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A novel method for quantitative determination of tea polysaccharide by resonance light scattering

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ABSTRACT

A new method for the determination of tea polysaccharide (TPS) in green tea (*Camellia sinensis*) leaves has been developed. The method was based on the enhancement of resonance light scattering (RLS) of TPS in the presence of cetylpyridinium chloride (CPC)–NaOH system. Under the optimum conditions, the RLS intensity of CPC was greatly enhanced by adding TPS. The maximum peak of the enhanced RLS spectra was located at 484.02 nm. The enhanced RLS intensity was proportional to the concentration of TPS in the range of 2.0–20 μg/ml. It showed that the new method and phenol–sulfuric acid method give some equivalent results by measuring the standard compounds. The recoveries of the two methods were 96.39–103.7% (novel method) and 100.15–103.65% (phenol–sulfuric acid method), respectively. However, it showed that the two methods were different to some extent. The new method offered a limit of detection (LOD) of 0.047 μg/ml, whereas the phenol–sulfuric acid method gives a LOD of 1.54 μg/ml. Interfered experiment demonstrated that the new method had highly selectivity, and was more suitable for the determination of TPS than phenol–sulfuric method. Stability test showed that new method had good stability. Moreover, the proposed method owns the advantages of easy operation, rapidity and practicability, which suggested that the proposed method could be satisfactorily applied to the determination of TPS in green tea.

1. Introduction

Green tea (*Camellia sinensis* L.) is the second most consumed beverage in the world and has caused great interest among researchers [1–4]. Polysaccharide from tea has shown a variety of bioactivities, such as immunostimulating [5], hypoglycemic [6,7], and anti-bacterial activities [8]. Obviously, tea polysaccharide (TPS) becomes popular day by day. However, accurately determinate of TPS is also an unsolved problem at present. Several methods such as colorimetric methods [9–12], high-performance liquid chromatography [13–15] and densitometric method [16] have been used to quantitatively determine carbohydrates. Among many colorimetric methods, phenol–sulfuric acid method [10] is the easiest and most reliable method for measuring total content of the carbohydrates. However, it has disadvantages, such as low selectivity, need of aggressive sulfuric acid and phenol (toxic mutagen category 3). HPLC method is limited by complex operation, time consuming and expensive apparatus. Although densitometric method is suitable for measuring total carbohydrates, it has no selectivity for carbohydrate. Not only this method has no selectivity, but also many other methods lack selectivity for carbohydrate. So far, selectivity for analysis of a certain kind of carbohydrate is also a great difficulty. Thus, it is necessary to establish a series of simple and sensitive methods for quantitative determination of various kinds of carbohydrates.

TPS is a kind of high molecular polysaccharide [7,17,18]. In order to accurate determination of it in *C. sinensis*, our previous work applied phenol–sulfuric acid method and modified the method [24]. The modified method has a good result in the determination of it. However, the modified method is complicated, which needs employ gas chromatography. The aim of this work is to develop a simple and sensitive method to accurately determine TPS in *C. sinensis*. The new method should be more alternative to phenol–sulfuric acid method. RLS is a useful technique for monitoring molecular aggregation. Since the technique was firstly established by Pasternack et al. in 1993 [19], RLS has been enjoyably practiced by numerous scholars for various quantitative events [20–22].

In this research, interaction of CPC with TPS under aqueous basic conditions was studied by RLS technique. The results showed that RLS signal of CPC was remarkably enhanced by adding a

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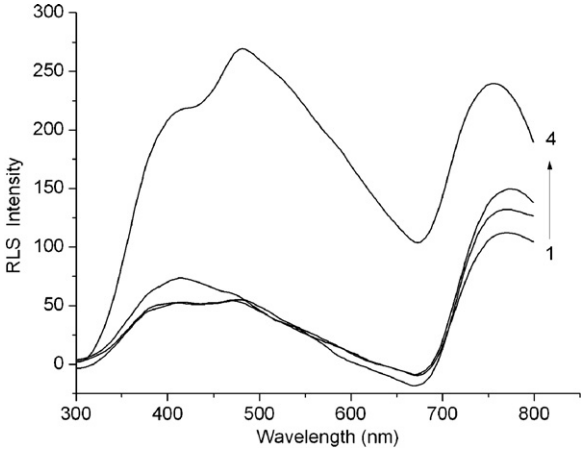

Fig. 1. RLS spectra of the systems: (1) TPS–NaOH; (2) CPC–NaOH; (3) NaOH; (4) TPS–CPC–NaOH. Conditions: NaOH, 0.5 mol/l; TPS, 1.0 μg/ml; CPC, 1.0 mmol/l; excitation and emission slit widths were 2.5 nm and 10 nm, respectively.

