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Concentration Determination of Individual Components in Methanol–Raffinose Mixtures Using Diffusion through Agar Membrane Attached to a Surface Plasmon Resonance Sensor

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A surface plasmon resonance (SPR) sensor with agar membrane was developed and applied to aqueous solutions containing methanol and raffinose. The concentrations of the components in the mixture were determined by fitting diffusion-based equations to the SPR time chart. Good agreement between the measured and expected concentrations was observed.

The SPR sensor is based on the interaction between incident light and the surface plasmon of a metal film at its interface. This interaction responds to the refractive index of a sample around the metal film surface in real time and can be applied to any chemical substance. Metal surface chemical modification also makes it possible to measure a desired specific substance.[1] Thus the SPR sensor is frequently used as a biosensor,[2] chemical sensor,[3] and for measurements of physical quantities such as humidity[4] and temperature.[5] The SPR sensor is simple in configuration. The basic components are a light source, metal film, prism, and a detector. Extensive work on the development of compact SPR sensors for field work[6] and medical[7] purposes is being carried out. In addition, improvements in miniaturization technologies have accelerated the development of compact SPR sensors.[8]

However, the SPR sensor responds only to the total refractive index of a sample solution, and cannot be used for determination of components in a mixture. High-performance liquid chromatography or gas chromatography may be used to separate components, but these systems are large and unsuitable for on-site analysis. Surface chemical modification is also useful as stated above, but is used for specific chemicals. Diffusion through a membrane is one of the most feasible methods to separate the individual chemicals in a mixture. For a general compact sensor, a membrane should be thin for the entire SPR sensor to be compact.

In this research, agar was used for the membrane because its refractive index is lower than the prism used. Methanol (MW: 32.0 g mol^{-1}) and raffinose (MW: 594 g mol^{-1}) were chosen as model compounds because of the large difference in molecular weights. The SPR-agar system was constructed and its time charts of standard aqueous solutions of 3.0 M methanol and 0.018 M raffinose were analyzed using Fick's law of diffusion.[9,10] Mixed solutions containing methanol and raffinose were prepared and analyzed using a curve fitting technique based on the standard solutions. The individual components were quantified.

A SPR sensor including an Au metal film (DKK Co., Ltd.) with an attached agar (Kanto Chemical Co., Inc., reagent grade) membrane was used. The agar membrane was prepared by spreading a 4% agar solution onto the Au film. 8% agar membrane had almost the same separation ability as 4% one. The thickness of this agar membrane was approximately 670 µm, based on the volume of agar solution used (20 µL) and area of Au film (30 mm^2). After washing the membrane, the SPR signal always goes back to the baseline. The same membrane can be used at least for 3 days provided that this is immersed in water, so far this is the maximum length used at present. Methanol and raffinose were purchased from Wako Pure Chemical Industries Ltd. as reagent grade. Aqueous solutions of 3.0 M methanol and 0.018 M raffinose were prepared and treated as standard solutions for the experiment. Methanol–raffinose mixtures with concentrations of 1.5 M methanol–0.0090 M raffinose, 1.5 M methanol–0.0045 M raffinose, and 0.75 M methanol–0.0090 M raffinose were prepared.

Diffusion phenomena are usually represented by Fick's second law. Applying the appropriate initial and boundary conditions for the SPR sensor to the studies done by Crank[10] yielded the following solution to Fick's second law:

$$C = C_0 - \frac{4C_0}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\,\mathrm{e}^{-D(2n+1)^2\pi^2 t/4l^2} \tag{1}$$

where C is the concentration of the sample on the Au film, t is time, D is diffusion coefficient, C_0 is the concentration of the sample prepared, and l is the thickness of the agar membrane. This equation describes the concentration of sample diffusing in the SPR-agar sensor system. The refractive index of the sample solution reaching the Au film surface through the agar membrane is correlated to the concentration of the sample solution on the Au film determined by using eq 1. Generally, the refractive index of a solution is linearly proportional to concentration in the low-concentration region.[11] The resonance angle of a surface plasmon (SPR signal) is related to the refractive index of a sample solution considering the SPR condition, and implies to be proportional to the refractive index of the sample in the low signal region using the SPR resonance equation.[1] For methanol aqueous solutions, the SPR signal was experimentally confirmed to be directly proportional to the concentration from 0.5 to 3.0 M, which is expected theoretically for less than 0.5 M.

