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Short communication

An improved technique for concentration measurement of galactomannan solutions by differential refractive index

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ABSTRACT

Galactomannans such as guar gum and locust bean gum are naturally occurring polysaccharides which have been widely used in various industries. One of the issues when dealing with these galactomannans is that they do not dissolve completely into water; therefore the actual dissolved concentration of the solution will always be ambiguous. In this article, we present an easy and robust method for determining the concentration of galactomannan solutions by utilizing a Differential Refractive Index (DRI) detector. Calibration charts for guar gum and locust bean gum were constructed for the DRI method, with the accuracy of results being compared to that of the traditional phenol–sulfuric acid method. The results of this investigation showed that the DRI method is a more accurate and reliable technique for determining the concentration of galactomannan solutions, having an average error of ±0.5% compared to ±5.0% for the phenol–sulfuric acid method.

1. Introduction

Guar gum is a naturally occurring polysaccharide obtained from the endosperm of the leguminous plant *Cyamopsis tetragonoloba*. It is one of the most common forms of galactomannan, consisting of a β-(1-4)-linked D-mannan backbone grafted with α-(1-6)-linked D-galactose side chains, with the ratio of mannose to galactose units being approximately 2:1. Guar gum is widely utilized in various industries due to its excellent thickening properties and relatively low cost. It is commonly used as a thickener and stabilizer in the food and cosmetic industries as well as being used extensively in the mining, textile, paper, and pharmaceutical industries.

As part of our research we have been investigating the solution properties of various galactomannans, guar gum in particular. However, like many other natural polysaccharides, guar gum does not dissolve completely in water. It is also a very hygroscopic material which will absorb moisture rapidly from air, making it difficult to accurately weigh out a known mass. Consequently, the amount of dissolved guar in solution will always be ambiguous. Since it is this soluble fraction of guar which determines many of the physical properties we are typically interested in as researchers, it is vitally important that we can accurately measure the actual concentration of guar dissolved in solution. Indeed, much of the disparity in the literature related to the study of galactomannans can be attributed to the difference between the calculated concentration and the actual dissolved concentration.

In the past, numerous methods have been invented to measure the concentration of polysaccharides, the most commonly applied one being the colorimetric method. This method involves the reaction of a polysaccharide with phenol and sulfuric acid followed by recording its absorbance via UV spectroscopy. The method is applicable to all carbohydrates with either a free or potential reducing group and is particularly useful for determining the concentration of sugars which have been separated by partition chromatography using phenol–water as the solvent (Dubois, Gilles, Hamilton, Rebers, & Smith, 1951, 1956). With some modifications to the procedure, the method can also accurately determine concentrations of oligosaccharides, complex type carbohydrates, and glycoconjugates (Saha & Brewer, 1994) as well as total soil carbohydrate content (Safarik & Santruckova, 1992). A simpler and more sensitive phenol–sulfuric acid assay using a 96-well microplate was also reported to suit analysis of a large number of samples (Masuko et al., 2005). Other colorimetric methods such as the anthrone-sulfuric acid (Laurentin & Edwards, 2003) and orcinol (Irwin & Leaver, 1956) methods also exist, however their procedures are slightly more involved.

During our testing, we found that the accuracy and repeatability of the phenol–sulfuric acid method on guar gum was not satisfactorily reproducible, with an error of around ±5%. For characterization experiments, a more reliable and robust method is required to accurately determine guar concentrations. In this communication,

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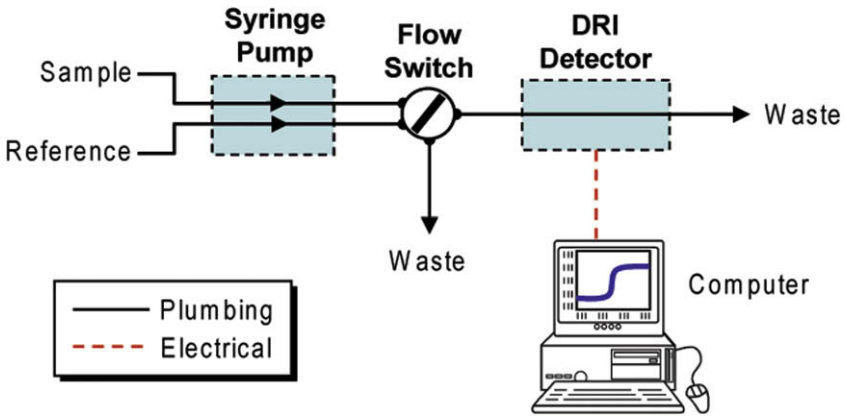

