Investigation of the Molecular Extraction Process in Single Subpicoliter Droplets Using a Near-Infrared Laser Raman Trapping System

ARTICLE *in* ANALYTICAL CHEMISTRY · NOVEMBER 2000

Impact Factor: 5.64 · DOI: 10.1021/ac0002994 · Source: PubMed

3 AUTHORS, INCLUDING:

Katsuhiro Ajito
Nippon Telegraph and Telephone
124 PUBLICATIONS **1,556** CITATIONS
SEE PROFILE

Keiichi Torimitsu
Tohoku University
138 PUBLICATIONS **2,504** CITATIONS
SEE PROFILE

Anal. Chem. **2000,** *72,* 4721−4725

Investigation of the Molecular Extraction Process in Single Subpicoliter Droplets Using a Near-Infrared Laser Raman Trapping System

Katsuhiro Ajito,* Masao Morita, and Keiichi Torimitsu

NTT Basic Research Laboratories, Nippon Telegraph and Telephone Corporation, 3-1, Morinosato-Wakamiya, Atsugi, Kanagawa 243-0198, Japan

The near-infrared (NIR) laser Raman trapping system was applied to study liquid−liquid extraction in a single droplet in a subpicoliter range. The system trapped a single subpicoliter toluene droplet in water using the NIR laser beam and provided time-dependent optical images of the droplet during liquid−liquid extraction. The size of the trapped droplet gradually increased after *p*-nonylphenol solution was added in water. The Raman spectra of the droplet prove that the increase of the droplet size was caused by the absorption of *p*-nonylphenol from the water. The distribution coefficient of *p*-nonylphenol in the subpicoliter droplet was much higher than that in bulk solution.

The laser trapping technique has made it possible to manipulate single organic or biological particles, such as latex beads, microdroplets in emulsions, and individual cells or clumps of cells in biological samples in the range from picoliters to femtoliters. A single particle can be trapped at the focused point of the laser beam where the force of gradient radiation pressure is generated.[1] Initially, a visible laser beam was used for laser trapping. Nowadays, a near-infrared (NIR) laser beam generated in the 700−1300-nm wavelength range from neodymium-doped yttrium−aluminum−garnet (Nd:YAG), neodymium-doped vanadate (Nd:YVO_4), or titanium-doped sapphire (Ti:sapphire) lasers is widely used, because its lower energy results in much less photochemical damage to organic and biological samples.[2−4]

The laser trapping technique has also been used in analytical chemistry and spectroscopy to detect molecules in single microparticles. Laser trapping enables us to handle single microdroplets easily. Mass and electron transfers in a single microdroplet have been studied using electrochemistry[5] and absorption spectroscopy.[6] In addition, an acid−base reaction in a single microdroplet has been studied using morphology-dependent resonance in a Raman spectrum.[7] Raman spectroscopy is advantageous as an analytical tool for trapped particles, because it provides a wealth of information about various kinds of molecules in a trapped particle with respect to composition, structure, conformation, and intermolecular interactions. Another advantage in terms of instrumentation for Raman spectroscopy is that the focused laser beam used for laser trapping can also be used as the excitation light source for Raman measurements. This enables us to analyze smaller volume samples ranging from subpicoliters to femtoliters, which is beyond the capability of other analytical tools, such as those using a capillary tube[8] or a vial.[9] Visible laser beams are commonly used in Raman measurements for trapped particles.[10−13] However, we demonstrated, for the first time, that an NIR laser beam is advantageous in Raman measurements; i.e., there is less fluorescence interference in a Raman spectrum of the trapped particles.[14] We also showed that increasing the laser power in the system produces a larger force for optical trapping and shortens the Raman spectrum acquisition time.[15] Moreover, the system can be used for a quantitative analysis of chemicals in a single droplet.[16]

This paper describes the first observation of extraction in a subpicoliter droplet, which was achieved using a new version of the NIR laser Raman trapping system. We trapped single subpicoliter toluene droplets in water and obtained the time-dependent optical images and Raman spectra of the droplets during liquid−liquid extraction of *p*-nonylphenol. The liquid−liquid extraction process in the droplet is discussed based on a comparison between the optical images and the Raman spectra of the trapped droplet.

EXPERIMENTAL SECTION

Figure 1A is a schematic of the new version of the NIR laser Raman trapping system and the optical setup for the cell. The

* Corresponding author: (fax) +81 46 270 2364; (e-mail) ajito@will.brl.ntt.co.jp.