Although eq 1 implies that the SPR signal will be a multiexponential function, signal analysis would be simpler if

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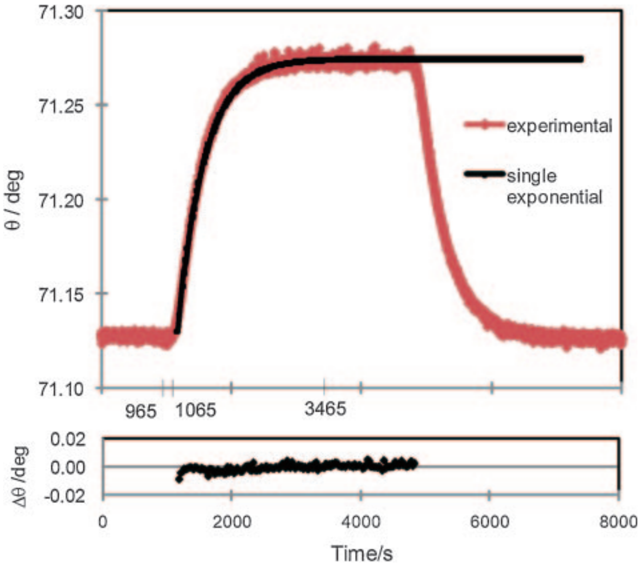

Figure 1. Experimental SPR time chart for 0.018 M raffinose and its fitted single exponential function, together with the difference between the experimental and fitted single exponential function ($\Delta\theta$).

the SPR signal could be represented by a single exponential function (i.e., if $n = 0$ in eq 1). The SPR signals for the standard solutions (3.0 M methanol and 0.018 M raffinose) were measured and their time charts fitted with single exponential functions. Figure 1 shows the experimental results, fitted function and differences for raffinose. The fitted single exponential curve was close to the experimental results at approximately 90% in the latter region. The 90% region refers to the region in the time chart where the values of the resonance angles of the experimental agree with the single exponential within 0.0010% in error. Thus the SPR signal time chart can be represented with single exponential function in the 90% region. This was also confirmed by numerical analysis of eq 1. Hence analysis using single exponential functions may be appropriate data processing for multi component sample solutions.

The data from $t = 965$ to 3465 s (where the resonance angle has almost reached the maximum) in Figure 1 have been analyzed further in Figure 2. The initial time has been set to $t = 1065$ s in these analyses. 1065 s is the initial time at which the sample is injected into the sample cell (this was determined experimentally). Beyond 3465 s, the signal changes were less than 0.0010% of the signal intensity. Figure 2 shows the differences between the maximum and the individual resonance angles ($\theta_{\max,i} - \theta_i$) (violet) where i can be any compound. The natural logarithm of this difference, $\ln(\theta_{\max,i} - \theta_i)$ is also plotted in Figure 2 (red), and is a linear function of time. All the SPR curves obtained for the various solutions were processed in this manner. The final $\ln(\theta_{\max,i} - \theta_i)$ curve for a mixed solution (1.5 M methanol–0.0045 M raffinose) is shown in Figure 3 together with those for the standard solutions (3.0 M methanol and 0.018 M raffinose). Both standard solutions give linear relationships, but the curve for the mixture has a change in slope, indicating the possible separation of the two components in the mixture. The standard solution curves have been fitted with logarithmic lines of single exponential functions (shown by the solid black lines).

