

Dye adsorption revisited: Application of the cationic dye adsorption method for the quantitative determination of the acidic surface groups of nanocellulose materials

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Research Article

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Abstract

The surface carboxyl and sulfate groups on cellulose nanowhiskers were quantified via the adsorption of toluidine blue O (TBO), a cationic dye. Here, simple and rapid protocols, such as mixing the nanowhisiker suspensions with a dye solution, separating the supernatants via centrifugation, and determining the excess dye concentration via visible light absorbance techniques, were used to obtain reproducible results comparable with those obtained via titration. In addition to facilitating the discrete quantification of the sulfate and carboxyl groups, the TBO adsorption method enabled carboxyl quantification in the presence of mercapto groups, which was difficult to achieve via titration. The adsorption of TBO onto the carboxyl groups was completed within 30 min, enabling the rapid treatment of many samples within a short period.

1. Introduction

Various nanocellulose materials, including cellulose nanowhiskers (CNWs) (Araki 2013; de Souza Lima and Borsali 2004; Eichhorn 2011), cellulose nanofibers (CNFs) (Isogai et al. 2011), and cellulose nanocrystals (CNCs) (Habibi et al. 2010), contain different types of surface functional groups, especially ionic groups. These ionic surface functional groups include sulfates (sulfate halfesters) which are introduced onto the surface of CNWs (Araki 2013; Araki et al. 1998; de Souza Lima and Borsali 2004; Eichhorn 2011) or CNCs (Habibi et al. 2010) during sulfuric acid-mediated hydrolysis. The carboxyl or phosphate groups can also be introduced via the surface oxidation of CNWs/CNFs (Araki 2013; de Souza Lima and Borsali 2004; Eichhorn 2011; Isogai et al. 2011) and the post-phosphorylation of CNWs (Araki et al. 2000a), respectively. These ionic moieties work as charged groups through their dissociation in water, which avoid the aggregation and sedimentation of the nanocellulose particles/fibers because of electrostatic repulsions, resulting in a good stability of the aqueous nanowhisiker dispersions (Araki 2013; de Souza Lima and Borsali 2004; Eichhorn 2011; Habibi et al. 2010; Isogai et al. 2011). Therefore, the introduction of these charged surface groups is crucial for achieving good dispersion without aggregation. Furthermore, various types and numbers of such charged surface groups affect the properties of the nanocellulose aqueous suspensions, including the suspension viscosity (Araki et al. 1998; Araki et al. 2000a), liquidcrystal formation (Araki et al. 2000b; Araki and Kuga 2001), or heat resistance (Fukuzumi et al. 2010). The controlled introduction of these surface functional groups will help in grafting other functional molecules and polymers as possible reactive sites (Araki et al. 2001; Araki et al. 2002). Given all the above factors, techniques for quantitatively determining the charged surface group contents are indispensable for targeted control of the properties of nanocellulose, thereby furthering research efforts on various functional materials containing nanocellulose.

Many techniques have been developed and utilized for the quantification of the charged surface groups of CNWs, CNFs, and CNCs. One of the most typical methods is acid–base titration in which the pH (Batmaz et al. 2014) and conductivity (Araki et al. 1998; Araki et al. 2000a; Bondeson et al. 2006; ISO 21400, 2018; Johnston et al. 2018) of the samples are monitored. Other methods, including headspace gas chromatography (Chai et al. 2003; Wu et al. 2018), gel permeation chromatography after fluorescent derivatization (Bohrn et al. 2006), ¹³C NMR spectroscopy (Kumar and Yang 1999), and zeta potential measurements (Zhu et al. 2013), were also examined. Among the various methods available, conductometric titration is the simplest and most widely used method because it facilitates the quantification of strong and weak acid moieties (e.g., sulfate and carboxyl groups, respectively). However, the titration method exhibits some disadvantages, including a lengthy measurement time with laborious procedures, the use of expensive apparatus for automation, and a lengthy and complicated pretreatment

procedure for the samples (e.g., ion exchange resin treatment may take several hours) (ISO 21400, 2018). Therefore, an alternative method is required for rapid and facile quantification using common analytical instruments.

