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Development, validation and influence factor analysis of a near-infrared method for the molecular weight determination of xanthan gum

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ABSTRACT

A practical molecular weight determination model of xanthan gum (XG), based on near-infrared (NIR) spectroscopy, was built in this study. Two sample measurement modules, integrating sphere module and fiber-optic probe module, were compared, and the best partial least square (PLS) regression model was based on fiber-optic probe module. The values of coefficient of determination in calibration (R^2_c), coefficient of determination in prediction (R^2_p), residual predictive deviation (RPD) and root mean square error of prediction (RMSEP) were 0.967, 0.975, 6.028 and 0.250×10^6 Da, respectively. The molecular weight range, linearity, accuracy and precision of the established method were also validated. Furthermore, influence factors on this method were discussed in order to establish an appropriate measurement protocol. Results showed that the proposed NIR method may be suitable for practical applications in manufacturing plants and probably be accepted as a good alternative approach for fast determination of molecular weight of XG in production process.

1. Introduction

Xanthan gum (XG) is a high molecular weight microbial heteropolysaccharide with a primary structure consisting of repeated pentasaccharide units formed by two D-glucose, two D-mannose, and one D-glucuronic acid. The molecular weight distribution of XG ranges from 2×10^6 to 20×10^6 Da (García-Ochoa, Santos, Casas, & Gómez, 2000). Its main chain contains a cellulosic backbone of linear linked (β-1,4) D-glucose. The trisaccharide side chains on every alternate glucose at C-3 contain a glucuronic acid residue linked (β-1,4) to a terminal mannose unit and (β-1,2) to a second mannose that connects to the backbone (Palaniraj & Jayaraman, 2011). XG is widely used in many industries mainly in the food, cosmetic, textile, oil, agricultural and pharmaceutical fields (García-Ochoa et al., 2000; Llamas-Moreno, Baiza-Durán, Saucedo-Rodríguez, & Alaníz-De la, 2013; Palaniraj & Jayaraman, 2011; Shalviri, Liu, Abdekhodaie, & Wu, 2010). Recently, studies have been carried out on the XG intra-articular injection in treating experimental osteoarthritis, including its pharmacodynamics and action mechanism. Intra-articular injection of XG had protective effect on the articular cartilage in a rabbit osteoarthritis model (Han et al., 2012b), significantly reduced osteoarthritis pain and alleviated the joint cartilage degradation in a rat osteoarthritis model (Shao et al., 2013). XG also exhibited protective effect on rabbit chondrocytes in the presence of interleukin-1β (Han et al., 2012a). The results suggested that intra-articular injection of XG was probably a new therapeutic method for osteoarthritis. Recent advances in medical and pharmaceutical fields have expanded its applications, therefore, high demand for purified XG is anticipated.

Molecular weight, one of the most fundamental parameters in XG characterization, is a key industrial output control variable for many applications of end products. XG of different molecular weights displays different physical and chemical properties, which may determine its final applications (Born, Langendorff, & Boulenguer, 2005). The current methods used for molecular weight determination of XG include intrinsic viscosity, size exclusion chromatography combined with multiangle laser light scattering (SEC-MALLS), asymmetrical flow field fractionation with multiangle laser light scattering (AFFF-MALLS) and electron microscopy

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(Born et al., 2005). However, these methods may be laborious and time consuming, which limit them as tools for routine analysis online in manufacturing plants. Hence, a rapid, straightforward and still accurate method is very desirable for the molecular weight determination of XG.

Near-infrared (NIR) spectroscopy is a rapid, cost-effective and non-destructive technique that requires little or no sample preparation. NIR spectroscopy offers many possibilities for a broad range of industrial analysis applications (Huang & Qu, 2011; Luypaert, Massart, & Vander Heyden, 2007; Xiao et al., 2012; Zamora-Rojas, Garrido-Varo, De Pedro-Sanz, Guerrero-Ginel, & Pérez-Marín, 2013). It has been adopted as a practical quality control method both in laboratories and in the production plants for online process control (Luypaert et al., 2007). With the development of NIR spectrometer, innovative handheld devices with small size, light weight, low cost and ease of use are now available in the market. Thereby, instead of taking the sample from the process and presenting it to the instruments, the NIR devices are placed on the process lines, making non-destructive in situ NIR spectroscopy analysis a reality (Zamora-Rojas et al., 2013). The NIR spectrum, with the wavelength ranging from 780 to 2500 nm, is mainly composed of overtones and the combinations of fundamental molecular vibrations caused by hydrogen bonds of X—H (e.g., N—H, C—H, O—H) (Luypaert et al., 2007). Many of these hydrogen bonds are present in XG molecules, making NIR spectroscopy suitable for investigating the physical or chemical properties of XG. The main difficulty of NIR technique in analysis is the complexity of the spectra due to their nature (overtones and combination bands of vibrational energy levels) (Luypaert et al., 2007). Overtones can be thought of as harmonics. And the fundamental will produce a series of absorptions at different wavelength. Combinations are more complicated. The fundamental/overtone absorptions are combined to make the NIR spectra to look rather uninteresting and to consist of a few broad absorption peaks. All these make it difficult to acquire relevant information about the feature of interest using measurements at only one wavelength from the raw spectra as is the case, for instance, like UV spectrophotometric or colorimetric analytical methods (Luypaert et al., 2007). So indirect calibration methods are needed to solve this problem and one of the most commonly used chemometric regression methods is partial least squares (PLS) regression. The PLS regression method, defined as a predictive two-block regression method based on estimated latent variables, is a method for relating two data matrices, X (matrix containing NIR spectra) and Y (matrix of response variables), by a linear multivariate mode (Wold, Sjöström, & Eriksson, 2001).