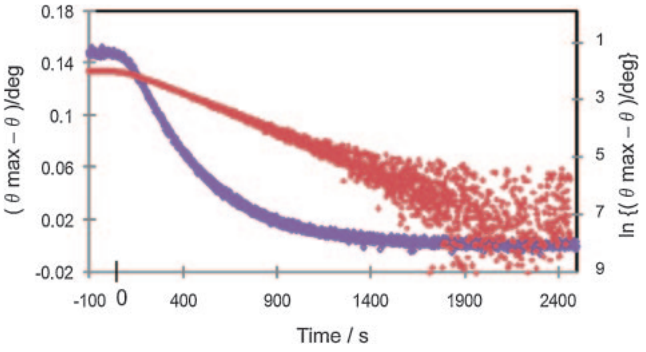

Figure 2. Data processing for 0.018 M raffinose. Difference between maximum and individual resonance angles ($\theta_{\max,i} - \theta_i$) (violet) and $\ln(\theta_{\max,i} - \theta_i)$ (red) which is linear.

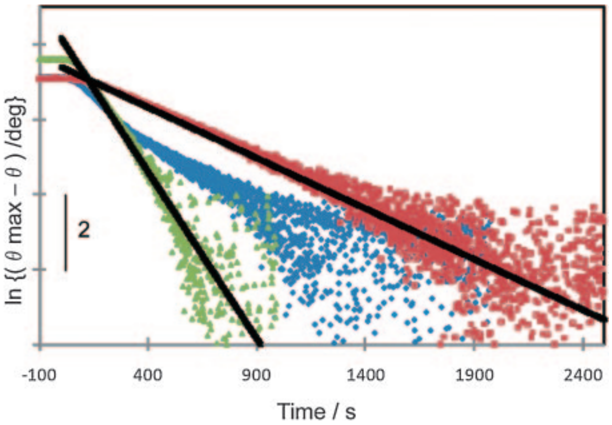

Figure 3. Comparison of the logarithmic functions of experimental SPR time charts for the standard solutions (3.0 M methanol (green) and 0.018 M raffinose (red)) which are linear and 1.5 M methanol–0.0045 M raffinose mixture (blue) which is nonlinear. The black solid lines are the fitted single exponential functions.

Two different methods were used to determine the concentrations of the individual components in the mixture: standard solution results (orange) and fitted single exponential results (black). The experimental results of the standard solutions of 3.0 M methanol (A) and 0.018 M raffinose (B) were used in the equation below to reproduce the mixture curve:

$$\ln[(\theta_{\max,A} - \theta_A)C'_A + (\theta_{\max,B} - \theta_B)C'_B] \tag{2}$$

where C'_i is the fitting coefficient for component i in the mixture. C'_i multiplied by the concentration of the standard solution of component i used is expected to be equal to the concentration of component i (C_i) in the mixture because generally the SPR signal of a solution is directly proportional to concentration in the low-concentration region as stated above. The values of the fitting coefficients of the individual components were adjusted to obtain the best correspondence to the experimental mixture result. The best agreement was obtained using $C'_{\text{methanol}} = 0.50$ and $C'_{\text{raffinose}} = 0.26$ as seen in Figure 4 (orange), using least square method, which gives final concentrations of 1.5 M (=0.50 × 3.0 M) for methanol and 0.0047 M (=0.26 × 0.018 M) for raffinose in the mixture.

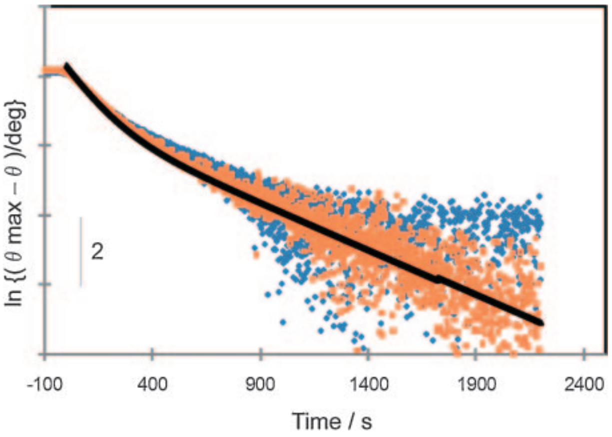

Figure 4. Experimental results for 1.5 M methanol–0.0045 M raffinose mixture (blue) and the fitted points based on the experimentally obtained standard solution results (orange) and the fitted curve (black), based on single exponential functions fitted to the standard solution results shown by the solid black lines in Figure 3.

