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Differentiation of Carbohydrate Gums and Mixtures Using Fourier Transform Infrared Spectroscopy and Chemometrics

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Guar gum, a nonionic galactomannan, is used as an economical thickener and stabilizer in the food industry and is often combined with xanthan, locust bean gum (LBG), or carboxymethylcellulose (CMC) to promote synergistic changes in viscosity or gelling behavior via intermolecular interactions; however, the adulteration of LBG with guar gum is a well-known industrial problem. The ability to identify the purity of gums and concentrations of individual gums in mixtures would be advantageous for quality control in the food industry. Fourier transform infrared spectroscopy (FTIR) methods are rapid and require minimum sample preparation. The objectives of this study were to evaluate the ability of FTIR techniques to (1) differentiate LBG with a variety of mannose/galactose (M/G) ratios, (2) differentiate guar, LBG, tara, and fenugreek gums, (3) differentiate pure guar gum from guar gum mixed with LBG, xanthan gum, or CMC, (4) quantify LBG, xanthan gum, and CMC in guar gum, and (5) quantify guar gum in LBG. Two FTIR methods were used: diffuse reflectance (DRIFT) on powdered gum samples added to KBr at 5%, w/w, and attenuated total reflectance (ATR) on 1%, w/w, gum solutions. Spectra were collected and then analyzed by multivariate statistical procedures (chemometrics). The DRIFT method provided better discrimination and quantitative results than the ATR method. Canonical variate analysis (CVA) of DRIFT spectra (1200−700 cm^{-1}) was able to classify LBG with various M/G ratios, pure galactomannans, and pure versus mixtures of gums with 100% accuracy. Quantification of an individual gum in gum mixtures (0.5−15%, w/w) was possible using partial least-squares (PLS) analysis of DRIFT spectra with $R^2 > 0.93$ and using this approach for quantifying guar gum added to LBG resulted in an $R^2 > 0.99$, RMSEC = 0.29, and RMSEP = 3.31. Therefore, the DRIFT FTIR method could be a useful analytical tool for quality control of select gums and gum mixtures used in the food industry.

KEYWORDS: Gums; adulteration; FTIR; DRIFT; ATR; Mahalanobis distance; chemometrics

INTRODUCTION

Galactomannans and cellulose-backbone polysaccharides are two groups of hydrocolloids (gums) that are widely used in the food industry as thickeners, binders, stabilizers, emulsifiers, and suspending or gelling agents because they are functional, available, and low in cost (*1*). Their functional and physical properties (including solubility, gelling behavior, and viscosity) are related to the molecular structure, sugar composition, degree and distribution of branching, and polymerization (*2*), and these properties are the focus of several reviews (*3*, *4*). The structural details of select gums are given in **Table 1**. Galactomannans consist of a backbone of (1→4)-linked β-D-mannopyranosyl units with chains of (1→6)-∝-D-galactopyranosyl groups. Guar gum, locust bean gum (LBG), tara gum, and fenugreek gum are galactomannans that have different mannose/galactose (M/G) ratios and distributions of galactopyranosyl units along the mannan chains. On the other hand, cellulose gums such as xanthan gum and sodium carboxymethylcellulose (CMC) have a common (1→4)-linked β-D-glucan backbone. The cellulose backbone of xanthan gum is substituted on alternate glucose residues with a trisaccharide side chain that contains pyruvic acid and an acetal group (*5*). The cellulose backbone of CMC can be substituted with sodium carboxymethyl groups at three available hydroxyl groups on each monomer of the chain.

The functional properties of galactomannans vary with the distribution of galactose side chains, source of seed (variety and growing conditions), and gum extraction processes. Guar gum produces highly viscous, thixotropic solutions at <1%, w/w, concentrations (*4*). LBG increases water-binding capacity and stabilizes food systems such as sherbet and cream cheese (*6*). Tara gum has a M/G ratio between guar and LBG and has physical properties similar to those of LBG and guar mixtures (*7*). Fenugreek gum lowers the surface tension of water and makes better oil-in-water emulsions than the other galactomannans (*8*). Xanthan gum has two main functions in foods: pseudoplasticity and gelation. Pseudoplasticity facilitates processes such as spreading, pumping, pouring, and spraying. Low