The use of surface adsorption techniques using cationic dyes, such as methylene blue (MB) or toluidine blue O (TBO), has gained popularity as another method for quantitative analysis of the carboxyl content of cellulosic materials. This method is based on the stoichiometric formation of ionic pairs between the dye molecules and the respective carboxyl groups. Quantification based on MB adsorption was developed early in the 1940s (Davidson 1948; Wilson and Mandel 1961) and was incorporated into the TAPPI guidelines for pulp and paper (TAPPI T237 om-93). Although the method was later excluded from the TAPPI standards because of its complexity and time-consuming nature, the recent TAPPI description agrees that the MB adsorption method is still useful for analyzing small sample quantities or samples with a very low carboxyl content. Further, the dye adsorption method was applied for quantification of the carboxyl contents on various solid supports (Rödiger et al. 2011; Sano et al. 1993; Tiraferri and Elimelech 2012). Majority of the reported protocols include multistep processes, including the addition of excess dye molecules relative to the carboxyl content to be determined, thorough washing of the unadsorbed dye, and quantitative liberation and measurements of the adsorbed dye. However, the method initially used to analyze cellulosic materials (Wilson and Mandel 1961) seems to be simpler and more straightforward; namely, the carboxyl content could be accurately quantified by directly determining the unadsorbed dye amounts in the supernatant after a reaction of the solid cellulosic materials in an excess dye solution. Although this method offers many advantages, it seems to be difficult to apply it to nanocellulose materials because of the difficulty associated with the separation of the supernatant dye solution from the dye-saturated nanocellulose solid particles. The filtration of the nanosized samples seems to be unreasonable, and collection via centrifugation seems to be implausible because majority of the nanocelluloses are well-dispersed colloids or nanofibers. Therefore, carboxyl groups quantification by the dye adsorption method have never been applied to nanocellulose materials.

Herein, the dye adsorption method was examined to quantify the anionic surface groups, especially carboxyl groups, of CNWs to establish optimized protocols. The developed methods exhibited excellent reproducibility, and the results were sufficiently comparable to those obtained via conventional titration methods. One major advantage is the trace amounts of the samples (0.5–2.5 mg per measurement) required for accurate results, as well as brief and facile procedures. In addition to the discrete quantification of the sulfate and carboxyl contents in CNW samples, which could also be achieved using the conventional titration methods, the newly developed method was extremely selective toward the carboxyl groups, even in a complex sample matrix containing other weakly acidic groups such as mercapto groups. Carboxyl quantification in such a complex sample matrix was improbable using the conventional titration method.

2. Materials And Methods

Complete experimental details about the measurement procedures, sample preparation, and analytical protocols are presented in the Supplementary Information. An overview of the protocol is presented in Scheme 1. Briefly, an aqueous suspension of the CNWs was mixed with a TBO standard solution of a known concentration in water (for quantifying the carboxyls or total anionic surface groups) or in aqueous HCl under pH = 1 (for quantifying strong acid groups). After a sufficiently long shaking period, the supernatant was separated from the dye-adsorbed CNWs via centrifugation. Then, the dye concentration in the pipetted supernatant was determined after being diluted using the appropriate dilution factor. The concentration of the dye in the supernatant was calculated, and the

results were subtracted from the initial dye content to obtain the content of the anionic surface groups in the CNWs. The relevant equation is presented in the Supplementary Information.

As noted in the Supplementary material, it is crucial to avoid the usage of glassware during the experiment because the positively charged TBO molecules are readily adsorbed onto the glass surface, causing the results to become inaccurate.

3. Results And Discussion

Figure 1 shows the relation between the carboxyl content obtained via the titration and TBO adsorption methods for CNWs, CCNWs with different degrees of oxidation, and CNW/CCNW100 mixtures containing various composition ratios. It should be noted that the titration measurements were not directly conducted for the series of CNW/CCNW100 mixed samples; instead, their “titration” values were calculated using the titration values of CNW and CCNW100 based on their mixing ratios. Figure 1 clearly indicates that the values obtained using the dye adsorption methods were consistent with those by titration. The developed method exhibited excellent reproducibility, as indicated by the narrow deviations in Fig. 1, which indicates sufficiently narrow deviations to be hidden by the symbols. Although previous studies performed the dye adsorption processes for the surface carboxyls under buffered or conditioned pH experimental conditions (Davidson 1948; Fardim et al. 2005; Rödiger et al. 2011; Sano et al. 1993; Tiraferri and Elimelech 2012; Wilson and Mandel 1961), the results of this study indicated that quite satisfactory results could be obtained (for the stoichiometric adsorption of TBO on the surface carboxyls) without an addition of electrolytes. The usage of electrolytes should be avoided because the presence of some types of electrolytes suppressed the dye adsorption process, affecting the experimental results (Soedjak 1994). Figure 1 indicates the results of similar dye adsorption measurements performed under “pH = 1” conditions (see Supplementary Information for details); here, the calculated carboxyl content results were almost negligible at pH = 1 under the employed measurement conditions.