Fig. 1. Schematic diagram of instrumental setup used for DRI detection.

we have developed a simple method to measure the concentration of galactomannan (guar gum and locust bean gum) in solution. Our method uses only a Differential Refractive Index (DRI) detector and galactomannan solutions. The accuracy of this method is extremely high, having a reproducibility error of only ±0.5%, and is inherently safe as it does not involve any chemical reactions or other variables that could lead to an error in the results.

2. Materials and methods

Food grade guar gum (Redox Chemicals Pty. Ltd.) and locust bean gum (Sigma–Aldrich Chemical Co.) were purified for calibration purposes by removing the insoluble component. Purification was achieved by stirring a 0.25% (wt/wt) aqueous solution of the raw galactomannan for 24 h in a thermostated room at 20 °C. The solution was then centrifuged for 10 min at 4400 rpm to remove the insoluble fraction, with the clear supernatant slowly being added drop wise into a solution of ethanol (1:10 ratio of supernatant to ethanol by volume). The precipitated galactomannan was then collected and dried under high vacuum (<1 mm Hg) before being ground into a fine powder and subjected to further drying.

A 150 mL stock solution of the purified galactomannan powder in distilled filtered (0.45 μm) water was made up at a concentration of 1.00 mg/mL. Part of the stock solution was then diluted with distilled filtered water to make up 0.8, 0.6, 0.4, 0.2, and 0.1 mg/mL solutions, each having a final volume of around 20 mL. All of the diluted solutions were shaken vigorously in lidded developing jars to ensure they were homogenously mixed.

Calibration of the differential refractive index detector (Shimadzu RID-10A) involved initially pumping distilled filtered water through the detector using a syringe pump to obtain a stable baseline which was zeroed to 0 V. Care was taken not to introduce any air bubbles into the system, with all air being purged from the syringe before connection was made to the detector. When a stable baseline was achieved, the injection was then swapped with the 0.2 mg/mL purified galactomannan solution. After a new constant voltage was reached and recorded, distilled filtered water was again injected into the detector until a constant baseline of 0 V was achieved. Higher concentration purified galactomannan solutions were injected in the same way, with distilled filtered water being injected between samples. Injections were repeated for all solutions and the averaged voltage readings were subsequently used to construct a concentration calibration chart.

Phenol–sulfuric acid tests were conducted using the method described by Masuko et al. (2005) using galactomannan samples purified in the manner described above. The absorbance measurements were performed using a Shimadzu 2101PC UV–Vis spectrophotometer with the maximum absorbance for guar gum occurring at λ_{max} = 490 nm.

3. Results and discussions

The instrumental setup for the DRI detection method is shown in Fig. 1. A syringe pump loaded with two 10 mL syringes, one containing the pure reference solvent and the other the sample to be analyzed, is connected directly to the DRI detector. A directional valve between the syringe pump and the detector allows for the flow to be switched between the reference solvent and the sample, with the unanalyzed flow stream being diverted to waste. In a typical experiment a flow rate of 0.5 mL/min is applied to the syringe pump with the reference solvent being passed through the detector until a stable baseline is achieved. The flow is then switched to the galactomannan sample and a new baseline established. This generates a step function (Fig. 2a) where ΔV is proportional to the concentration of the sample.

