Detection and Identification of Aqueous Saccharides by Using Surface-Enhanced Raman Spectroscopy

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The sensitive detection and characterization of carbohydrates by means of a strategy based on surface-enhanced Raman spectroscopy is demonstrated. Spectra are obtained after injecting a small amount of saccharide solution onto a roughened silver substrate, with subsequent deposition of silver colloid. The sensitivity achieved by this two-step approach enables high-quality Raman spectra to be obtained for small amounts of aqueous saccharides (5 μL of a 10^{-2} M solution) utilizing minimal laser power and small signal acquisition times (a few seconds). Spectral "fingerprints" obtained for seven structurally similar monosaccharides demonstrate clearly an effective means by which each sugar can be identified. The application to more complex analyses is demonstrated for monosaccharide mixtures and a disaccharide, whereby the SERS fingerprints aid in the determination of components.

Carbohydrates comprise one of the widest classes of organic molecules and have been found to play major roles in a variety of biological functions. The importance of carbohydrates in biological phenomena is reflected by the numerous investigations regarding the structure and function of these molecules. In aqueous solution, monosaccharides equilibrate among various isomers, including pyranose and furanose ring structures, a variety of ring conformers, and α and β anomeric configurations.[1]

Of the analytical techniques available, vibrational spectroscopy has proven invaluable in obtaining structural information for various aqueous saccharides.[2] Infrared (IR) and Raman spectroscopies have been employed to distinguish between different carbohydrate samples or structural configurations and to identify spectral regions and frequencies in terms of likely vibrational modes.[2] The latter has been greatly facilitated by theoretical modeling, since the vibrational modes of saccharides are known to be highly coupled, and their configuration and conformations are sensitive to the environment.[2] In addition, IR spectroscopy has been utilized to monitor spectral changes as a function of time for anomeric forms of D-glucose and D-fructose, providing insight into mutarotation behavior.[3] The technique of vibrational Raman

optical activity has also provided significant stereochemical information on carbohydrates in solution.[4] Furthermore, bands characteristic of glycosidic linkages in oligosaccharides have been identified.[5] More recently, IR resolution enhancement for carbohydrates was achieved by deconvolution methods, enabling the separation of a larger number of spectral bands for mono-, di-, and polysaccharides.[6]

Most noteworthy from an analytical perspective, however, is the observance of vibrational spectral "fingerprints" for individual monosaccharides. A far-infrared study (500−100-cm^{-1} range) along these lines showed that each saccharide exhibited a characteristic spectral pattern, and development of an "encoding" method based on a sequence of relative intensities enabled the discrimination of different saccharides.[7] A recent report utilized normal Raman spectroscopy for the identification and characterization of solution-phase oligosaccharides.[8] Unique Raman spectral fingerprints were observed for each anomer of several monosaccharides, enabling the determination of disaccharide composition.[8] However, reflecting the insensitivity of normal Raman spectroscopy, relatively high concentrations, high laser powers, long acquisition times, and background subtraction were necessary to obtain acceptable spectra for aqueous mono- and disaccharide solutions.

The well-known phenomenon of surface-enhanced Raman scattering (SERS) involves very large enhancements in the effective Raman cross section of species located at (or close to) certain roughened metal surfaces or large nanoparticles, usually of coinage metals.[9] The signal enhancements are usually sufficient

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(1) Moran, L. A.; Scrimgeour, K. G.; Horton, H. R.; Ochs, R. S.; Rawn, J. D. In *Biochemistry*, 2nd ed.; Pratt, C., Ed.; Neil Patterson Publishers/Prentice Hall: Englewood Cliffs, NJ, 1994; Chapter 9.

(2) For a review, see: Mathlouthi, M.; Koenig, J. L. *Adv. Carbohydr. Chem. Biochem.* **1986**, *44*, 7−89.

(3) Back, D. M.; Michalska, D. F.; Polavarapu, P. L. *Appl. Spectrosc.* **1984**, *38*, 173−180.

(4) (a) Barron, L. D.; Gargaro, A. R.; Wen, Z. Q. *Carbohydr. Res.* **1991**, *210*, 39−49. (b) Wen, Z. Q.; Barron, L. D.; Hecht, L. *J. Am. Chem. Soc.* **1993**, *115*, 285−292. (c) Bell, A. F.; Hecht, L.; Barron, L. D. *J. Raman Spectrosc.* **1993**, *24*, 633−635. (d) Bell, A. F.; Hecht, L.; Barron, L. D. *J. Am. Chem. Soc.* **1994**, *116*, 51555161. (e) Bell, A. F.; Hecht, L.; Barron, L. D. *Spectrochim. Acta A* **1995**, *51*, 1367−1378.

(5) (a) Dauchez, M.; Derreumaux, P.; Lagant, P.; Vergoten, G. *Spectrochim. Acta* **1994**, *50A*, 87−118. (b) Dauchez, M.; Derreumaux, P.; Lagant, P.; Vergoten, G. *Spectrochim. Acta* **1994**, *50A*, 105−118. (c) Sekkal, M.; Dincq, V.; Legrand, P.; Huvenne, J. P. *J. Mol. Struct.* **1995**, *349*, 349−352. (d) Cadet, F.; Offman, B. *Spectrosc. Lett.* **1996**, *29*, 523−536. (e) Kacurakova, M.; Mathlouthi, M. *Carbohydr. Res.* **1996**, *284*, 145−157. (f) Nikonenko, N. A.; Buslov, D. K.; Sushko, N. I.; Zhbankov, R. G. *Biopolymers* **2001**, *57*, 257−262.

