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Analysis of mono- and oligosaccharides by multiwavelength surface plasmon resonance (SPR) spectroscopy

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Abstract

Surface plasmon resonance (SPR) spectra of different saccharides were collected using a home-made multiwavelength SPR apparatus. Pentoses, hexoses, disaccharides and a trisaccharide were distinguished from one another according to their SPR spectra collected at the same concentration. The spectra were also used for the quantitation of sugars by exploring the linear relationship between resonance wavelength and solute concentration. The dynamic linear ranges for the determination of glucose, sucrose and raffinose are 0.01–0.2, 0.005–0.1 and 0.0025–0.1 mol/L, respectively. The SPR spectrum of a mixture of two components was investigated. While the experiments have not been carried out, the implications from this work are that the technique would be applicable to mixtures containing more than two components.

Keywords: Surface plasmon resonance (SPR); Saccharide identification; Quantitation of sugars

1. Introduction

As a surface-sensitive spectroscopic technique, surface plasmon resonance (SPR) is very suitable for monitoring changes in the medium adjacent to a metal film attached to a glass prism. Variation in the refractive index of the medium leads to a shift of the resonance wavelength or the resonance angle. SPR is regarded as a simple optical technique for surface and interfacial studies[1] and shows great potential for investigating biomolecules. For example, SPR has been used to study the interactions between DNA and DNA,[2,3] ligands and receptors,[4] phosphotyrosine and protein,[5] fusion proteins and T-cells,[6] and antibiotics and bacteria,[7] among other examples.

Although at present most of the SPR reports focus on recognition of biomolecules, other applications should also be possible. SPR can be regarded as a significantly useful tool for analyzing saccharides as the solutions of saccharides commonly have high refractive indices. Moreover, solutions of saccharides can be detected directly, obviating the need for chemical reactions. Although sucrose is commonly used as a testing chemical to evaluate some SPR systems,[8] and glucose has been studied with SPR,[9] a systematic study of SPR spectra of mono- and oligosaccharides has not been reported. In this paper, a systematic study of the saccharides has been carried out, which demonstrate that the SPR technique

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can not only distinguish among pentoses, hexoses, disaccharides and a trisaccharide, but can also analyze them quantitatively.

2. Results and discussion

Selection of incident angle.—In the multi-wavelength SPR technique, attention should be paid to the incident angle, the alteration of which has significant influence on the resonance wavelength. It has been reported that the resonance wavelength is shifted to longer wavelengths as the incident angle decreases,[10] which allows for improved sensitivity, as greater sensitivities can be obtained at longer wavelengths according to Eq. (1):[11]

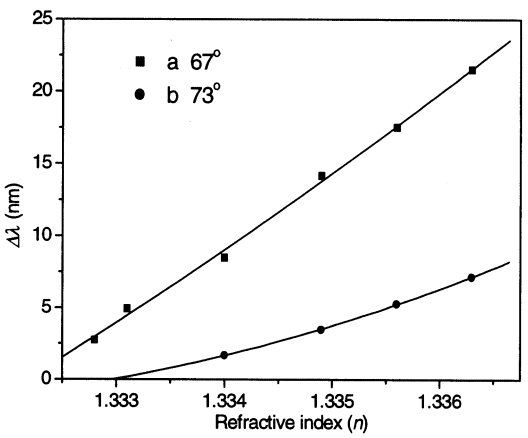

Fig. 1. Resonance wavelength versus refractive index for sucrose solutions at the incidence angles of 67 and 73° obtained with gold film B.