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Table 1. Chemical Structure and Functionality of CMC, Xanthan, Guar, LBG, Tara, and Fenugreek (*1*–*10*)

	structure								
gum	backbone	side chains	M/G ratio	branch distribution	molecular weight	viscosity (cP) (1% solutions)	functionality	synergy	source
guar	(1→4)-β-D-Man*p* branched every other Man*p* at O-6	–(6→1)-α-D-Gal*p*	1.8:1	nonuniform	220 000	4600	high viscosity and pseudoplastic at low concentrations; formulation aid, stabilizer, thickener, emulsifier, firming agent	xanthan gum, starches, cellulose, agar, K-carrageenan	*Cyamopsis tetragonolobus*
LBG	(1→4)-β-D-Man*p* branched in clusters in Man*p* at O-6	–(6→1)-α-D-Gal*p*	3.9:1	clustered	310 000	2400–2300	thixotropic, binder, lubricator, and stabilizer; provides heat-shock resistance in ice cream products; speeds coagulation of cheeses	agar, K-carrageenan, xanthan	*Ceratonia siliqua*
tara	(1→4)-β-D-Man*p* branched in clusters in Man*p* at O-6	–(6→1)-α-D-Gal*p*	2:1				higher viscosity than guar or LBG, water holding capacity, protective colloidal properties, interfacial tension activity	carrageenan and agar	*Cesalpina spinosum*
fenugreek	(1→4)-β-D-Man*p* branched in clusters in Man*p* at O-6	–(6→1)-α-D-Gal*p*	uniform, 1:1			mucilaginous solutions	lower viscosity than guar and LBG at the same concentration and molecular weight; reduces surface tension		*Trigonella foenumgraecum*
CMC	(1→4)-β-D-Glc*p* branched in clusters or evenly at O-2, O-3, and O-6	–O–CH_2–CO_2–Na^+		nonuniform and uniform	90 000–700 000		stabilizes protein dispersions, especially at pI; provides viscosity and clear solutions; fast solubility		chemically modified cellulose
xanthan	(1→4)-β-D-Glc*p* branched every other Glc*p* at O-3	–(3→1)-6-*O*-acetyl-α-D-Man-(2→1)-β-D-GlcA-(4→1)-β-D-Man Half of β-D-Man has PyrA attached as a 4–6 cyclic acetal		uniform	2×10^6	600 (min)	very stable to heat, acid, and alkali; high solubility; high viscosity at low concentrations and "weak gel" properties pseudoplastic stabilizes emulsions and suspensions	with guar and LBG is an excellent stabilizer of ice cream frozen novelties	bacteria *Xanthomonas campestris*

concentrations of xanthan can form weak gels (fluid gels), which are useful for stabilizing emulsions, foams, and particulate suspensions. Gels are formed when a mixture of xanthan and galactomannnans is used in foods (*9*, *10*). CMC is used to thicken solutions, bind moisture, texturize a wide range of food products, reduce caloric content, aid in extrusion, and retard crystal growth (*1*). The rheological behavior of CMC depends on its degree of substitution and molecular weight (*11*).

The interaction of gums is of vital importance in the food industry because synergistic effects are manifested as increased viscosity and/or firmer gels that can improve the mouthfeel and texture of many products (*12*). For example, mixtures of xanthan with guar gum show a unique enhancement of viscosity; mixtures of guar with other galactomannans alter their viscosity and rheological behaviors; and mixtures of CMC (anionic) and guar gum (nonionic) may show significant synergistic effects depending on the ratios of the gums used (*13*). If the wrong types of gums are used for a specific food application or adulteration or contamination of gums occurs, then the desired functionality will not be observed in the food product. An example of this is the lower than expected viscosity produced when unexpected mixtures of guar gum and LBG are used in place of pure LBG (*14*). The adulteration of LBG with the less expensive guar gum is a well-known commercial and illegal problem. Functionality is dependent on the purity of the gum or the ratio of gums present in a mixture. Therefore, the ability to measure gum purity or concentrations of individual gums present in a mixture is important for quality control.