(6) Buslov, D. K.; Nikonenko, N. A.; Sushko, N. I.; Zhbankov, R. G. *Appl. Spectrosc.* **2000**, *54*, 1651−1658.

(7) Yang, L.; Weng, S.; Ferraro, J. R.; Wu, J. *Vib. Spectrosc.* **2001**, *25*, 57−62.

(8) Arboleda, P. H.; Loppnow, G. R. *Anal. Chem.* **2000**, *72*, 2093−2098.

to yield readily measurable vibrational spectra for a myriad of species without the need for solution-phase background subtraction. Our laboratory has long been harnessing SERS to explore metal interfacial phenomena in both electrochemical and gaseous environments.[9c,d] Detailed studies have been made on probing the interactions of atoms and molecules on intrinsically SERS-active copper, silver, and gold surfaces, as well as transition-metal surfaces deposited as uniform ultrathin films onto SERS-active gold substrates.[10]

A related yet quite distinct application concerns *bulk-phase* analyses, in which introducing SERS-active metal nanoparticles can selectively amplify Raman signals for solid-state, solution, or gaseous species. Such tactics have proven valuable, for example, in the characterization of biological materials, so that SERS is increasingly being employed in such studies.[11] The attributes of this technique have enabled detailed vibrational information to be obtained for extremely small amounts of materials such as amino acids, nucleic acid components and DNA, and proteins.[11]

Given these virtues, SERS would appear to offer substantial potential for improved sensitivity in the detection and characterization of carbohydrates. Described herein is an initial study along these lines. The basic strategy employed is to obtain SER spectra for several simple saccharides and evaluate this method as an analytical tool for the identification and characterization of carbohydrates. Spectra are obtained after injecting a small amount of saccharide solution onto a roughened silver substrate, greatly enhanced signals being obtained upon subsequent deposition of silver colloid onto the sample. In each case, the solvent is allowed to evaporate before measurement of the SER spectrum. Although strictly speaking not an "in situ" analysis of the aqueous saccharide, the spectra of the dried samples correlate well with the bulk Raman spectra for the corresponding aqueous (rather than crystalline) sugars. The greatly enhanced sensitivity of this approach enables high-quality Raman spectra to be obtained for even small quantities (5 μL of 10^{-2} M) of aqueous saccharide solutions using ~50-mW laser power and 30-s spectral acquisition times. Spectral fingerprints are obtained for each monosaccharide, furnishing an effective sensitive means by which each sugar can be distinguished.

EXPERIMENTAL SECTION

Most details of our SERS experimental arrangement are given in ref 10c. Briefly, the Raman excitation, from a Spectra Physics Stabilite model 2017 Kr$^+$ laser operated at 647.1 nm, was fiber-optically coupled to a custom-built Raman Microprobe System (SpectraCode, West Lafayette, IN). Scattered light was collected by the microprobe and directed through a fiber bundle coupled to an Acton SP 300i monochromator equipped with a Princeton Instruments Spec-100 400BR Digital CCD camera.

Solid monosaccharides (D-glucose, D-galactose, D-mannose, D-ribose, D-arabinose, D-xylose, D-lyxose), including four methylated pyranosides (methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, methyl α-D-galactopyranoside, methyl β-D-galactopyranoside) and one disaccharide (α-lactose), were all procured from Sigma. Aqueous saccharide solutions were prepared using ultrapure water from a Millipore MilliQ Plus system and allowed to equilibrate overnight before use, so that isomeric composition would be stable during measurements.

The Ag colloid was prepared according to the method in ref 12a, by aqueous reduction of AgNO$_3$ with sodium citrate, which typically yields a size distribution of particles between 30 and 60 nm in diameter.[12b] The Ag electrode substrates were of rotating-disk construction, consisting of 4-mm disks sheathed in Teflon. They were roughened to engender stable SERS activity by means of five potential steps from -0.4 to 0.4 V versus SCE and return in 0.1 M KCl as outlined in ref 13. The surface was then rinsed thoroughly with Millipore water and dried in a stream of nitrogen gas. A $5-10$-μL volume of the saccharide of interest (typically 10^{-2} M) was injected onto the roughened Ag substrate, and the solvent was allowed to evaporate. A drop of Ag colloid was then placed onto the dried saccharide sample and allowed to dry in air, and the Raman spectrum collected (vide infra). Laser power was ~50 mW at the sample, and spectral acquisition times were typically 30 s. All spectra were reproduced at least 5 times, preparing new samples each time.

RESULTS AND DISCUSSION

Spectra a and b in Figure 1 show the Raman spectra of D-glucose in the crystalline state and in aqueous solution, respectively; these correspond closely to previous reports.[14,15] The wavenumber range shown between 1600 and 300 cm^{-1} is most instructive for our purposes, containing the majority of vibrational information available. (The C$-$H and O$-$H stretching regions located at about $3000-2800$ and $3600-3100$ cm^{-1}, respectively, are not considered here; the vibrational bands tend to be broad and ill-defined.) Comparison of (a) and (b) reveals a considerable difference in the Raman spectra obtained in the solid versus solution state, presumably due to hydrogen-bonding influences in the latter and variations in isomeric composition.[2]

Most of the normal vibrational modes of carbohydrate molecules in this spectral region are highly coupled and, hence, difficult to identify. However, the combination of spectroscopic measurements with vibrational frequencies calculated from normal coordinate analysis has yielded insight into the nature of these

(9) For recent reviews, see: (a) Campion, A.; Kambhampati, P. *Chem. Soc. Rev.* **1998**, *27*, 241−250. (b) Vo-Dinh, T. *Trends Anal. Chem.* **1998**, *17*, 557−582. (c) Weaver, M. J.; Zou, S.; Chan, H. Y. H. *Anal. Chem.* **2000**, *72*, 38A−47A. (d) Weaver, M. J.; Zou, S. In *Spectroscopy for Surface Science*; Clark, R. J. H., Hester, R. E., Eds.; Advances in Spectroscopy 26; Wiley: Chichester, U.K., 1998; Chapter 5.