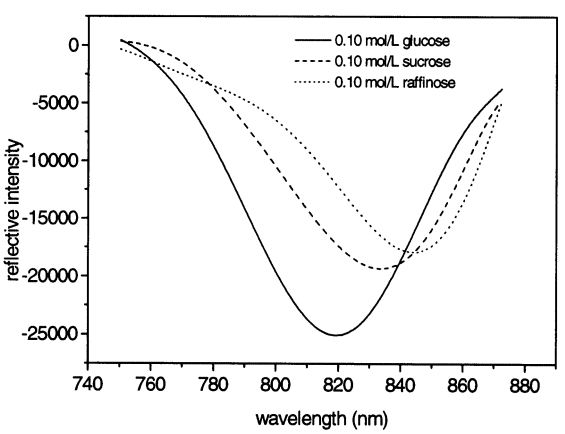

Fig. 2. SPR spectra of glucose, sucrose and raffinose obtained with gold film A.

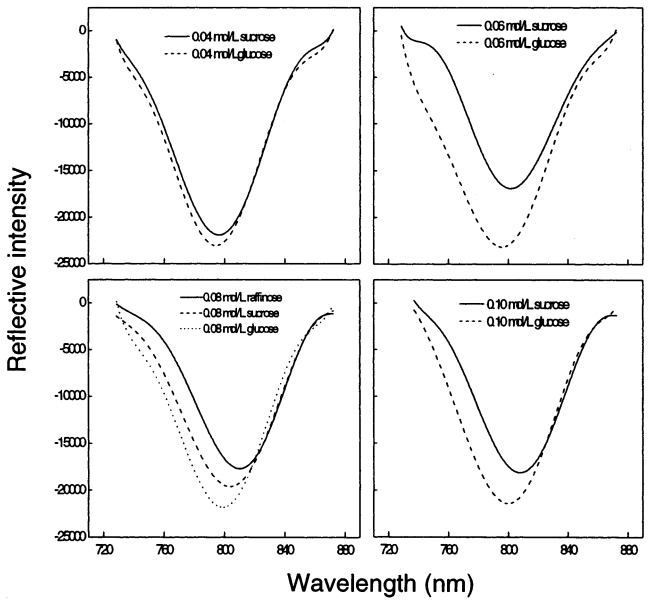

Fig. 3. The SPR spectra of glucose, sucrose and raffinose of different concentrations using gold film B.

$$s_{p\lambda} = \frac{d\lambda}{dn_a} = \frac{\varepsilon_{mr}^2}{\frac{n_a^3}{2}\left|\frac{d\varepsilon_{mr}}{d\lambda}\right| + (\varepsilon_{mr} + n_a^2)\varepsilon_{mr}\frac{dn_p}{d\lambda}\frac{n_a}{n_p}} \quad (1)$$

where $s_{p\lambda}$ is the sensitivity, ε_{mr} is the real part of the dielectric constant of the metal and n_a and n_p are the refractive indices of the analyte and the prism, respectively. Therefore, smaller incident angles are preferable. In our case, the relationship between the sensitivity and the incident angle can easily be seen from Fig. 1. Shown in Fig. 1 is the dependence of the resonance wavelength on the refractive index of sucrose solutions at incident angles of 67 and 73°, and the phenomenon that a smaller incident angle resulted in higher sensitivity becomes obvious. The rest of the experiments were conducted at 67° unless otherwise stated.

SPR spectra of different saccharides.—The SPR spectra of aqueous solutions of mono- and oligosaccharides at a concentration of 0.1 mol/L were collected at incident angles of 67 and 73°. Fig. 2 shows the SPR spectra of glucose, sucrose and raffinose obtained with gold film A. It is evident that the depth of the resonance increases with the decrease of the number of carbons in a saccharide. Similar resonance curves were also obtained with gold film B (Fig. 3). The difference in the resonance wavelengths between sample solutions and

water can be seen clearly from Fig. 4. As shown in Fig. 4(B), the resonance wavelength of pentose was only a little larger than that of water, while in Fig. 4(A), the $\Delta\lambda$ of pentoses became discernible. At the incident angle of 67°, hexoses, disaccharides and trisaccharide also had larger SPR responses. It is interesting that sugars with the same number of carbons yield similar SPR responses at the same incident angle and the same concentration. Since SPR is sensitive to the refractive index of the medium, the phenomenon above may be explained by the comparison of refractive indices which are closely related to the mean polarizabilities (α). Moreover, the polarizability of a molecule is determined by the polarizabilities of all the constituent atoms. The