The array of galactomannan fine structures, the wide range of use levels, and their mixtures with cellulose-backbone gums are a challenge to quality analysts. Most analytical methods are designed for quantitative measurements of single gums when the identity of the gum is already known (*16*), and these traditional methods are time-consuming and require sophisticated sample preparation steps. A colorimetric method based on the determination of pyruvate can be used to quantify xanthan and CMC (*17*). For blends of xanthan gum with galactomannans, decarboxylation of the uronic acid group in xanthan can be used to differentiate it from guar gum (*15*). However, using a colorimetric assay based on the oxidation of galactomannans has limitations in distinguishing guar from LBG and other galactomannans because of the similarity in compositions (varying ratios of galactose and mannose) (*18*). Other qualitative analyses for galactomannans include a method based on galactomannan gel formation with borate, a visual discrimination between solutions of gums with different M/G ratios at different temperatures, and cold viscosity development of 1%, w/w, LBG solutions. The analytical techniques for unknown gum samples include (1) chromatography or electrophoresis, limited to gums that are polydisperse and (2) hydrolysis with subsequent determination of the monosaccharide composition (*15*). However, a drawback of hydrolysis is that it often cannot be taken to completion. The lack of sensitivity and time-consuming nature of many of these methods make routine analyses for quality control of gums and gum mixtures difficult.

Fourier transform infrared spectroscopy (FTIR) is a rapid analytical method that is sensitive to the presence of chemical functional groups (structural fragments) in a molecule (*19*). The functional groups in a molecule will absorb energy at specific wavelengths, and every molecule will have a unique spectrum in the mid-infrared region of 4000−400 cm^{-1}. Infrared analysis is used for the determination of unknowns, confirmation of identities, and measurement of concentrations (*19*). The advantages of FTIR include the ability to analyze powders, liquids, gases, semisolids, and polymers with minimum sample preparation; information on chemical groups present in analytes is contained in the spectra; chemometrics can be used for qualitative and quantitative analyses of the same spectra; and FTIR is a relatively fast and simple technique to use on a routine basis. Because of the structural differences between the gums selected

for this study (shown in **Table 1**), differences in spectra are expected. Using multivariate statistics to analyze the differences in the FTIR spectra could therefore be suitable for the qualitative and quantitative analysis of polysaccharide gums.

The objectives of this study were to explore the feasibility of using FTIR techniques in combination with multivariate statistical analysis (chemometrics) to (1) differentiate LBGs with a variety of M/G ratios, (2) differentiate pure guar, LBG, tara, and fenugreek gums, (3) differentiate pure guar gum from guar gum mixed with LBG, xanthan gum, or CMC, (4) quantify LBG, xanthan gum, and CMC added to guar gum, and (5) quantify guar gum added to LBG.

MATERIALS AND METHODS

Samples. LBG with M/G ratios of 2.3, 2.9, 3.8, and 6.0 were provided by Dr. James BeMiller at Purdue University (West Lafayette, IN). Fenugreek extract was provided by Acatris (Minneapolis, MN). Tara gum was given by Carob S.A. (Morristown, NJ), AEP Colloids (Saratoga Springs, NY), and Gum Technology (Tucson, AZ). Three types of guar gum, two types of xanthan gum, and two types of CMC were provided by PL-Thomas (Morristown, NJ). Akzo Nobel (Stratford, CT) provided five types of CMC; Wolff Cellulosics (Walsrode, Germany) provided two types of CMC; and Montello (Tulsa, OK) provided one type of CMC. For experiments on gum mixtures, blends of pure gums were prepared from equal amounts of each source of gum. The blend of LBG was added to the blend of guar at 0.5, 0.8, 1, 2, 5, 7.5, 10, and 15% (w/w). Using the same concentrations, the blend of xanthan gum and the blend of CMC were individually added to the blend of guar gum, and the blend of guar was added to the blend of LBG.

FTIR Spectroscopy. Spectra of samples were collected using a ThermoNicolet Nexus 670 FTIR, equipped with a mercury cadmium telluride A (MCTA) detector and KBr beamsplitter (ThermoNicolet Analytical Instruments, Madison, WI). Two sampling techniques were used: diffuse reflectance (DRIFT) spectroscopy for powdered samples, and multibounce attenuated total reflectance (ATR) with a ZnSe crystal for liquid samples. For DRIFT and ATR methods, duplicate spectra of each sample were obtained in the mid-infrared region (wavenumbers of 4000−650 cm^{-1}) and used for multivariate analysis. A total of 128 scans at 4 cm^{-1} resolution were coadded for each spectrum.