(10) For example, see: (a) Zou, S.; Williams, C. T.; Chen, E. K.-Y.; Weaver, M. J. *J. Phys. Chem. B* **1998**, *102*, 9039−9049. (b) Mrozek, M. F.; Weaver, M. J. *J. Am. Chem. Soc.* **2000**, *122*, 150−155. (c) Mrozek, M. F.; Weaver, M. J. *J. Phys. Chem. B* **2001**, *105*, 8931−8937. (d) Mrozek, M. F.; Wasileski, S. A.; Weaver, M. J. *J. Am. Chem. Soc.* **2001**, *123*, 12817−12825.

(11) For reviews, see: (a) Cotton, T. M. In *Spectroscopy of Surfaces*; Clark, R. J. H, Hester, R. E., Eds.; Wiley: New York, 1988; pp 91−153. (b) Cotton, T. M.; Kim, J.-H.; Chumanov, G. D. *J. Raman Spectrosc.* **1991**, *22*, 729−742. (c) Nabiev, I. R.; Sokolov, K. V.; Manfait, M. In *Biomolecular Spectroscopy*; Clark, R. J. H., Hester, R. E., Eds.; Wiley: Chichester, U.K., 1993; Vol. 21, Chapter 7. (d) Nabiev, I.; Chourpa, I.; Manfait, M. *J. Raman Spectrosc.* **1994**, *25*, 13−23.

(12) (a) Lee, P. C.; Meisel, D. *J. Phys. Chem.* **1982**, *86*, 3391−3395. (b) Rivas, L.; Sanchez-Cortes, S.; Garcia-Ramos, J. V.; Morcillo, G. *Langmuir* **2001**, *17*, 574−577.

(13) Roth, E.; Hope, G. A.; Schweinsberg, D. P.; Keifer, W.; Fredericks, P. M. *Appl. Spectrosc.* **1993**, *47*, 1794−1800.

(14) For example: (a) Wells, H.; Atalla, R. H. *J. Mol. Struct.* **1990**, *224*, 385−424. (b) Soderholm, S.; Roos, Y. H.; Meinander, N.; Hotokka, M. *J. Raman Spectrosc.* **1999**, *30*, 1009−1018.

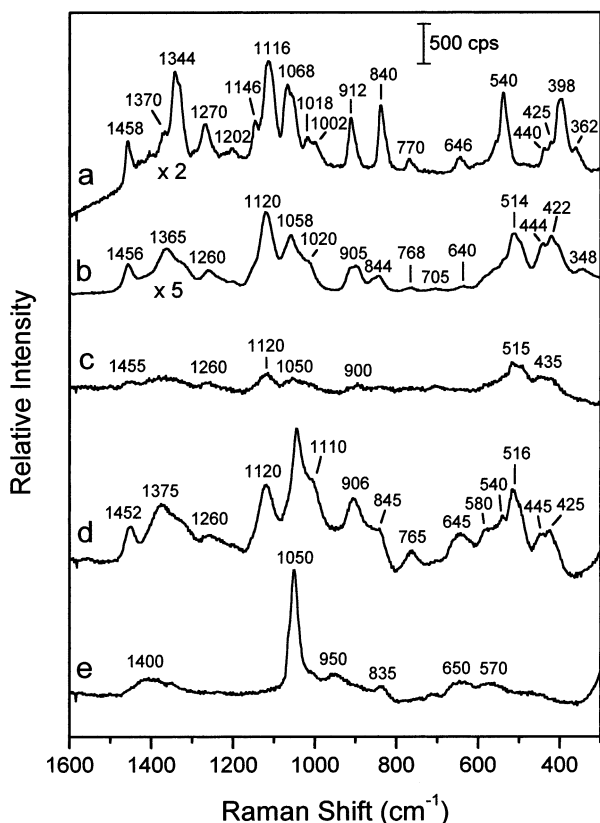(15) For example: Mathlouthi, M.; Luu, D. V. *Carbohydr. Res.* **1980**, *81*, 203−212.

Figure 1. (a) Normal Raman spectrum of crystalline D-glucose; (b) normal Raman spectrum of aqueous D-glucose (1 M); (c) SER spectrum of a 5-μL aliquot of a 10^{-2} M D-glucose solution on a roughened Ag substrate, after evaporation of solvent; (d) same as in (c) but after addition of a droplet of Ag colloid, again allowing the solvent to evaporate in air; (e) "blank" spectrum of Ag colloid on roughened Ag substrate (after evaporation of solvent).

highly coupled vibrations.[2] Characteristic wavenumber regions that encompass vibrational spectra of carbohydrates have been identified for a number of specific vibrational modes: (a) 3600–2800 cm^{-1}, CH and OH stretching; (b) 1500–1200 cm^{-1}, deformational vibrations such as involving HCH and CH$_2$OH functionalities; (c) 1200–950 cm^{-1}, C–O stretching region (with some C–C contribution); (d) 950–700 cm^{-1}, side-group deformational region (COH, CCH, OCH) containing "fingerprint" or anomeric bands (also C–C stretching); (e) below 700 cm^{-1}, skeletal region containing exocyclic (700–500 cm^{-1}) and endocyclic (below 500 cm^{-1}) deformations.[2] However, a detailed analysis of band assignments is beyond the scope of the present work.

