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Detection Method Based on a Surface Plasmon Resonance and Its Application to Flow Injection Analysis and Liquid Chromatography

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A simple surface plasmon resonance (SPR) experimental set-up has been made for investigating further applications of the SPR detection method in analytical chemistry. In the home-made SPR experimental set-up, a Ag film with a thickness about 50 nm was deposited on the flat surface of a hemicyclindrical prism, and a flow cell was attached directly to the flat surface of the prism. It has been confirmed that SPR occurred in the experimental setup using model samples of water and alcohols. The SPR detection modes, based on changes in the coupling angle and reflectivity at a fixed angle, were investigated both theoretically and experimentally. It has been shown that the SPR detection mode based on the reflectivity change is much more sensitive than that based on a change in the coupling angle, for the SPR experimental set-up. A preliminary investigation indicates that the SPR method can be used as a universal detector for HPLC or FIA or other analytical methods. Its problems, improvements, and future applications in analytical chemistry are also discussed.

Surface plasmon resonance (SPR) is a well-known physical phenomenon occurring in a metal-dielectric interface.[1,2] Various methods can be used to generate SPR. Among them, prism-based SPR with the Kretchmann configuration[2] might be the most common, because of its simplity and easiness. In a prism-based SPR experiment, a thin metal film, such as Au or Ag, is usually directly deposited onto the surface of a prism, and a sample is arranged to contact with the metal surface. A p-polarized light is incident onto the prism/metal interface at an angle larger than the critical angle, and total internal reflection (TIR) occurs. Under TIR conditions, an evanescent electromagnetic field still exists in both the metal film and sample. At a certain incidence angle, the wave vector of the evanescent field matches that of the surface plasmon in the metal film. As a result, the energy of the p-polarized incident light is coupled to the surface plasmon, and the reflectivity of the incident light becomes minimum. The coupling angle at which SPR occurs, or the coupling efficiency of the energy of the incident light, is sensitive to the refractive index of the sample. Therefore, in principle, a change in either the coupling angle or reflectivity can be used as an SPR signal. In other words, two SPR detection modes can be carried out: one is based on a measurement of a change in the coupling angle;[3—7] the other is based on a measurement of the reflectivity change at a fixed angle.[8—12] So far, most SPR studies have been based on a measurement of the change in the coupling angle. Especially, with the use of a multichannel detection system,[3,4] it has achieved commercial success.[5—7]

Although the SPR method has been widely applied for chemical sensing,[3,4,8—10,12—17] biosensing,[18—20] characterization of thin films,[21,22] and the analysis of the molecular interaction in a solid/liquid interface,[6,7,23—25] few applications[11] have been reported in other fields of analytical chemistry, such as flow injection analysis (FIA) and liquid chromatography (LC). On the other hand, since the SPR detection method measures any change in the refractive index of a sample, a change in either the concentration or composition of the sample gives an SPR signal. Therefore, it is expected to have more applications in analytical chemistry, especially as a universal detector for FIA and separation methods. Our aim is to develop a universal SPR detector for FIA and LC. In this work, we first made a simple SPR experimental set-up, and confirmed the SPR phenomena using this set-up. Secondly, SPR detection modes based on changes in the coupling angle and reflectivity at a fixed angle were compared both theoretically and experimentally for application as a detector. Thirdly, it was demonstrated that the SPR method can be used as a detector for flow injection analysis (FIA) and high performance liquid chromatography (HPLC). Its problems and further developments are also discussed.

Experimental

Experimental Set-Up: Figure 1A shows the experimental set-up. A He–Ne laser (632.8 nm, 0.5 mW) provided p-polarized excitation light for the SPR. The excitation light was first separated into a reference and signal beams by a half mirror. The intensity of the reference beam was monitored by a photodiode. The signal beam was focused onto the focal plane of a hemicylindrical prism ($n = 1.88$) through lens 1 (focal length: 10 cm). The reflection light was focused to another photodiode with lens 2 (focal length: 3 cm). The signals from the two photodiodes were fed into a multichannel digital meter (Iwatsu Corp., Japan), and recorded in a computer. The sampling rate was 1 data per second.