Powdered gum samples for DRIFT analysis were prepared by first ball-milling each gum (0.5 g) to have a particle size of ≤ 5 μm, so that particles did not absorb all of the infrared light during the analysis. For milling, each gum was placed in a 5-mL grinding jar (serial number 14034, Retsch, Germany) with two steel balls of 7 mm in diameter and ball-milled (model MM2, Retsch, Germany) for 3 min at setting 5 on a 10-point scale. Samples for gum mixtures and pure gums were added to KBr at 5%, w/w, and mixed in the ball-mill canister without balls for 1 min at setting 5 on a 10-point scale prior to placement in the DRIFT sampling apparatus. An Avatar Diffuse Reflectance Smart Accessory (ThermoElectron Corp., Madison, WI) was used for DRIFT analysis of powdered gums. The background for DRIFT spectra was the spectrum of pure KBr, which was subtracted from all sample spectra prior to statistical analysis. Liquid gum samples for the ATR method were prepared by (1) blending gums in the powdered state, (2) preparing aqueous 1% (w/w) gum solutions in deionized water by stirring the solution for 15 min at ambient temperature, heating at 80 °C for 40 min, and then stirring for 15 min at ambient temperature, and (3) degassing gum solutions by centrifugation at 25000*g* for 30 min at 20 °C using a centrifuge (Beckman Coulter, Palo Alto, CA). A ZnSe multiple bounce ATR accessory (ThermoElectron Corp., Madison, WI) was used to analyze liquid samples. For ATR, an air background was collected before every sample and subtracted from the sample spectrum prior to statistical analysis.

Statistical Analysis. For DRIFT and ATR methods intended for qualitative analysis, four spectra (two spectra from two preparations) were generally obtained for all samples, six spectra (two spectra from three preparations) of guar and tara gums were collected for the pure galactomannans study, and two spectra from triplicate samples were collected at each concentration for the quantitative analysis of the gum mixture study. The spectral region of 1200−700 cm^{-1} was chosen for analysis because it encompassed the carbohydrate and fingerprint regions of infrared spectra and contained the regions that the TQ Analyst program (ThermoElectron Corp., Madison, WI) identified as having differences between samples. Spectra were analyzed using qualitative analytical methods [canonical variate analysis (CVA), discriminant analysis (DA), and principal component analysis (PCA)] and a quantitative analytical method (PLS). CVA was used to create a graphical image of the separation between various types of gums based on spectral differences, after the spectra were compressed by PCA using Win-DAS software (John Wiley and Sons, Inc., Chichester, U.K.). Vertical and horizontal lines on CVA plots showed 95% confidence intervals, while circles around each group indicate 95% tolerance regions.

The Mahalanobis distances were measured in the 1200−700 cm^{-1} spectral region to quantify the extent of separation between gums using the DA function of the Win-DAS software. The Mahalanobis distance was defined as $D_M = ((X_1 - X_2)W^{-1}(X_1 - X_2)^T)^{0.5}$, where W was the pooled estimate of the within group covariance matrix, X_1 and X_2 were mean vectors for the two groups, and T was the transpose (*20*). The Mahalanobis distance is a useful means of determining the similarity of a set of values from an unknown sample to a set of values measured from known samples, and this distance represents standard deviations from the mean of one set of samples to another sample (*21*). Therefore, the distance gives a statistical measure of how well the spectrum of the unknown sample matches the spectra of the known samples.

TQ Analyst Software was used to perform a quantitative analysis (PLS) of gum mixtures. PLS condenses relevant concentration and spectral information in the selected regions of the calibration standards into a number of factors. Each factor represents a source of variation in the data. PLS was chosen because its calibrations have shown better predictability of component concentrations in a mixture than other quantitative chemometric methods (*22*). In this study, with the aim to quantify LBG, xanthan gum, and CMC in guar gum or guar gum in LBG, PLS was used to plot curves of predicted mixture concentrations versus actual concentrations. The developed methods were confirmed by internal cross validation techniques including R^2, root-mean-square error of calibration (RMSEC), and cross validation. The R^2 is the proportion of variability in the data explained by the analysis of variance of the model (*23*). RMSEC refers to the uncertainty of calibration for a selected component. Small RMSEC values show that the calibration has less error (*23*). Cross validation helps to identify standards that may be outliers and serves to validate a method that does not have validation standards. The root-mean-square error of prediction (RMSEP) values indicate how well the developed model will perform on new samples (*23*).