The use of DRI to determine solution concentration has been around for many years and is now utilized routinely in size exclusion chromatography (SEC) as an inline analysis technique. In the inline SEC case a peak is obtained (Fig. 2b) rather than a step function. Providing that the dn/dc of the polysaccharide is known, the area under the peak can be used in conjunction with the injection volume to calculate the sample concentration. However, this route was found to be less accurate than the offline technique, with baseline noise making it difficult to accurately integrate the peak area. The poor signal to noise ratio is due to the low concentration of the injected guar solution, typically less than 0.1 wt%, with higher concentrations

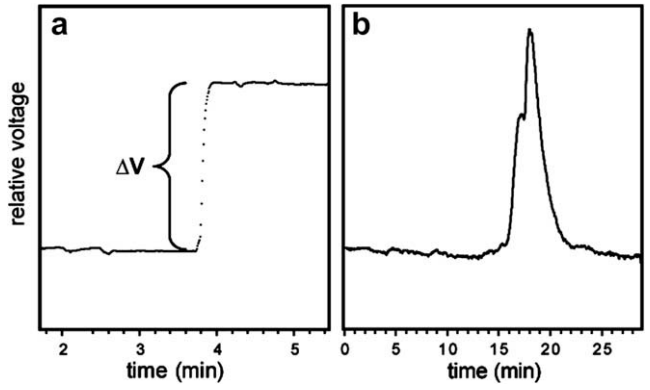

Fig. 2. Typical DRI traces obtained for galactomannan solutions using (a) offline syringe pump method and (b) inline SEC method.

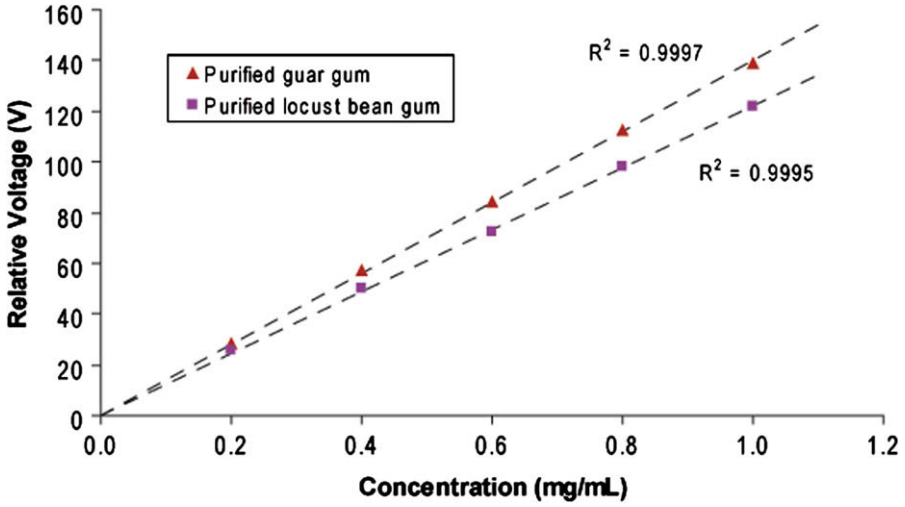

Fig. 3. Calibration chart for purified guar gum and purified locust bean gum using DRI method.

resulting in limited solubility and very high solution viscosity which causes flow difficulties in the inline SEC setup. This problem of low soluble guar concentration and high solution viscosity combined with the hydroscopic nature of guar makes it very difficult to accurately determine the guar solution concentration. However, the offline DRI measurement technique overcomes these issues since: (1) baseline noise is averaged out by only measuring the differential voltage between the baselines, allowing a high degree of accuracy at low concentrations; (2) it is an in situ measurement and therefore, negates the hydroscopic problem; (3) the simplicity of the procedure reduces the possibility of errors. This method demonstrates for the first time a very accurate and simplistic technique for guar solution concentration measurement.

The DRI calibration graph for guar gum and locust bean gum, two different galactomannans having mannose to galactose ratios of 2:1 and 4:1 respectively, is shown in Fig. 3. The galactomannan samples used to generate the calibration graph were purified according to the previously described methodology. The samples were thoroughly dried prior to weighing in order to minimize the volatile content, ensuring that accurately known sample masses were used in the preparation of the calibration standards. Each standard was analyzed multiple times to test the reproducibility of the DRI technique, with a variation of no more than 1.0 relative voltage units being observed over a series of three injections. This corresponds to an average error of only ±0.5%, showing the high degree of reproducibility afforded via this technique. An examination of Fig. 3 shows that both galactomannan samples displayed a linear relationship between relative voltage and concentration as expected, with the coefficient of determination (R^2) being close to unity. A difference in slopes for the two galactomannan samples can also be observed (139.9 and 122.3 for guar and LBG respectively), indicating a slight difference in the refractive indices of guar gum and locust bean gum.