The basic measurement protocol employed here was to examine the SER spectra for several monosaccharides. Our initial attempts at obtaining SER spectra of these molecules, by adding a small amount of the analyte to a colloidal suspension of Ag or Au nanoparticles, proved unfruitful; significant spectral interference was obtained in each case from species associated with the colloid, thwarting the detection of saccharide spectral features. Similar failure was also reported by Arboleda and Loppnow in their attempts at obtaining SERS for monosaccharide samples.[8] Furthermore, conventional solution studies employed in our laboratory using Ag or Au electrode substrates immersed in the analyte solution also yielded inadequate SERS for sensitive carbohydrate detection. Saccharides, unlike unsaturated molecules or species

with available lone pairs, are not anticipated to interact to any great extent with the metal surface. Our difficulties in obtaining intense SER spectra for saccharides adsorbed from solution therefore probably reflect weak binding of these molecules at metal surfaces.

An eminently more successful tactic explored subsequently involves initially placing a small aliquot ($\sim$5–10 μL) of the saccharide solution of interest (typically 10^{-2} M) onto a SERS-active Ag substrate and, after evaporating the solvent in air, acquiring a SER spectrum. This method clearly circumvents the lack of spontaneous binding of the analyte onto the SERS-active surface. A similar approach has been utilized by Fredericks et al. to obtain SER spectra of insoluble organic compounds, amino acids, and peptides and proteins.[13,16] Spectrum c in Figure 1, obtained for D-glucose in this manner, illustrates how this approach facilitates the detection of additional (albeit weak) Raman spectral features. Several bands, most notably at 1120, 1050, 515, and 435 cm^{-1}, are evident in spectrum c that correlate well with those in the normal Raman spectrum of aqueous D-glucose (b). No vibrational features were observed by utilizing an unroughened Ag substrate, confirming the origin of the SERS bands in (c). However, the relatively low Raman intensities and signal-to-noise ratio prompted us to pursue modified tactics to improve the spectral quality.

To this end, we then placed a small droplet of the Ag colloid solution onto the dried saccharide sample and allowed the solvent to evaporate in air, yielding a Ag nanoparticle/saccharide mixture on the roughened Ag substrate. Such nanoparticle doping of surfaces was previously shown to yield surface Raman enhancement for species on smooth substrates.[17] The resulting spectrum (d in Figure 1) for D-glucose shows marked improvement in the observed Raman intensities and hence the signal-to-noise ratio, the features again being similar to those observed in the bulk Raman spectrum of *aqueous* D-glucose (b). It is noteworthy that although the solvent is allowed to evaporate from the sample before measurement, the SER spectrum correlates more closely to the solution-phase rather than solid-state Raman spectrum (b versus a, respectively). This suggests that either the monosaccharide is still solvated or the conformational structure of the monosaccharide in aqueous solution is largely retained following solvent evaporation within the Ag nanoparticle matrix.

To check that the observed spectral features are not partly originating from species associated intrinsically with the Ag colloid matrix, a "blank" spectrum, consisting of a drop of Ag colloid evaporated onto the SERS-active Ag substrate, was measured, resulting in spectrum e in Figure 1. A notable band at 1050 cm^{-1} is evident, along with a few other weak features at about 1400, 950, 835, 650, and 570 cm^{-1}. These bands are most likely due to species such as citrate and nitrate utilized in the colloid preparation. By comparison to a previous report on SER spectra utilizing metal colloids,[18] the weaker features can be attributed to residual citrate. The more intense 1050-cm^{-1} band seen in spectrum e,

(16) (a) Stewart, S.; Fredericks, P. M. *Spectrochim. Acta A* **1999**, *55*, 1615–1640. (b) Stewart, S.; Fredericks, P. M. *Spectrochim. Acta A* **1999**, *55*, 1641–1660. (c) Stewart, S. D.; Fredericks, P. M. *J. Raman Spectrosc.* **1995**, *26*, 629–635.

(17) Muniz-Miranda, M.; Sbrana, G. *J. Mol. Struct.* **2001**, *565–566*, 159–163.

(18) Sanchez-Cortes, S.; Garcia-Ramos, J. V. *J. Raman Spectrosc.* **1998**, *29*, 365–371.

however, is probably due to the symmetric stretching vibration of nitrate.[19]

A comparison of spectra d (with D-glucose) and e (without D-glucose) confirms that most of the bands observed in spectrum d are indeed originating from the saccharide, and not the colloid solution. However, the intense 1050-cm^{-1} band is also evident in the spectrum for D-glucose (d), nearly identical to that in the blank spectrum (e). Nonetheless, a weak feature is observed at this frequency in (c), without the addition of Ag colloid to the dried saccharide sample, and similar bands are evident in the bulk-phase Raman spectra of D-glucose (a and b in Figure 1), as well as other monosaccharide solutions, typically associated with a C−O stretching mode.[5d,e,15] However, the similar appearance of the 1050-cm^{-1} band in both spectra d and e indicates that it originates primarily from the colloid material. Still, the shoulders evident on this feature in spectrum d suggest that unresolved bands due to saccharide vibrations are also present.