RESULTS AND DISCUSSION

Spectra collected using the DRIFT method for gums used in this study are shown in **Figure 1**. The 1200−700 cm^{-1} spectral region was selected for analysis because it encompasses the carbohydrate region (1200−900 cm^{-1}), the "fingerprint" or anomeric region of carbohydrates (900−700 cm^{-1}), and specific peaks that correspond to α and β forms of galactose (871 ± 7 cm^{-1}), mannose (876 ± 9 cm^{-1}), and glucose (915 ± 5, 874 ± 6, and 767 ± 8 cm^{-1}) as well as vibrations of groups of atoms peripheral to pyranoid or furanoid rings of sugars (*24*−*27*). The gums used in this study have a variety of M/G ratios as well as other structural differences (refer to **Table 1**) that will contribute to differences in absorbance spectra in the 1200−700 cm^{-1} region. Additionally, the TQ Analyst software also identified the 1200−700 cm^{-1} region as having the majority of spectral differences between samples.

Qualitative Analysis of LBGs with Different M/G Ratios. Because the M/G ratios in the galactomannans vary, it is important to be able to discriminate between samples based on the M/G ratio. Using LBG samples with known M/G ratios (2.3,

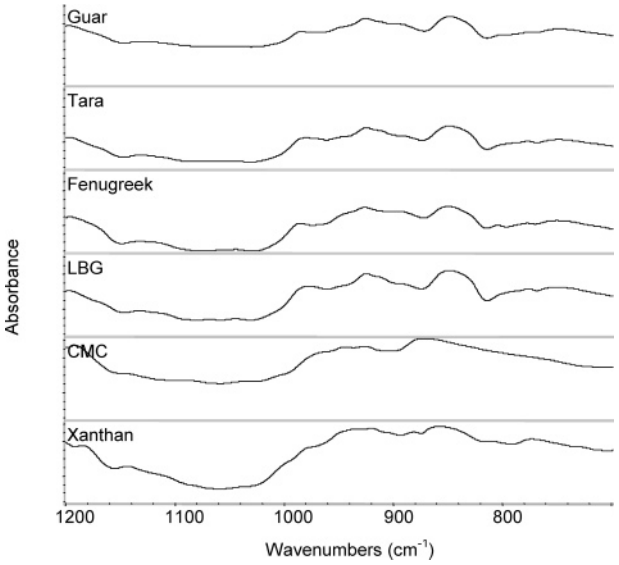

Figure 1. Spectra of guar gum, CMC, LBG, xanthan, tara, and fenugreek gums using the DRIFT method in the spectral region of 1200–700 cm^{-1}, which encompasses the carbohydrate region (1200–900 cm^{-1}) and the fingerprint region (900–700 cm^{-1}).

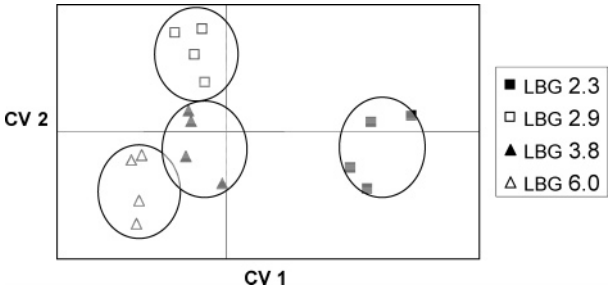

Figure 2. Clustering of LBG with mannose/galactose ratios of 2.3, 2.9, 3.8, and 6.0 based on CV 1 and CV 2 using CVA of DRIFT spectra in the 1200–700 cm^{-1} spectral region. Horizontal and vertical lines indicate a 95% confidence interval. Circles for each group indicate 95% tolerance regions.