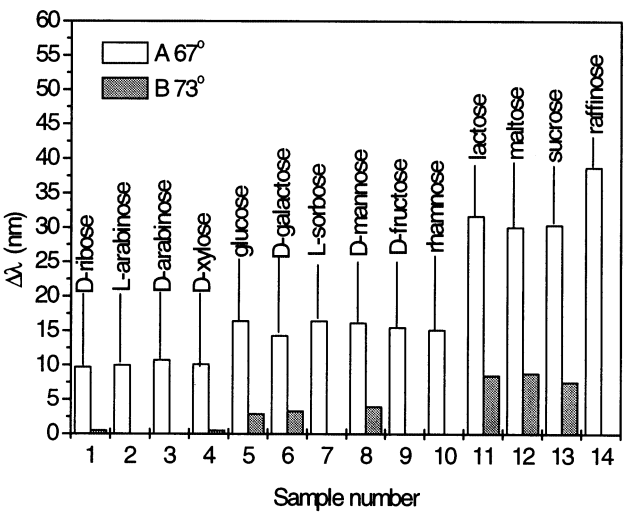

Fig. 4. Shift in SPR wavelengths for different saccharides at incident angles of 67 and 73° using gold film A.

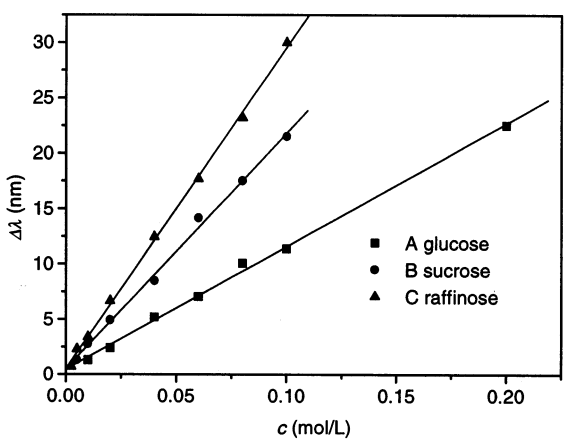

Fig. 5. Plot of resonance wavelength versus the concentration of glucose, sucrose and raffinose using gold film B.

Lorentz–Lorenz equation describes the relationship between molar refractivity (A) and mean polarizability as follows:[12]

$$A = \frac{4}{3}\pi N_m \alpha \quad (2)$$

Here, N_m is Avogadro's number. For a mixture of substances, the refractive index contains contributions from all of the components as shown in Eq. (3).

$$A_{mix} = \frac{\sum_i A_i N_i}{\sum_i N_i} \quad (3)$$

where A_i and N_i are the molar refractivity and the number of moles per unit volume of component i, respectively. Sugar solutions with the same molar concentration have the same N_i, but different polarizabilities result in different refractive indices. Similar structures of glucose, sorbose, mannose and galactose give them similar refractive indices, and thus similar resonance wavelengths except for galactose. The underlying reason for the behavior of galactose is still unclear. The lower polarizability of C=O in fructose and the lack of one hydroxyl in rhamnose result in fructose and rhamnose having lower polarizabilities than the other six-carbon sugars. Therefore, it is natural to observe smaller resonance wavelengths with fructose and rhamnose. More hydroxyls in the disaccharides and in the trisaccharide give them higher refractive indices and thus larger resonance wavelengths. The fact that aqueous solutions of saccharides with the same number of carbons at the same concentration have similar resonance wavelengths can be applied to size determination.