A calibration graph for guar gum based on the phenol–sulfuric acid method described by Masuko et al. (2005) (Fig. 4) was generated as a comparison for the DRI technique. Once again, each calibration sample was analyzed multiple times to examine the reproducibility, with a maximum variation of 0.1 units in absorption being observed over three repeated experiments. This variation corresponds to an average error of ±5.2% for the phenol–sulfuric acid method, being much higher than that obtained using the DRI method (±0.5%). In addition to this, it can be seen that the linear fit for the phenol–sulfuric acid method (R^2 = 0.9803) is not as accurate as that generated for DRI method (R^2 = 0.9997).

A comparison of the two techniques shows that the DRI method is capable of determining galactomannan concentrations with a higher degree of accuracy than the phenol–sulfuric acid method. It is also a relatively fast and versatile technique, allowing direct

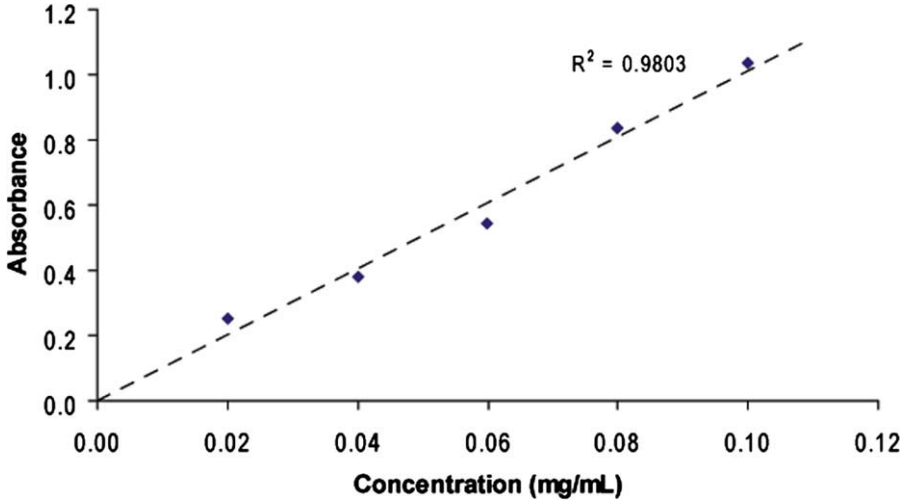

Fig. 4. Calibration chart for purified guar gum using phenol–sulfuric acid method.

measurement of the galactomannan solution and eliminating the need for pretreatment reactions (such as those required for the phenol–sulfuric acid method) which can potentially act as additional sources of error. The larger detection range of the DRI detector (0.1–1.6 mg/mL) compared to the UV–Vis spectrophotometer (0.01 mg/mL–0.2 mg/mL) makes the DRI method more versatile, reducing the need for dilution of concentrated samples to avoid "off-scale" readings.

In order to determine the concentration of raw (i.e. unpurified) galactomannan solutions using the DRI method, the sample has to be centrifuged prior to measurement to remove the insoluble component. For raw guar the insoluble component can contribute up to 25% of the mass, however this is not seen as an issue because it is generally the concentration of the soluble component which is of greatest interest, being directly responsible for the majority of desired application properties.

Like the colorimetric methods, the DRI analysis technique is not suitable for detecting the concentration of unknown polysaccharides or mixtures of polysaccharides due to the requirement for a calibration curve. Care has to be taken when using the DRI technique that the analysis is performed under the same conditions as the calibration, with additional soluble components (e.g. salts) affecting the refractive index of the solution. Fouling of the DRI detector can also affect the measured refractive index, with periodic cleaning being required to minimize the high fouling characteristics of the galactomannan solutions.

4. Conclusions

The offline DRI method was used to generate standard calibration curves for purified guar gum and purified locust bean gum with high level of accuracy, surpassing that of the phenol–sulfuric acid method or any other known methods. The DRI method is fast, easy and extremely reproducible, allowing for the direct analysis of hard to measure polysaccharide solutions without the need for prior chemical treatment. A larger detection range compared to the colorimetric methods also allows for this method to analyze polysaccharide solutions with higher concentrations up to about 1.6 mg/mL without dilution.

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