Table 2. Mahalanobis Distances between LBG with Different Ratios of Mannose/Galactose (2.3, 2.9, 3.8, and 6.0) Calculated Using Spectra from the DRIFT Method in the 1200–700 cm^{-1} Spectral Region

	LBG 2.3	LBG 2.9	LBG 3.8	LBG 6.0
LBG 2.3	3.6			
LBG 2.9	76.4	2.1		
LBG 3.8	97.2	7.4	3.3	
LBG 6.0	159	26.8	21.6	3.0

2.9, 3.8, and 6.0), a 100% correct identification of LBG samples at each M/G ratio was possible using CVA of spectra (1200−700 cm^{-1}) collected using the DRIFT method. CVA of spectra collected using the ATR method resulted in a 56% correct identification of the LBGs. Therefore, ATR spectral differences were not sufficient to classify LBGs according to their M/G ratios. The graph generated using CVA of the DRIFT spectra clearly shows that LBGs with different M/G ratios can be identified (**Figure 2**). The Mahalanobis distances between types of LBG show the extent of the separation between LBGs with different M/G ratios (**Table 2**). All of the Mahalanobis distances were large enough to enable differentiation between the types of LBG. A higher numerical value for the Mahalanobis distance indicates a greater difference between LBGs, and separations between groups can be made when intergroup distances are

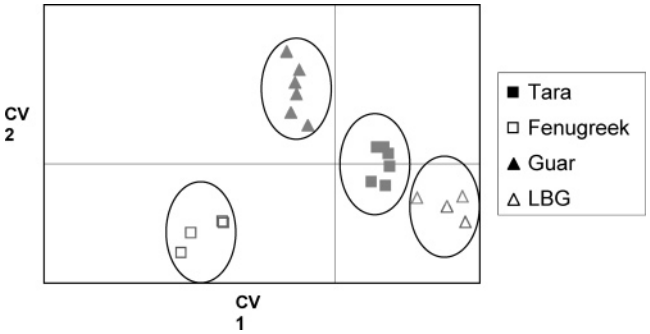

Figure 3. Clustering of tara gums, fenugreek gums, guar gums, and LBGs based on CV 1 and CV 2 using CVA of DRIFT spectra in the 1200–700 cm^{-1} spectral region. Horizontal and vertical lines indicate a 95% confidence interval. Circles for each group indicate 95% tolerance regions.

Table 3. Mahalanobis Distances between Tara Gums, Fenugreek Gums, Guar Gums, and LBGs Calculated Using Spectra from the Diffuse DRIFT Method in the 1200–700 cm^{-1} Spectral Region

	tara	fenugreek	guar	LBG
tara	3.8			
fenugreek	78.2	4.1		
guar	33.0	41.9	1.5	
LBG	40.8	191	132	4.0

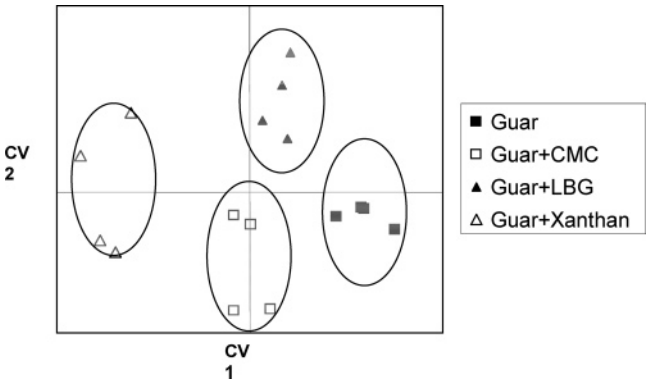

Figure 4. Clustering of pure guar gum and guar gum mixed with CMC, LBG, or xanthan gum based on CV 1 and CV 2 using CVA of DRIFT spectra in the 1200–700 cm^{-1} spectral region. Horizontal and vertical lines indicate a 95% confidence interval. Circles for each group indicate 95% tolerance regions. The percentage of CMC, LBG, and xanthan in guar gum was 15% (w/w).

greater than intragroup distances. This study indicated that the DRIFT method was better suited for differentiationg M/G ratios than the ATR method and also showed that spectral differences occurred in the expected regions (encompassed by the 1200−700 cm^{-1} region used for analysis). Results indicate that CVA of spectra generated using the DRIFT method could be useful for identifying the M/G ratio in a LBG by assigning the unknown gum to one of the classes used in developing the model. While this might be of trivial interest to the food industry because the majority of LBG contains a M/G ratio near 3.9:1, this study demonstrated that the FTIR method could be used to differentiate samples with similar chemical structures, a problem that impacts the sensitivity of other methods used for gum analysis.

