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Application News

Quantitative Determination of Surface Functional Groups of Cellulose Nanofibers Using Toluidine Blue O Adsorption Method

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User Benefits

- Simple quantitative determination of the surface functional groups of cellulose nanofibers (CNF) is possible with a small sample amount on the order of a few mg[1)] per measurement.
- Quantitation results with high repeatability and low dependence on individual skill can be obtained.
- Due to the extremely short time of one measurement with a UV-Vis spectrophotometer, it is possible to conduct quantitative analyses of 50 specimens in about 2.5 hours*.

* Here, 2.5 hours means the actual measurement time with the UV-Vis spectrophotometer, and does not include the time required for sample preparation procedure and time spent in cell insertion/removal before and after measurements.

■ Introduction

Cellulose is one of the polysaccharides which are the main components of plant cell walls. Among the various types of cellulose, those with a fiber diameter of 4 to 100 nm, length of several µm, and aspect ratio of 100 or more are called cellulose nanofibers (CNF), and have attracted attention as a leading-edge biomass material. In addition to the light weight and high strength of CNF, they also have other excellent functions, including a high gas barrier property, adsorption and transparency, and as a plant-derived material, the environmental impacts of production and disposal are small. In the future, use in automotive components, electronic materials, packaging materials and other applications are expected.

The surface of nanocellulose materials can be modified with surface functional groups such as the sulfate groups and carboxy groups to add various functions. In water, the ionic parts of these surface functional groups function as charged groups, improving water dispersibility. Conventionally, the conductometric titration method had been used in quantitative analysis of these surface charged groups. Although this is a general-purpose technique, it has a number of problems, including ① a large amount (several hundred mg) of sample material is required, ② the measurement time is long, ③ visual confirmation is necessary, and ④ the results differ depending on the analyst. Therefore, a simple method which does not depend on the skill of the individual analyst has been needed to solve these problems. This article introduces an example of a quantitative analysis of surface functional groups by the toluidine blue O (TBO) adsorption method using a Shimadzu ultraviolet-visible light (UV-Vis) spectrophotometer. This experiment was carried out with the cooperation of Prof. Jun Araki of Shinshu University.

■ Toluidine Blue O (TBO) Adsorption Method

TBO is a basic dye which is used in staining various types of specimens such as cell substrates. Fig. 1 shows the structure of TBO.

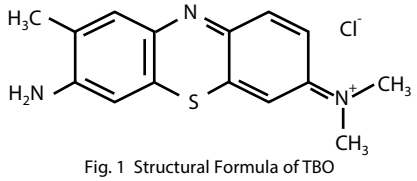


Fig. 1 Structural Formula of TBO

Quantitative analysis of surface functional groups is possible by adsorbing the basic dye TBO on anionic surface groups (here, the carboxy groups) on the surface of CNF[1), 2), 3)]. If this method is used, the amount of sample material necessary for quantitative analysis is very small, being on the order of a few mg[1)]. Furthermore, because a UV-Vis spectrophotometer is used, the time required for one measurement is short, and quantitative analysis of 50 specimens in around 2.5 hours is possible. It should be noted that this time is the time required for the actual measurement with the UV-Vis spectrophotometer, and does not include the time necessary for the sample preparation procedure and time spent in insertion/removal of the cells before and after measurements.

Fig. 2 shows the sample preparation procedure in the TBO adsorption method. The CNF suspension used in this experiment was a commercially-available viscous product.

■ Sample Preparation Procedure

① Dilute the CNF suspension with water to a sample concentration of 2.5 wt/%, and stir for 10 min using ultrasonic, a shaker, or the like so as to obtain a uniform aqueous suspension.

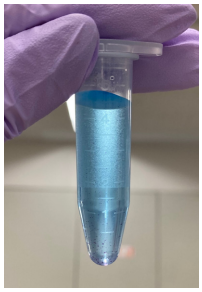
② Add 0.1 ml of the uniform CNF aqueous suspension prepared in ①, 2.9 ml of pure water, and 2 ml of a TBO solution to a 5 ml microcentrifuge tube.

③ Shake for 2 h using a shaker.

④ Conduct centrifugal separation at 3500 rpm or higher for 10 min using a centrifugal separator to sufficiently precipitate the suspended solids.

Dye-adsorbed CNF aqueous suspension after centrifugal separation

⑤ Using a micropipette, take 0.45 ml of the supernatant in ④, and dilute 10x with 4.05 ml of pure water.