Similar measurements were undertaken using smooth (SERS-inactive) Ag substrates. Comparable Raman intensities were generally obtained with either roughened or smooth Ag substrates *after* the addition of Ag colloid to the dried saccharide sample, indicating that most (if not all) of the observed enhancement originates from the colloid. Samples were also prepared along the same lines, but by first depositing colloid onto the roughened (or smooth) Ag substrate and then adding the saccharide solution. Qualitatively similar results were again obtained. However, all of the spectra reported here were obtained by addition of the saccharide solution to a roughened Ag substrate and, after solvent evaporation, addition of the Ag colloid. This approach reproducibly yielded stable, high-quality spectra and, furthermore, enabled us to acquire an initial spectrum of the saccharide (before adding Ag colloid) without interference from the 1050-cm^{-1} nitrate band.

Most importantly, the SER spectrum for D-glucose obtained here (spectrum d in Figure 1) can be utilized as a "fingerprint" by which this monosaccharide can be identified. Figure 2 displays the SER spectrum of D-glucose (reproduced from spectrum d in Figure 1) with that of two other hexoses, D-galactose and D-mannose (a−c, respectively). Again, an aliquot of the saccharide solution of interest was injected onto a roughened Ag substrate and the solvent allowed to evaporate in air. A droplet of Ag colloid solution was then placed onto the sample and allowed to dry, and the SER spectrum was measured.

A detailed examination of the SER spectra for the three hexoses in Figure 2 is instructive. At first sight, the spectra appear closely similar above 1000 cm^{-1}, although wavenumber differences are discernible for the bands at ~1115−1135 cm^{-1}. As noted above, this spectral region encompasses deformational vibrations such as HCH and CH$_2$OH (1500−1200 cm^{-1}) and also C−O stretching (1200−950 cm^{-1}).[2] Significantly, however, the region below 1000 cm^{-1} appears to provide a *unique* fingerprint for each monosaccharide. For example, spectrum a of D-glucose contains bands at 906, 845, 645, and 516 cm^{-1}, along with two overlapping features at about 445 and 425 cm^{-1}, yet these bands are absent (or located at different frequencies) in spectra b (D-galactose) and c (D-mannose). The SER spectrum for D-galactose (spectrum b
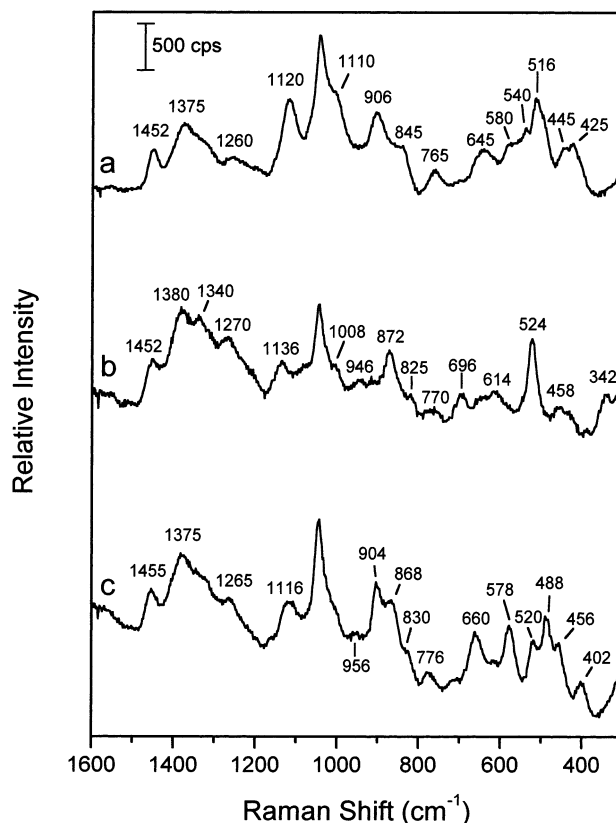

Figure 2. SER spectra obtained following the described procedure for 5-μL aliquots of 10^{-2} M solutions of (a) D-glucose (as in spectrum c, Figure 1), (b) D-galactose, and (c) D-mannose.

in Figure 2) most notably contains bands at 872, 696, 524, and 342 cm^{-1}. That for D-mannose, however, displays bands at 904 and 868 cm^{-1}, at 660 and 578 cm^{-1}, and three closely spaced peaks at 520, 488, and 456 cm^{-1}, along with a feature at 402 cm^{-1}. Consequently, it is feasible to distinguish between the three hexoses in Figure 2 by examining such characteristic band frequencies.

D-Glucose, D-galactose, and D-mannose have the same chemical formula and have identical molecular masses; the structural differences for these hexoses merely involve the spatial arrangement of the hydroxyl groups on C(2) and C(4) atoms. (Some variation in isomeric composition also exists in aqueous solution; equilibrium compositions of the monosaccharides most pertinent to the work here are given in ref 20.) However, comparison of the three spectra in Figure 2 reveals remarkable differences in the frequencies and intensities of several bands. As noted above, the 950−750-cm^{-1} segment, generally referred to as the "fingerprint region", contains side-group deformational (and C−C stretching) vibrations that are especially sensitive to hydroxyl position.[2] Since the normal vibrations of saccharides are highly coupled, noticeably different vibrational spectra may therefore be obtained even for slight variations of the hydroxyl position in similar saccharide structures.

This sensitivity of the vibrational spectrum to small structural differences of monosaccharides is further exemplified in Figure 3, displaying the SER spectra obtained for α and β anomers of

(19) (a) Degen, I. A.; Newman, G. A. *Spectrochim. Acta* **1993**, *49A*, 859−887. (b) Lombardi, D. R.; Wang, C.; Sun, B.; Fountain, A. W., III; Vickers, T. J.; Mann, C. K.; Reich, F. R.; Douglas, J. G.; Crawford, B. A.; Kohlasch, F. L. *Appl. Spectrosc.* **1994**, *48*, 875−883.