The hemicylindrical prism was 1 cm in diameter and 2 cm in length. Its flat plane was coated with a thin film of Ag by a vacuum evaporation method. The thickness of the Ag film was about 50

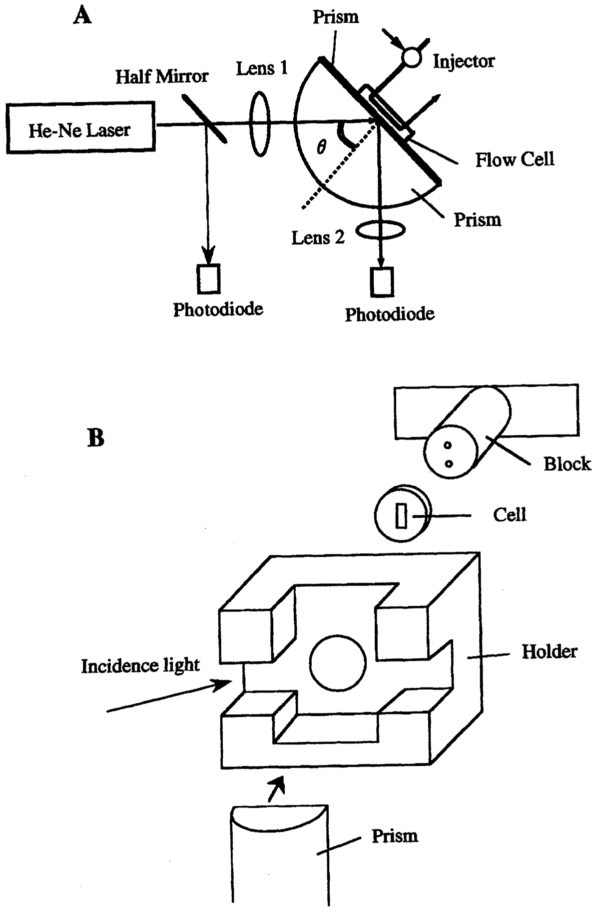

Fig. 1. Illustration of experimental set-up (A) and the construction of flow cell and holder (B).

nm. A sheet of Teflon® with a thickness of 0.5 mm, where a ditch with a size of 7 mm × 1 mm was opened (as shown in Fig. 1B), was pressed directly to the flat plane coated with Ag film by a brass block. The ditch was used as a flow cell; its volume was calculated to be 3.5 µl. Two holes were opened in the brass block, either for the inlet and outlet of the cell in a FIA experiment, or for introducing the sample into the cell in a batch experiment. The prism was held by a brass holder, as shown in Fig. 1B. The holder was mounted onto a rotating stage (angular resolution: 0.01 degree), so that the incidence angle could be changed.

In FIA experiments, a 20 µl loop-injector and a HPLC pump were connected to the flow cell. A Teflon tube (i.d.: 1 mm) was used for the flow line. In HPLC experiments, an ODS column (15 cm × 0.3 cm) was further connected between the injector and the flow cell. Water was used as the mobile phase. The model sample was the mixture of ethanol and 2-propanol dissolved in water.

Reagents: All of the reagents used in the experiments were of analytical grade. Water was deionized water.

Experimental Procedure: 1) Determination of the coupling angle in a batch experiment. Samples, such as water and alcohols, were introduced into the cell by a microsyringe. The incidence angle was changed by the rotating stage from 25 to 75 degrees, and the corresponding intensity of the reflection light was measured. When the incidence angle was changed, the positions of lens 2 and photodiode in Fig. 1 were also changed, so that the intensity of the reflection light could be measured accurately.

2) FIA and HPLC experiments. The incidence angle was fixed at 50.00 degrees. Alcohol samples with certain concentrations were injected into a flow line of water with the 20 µl injector, and the intensity of the reflection light was monitored. In FIA experiments, the flow rate was 0.1 ml min^{-1}. In HPLC experiments, the flow rate was 0.4 ml min^{-1}.

Results and Discussion

1) Generation of SPR in the Experimental Set-Up. Firstly, the generation of SPR was confirmed in the experimental set-up using a batch experiment. Figure 2A shows the experimental results of the dependence of the reflectivity on the incidence angle when cell is filled with water. When the incidence angle was increased to about 45 degrees, reflectivity became maximum, because of the occurrence of total internal reflection (the critical angle was 45.03 degrees for the prism/water interface). When the incidence angle was about 50 degrees, the reflectivity decreased remarkably. This indicates the occurrence of the SPR.