At pH = 1, the TBO adsorption onto the surface carboxyls, which are present in their acidic form ($-\text{COOH}$), is suppressed. In contrast, the surface sulfate groups are expected to still interact with TBO under these conditions because they are dissociated. Therefore, the sulfate and carboxyl contents can be estimated by focusing on the differences in the dye adsorption values obtained under “neutral water” and “pH = 1” conditions. Thus, the dye adsorption measurements were performed both in water and at pH = 1 for several samples obtained via sulfuric acid-mediated hydrolysis under various hydrolyzing conditions containing different amounts of surface sulfate/carboxyl groups (Table 1).

The content of the surface sulfate/carboxyl groups in the nanocellulose materials was estimated based on the protocol described in the Supplementary material (also see Table 1). The values obtained via TBO adsorption were consistent with those obtained using the conventional titration technique for majority of the tested samples. However, there were some entries exhibiting slight differences in their results. This variation seems to be observed in samples with low total surface sulfate/carboxyl group contents. However, it is still difficult to obtain a consistently reasonable explanation for this variation. All the results presented in Table 1 exhibited general agreement between the two quantitative methods within a comparable order of magnitude, indicating that the TBO adsorption method could be applied for the quantification of the surface sulfate/carboxyl groups of nanocellulose materials with high accuracy and reproducibility.

Table 1

Surface sulfate and carboxyl group contents of various SCNWs, as determined via the titration and TBO adsorption methods, and the starting cellulose materials and hydrolysis conditions for each SCNW.

SCNWs	Cellulose starting materials	Hydrolysis conditions		Sulfate group content (mmol/g)		Carboxyl group content (mmol/g)	
		Temperature (°C)	Time (min)	by titration	by TBO adsorption	by titration	by TBO adsorption
C-70C20M	Cotton powder	70	20	0.231	0.171 ± 0.000	0.027	0.105 ± 0.001
C-45C30M	Cotton powder	45	30	0.130	0.065 ± 0.004	0.050	0.058 ± 0.004
C-45C60M	Cotton powder	45	60	0.112	0.133 ± 0.001	0.036	0.048 ± 0.004
C-50C60M	Cotton powder	50	60	0.181	0.209 ± 0.007	0.042	0.020 ± 0.004
C-60C60M	Cotton powder	60	60	0.271	0.257 ± 0.005	0.069	0.012 ± 0.007
W-45C60M	MCC	45	60	0.273	0.175 ± 0.003	0.058	0.070 ± 0.005
W-70C10M	SBKP	70	10	0.258	0.264 ± 0.004	0.057	0.084 ± 0.007

The TBO adsorption method offers another advantage over the traditional titration method because it facilitates the quantification of carboxyl content in the presence of other weakly acidic groups such as mercapto groups, which is considerably difficult to achieve using the titration method. Previously, surface mercapto groups were introduced onto the PEG-grafted CCNWs by grafting 2-aminoethanethiol into the surface carboxyl groups (Urata and Araki 2020). It was difficult to determine the carboxyl and mercapto contents separately by titration, because both functional groups were weakly acidic. Therefore, it was almost impossible to differentiate the results obtained using the grafted 2-aminoethanethiol and those obtained using the residual carboxyl groups. Because these two functional groups had different pK_a values, i.e., 3–4 and 11–13 for the carboxyl and mercapto groups, respectively, it was theorized that maintaining the pH of the system within a defined range (e.g., approximately 7) would promote the preferential dissociation of one group (in this case, the carboxyls) over the other group. At pH 7, the mercapto groups remain protonated, whereas the carboxyl groups are dissociated. Thus, TBO would only adsorb onto the carboxyls under pH 7, whereas the mercapto groups would remain unaffected.

This strategy was verified by mixing the CCNW50 sample with various amounts of silica containing surface –SH groups and subjecting the mixtures to both the quantitative methods. The results are summarized in Table 2. The values obtained via the titration method are listed as “weakly acidic group contents” and not as “carboxyl contents.” The conductometric titration curves of these samples indicated that there were only two inflection points that corresponded to the neutralization (end point) of the strongly and weakly acidic groups. The former corresponded to the known concentration of HCl added prior to titration, whereas the latter indicated the sum of the carboxyl and mercapto groups because of no differentiation within the weakly acidic region. Based on Table 2, the amount of weakly acidic groups quantified via titration was not equal to the sum of the carboxyl and mercapto groups. For CCNW-S5.00, which was the sample with the highest –SH content, the value was lower than

that obtained for the carboxyl content of CCNW50 even after the addition of mercapto groups. Although a reasonable explanation for this observation has not yet been obtained, Table 2 indicates the unnegligible effect exerted by the mercapto groups on carboxyl quantification. The carboxyl content values obtained via TBO adsorption were consistent with the results obtained using the CCNW50 sample regardless of the mixing ratios because there was no interference due to the presence of the mercapto groups. These observations indicate the superiority of the TBO adsorption method because it facilitated accurate carboxyl quantification even in the presence of mercapto groups, which was an impossible task when using the titration method.