Qualitative Analysis of Pure Galactomannans. The graph generated by CVA of spectra (1200−700 cm^{-1}) of the pure galactomannans (LBG, guar, tara, and fenugreek) from the DRIFT method is shown in **Figure 3**. A 100% correct classification of the pure galactomannans was obtained using

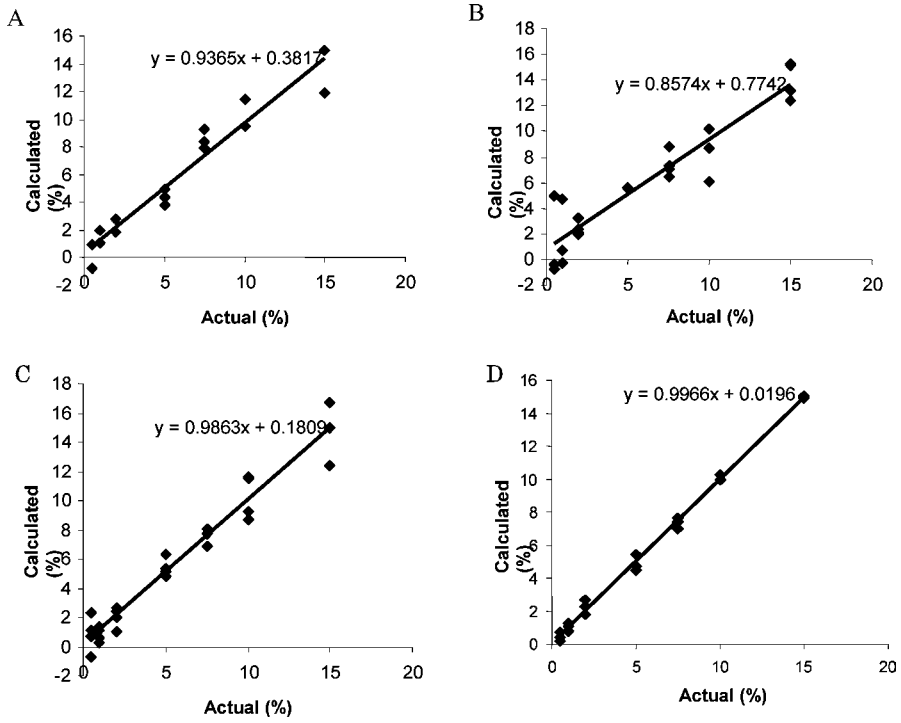

Figure 5. PLS curves of actual concentration versus predicted concentration (0.5–15%, w/w) of CMC (A), LBGs (B), and xanthan gums (C) in guar gums and guar gums (D) in LBGs using spectra collected by the DRIFT method in the 1200–700 cm^{-1} spectral region.

Table 4. Mahalanobis Distances between Pure Guar Gums and Guar Gums Mixed with CMC (15%, w/w), LBGs (15%, w/w), or Xanthan Gums (15%, w/w) Calculated Using Spectra from the DRIFT Method in the 1200–700 cm^{-1} Spectral Region

	guar	guar and CMC	guar and LBG	guar and xanthan
guar	3.6			
guar and CMC	21.8	1.8		
guar and LBG	26.4	9.6	2.5	
guar and xanthan	110	42.6	40.0	4.2

the CVA of the DRIFT spectra. CVA of spectra collected using the ATR method resulted in only an 85% correct classification of the pure galactomannans. Mahalanobis distances between the galactomannans were large enough to enable differentiation of the gums using spectra generated by the DRIFT method (**Table 3**), because intergroup distances were much larger than intragroup distances. The DRIFT FTIR method could be useful for identifying an unknown pure galactomannan by using CVA of the spectra to assign the unknown gum to one of the known classes used in developing the model. The success of this study was expected because of the ability of the DRIFT FTIR method to differentiate between the LBGs with varying M/G ratios. The most common M/G ratios for the gums used are LBG, 3.9:1; tara, ~3:1; guar, 1.8:1; and fenugreek, ~1:1 (*1*−*3*).