It is well known that the dependence of the reflectivity on the incidence angle can be described by Fresnel's reflection equation.[26)] Figure 2B shows the calculated results concerning the reflectivity. The solid and dashed lines are the calculated results for the refractive index of a Ag film of $0.05+4.27i$[27)] and $0.05+2.87i$,[28)] respectively. The other parameters used in the calculation were the refractive indices of the prism (n_p: 1.88) and water (n_w: 1.33), Ag film thickness (d: 50 nm), and wavelength of the incidence light (λ: 632.8 nm). Figure 2B shows that both the shape of the reflectivity curve and the coupling angle depend on the refractive index of the Ag film. Regarding the refractive index of a metal film, such as the Ag film, different values have been reported by different researchers. It has been reported that many factors, such as the film thickness, surface roughness of the metal film, and preparation conditions, affect the refractive index of metal film.[28,29)] For the present experiment, since the real value of the refractive index of the Ag film is unknown, it is difficult to compare the measured reflectivity curve with the calculated one. However, the shape of the measured reflectivity curve is similar to the calculated ones.

In addition to water, alcohols such as ethanol and propanol were also used as model samples; their reflectivity dependence on the incidence angle were also measured in batch experiments. Their results are similar to those shown in Fig. 2A, but with different coupling angles. Table 1 shows the coupling angles of the alcohol samples. As stated above, since the real value of the refractive index of the Ag film is unknown, it is difficult to calculate the theoretical coupling

Table 1. Experimental Results of Coupling Angles of Model Samples

Sample	Coupling angle (degree)
Water	50.0
Methanol	49.9
Ethanol	52.7
2-Propanol	54.1
o-Xylene	60.8

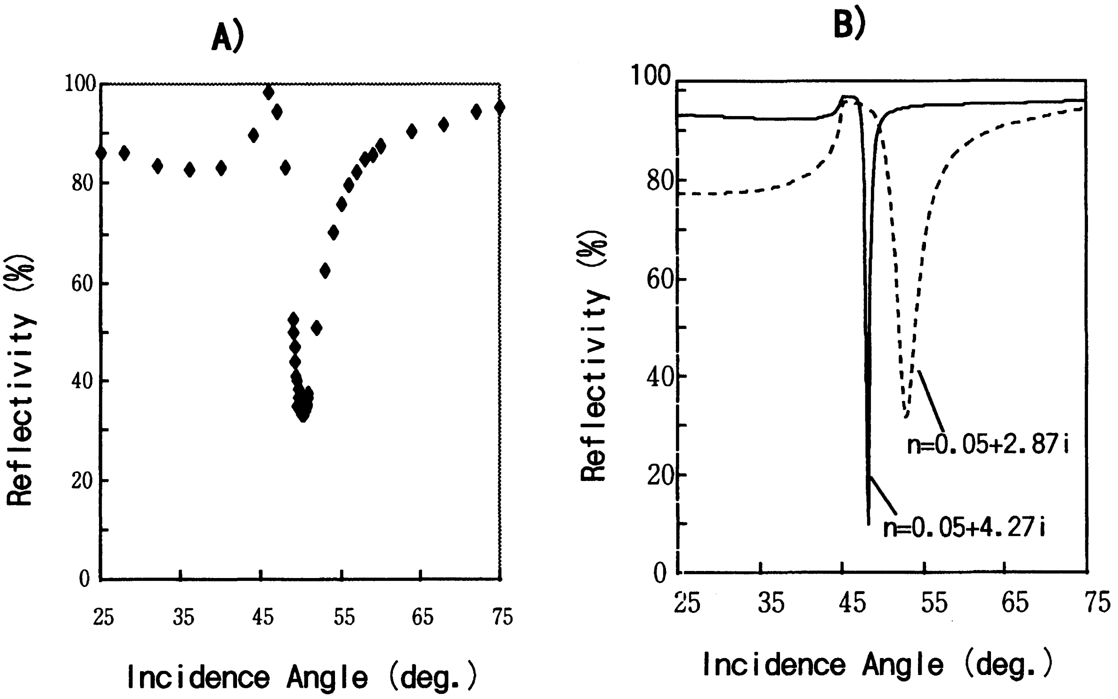

Fig. 2. Dependence of reflectivity on incident angle. A: experimental results; B: calculated results. Solid and dashed lines are the results for different refractive index of Ag film.

