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# Determination of reducing-end sugar in water-soluble chitosan - Spectrophotometric method

水溶性壳聚糖中还原端基糖的测定 分光光度法

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## 1 Scope

This document specifies the spectrophotometric method for the determination of reducing-end sugar in water-soluble chitosan.

This document applies to the detection of reducing-end sugar in water-soluble chitosan (content ≥90%, degree of deacetylation ≥90%).

The detection limit of this method is 0.006 mmol/g; the quantitation limit is 0.023 mmol/g.

## 2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. For dated references, only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable to this document.

GB/T 6682, Water for analytical laboratory use - Specification and test methods

## 3 Terms and definitions

### 3.1 Chitosan

The derivative, which is obtained by deacetylation of chitin, that is insoluble in water and soluble in most dilute acids. The chemical name is β-1,4-2-amino-2-deoxy-D-glucan.

### 3.2 Degree of deacetylation

The percentage of the number of sugar residues that are removed from the acetyl group in the chitosan molecule TO the total number of sugar residues in the chitosan molecule.

### 3.3 Water-soluble chitosan

Chitosan of low degree of polymerization, which is soluble in acidic, neutral and alkaline solutions.

## 4 Principle

The reducing-end sugar at the end of the chitosan molecule reacts with 3-methyl-2-MBTH under alkaline conditions to form azine. Under acidic conditions, excess MBTH is oxidized by $Fe^{3+}$ to cations; then, it reacts with azine to produce a cyan compound. The concentration of the reducing-end sugar is in a linear relationship with its absorbance at 590 nm within a certain range. Quantify by external standard method by measuring the absorbance value at 590 nm.

## 5 Reagents and materials

Unless otherwise specified, the reagents which are used in this method are analytical reagents. The water is grade-3 water that meets the requirements of GB/T 6682.

**5.1** 3-Methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH, $C_8H_9N_3S \cdot HCl \cdot H_2O$): CAS number: 38894-11-0, purity ≥98%.

**5.2** DL-Dithiothreitol [DTT, $HSCH_2CH(OH)CH(OH)CH_2SH$]: CAS number: 3483-12-3, purity ≥98%.

**5.3** D-Glucosamine hydrochloride ($C_6H_{13}NO_5 \cdot HCl$): CAS number: 66-84-2, purity ≥99%, or a standard substance that is certified by the country and awarded with a certificate of standard substance.

**5.4** Concentrated hydrochloric acid.

**5.5** Sodium hydroxide solution (0.5 mol/L): Weigh 2 g of sodium hydroxide; add water to dissolve; use water to dilute and fix volume to 100 mL; mix well.

**5.6** MBTH solution: Weigh 0.3 g of MBTH; add water to dissolve and dilute to 100 mL; mix well; store in the dark at 4 °C; it is valid for 10 days.

**5.7** DTT solution: Weigh 0.080 g of DTT; add water to dissolve and dilute to 100 mL; mix well; store in the dark at 4 °C; it is valid for 10 days.

**5.8** MBTH reagent: Mix MBTH solution (5.6) and DTT solution (5.7) in equal volumes to obtain MBTH reagent. Prepare when necessary.

**5.9** Ferric ammonium sulfate reagent: Weigh 5 g of ferric ammonium sulfate [$NH_4Fe(SO_4)_2 \cdot 12H_2O$] and 5 g of sulfamic acid; add 41.8 mL of concentrated

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