CNF aqueous suspension after 10x dilution of the supernatant

Fig. 2 Sample Preparation Procedure Using Toluidine Blue O Adsorption Method

■ Measurement of Spectrum of Dye-Adsorbed CNF Supernatant Solution

The supernatant of the CNF aqueous suspension sample prepared by the protocol described above was measured using a Shimadzu UV-1900i UV-Vis spectrophotometer. The surface of the CNF measured in this experiment was modified with the carboxy groups by the 2,2,6,6 tetramethylpiperidine1-oxyl free radical (TEMPO) oxidation method using the TEMPO, which is a stable nitroxyl radical. Fig. 3 shows the appearance of the instrument, and Table 1 gives the measurement conditions. Fig. 4 shows the absorption spectrum of the supernatant solution of the TEMPO-oxidized CNF (TOCN). Since there is a possibility that the TBO used in quantitation may be adsorbed on quartz glass, which is used in measurements, use of disposal-type cells is recommended if multiple measurements are to be conducted.

Fig. 3 Appearance of UV-1900i

Table 1 Measurement Conditions

Instrument	: UV-1900i
Measurement Wavelength Range	: 400 - 900 nm
Data Interval	: 1 nm
Scanning Speed	: High
Slit Width	: 1 nm
Light Source Switching Wavelength	: 340 nm

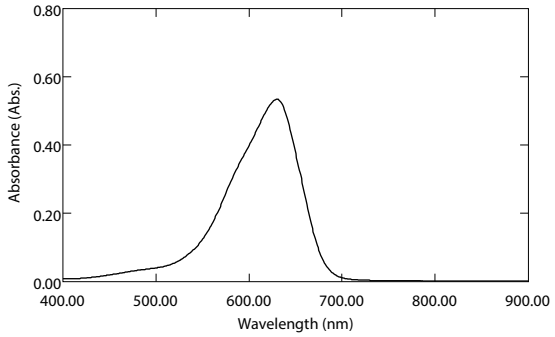


Fig. 4 Absorption Spectrum of TEMPO-Oxidized CNF Supernatant Solution

From Fig. 4, it can be understood that the supernatant solution shows its absorption maximum at 628 nm. It is possible to quantify the surface functional groups by using the absorbance value at this 628 nm wavelength.

■ Quantification of Surface Functional Groups

The surface carboxy groups were quantified by calculation using Eq. (1) [1].

$$S_{COOH} = \frac{(c_{TBO} \times 2 \times 10^{-3}) - (\frac{A_w}{\varepsilon_w} \times 5 \times 10^{-2})}{c_{CNF} \times v \times 10^{-3}}$$

$$= \frac{(c_{TBO} \times 0.2) - (\frac{A_w}{\varepsilon_w} \times 5)}{c_{CNF} \times v} \times 10^4 \quad \ldots (1)$$

where, S_{COOH}: concentration of surface carboxy groups of CNF, C_{TBO}: concentration of TBO solution at time of mixing, ε_w: molar absorption coefficient, C_{CNF}: concentration of CNF suspension, v: volume of CNF suspension, A_w: absorbance at 628 nm. Since this sample contained only the carboxy groups, the total number of surface charged groups corresponds to the total carboxy groups. It may be noted this calculation was done using Excel®, which enables quantification simply by inputting the absorbance value and concentrations of the CNF suspensions[1].

Table 2 shows the carboxy group concentration S_{COOH} calculated by using the above-mentioned equation and the absorbance value at 628 nm measured by the UV-Vis spectrophotometer. The measurements and calculations were repeated 5 times. The coefficient of variation (CV) in the table is one index showing the variation of data. Here, it was found that the CV of the carboxy group concentration was 0.0324.

Table 2 Quantification Results

Repetition No.	Absorbance value at 628 nm (Abs.)	S_{COOH} (mmol/g)
1	0.557	2.098
2	0.568	2.050
3	0.554	2.112
4	0.550	2.129
5	0.592	1.944
Average value		2.067
Standard deviation (SD)		0.067
Coefficient of variation (CV)*		0.0324

* Coefficient of variation = Standard deviation / Average value

■ Conclusion

Quantitative determination of the surface functional groups of CNF was carried out using the toluidine blue O (TBO) adsorption method. Simple and quick quantification of the carboxy groups was possible by using a UV-Vis spectrophotometer and a TBO solution. Higher efficiency in quality control of CNF-based products can be expected by using this technique.

<Acknowledgement>

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<References>

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