Table 1. Concentrations of Components of Various Methanol–Raffinose Mixtures[a)]

Prepared concentration of methanol–raffinose /M	By SSR		By FSEF	
	C_{methanol} /M	$C_{\mathrm{raffinose}}$ /M	C_{methanol} /M	$C_{\mathrm{raffinose}}$ /M
1.5–0.0090	1.6	0.0072	1.6	0.0072
1.5–0.0045	1.5	0.0047	1.5	0.0047
0.75–0.0090	0.63	0.010	0.69	0.0094
1.5–0.0020	1.4	0.002	1.4	0.002

a) C_i determined from the standard solution results (SSR) and from the fitted single exponential functions (FSEF).

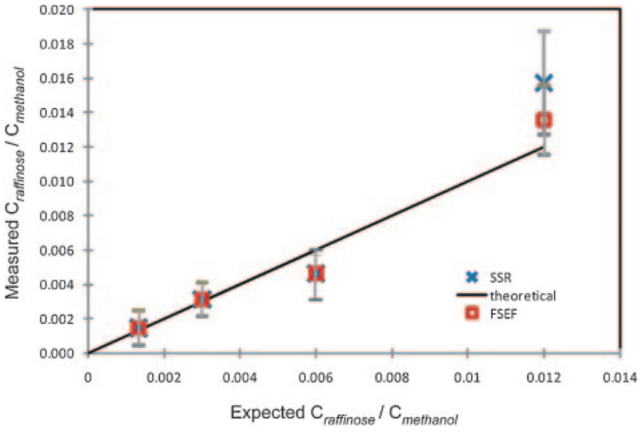

Figure 5. Calibration curve of the expected and measured concentration ratios $C_{\mathrm{raffinose}}/C_{\mathrm{methanol}}$, determined from the standard solution results (SSR) (blue) and from the fitted single exponential functions (FSEF) (red).

These values agree well with the concentrations of components in the original mixture (1.5 M methanol and 0.0045 M raffinose). Experiments were carried out for other compositions, and the concentration values obtained are summarized in Table 1, which shows good agreement between measured and expected values. The concentration ratios of raffinose to methanol are shown in Figure 5 as a calibration curve to show the accuracy between the expected and measured ratios. Both were fitted within experimental error. Thus knowledge of the sensor response to the components of the mixture makes it possible to determine the concentrations of the components with high accuracy.

However it is more convenient to analyze the mixture results using only the single exponential lines rather than the full experimental curves for each component. Thus, the experimental results for mixtures were also analyzed by using the single exponential lines fitted to the individual standard solutions shown by the solid lines in Figure 3. The best agreement was obtained using $C'_{\mathrm{methanol}} = 0.50$ and $C'_{\mathrm{raffinose}} = 0.26$ using least square method as seen in Figure 4 (black), which resulted in concentration values of 1.5 M (=0.50 × 3.0 M) for methanol and 0.0047 M (=0.26 × 0.018 M) for raffinose. These values were also close to those of the concentrations of the components in the prepared mixture. The SPR-agar sensor system time chart can be analyzed using fitted single exponential functions to determine the concentrations of the components. Numerical calculations[12,13] would make it possible to separate more than 2 components.

In conclusion, an SPR-agar sensor system was developed and this system was able to separate the components in a methanol–raffinose mixture by diffusion. The concentrations of the components in the mixture were determined using a curve fitting technique. Best agreement was obtained between the expected and measured concentrations. This system forms a useful basis for further development of compact SPR sensors.

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