(20) Boons, G.-J. In *Carbohydrate Chemistry*; Boons, G.-J., Ed.; Thompson Science: London, 1998; Chapter 1, p 14.
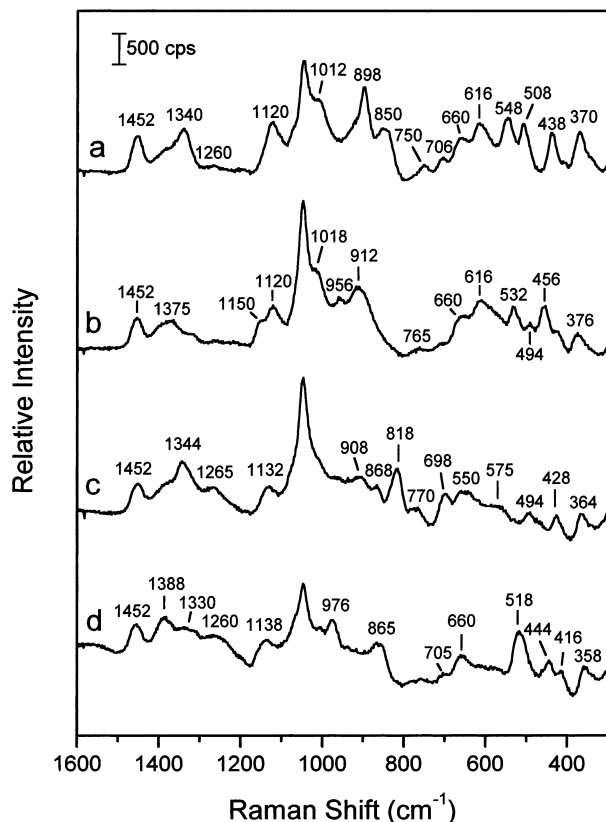
Figure 3. SER spectra for 5-μL aliquots of 10^{-2} M solutions of (a) methyl α-D-glucopyranoside, (b) methyl β-D-glucopyranoside, (c) methyl α-D-galactopyranoside, and (d) methyl β-D-galactopyranoside.

Figure 4. SER spectra for 5-μL aliquots of 10^{-2} M solutions of (a) D-ribose, (b) D-arabinose, (c) D-xylose, and (d) D-lyxose.

two of the hexoses examined above: D-glucose and D-galactose (a and b, and c and d, respectively). A detailed investigation of the vibrational spectra of these monosaccharides was previously described by Wells and Attalia.[14a] Methylated pyranosides were utilized to prevent racemization of the anomeric carbon in aqueous solution, thus allowing the SER spectra of the individual α and β anomers to be obtained. Again, most noteworthy are the differences in the region below 1000 cm^{-1}. The α-glucoside (spectrum a) yields a sharp band at 898 cm^{-1}, along with another feature at 850 cm^{-1}. These bands are not observed, however, in the SERS of the β-glucoside (spectrum b), the latter containing a band at 912 cm^{-1}. Spectrum a furthermore reveals bands at 548, 508, and 438 cm^{-1} for the α anomer, while bands at 532, 494, and 456 cm^{-1} are noted for the β anomer (spectrum b). Similar results are also obtained for the α- and β-galactosides, as shown in spectra c and d in Figure 3, respectively. Most notable distinctions are the 818-cm^{-1} band for α-galactoside (spectrum c) and the 976-, 865-, and 518-cm^{-1} bands in spectrum d for β-galactoside. Remarkably, then, clear-cut spectral differences are evident for the α and β anomers of each monosaccharide, even though the only structural dissimilarity is a change in the C(1) hydroxyl conformation.

Several other monosaccharides were examined in order to explore the broader applicability of this method for saccharide identification. Four pentoses (D-ribose, D-arabinose, D-xylose, D-lyxose) were chosen, each structure differing in the spatial arrangement of hydroxyl groups at the C(2) and C(3) positions. (Again, variations in isomeric composition also exist at solution-phase equilibrium.[20]) Figure 4 displays the SER spectra obtained
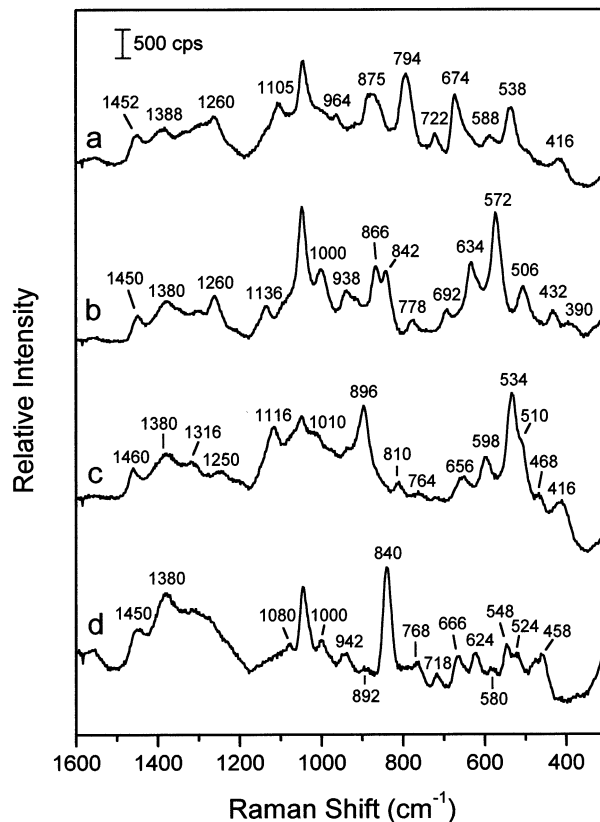
for each pentose here. Again, these correspond closely to the aqueous-phase Raman spectra as given in ref 4b. Examination of Figure 4 reveals distinct differences in the band frequencies and intensities for the four pentoses. For further clarification, Table 1 lists the major characteristic band frequencies and relative intensities observed for each pentose, along with those for the three hexoses described above.