Table 2

Carboxyl or weakly acidic group contents determined via titration or TBO adsorption for different mixing ratios of CCNW50 and SiliaMetS® thiol.

Sample	Weight ratio of CCNW50: SiliaMetS® Thiol	Mixtures for titration measurements		Mixtures for TBO adsorption measurements		Weakly acidic group contents by titration (mmol/g)	Carboxyl contents by TBO adsorption (mmol/g)
		CCNW50 suspension (mL) ^a	SiliaMetS® thiol (mg)	CCNW50 suspension (mL)	SiliaMetS® thiol (mg)		
CCNW50	1:0	50	0	0.5	0	1.107	1.028 ± 0.008
CCNW-S1.00	1:0.2	50	50	10	1.00	1.126	0.992 ± 0.004
CCNW-S2.80	1:0.5	50	125	10	2.80	1.169	1.007 ± 0.007
CCNW-S5.00	1:1	50	250	10	4.96	0.942	1.001 ± 0.007

^a The solid content of CCNW50 was 5 mg/mL for all mixtures.

Finally, the changes in the adsorbed amount of TBO in the given mixing period were examined. Here, aliquots were sequentially pipetted from one stock reaction vessel at a given time, followed by the analysis of each aliquot to determine the adsorption amounts and the apparent carboxyl content with respect to the reaction/mixing time. If the TBO adsorption required considerable reaction/mixing times, the initial adsorption process became slower with a gradual increase as time progressed, affecting the apparent carboxyl content. Figure 2 showed that the apparent carboxyl content values were similar to the actual carboxyl content values 30 min after mixing and that the value remained constant for a further 24 h. The carboxyl content values after the 24h TBO adsorption process are expressed using the dotted lines and numbers in Fig. 2. The results showed that the interactions between the surface carboxyl groups and TBO were complete after 30 min. Although all of the TBO adsorption results in the above-mentioned studies were obtained after overnight treatment, Fig. 2 clearly indicates that a 2h mixing time is sufficient for the stoichiometric adsorption of TBO.

Carboxyl quantification utilizing the TBO adsorption method developed in this study exhibited various advantages that the titration protocol could not offer. The TBO method yielded reproducible, quantitative results using trace samples of 0.5–2.5 mg of solid per measurement, and the values obtained were comparable with those obtained via titration. Unlike the lengthy, laborious efforts necessary for the titration method, the TBO adsorption required just a simple mixing of the samples with the dye solution and a subsequent 2h shaking process that could be easily automated. The simple, rapid post-procedure protocols of dilution and measurement using a common and

inexpensive UV–Vis spectrophotometer (unlike the highpriced automatic titrator) enabled the highthroughput analysis of large numbers of samples. Several tens of measurements can be conducted within a day, which was totally impossible to achieve using the titration method. Further, the TBO adsorption method facilitated the separate quantification of strongly and weakly acidic groups, and the targeted carboxyl quantification could be performed in the presence of different weakly acidic moieties.

The nature of TBO adsorption indicated that all the stages, ranging from the mixing of the samples with the dye solutions to the application of the measurement protocols, provided the results automatically and arithmetically, thereby making the introduction of a human error into the entire process difficult. On the other hand, the titration method is susceptible to biases because the interpretations of the results were influenced by the operator. The author anticipate that the TBO adsorption method will find widespread applicability for the rapid and precise quantification of the surface functional groups in nanocellulose materials.

Supplementary Information

Full experimental details, including the starting materials and reagents as well as protocols for conducting the TBO adsorption measurements “in water” and under “pH = 1,” are reported in the Supplementary material. An example of the calibration curves indicating the relations between TBO concentrations and the absorbance at 628 nm both “in water” and in “pH = 1” are shown in the Supplementary Information.

The following files are available free of charge.

An Excel worksheet for automatic calculation of surface carboxyl/sulfate groups (Worksheet for dye adsorption.xlsx).

These materials are available free of charge via the Internet.

Declarations

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