Quantitative analysis of mono- and oligosaccharides.—A linear relationship was found to exist between the resonance wavelength and solute concentration (Fig. 5). Glucose, sucrose and raffinose were selected as examples of mono-, di- and trisaccharides. The linear equations for them can generally be expressed as:

$$\Delta\lambda = a + bc \quad (4)$$

where c is the concentration of saccharide and a and b are constants. The linear regression coefficients/dynamic linear ranges for glucose, sucrose and raffinose were 0.9959/0.01–0.2, 0.9979/0.005–0.1 and 0.9997/0.0025–0.1 mol/

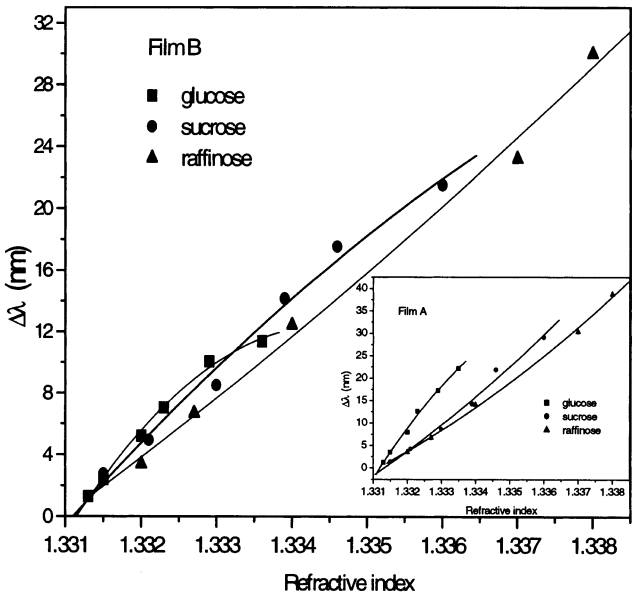

Fig. 6. Plot of resonance wavelength versus refractive index.

L, respectively. Included in Fig. 6 are two graphs of the resonance wavelengths versus the refractive indices for glucose, sucrose and raffinose obtained using gold film A and B. It seems from Eq. (1) that the sensitivity is independent of the composition of the medium when the refractive index is kept unchanged. However, the results here display that the sensitivities for these three sugars are greatly different, although their solutions possess the same refractive indices.

Further studies found that the SPR response of a mixture of two saccharide solutions equaled approximately the sum of the separate responses of the two unmixed solutions and so were the increments in refractive indices, Δn. Listed in Table 1 are three sets of experimental data from solutions of two components. The results can be summarized as:

$$\Delta\lambda_{\text{mix}} = \sum_i \Delta\lambda_i = \sum_i a_i + \sum_i b_i c_i \quad (5)$$

The parallel relation demonstrated in refractive indices can explain why the shift of the resonance wavelength is summable.

According to the results listed in Table 1, in order to determine the concentrations of two components in a mixture, the SPR spectra collected at two incident angles are required. Similarly, for a solution with n components, n independent equations or measurements are required to solve the concentration of the target component. This can be realized by measuring the resonance wavelengths at n incident angles. Further experiments are being performed and will be discussed elsewhere. However, a troublesome question was encountered in carrying out the research for this paper. The thickness of gold films prepared at different times is not exactly the same because the evaporating conditions are difficult to control precisely. Thus, different sensitivities are generated for different films.

3. Experimental

Apparatus.—There are several optical arrangements available for SPR.[13] Some com-

Table 1
Comparison of the resonance wavelengths and the refractive indices between the mixed and separated solutions [a]