Qualitative and Quantitative Analysis of Gum Mixtures. CVA was used to differentiate between pure guar gum and pure CMC, xanthan, and LBG (100% correct classification, data not shown) and then mixtures of guar gum with CMC, xanthan, and LBG (**Figure 4**). A 100% correct classification for the pure guar gum and guar mixtures containing 15% (w/w) CMC, xanthan, or LBG was obtained by analyzing spectra from the DRIFT method. Only a 75% correct classification was obtained from CVA of the ATR spectra. Mahalanobis distances calculated from CVA of the DRIFT spectra were again large enough to differentiate between the pure gum and guar mixtures (**Table 4**). This approach was taken to determine the feasibility of using FTIR methods to qualitatively differentiate between mixtures of gums prior to conducting a quantitative study. A qualitative analysis such as CVA for discriminating between pure guar gums and guar mixtures with other gums could be useful for general quality control; however, the ability to quantify a single

Table 5. PLS Analysis for Assessing the Concentration (0.5–15%, w/w) of CMC, LBG, and Xanthan Gum in Guar Gums and Guar Gums in LBG Using Spectra from the DRIFT Method in the 1200–700 cm^{-1} Spectral Region

	spectral regions (cm^{-1})[a]	number of samples for calibration	number of validation samples	number of PLS factors	R^2	RMSEC[b]	RMSEP[c]
CMC in guar	1070–950	20	7	3	0.9660	1.19	3.97
LBG in guar	1159–1112 919–892 804–788	21	7	5	0.9366	1.82	3.36
Xanthan in guar	1168–1122 1116–1035 914–890	21	7	4	0.9670	1.29	0.60
Guar in LBG	1200–700	21	7	8	0.9983	0.29	3.31

[a] Spectral regions identified by TQ Analyst software as regions containing differences between sample spectra. [b] Root-mean-square error of calibration. [c] Root-mean-square error of prediction.

gum in a mixture of gums could be even more useful for the food industry because functionality of the gums is dependent on the ratios of the gums used.

After the success of CVA in differentiating the pure guar gum and mixtures of guar with LBG, CMC, and xanthan, PLS was used to evaluate the concentration of a gum (0.5−15%, w/w) in mixtures of two gums. Data in **Figure 5** and **Table 5** indicate that PLS analysis of spectra collected using the DRIFT FTIR method could be a useful approach for determining the concentration of a gum in a mixture of gums. The 1200−700 cm^{-1} spectra region was again chosen for analysis because it not only encompassed regions of expected structural differences but also contained the spectral regions identified by the software as containing the greatest differences between samples (**Table 5**). The R^2 values for PLS of DRIFT spectra were 0.966, 0.937, and 0.967 for CMC, LBG, and xanthan in guar, respectively, and 0.998 for guar in LBG, with reasonable RMSEC and RMSEP values. Increasing the number of samples used for calibration and validation could improve the model by reducing the RMSEC and RMSEP values.

The ability to not only qualitatively identify a single gum or gum mixture but also quantify a gum in a mixture from the same FTIR spectra would be advantageous for quality control applications. The adulteration of industrial gums, especially for LBG adulterated with guar, has traditionally been investigated using cold versus hot water viscosity tests; however, results may be uncertain because of differences in gum sources and sample preparations (*28*). The adulteration of LBG with guar gum has also been investigated using both capillary electrophoresis and polarized light microscopy (*29*). Using a combination of these two methods permitted the differentiation of LBG from guar, even though the methods were not designed for quantitative analysis. However, these methods are inefficient and time-consuming, requiring complex protocols such as protein extraction for capillary electrophoresis and staining techniques for the polarized light microscopy. The DRIFT FTIR method developed in this study required minimum sample preparation (grinding in KBr) and was useful for both qualitative and quantitative applications.

Conclusions. The DRIFT FTIR method and qualitative statistical analyses (CVA, DA, and Mahalanobis distances) used in this study were able to classify LBGs with various M/G ratios, classify pure galactomannans, and classify mixtures of guar with LBG, xanthan, or CMC with 100% accuracy. Analysis of spectra from the ATR FTIR method did not yield good results for these applications (56−85% correct classifications). The DRIFT FTIR method and quantitative statistical analysis (PLS) of spectra were useful for predicting the concentration of xanthan, LBG, or CMC in guar gum ($R^2 > 0.96$, 0.93, and 0.96, respectively). For predicting the concentration of guar gum in LBG (0.5−15%, w/w), PLS analysis of DRIFT spectra resulted in $R^2 > 0.99$, RMSEC = 0.29, and RMSEP = 3.31. This study suggests that DRIFT FTIR could be used as a routine analytical method for quality control in the gum and food industries with much less time and sample preparation required than for other currently used analytical methods.

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