Carbohydrate Polymers 77 (2009) 150–153

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

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

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ARTICLE INFO

Article history:
Received 17 September 2008
Received in revised form 18 December 2008
Accepted 23 December 2008
Available online 14 January 2009

Keywords:
Galactomannan
Guar gum
Concentration
Refractive index

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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doi:10.1016/j.carbpol.2008.12.028

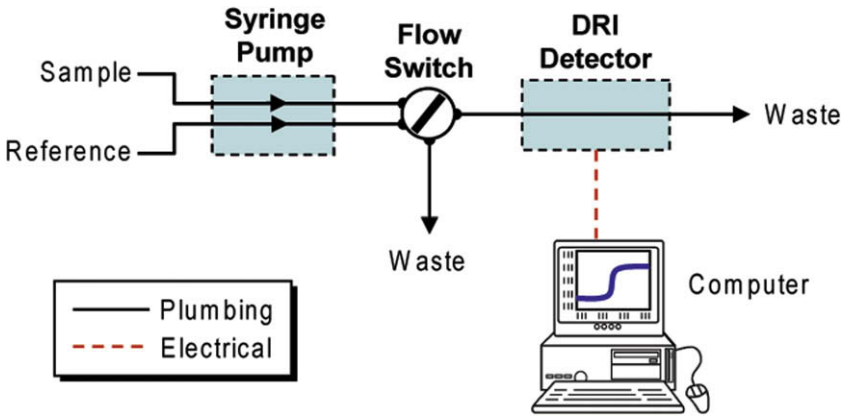

Fig. 1. Schematic diagram of instrumental setup used for DRI detection.

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

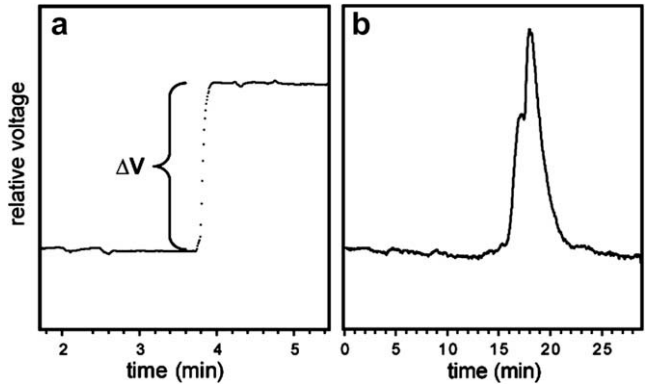

Fig. 2. Typical DRI traces obtained for galactomannan solutions using (a) offline syringe pump method and (b) inline SEC method.