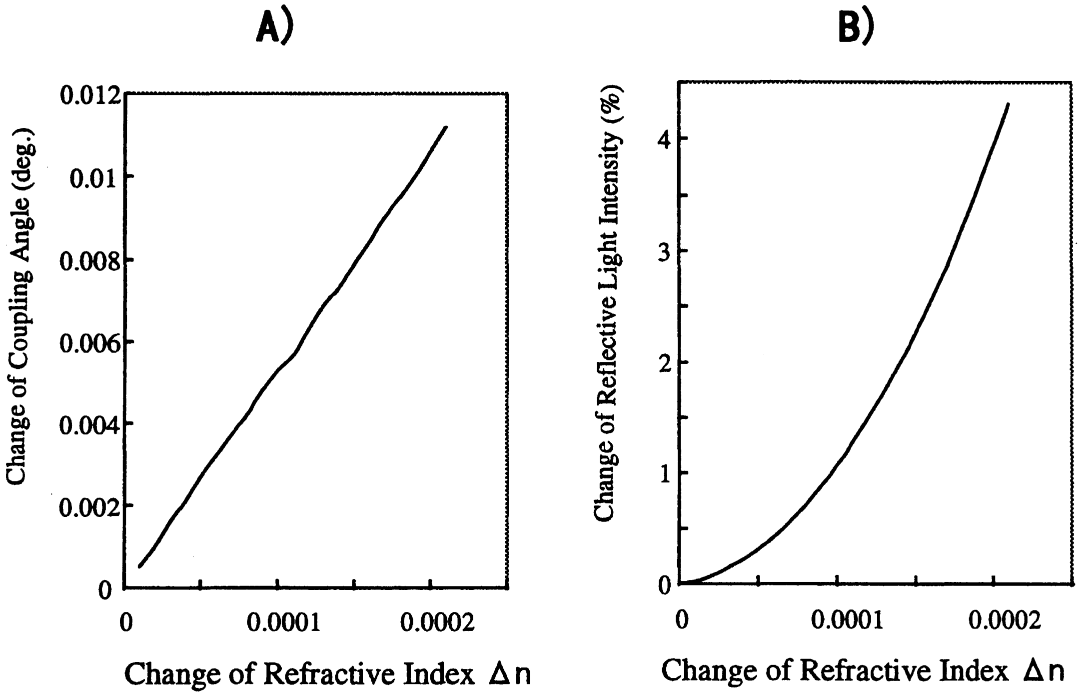

Fig. 3. Simulation results for the two SPR detection modes based on the changes in coupling angle (A) and intensity of reflection light (B). Parameters used in calculations are the same as the one in Fig. 2B except that refractive index of aqueous solution is assumed to be $1.3300+\Delta n$.

angles of samples. If the theoretical coupling angles could be calculated, an evaluation of the experimental setup would be possible. It is therefore important to prepare a Ag film with a known refractive index in SPR experiments.

2) Comparison of the Two SPR Detection Methods. Although the real value of the refractive index of Ag is unknown, an investigation of the SPR detection method is still possible for the present experimental setup. As stated in the introduction part, there are two SPR detection modes. One is based on a change in the coupling angle; the other is based a change in the reflectivity at a fixed angle. For the detection mode based on a change in the coupling angle,

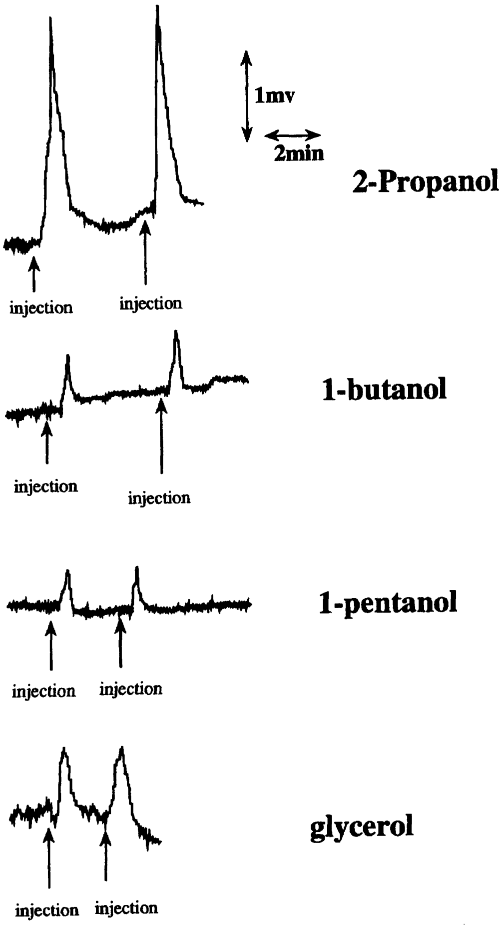

Fig. 4. SPR signals for alcohol samples in FIA experiment obtained by the SPR detection mode based on the change in intensity of reflection light. Concentration of samples are 0.5%.