Test [b]	Solution A	Solution B	Solution A + Solution B	Mixture
1	$c_g = 0.02$ mol/L $\Delta\lambda_g = 2.58$ nm $\Delta n_g = 0.0005$	$c_s = 0.02$ mol/L $\Delta\lambda_s = 4.87$ nm $\Delta n_s = 0.0011$	 $\Delta\lambda = 7.45$ nm $\Delta n = 0.0016$	$c_g = 0.02$ mol/L, $c_s = 0.02$ mol/L $\Delta\lambda = 8.03$ nm $\Delta n = 0.0015$
2	$c_g = 0.04$ mol/L $\Delta\lambda_g = 5.20$ nm $\Delta n_g = 0.0010$	$c_s = 0.04$ mol/L $\Delta\lambda_s = 8.49$ nm $\Delta n_s = 0.0020$	 $\Delta\lambda = 13.69$ nm $\Delta n = 0.0030$	$c_g = 0.04$ mol/L, $c_s = 0.04$ mol/L $\Delta\lambda = 13.55$ nm $\Delta n = 0.0029$
3	$c_s = 0.05$ mol/L $\Delta\lambda_s = 11.20$ nm $\Delta n_s = 0.0025$	$c_r = 0.02$ mol/L $\Delta\lambda_r = 6.51$ nm $\Delta n_r = 0.0017$	 $\Delta\lambda = 17.71$ nm $\Delta n = 0.0042$	$c_s = 0.05$ mol/L, $c_r = 0.02$ mol/L $\Delta\lambda = 17.65$nm $\Delta n = 0.0039$

[a] The refractive index (n) was measured at 30 °C, $\Delta n = n_{\text{sample}} - n_{\text{water}}$.
[b] g, glucose; r, raffinose; s, sucrose; m, mixture.

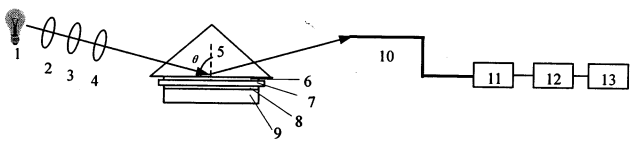

Fig. 7. The schematic diagram of the homemade SPR apparatus. 1, Light source; 2, Lens 1; 3, Lens 2; 4, polarizer; 5, prism; 6, matching oil; 7, glass slide; 8, gold film; 9, cuvette; 10, optic fiber; 11, monochromator; 12, ICCD; 13, computer.

mercial instruments measure the resonance angle, while others measure the resonance wavelength. The device described in this paper was built based on the second measuring mode because the measurement of wavelength in real time becomes much easier nowadays by using a charge coupled device (CCD) as the detector rather than the measurement of the angle, which requires a complex apparatus to measure angles precisely.

The practical setup is shown in Fig. 7, where a halogen bulb serves as the light source covering a wide range of the spectrum. The incident light is collimated through Lens 1 and Lens 2 and polarized with the polarizer. Then, the incident light is illuminated onto the bottom of the prism at an incident angle of total internal reflection. The reflected light is introduced into the monochromator through an optic fiber and is then measured by an image intensified charge coupled device (ICCD) (model ICCD-576/G1, Princeton Instruments, Inc., USA) which simultaneously monitors the reflective intensities as a function of the wavelengths. A range of 145 nm is covered in each spectrum as determined by the linear dispersion of the monochromator and the size of the ICCD. The 200-μL analytical cell is constructed by sealing a glass slide and Teflon cell with a silicone rubber gasket. The glass slide, which is coated with a film of gold (approx 50 nm), is brought in close contact with the glass prism ($n = 1.516$) using an index-matching oil ($n = 1.516$). The gold film is prepared by evaporating gold onto the glass slide. In this paper, two gold films (film A and film B) were prepared under slightly different conditions and were compared.

Reagents.—All saccharides of analytical grade were purchased from Beijing Chemical Reagent Company, and sample solutions were all prepared with doubly distilled water.

SPR detection.—The spectrum in air was first taken as the background, as no SPR phenomenon was shown to occur for air under the experimental conditions applied here. The sample solution was then injected into the analytical cell, and its SPR spectrum was collected from which the air spectrum was subtracted. The final SPR spectrum is shown in Fig. 2. The wavelength at which the reflective intensity has the minimum is the resonance wavelength of the sample solution, $\lambda_{sp(sample)}$. As water was the solvent, the shift of the resonance wavelength was calculated as:

$$\Delta\lambda = \lambda_{sp(sample)} - \lambda_{sp(water)} \quad (6)$$

where $\lambda_{sp(sample)}$ and $\lambda_{sp(water)}$ are the resonance wavelengths of sample and water, respectively.

Acknowledgements

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