Inspection of Table 1 along with the spectra in Figures 1–4, demonstrates further the unique nature of the SER response for each monosaccharide. In some cases, only one characteristic vibrational band can be sufficient to identify the monosaccharide, for example, the very strong band at 840 cm^{-1} for D-lyxose (spectrum d in Figure 4). More generally, however, the collective examination of the frequencies and relative intensities of several characteristic bands (as given in Table 1) provides a reliable determination of the monosaccharide identity. These spectral "fingerprints" therefore furnish an extremely effective means by which saccharides in aqueous solution can be identified.

Although a related approach utilizing the bulk-phase Raman spectra of aqueous saccharide solutions has recently been touted as a "carbohydrate discovery tool",[8] high laser powers (2–3 W), lengthy spectral acquisition times (30 min), and background water subtraction, along with typically higher analyte concentrations, were necessary to obtain reasonable-quality Raman spectra. In contrast, the SERS method described here permits high-quality spectra to be obtained using minimal laser power ($\sim$50 mW used here), spectral acquisition times on the order of seconds, and no need for background subtraction. Spectral detection and identification are readily achieved by utilizing 5 μL of 10^{-2} M saccharide

Table 1. Selected SER Band Frequencies, cm^{-1}, and Relative Intensities Characteristic of Several Monosaccharides

monosaccharide	vibrational frequency (cm^{-1})[a]
pentoses	
D-ribose	1105 (m), 964 (w), 875 (m), 794 (s), 722 (w), 674 (s), 588 (w), 538 (m), 416 (w)
D-arabinose	1136 (m), 1000 (m), 938 (m), 866 (s)/842 (s), 778 (w), 692 (w), 634 (m), 572, (vs), 506 (m), 432 (w), 390 (w)
D-xylose	1116 (m), 896 (s), 810 (w), 656 (w), 598 (m), 534 (vs)/510 (sh), 468 (w), 416 (m)
D-lyxose	1080 (w), 942 (w), 892 (w), 840 (vs), 718 (w), 666 (m), 624 (m), 548 (m)/524 (m), 458 (m)
hexoses	
D-glucose	1120 (s), 906 (s)/845 (sh), 765 (m), 645 (m), 580 (sh)/540 (w)/516 (s), 445 (m)/425 (m)
D-galactose	1136 (m), 946 (w), 872 (s), 770 (w), 696 (m), 614 (m), 524 (s), 458 (m), 342 (m)
D-mannose	1116 (m), 956 (w), 904 (s)/868 (m)/830 (sh), 776 (m), 660 (s), 578 (s), 520 (m)/488 (s)/ 456 (m), 402 (m)

[a] The relative band intensity is designated by (vs), (s), (m), and (w), corresponding to very strong, strong, medium, and weak, respectively. A shoulder on a band is designated by (sh).

solution, corresponding to less than 10 μg of analyte. However, the distribution of analyte after evaporation of the solvent is most likely not uniform, and the laser spot size (120 μm) is much smaller than the size of the substrate employed here. This indicates that only a fraction of the overall sample is interrogated in this manner. Consequently, optimization of the present method in this report should lead to impressively low detection limits (see below).

The applicability of this SERS approach to the analysis of saccharide mixtures and disaccharide composition was also addressed. Along these lines, we first acquired SERS data for a 1:1 mixture of two arbitrarily chosen monosaccharides, D-glucose and D-ribose, as displayed in spectrum a of Figure 5. We then compared this result with a sum of the individual spectra of D-glucose and D-ribose (see Figures 2 and 4 for the latter data), as displayed in spectrum b in Figure 5. Examination of spectra a and b reveals an *almost exact* reproduction of that obtained for the 1:1 mixture by adding the two components. It therefore should be feasible to analyze a complex mixture of saccharides by reference to individual SER spectra. A further demonstration along these lines is shown in spectra c and d of Figure 5. The former refers to a 1:1 mixture of α-glucoside and β-galactoside, whereas the latter represents the addition of the individual spectra for the corresponding monosaccharide units (see spectra a and d in Figure 3). Again, the addition of the individual component spectra accurately simulates that observed for the mixture.