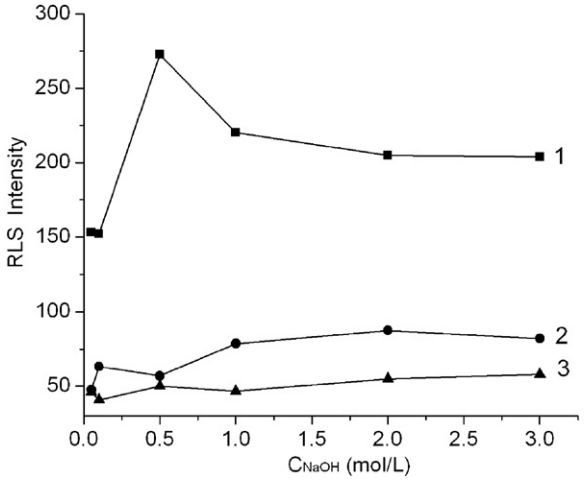

Fig. 2. Effect of NaOH concentration on the resonance light scattering (RLS) intensity. Curves: (1) TPS–CPC–NaOH; (2) CPC–NaOH; (3) TPS–NaOH. Conditions: C_{CPC} = 1.0 mmol/l; C_{TPS} = 1.0 μg/ml; excitation and emission slit widths were 2.5 nm and 10 nm, respectively.

trace amount of TPS. Meanwhile, enhanced intensity of RLS was in proportion to the concentration of TPS. Thus, a new method for determination of TPS was established and interaction mechanism of the system was initially discussed in the paper.

2. Materials and methods

2.1. Apparatus and reagents

The RLS spectrum was recorded on a LS-55 Fluorescence spectrophotometer (PerkinElmer, USA). The absorption spectra were recorded by a T6 UV-VIS spectrophotometer (Beijing Purkinje General Instrument Co. Ltd, China). Tea leaves were purchased from Hubei province of China. D-arabinose, D-xylose, D-mannose, D-glucose(Glc), D-galactose, sucrose and inositol were purchased from Sigma (MO, USA). Bovine serum albumin (BSA), EGCG, EGC, glycine, glutamine, vitamin C(Vc), caffeine, theanine and CPC were purchased from China National Medicines Corporation Ltd. TPS (purity ≥99%) was extracted and purified according to our laboratory[25]. All other reagents and solvents were of analytical grade and used without further purification unless otherwise noted. All aqueous solutions were prepared using newly double-distilled water.

2.2. Phenol–sulfuric acid method

Pipette 1.2 ml of TPS (or other samples) into a tube, add 1.0 ml of phenol solution and mix. Add 2.5 ml sulfuric acid from a fast-flowing pipette to the tube and mix the contents rapidly. After 20 min, absorbance of sample solution was measured at 480 nm. The reaction system without polysaccharide was used as blank test [10].

2.3. RLS method

Pipette 2.0 ml of TPS (or other samples) into a tube and add 1.0 ml of CPC solution. Add 0.5 ml of NaOH to the tube and mix the contents rapidly. Then the above solution was removed from the test tube to a cuvette for RLS.

The RLS spectra were obtained by scanning simultaneously excitation and emission monochromators of the spectrofluorometer from 300.0 to 800.0 nm with $\Delta\lambda = 0$ nm. According to the spectra, analytical wavelength of the RLS measurements was 484.02 nm. The enhanced RLS intensity of CPC was represented as $\Delta I_{RLS} = I_{RLS} - I^0_{RLS}$ (I_{RLS} and I^0_{RLS} were the RLS intensities of the systems with and without TPS).

3. Results and discussion

3.1. Spectral characteristics of RLS

The RLS spectra of NaOH, TPS–NaOH, CPC–NaOH and TPS–CPC–NaOH are shown in Fig. 1. Comparatively, the RLS signal of the aqueous TPS–CPC–NaOH system was remarkably enhanced. The maximum peak of the RLS spectra was located at 484.02 nm. Therefore, 484.02 nm was selected as analytical wavelength for further study. The reason for enhancement of RLS intensity may be that larger particles were produced in the TPS–CPC–NaOH system. It was reported that TPS is a kind of polyanion polysaccharide, which could combine with CPC to form an aggregation [23]. And because of strong hydrophobic interaction and intra molecular forces, this initial aggregation had a further aggregation to form even bigger particles. According to the Rayleigh formula [19], the larger the size of the particles, the stronger the signal of the scattering light could be obtained. Thus, the RLS signal intensity of CPC was enhanced by interacting with TPS. So TPS–CPC–NaOH system was selected as our analytical model. Then the optimized condition for determination of TPS was studied as follows.

3.2. Effect of NaOH concentration

The alkalinity of system on relative RLS intensities was investigated. When NaOH concentration changed in the range of 0–3.0 mol/L, RLS signal of the assay system was significantly affected. As shown in Fig. 2, RLS intensity of TPS–CPC–NaOH system was the strongest, and CPC–NaOH system was more sensitive than TPS–NaOH system. The maximum ΔI_{RLS} was obtained when concentration of NaOH was 0.5 mol/L. And concentration of NaOH is higher or lower than the optimum range, ΔI_{RLS} decreases. The reason for this phenomenon may be that the strong base could induce TPS to take more negative charge, making electrostatic interaction between TPS and CPC stronger. When excess alkali was added, TPS might be degraded. Thus it would lead the ΔI_{RLS} to decrease naturally. Therefore, NaOH concentration of 0.5 mol/L was chosen for further assay in the research.