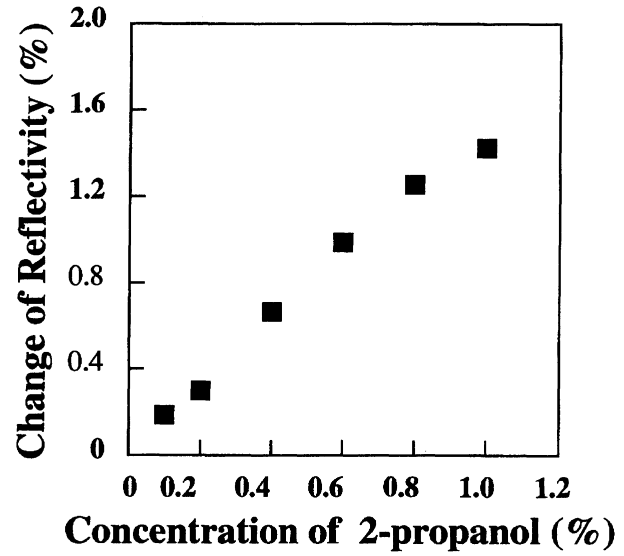

Fig. 5. Calibration curve for 2-propanol.

reflectivity curves (dependence of reflectivity on incidence angle) are measured before and after the addition of a sample into the cell; then, changes in coupling angles are calculated.

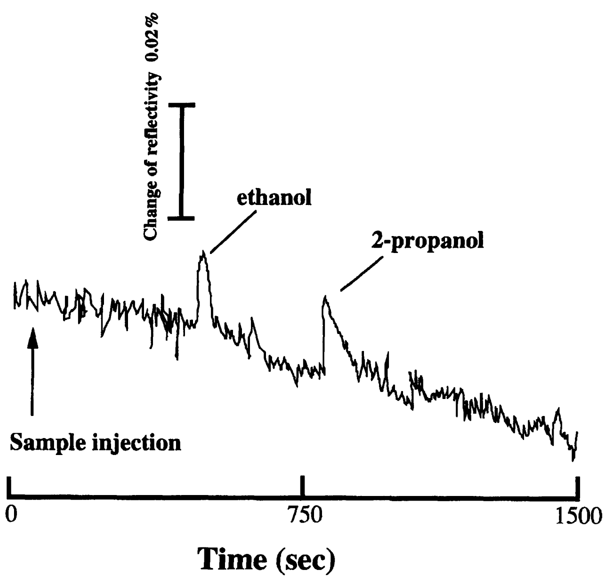

Fig. 6. Chromatogram for the mixture of ethanol and 2-propanol obtained by the SPR detector. Concentrations of ethanol and 2-propanol: 1%.

On the other hand, for the SPR detection mode based on a reflectivity change, the incidence angle of the excitation light is fixed at the coupling angle of the solvent medium, and intensity of reflection light is monitored both before and after the addition of a sample into the cell. Here, these two detection modes of the SPR method are first compared by a simulation.

For a prism/Ag film/aqueous solution system, when a sample is added into the aqueous solution, refractive index of the aqueous solution is assumed to be changed by Δn. This change in the refractive index of the aqueous solution will result in SPR signals for both of the SPR detection modes. Figure 3A) and 3B) show the corresponding SPR signals to the Δn for the two detection modes, respectively. The linearity in Fig. 3A) is better than that in Fig. 3B). This means that the SPR detection mode based on a change in the coupling angle has a better linearity with a refractive index change.

As shown in Fig. 3A), when Δn is 10^{-4}, the change in the coupling angle is about 0.004 degree. This means that a rotating stage with high resolution on the level of 0.001 degree is required for measuring the refractive index change at a level of 10^{-4}. On the other hand, the reflection light intensity is changed by about 1% for a change of 10^{-4} in the refractive index. The detection of a 1% change in the light intensity is not difficult for a double beam detection system (a signal beam and a reference beam, as shown in Fig. 1). Therefore, for an SPR experimental setup employing a cheap rotating stage with an angular resolution above 0.001 degree, the SPR detection mode based on measuring change in the reflectivity is much more sensitive than that based on a change in the coupling angle.