Finally, the feasibility of discerning by this means the nature of the monomeric saccharide units comprising a given disaccharide was investigated. Joined together via a glycosidic bond, the α anomer of D-glucose and the β anomer of D-galactose comprise the disaccharide α-lactose. A typical SER spectrum obtained for this disaccharide is shown in (e) of Figure 5. Although the observed SER spectrum for α-lactose is composed several broad bands (the 1050-cm^{-1} feature is again attributed to the Ag colloid solution), it is relatively similar to the aqueous-phase Raman spectrum previously reported.[5e,21] Comparison with spectra c (the 1:1 mixture of components) and d (addition of component spectra) reveals some noteworthy similarities. First, the vibrational fingerprint observed between 1500 and 1200 cm^{-1} is remarkably similar in the three cases (Figure 5c−e), as anticipated from the
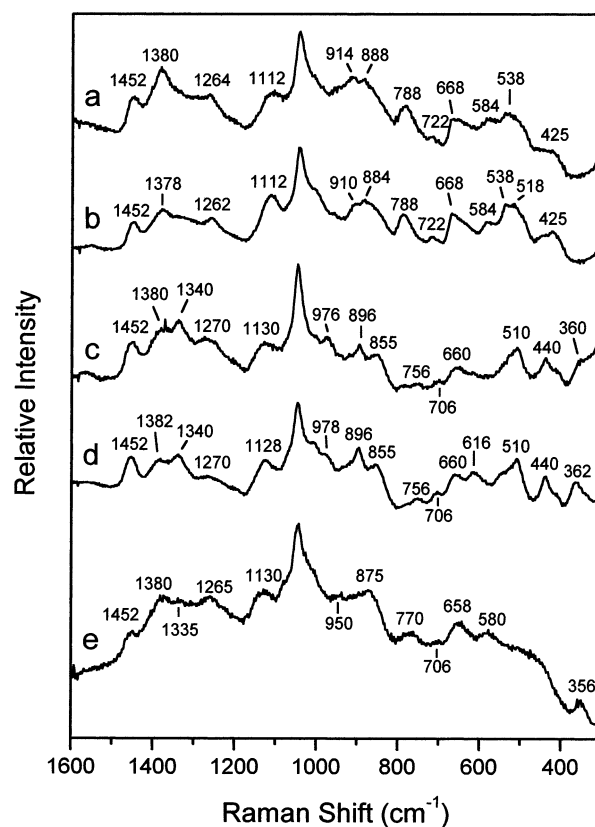
(21) Barret, T. W. *Spectrochim. Acta* **1981**, *37A*, 233−239.

Figure 5. (a) SER spectrum for 5 μL of a solution containing a 1:1 mixture of 10^{-2} M D-glucose and D-ribose, (b) mathematical addition of individual D-glucose and D-ribose spectra (taken from Figure 2 and Figure 4), (c) SER spectrum for 5 μL of a solution containing a 1:1 mixture of 10^{-2} M methyl α-D-glucopyranoside and methyl β-D-galactopyranoside, (d) mathematical addition of individual methyl α-D-glucopyranoside and methyl β-D-galactopyranoside spectra (taken from Figure 3), and (e) SER spectrum of a 5-μL aliquot of 10^{-2} M solution of α-lactose.

insensitivity of this spectral region to small differences is saccharide structure (vide supra). In addition, a band at 1130 cm^{-1} is observed in all three spectra. For the disaccharide, a band at ~875 cm^{-1} is obtained (e), yet two separate peaks, at 896 and 854 cm^{-1}, are evident in (c) and (d) for both the monosaccharide mixture and the addition of component spectra. However, the broadness of the α-lactose 875-cm^{-1} band in spectrum e suggests that additional unresolved features are masking such vibrational

components. Furthermore, bands at 658 and 356 cm^{-1} are observed for the disaccharide, corresponding closely with those (at 660 and 360 cm^{-1}) in spectra c and d. These similarities therefore indicate that it is indeed feasible to identify the monomeric units of a di- or oligosaccharide by using this approach.

However, as might be expected, neither the SER spectrum for the monosaccharide mixture nor the mathematical addition of monosaccharide spectra *completely* matches that of the disaccharide. Differences are evident in comparing spectra c−e, especially below ∼600 cm^{-1}, where several resolved bands are observed in the former two spectra but not the latter. Such spectral differences for a given disaccharide versus that of the individual monosaccharide units probably reflect the presence of the glycosidic linkage between the two monomeric units comprising the disaccharide. For example, a band at 580 cm^{-1} is evident in the SER spectrum of α-lactose (e) yet is absent in spectra c and d, referring to the individual monosaccharide units. A similar band at 600 cm^{-1} was previously noted in the Raman spectrum of another disaccharide, sucrose, but not in that of the monosaccharide components (D-glucose and D-fructose), and was assigned to a O−C−O bending mode about the glycosidic bridge.[15] In addition, the individual component spectra measured here actually refer to the methylated pyranosides of D-glucose and D-galactose, so that the α and β anomers could be measured separately in aqueous solution. Clearly, this necessary tactic could yield spectral features that are subtly different from that observed for the disaccharide, in which the individual monosaccharides are not methylated. Further SERS examination of di- and oligosaccharides may therefore be useful in identifying those bands characteristic of glycosidic linkages.

CONCLUDING REMARKS

The present findings illustrate the practical utility of our SERS strategy for the sensitive detection and characterization of carbohydrates. A clear analytical advantage of this approach is the ability to obtain high-quality spectra for small amounts of aqueous saccharide solutions (microliters of 10^{-2} M or less), employing minimal laser power and short signal acquisition times (∼30 s or less). The SER spectral fingerprints provide an effective, albeit semiempirical, means by which monosaccharides can be distinguished as well as detected. Furthermore, the present results obtained for monosaccharide mixtures, as well as that for the disaccharide examined here, demonstrate the potential of this method for undertaking more complex carbohydrate analyses. Further studies along these lines for di-, oligo-, and polysaccharides would therefore be worthwhile.

Clearly, further optimization and modification of the present SERS strategy could lead to substantial improvements in spectral quality and detection limits. For example, an improved method of depositing the analyte solution onto the substrate, as well as the use of a smaller metal substrate, comparable in size to the focused laser spot, would permit a reliable assessment of detection limits. In addition, the Ag colloid utilized here, although yielding adequate enhancement when excited with our Kr^{+} laser (647.1 nm), can be expected to provide more optimal SERS activity with green excitation, hence yielding even more sensitive analyses.

Overall, then, it is evident that our SERS approach constitutes a valuable means by which the sensitive detection and characterization of carbohydrates can be achieved. Further strides along these lines could lead to SERS becoming a viable tool in glycobiology.