In recent years, NIR spectroscopy has shown its successful potential for monitoring or controlling polymer molecular weights (Cherfi, Fevotte, & Novat, 2002; Othman, Févotte, Peycelon, Egraz, & Suau, 2004), and for non-destructive determination of cellulose molecular weight in pulp hand sheets and historic papers (Henniges, Schwanninger, & Potthast, 2009). Furthermore, NIR spectroscopy has been proved in the molecular weight determination of hyaluronic acid using sample cup as a sampling accessory (Dong et al., 2010), which was based on the presupposition that hyaluronic acid of different molecular weights might possess different amount of hydroxyl end-groups per unit, which would be reflected in the NIR spectra (Dong et al., 2010). However, there is little discussion associated with the validation characteristics and influence factors of this method. Moreover, diffuse reflection (load sample into a sample cup) analysis may have limitations for practical applications in manufacturing plants. It may not be suitable for the in situ analysis of the sample. Industries are interested in simplification of the sampling method for in situ measurement on the process line. NIR spectroscopy has been studied for these purposes by using fiber-optic instrumentations (Claps & Virojanapa, 2013; Rodionova, Balyklova, Titova, & Pomerantsev, 2013; Xiang et al., 2009). To the best of our knowledge, there was no report on using NIR spectroscopy to determine the molecular weight of XG.

Table 1
Weight-average molecular weight (M_w) of the 54 samples.

Sample number	M_w ($\times 10^6$ Da) (relative error, %)	Sample number	M_w ($\times 10^6$ Da) (relative error, %)
1	5.962 (5)	28	2.908 (5)
2	5.656 (5)	29	2.805 (3)
3	5.571 (5)	30	2.730 (5)
4	4.913 (5)	31	2.694 (4)
5	4.874 (5)	32	2.617 (4)
6	4.755 (4)	33	2.592 (3)
7	4.754 (4)	34	2.384 (4)
8	4.536 (4)	35	2.209 (4)
9	4.384 (4)	36	2.099 (3)
10	4.205 (5)	37	2.087 (4)
11	3.564 (4)	38	1.951 (3)
12	3.367 (5)	39	1.906 (5)
13	5.074 (5)	40	1.761 (4)
14	5.059 (5)	41	1.736 (4)
15	4.968 (5)	42	1.631 (3)
16	4.586 (4)	43	1.522 (3)
17	4.227 (4)	44	1.515 (3)
18	4.145 (4)	45	1.512 (3)
19	3.848 (5)	46	1.452 (3)
20	3.802 (4)	47	1.267 (3)
21	3.698 (4)	48	1.265 (3)
22	3.530 (4)	49	1.146 (3)
23	3.514 (4)	50	1.097 (3)
24	3.462 (4)	51	1.083 (2)
25	3.275 (4)	52	1.057 (2)
26	3.164 (4)	53	0.968 (2)
27	3.062 (3)	54	0.844 (2)

In this paper, the feasibility to determine the molecular weight of XG by NIR spectroscopy coupled with PLS algorithm was attempted. Through PLS method the useful information of the NIR spectra was extracted and a new orthogonal matrix was set to represent the raw spectral data. The new data set was related to the molecular weight, which was used to create the PLS model. Two sampling modules, integrating sphere and fiber-optic probe, were used and compared according to the performance of the best PLS regression models. Several validation characteristics (range, linearity, accuracy and precision) of the method were evaluated. The influence factors of the method were also discussed.

2. Materials and methods

2.1. Sample preparation

Twelve batches of purified XG powder samples (99.6% purity) were prepared according to the previously described method (Han et al., 2012b). These samples were collected over a period of one year, so that the batches included could be thought to be sufficiently representative to cover the normal variation of these samples. To acquire more samples with different molecular weights, we prepared another 42 samples by ultrasonic degradation with a modified method of Milas, Rinaudo, and Tinland (1985). Four batches of samples were randomly selected and dissolved to 0.5% (w/v) in 0.4% (w/v) aqueous NaCl solutions. The native samples were then degraded by ultrasonicating for different times from 10 min to 4 h using a JY92-2D Sonifier (Xinzhi Scientific Corp., China) with ultrasonic power of 100 W. Each of the ultrasonicated solutions was poured to a large quantity of isopropanol to re-precipitate the XG. In this way, 42 samples of different molecular weights, designated as sample 13–54, were prepared (Table 1). All samples were ground and passed through a 40 mesh screen, then dried in vacuum oven at 45 °C for 24 h before analysis.