The two SPR detection modes were further compared experimentally. In batch experiments, the coupling angles of aqueous alcohol solutions were measured and compared with water. For the present SPR experimental set-up, the difference in the coupling angles between water and 1% alcohols could not be distinguished. However, for SPR detection mode based on the change in the intensity of reflection light, injections of 0.5% alcohols into a flow line of water gave SPR signals, as shown in Fig. 4. This means that the SPR detection mode based on a measurement of the reflectivity change is more sensitive than that based on a change in the coupling angle in our experimental setup.

In Fig. 4, two injections of a sample gave nearly the same SPR signals. On the other hand, different samples gave different SPR signals. This is because that the refractive indices of the samples are different. Furthermore, the quantitative relation between the SPR signal and the concentration was also investigated using 2-propanol as a model sample. Figure 5 shows an approximated linearity between the SPR signal and the concentration of 2-propanol in the 0.1%—1% range. Therefore, the SPR detection method could be used as a detector for FIA.

3) Application of the SPR Detection Method to HPLC. Furthermore, the application of the SPR method as a detector for separation techniques, such as HPLC, was also investigated. Figure 6 shows a chromatogram obtained by the SPR detector for a mixture of 1% ethanol and 1% 2-propanol separated on an ODS column. Therefore, the SPR method could also be used as a detector for HPLC.

4) Comparison with the Commercial Refractive Index Detector and Its Problems. Both Figs. 4 and 6 show an unstable baseline. This might be due to the adsorption of the samples or impurities in the flow line on the surface of Ag film. This adsorption would change the optical properties of the Ag film, such as the refractive index. Therefore, it is important and necessary to eliminate the adsorption for the SPR detector. Some special treatment of the Ag film might be effective for the elimination or decrease of the adsorption. For example, coating with a polymer or an organic film on the Ag film might eliminate or decrease the adsorption of some samples. Another factor that contributed to the shift of baseline might be changes in the temperature or flow rate in the cell.

In addition to the problem of adsorption, the concentration sensitivity of the present SPR detection method is not yet sufficient, compared with a commercial refractive index detector with a temperature controller. Control of temperature around the cell, suppression of a pressure change induced by the flow, the use of a highly stable excitation light source, the selection of a metal film and the wavelength of the incidence light, and an improvement in the photodiode detection system are expected to stabilize the baseline, lower the noise level, and thus improve the sensitivity. Another important factor to improve the concentration sensitivity is the construction and size of the flow cell. Since the SPR is excited by the evanescent wave of incidence light, which exists only in the prism/Ag (sample) interface for a distance on the order of the incidence light wavelength, samples only in this interface region contribute to the SPR signal. For example, since in the present experiments the wavelength of the incidence light is 632.8 nm, the interface region contributed to the SPR signal was estimated to be about 1 μm from the Ag/solution interface. However, the actual size of the cell in the depth direction from the Ag/solution interface is 0.5 mm. This means that only 1/500 of the sample injected in the cell is probed by the SPR method. Therefore, the absolute amount of the sample probed by the SPR is very small, i.e., the absolute sensitivity of the SPR method is very high.

5) Further Development and Its Future Application. Since a conventional cell used in FIA or HPLC or other analytical method is usually much larger than the wavelength of light, the use of a conventional cell could not provide a high concentration sensitivity for the SPR detection method. Therefore, in order to improve the concentration sensitivity of the SPR method, the size of the cell should be on the order of μm. One trend in analytical chemistry is the development of various micro analytical techniques. For example, HPLC using a column with a diameter on the order of μm has been reported.[30)] Capillary electrophoresis with a capillary whose internal diameter is 2 μm has been used for the analysis of a single cell.[31)] Also, miniaturized devices with features of μm size have found wide applications in analytical chemistry.[32,33)] Since the sensitivity of an UV detector and the conventional refractive index detector are proportional to the pass length of light, their sensitivity is usually not satisfactory for micro analytical techniques. However, as stated above, since the SPR method probes the interface region at a distance on the level of μm with high sensitivity, it is expected to be a powerful universal detector for future micro analytical techniques.

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