min. The HPLC chromatograms of GAH standard compound (a), hydrolysates of chitosan-Schiff bases bearing benzaldehyde (BCSB) (b) and chitosan-Schiff base bearing propionaldehyde (PCSB) (c) were shown in Fig. 1.

### 3.2. Determination of chitosan content

The acid hydrolysis solution of chitosan-Schiff bases was measured and quantified by HPLC and datum processing were calculated on the basis of Eq. (1), Eqs. (2), and (3). The detailed results were shown in Tables 1 and 2. From the tables, we found that the total actual mass of the prepared chitosan-Schiff bases (BCSB or PCSB) was higher than the mass of BCSB/PCSB with complete substitution, because of the residual aldehydes (benzaldehyde or propionaldehyde) in Schiff base derivatives. Therefore, an impurity coefficient $\gamma$ ($\gamma = 0.9$) was introduced. The degree of hydrolysis was improved, when chitosan with different degrees of deacetylation was derived into chitosan-Schiff base, and the mass of GAH in hydrolysis solution was quantified and calculated. Then, total mass of GAH ($m$) could be obtained on basis of the total actual mass of the prepared chitosan-Schiff bases. Theoretical mass of chitosan was subject to the total mass of GAH ($m$), the molar mass of GAH ($Mr_1$) and average deacetylation degree (DD) of chitosan. Finally, contents of chitosan ($w$), which were close to 100%, were calculated

**Table 3**
Results of validation of the Schiff base bearing benzaldehyde (BCSB) method.

| DD of chitosan/% | Mass of chitosan sample/g | Total mass of prepared BCSB/g | Total mass of GAH/g | Theoretical mass of chitosan/g | Content of chitosan/% ($w$) | Average value of $w$/% | RSD/% |
|---|---|---|---|---|---|---|---|
| 81.35 | 1.0006 | 1.5377 | 1.2613 | 0.9887 | 98.81 | 98.94 | 0.12 |
| | 1.0021 | 1.5401 | 1.2661 | 0.9925 | 99.04 | | |
| | 1.0018 | 1.5413 | 1.2648 | 0.9915 | 98.97 | | |
| 88.98 | 1.0090 | 1.6096 | 1.3019 | 1.00125 | 99.23 | 99.25 | 0.18 |
| | 1.0013 | 1.5988 | 1.2905 | 0.9925 | 99.12 | | |
| | 1.0054 | 1.6016 | 1.2996 | 0.9995 | 99.41 | | |
| 95.84 | 1.0026 | 1.6472 | 1.3120 | 0.9915 | 98.89 | 99.18 | 0.33 |
| | 1.0009 | 1.6503 | 1.3189 | 0.9967 | 99.58 | | |
| | 1.0015 | 1.6514 | 1.3129 | 0.9922 | 99.07 | | |

**Table 4**
Results of validation of the Schiff base bearing propionaldehyde (PCSB) method.

| DD of chitosan/% | Mass of chitosan sample/g | Total mass of prepared PCSB/g | Total mass of GAH/g | Theoretical mass of chitosan/g | Content of chitosan/% | Average value of chitosan content/% | RSD/% |
|---|---|---|---|---|---|---|---|
| 81.35 | 1.0030 | 1.3164 | 1.2624 | 0.9896 | 98.66 | 98.96 | 0.26 |
| | 1.0026 | 1.3057 | 1.2680 | 0.9940 | 99.14 | | |
| | 1.0038 | 1.3111 | 1.2686 | 0.9945 | 99.07 | | |
| 88.98 | 1.0001 | 1.3144 | 1.2889 | 0.9912 | 99.11 | 99.09 | 0.14 |
| | 1.0049 | 1.3200 | 1.2928 | 0.99422 | 98.94 | | |
| | 1.0003 | 1.3156 | 1.2905 | 0.9925 | 99.22 | | |
| 95.84 | 1.0052 | 1.3599 | 1.3179 | 0.9960 | 99.08 | 99.03 | 0.15 |
| | 1.0022 | 1.3507 | 1.3149 | 0.9937 | 99.15 | | |
| | 1.0008 | 1.3582 | 1.3094 | 0.9895 | 98.87 | | |

and obtained accurately by combining the theoretical mass and the sample mass of chitosan.

The results demonstrated that the prepared chitosan-Schiff bases with aldehydes (benzaldehyde or propionaldehyde) have exhibited better acid hydrolysis effect compared with chitosan, which could be used for the determination of chitosan content. It maybe because the acid hydrolysis degree of chitosan was subject to the charge density of the glycoside center [6]. When chitosan-Schiff base derivatives were synthesized, the amino groups at the C-2 positions were protected and the positive charge density decreased on the reaction center, which stabilised the oxocarbonium ion transition state, together with the anchimeric catalytic assistance of phenyl groups or ethyl groups of aldehydes, resulting in their acid hydrolysis was facilitated and enhanced [1,21,23,51,52]. The advantage of this Schiff base method was that there was no need to deal the derivative products with purification and washing before the acid hydrolysis. In addition, the degree of substitution of derivatives did not need to be confirmed and data processing was easy. Hence, this Schiff base method was effective and simple, and it supplied a preeminent thought and method for the determination of chitosan content.

### 3.3. Method validation

The HPLC method was validated for linearity, accuracy, precision and solution stability. The detector response for GAH showed linearity over the selected concentration range from 0.5 to 3.5 $mg \cdot mL^{-1}$ with the correlation coefficient ($R^2$) 0.9998. The relative standard deviation (RSD) of GAH standard recovery was 3.04%, the RSD of the repeatability and reproducibility were 0.96% and 2.03% respectively, and the RSD of solution stability was 1.63%. This demonstrated that the HPLC method presented excellent linear relationship, quite high recovery, both good repeatability and reproducibility, and commendable stability. Consequently, the HPLC method was successfully used for quantitative analysis of GAH in hydrolysis solution of chitosan-Schiff bases.

The results of validation of the Schiff base method were showed in Tables 3 and 4. Chitosan with the average DD values 81.35%, 88.98% and 95.84% were derived into their Schiff base derivatives, severally. Those prepared derivative were hydrolyzed and determined according to the method in the previous sections. By data processing, the relevant masses were calculated and obtained in turn. Then, the average *w* values (BCSB method) of 98.94%, 99.25%, 99.18% were obtained, which were around their real values (98.95%, 99.13% and 99.08%) respectively. Simultaneously, the average *w* values (PCSB method) of 98.96%, 99.09%, 99.03% were around their real values as well. The relative standard deviation (RSD) was 0.12, 0.18, 0.33, 0.26, 0.14, 0.15, respectively. The validation results displayed that the Schiff base method could quantify the content of chitosan accurately.

## 4. Conclusions

In this work, an effective and simple Schiff base and HPLC method to quantify chitosan content was developed. Two kinds of aldehydes, benzaldehyde and propanal were used to synthesize chitosan-Schiff base derivatives (BCSB and PCSB). By data processing, the relevant qualities were calculated and deduced in order, and the chitosan content could be calculated and obtained by the specific value of theoretical mass and the sample mass of chitosan. The advantage of this Schiff base method was that there was no need to deal the derivative products with tedious purification and washing procedure before the acid hydrolysis. In addition, the degrees of substitution of chitosan-Schiff base derivatives did not need to be confirmed and the data processing was easy. Hence, this Schiff base method proposed here was effective and simple, supplying a preeminent thought and route for the determination of chitosan content.

## Declaration of competing interest

The authors have declared no conflicts of interest.

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