(1) Ashkin, A.; Dziedzic; J. M.; Bjorkholm, J. E.; Chu, S. *Opt. Lett.* **1986**, *11*, 288−290.
(2) Ashkin, A.; Dziedzic; Yamane, T. *Nature* **1987**, *330*, 769−771.
(3) Berns, M. W.; Aist, J. R.; Wright, W. H.; Liang, H. *Exp. Cell. Res.* **1992**, *198*, 375−378.
(4) Svoboda, K.; Block, S. M. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 247−285.
(5) Nakatani, K.; Uchida, T.; Misawa, H.; Kitamura, N.; Masuhara, H. *J. Phys. Chem.* **1993**, *97*, 5197−5199.
(6) Kim, H.-B.; Yoshida, S.; Miura, A.; Kitamura, N. *Anal. Chem.* **1998**, *70*, 111−116.
(7) Trunk, M.; Popp, J.; Lankers, M.; Kiefer, W. *Chem. Phys. Lett.* **1997**, *264*, 233−237.
(8) Nakatani, K.; Sudo, M.; Kitamura, N. *Anal. Chem.* **2000**, *72*, 339−342.
(9) Clark, R. A.; Ewing, A. G. *Anal. Chem.* **1998**, *70*, 1119−1125.
(10) Crawford, K. D.; Hughes, K. D. *J. Phys. Chem. B* **1998**, *102*, 2325−2328.
(11) Musick, J.; Popp, J.; Trunk, M.; Kiefer, W. *Appl. Spectrosc.* **1998**, *52*, 692−701.
(12) Kaizer, T.; Roll, G.; Schweiger, G. *J. Opt. Soc. Am. B* **1995**, *12*, 281−286.
(13) Esen, C.; Kaiser, T.; Schweiger, G. *Appl. Spectrosc.* **1996**, *50*, 823−828.
(14) Ajito, K. *Appl. Spectrosc.* **1998**, *52*, 339−342.
(15) Ajito, K. *Thin Solid Films* **1998**, *331*, 181−188.
(16) Ajito, K.; Morita, M. *Surf. Sci.* **1999**, *427/428*, 141−146.

10.1021/ac0002994 CCC: $19.00
Published on Web 08/31/2000

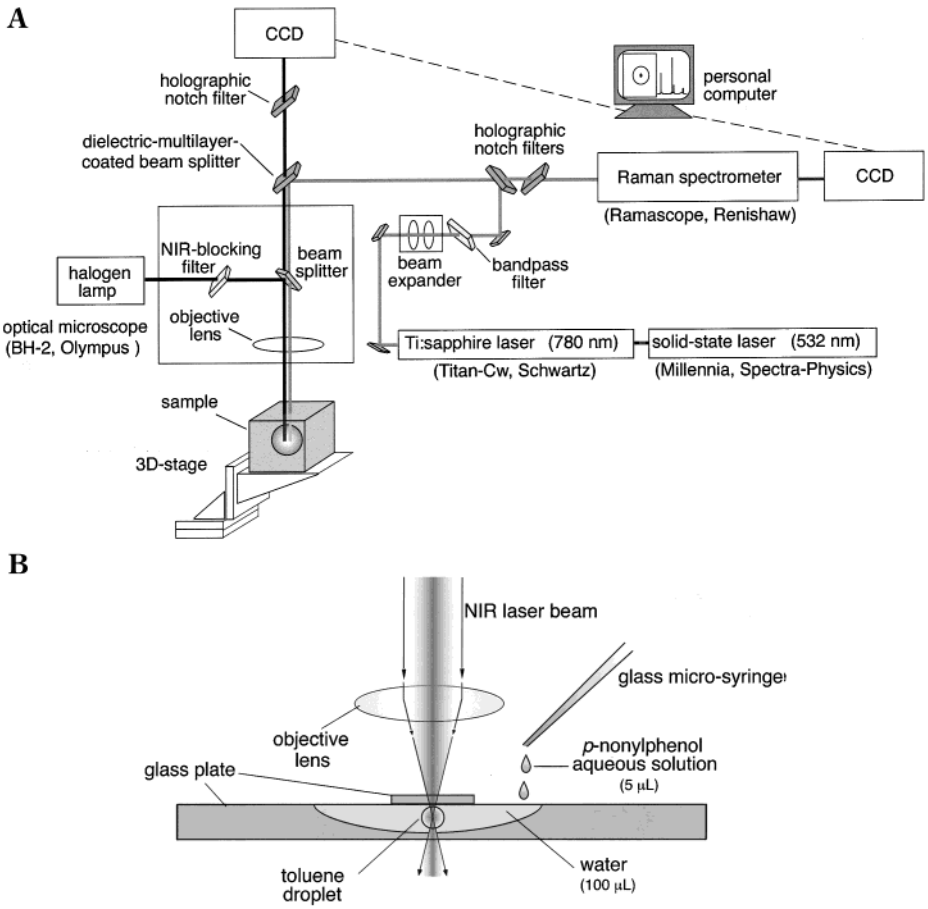

Figure 1. Diagrams of the near-infrared laser Raman trapping system: (A) the complete system and (B) the optical arrangement for the sample cell.

system is very similar to the original.[15] The main difference is the addition of a NIR-blocking filter attached to a halogen lamp, which enables us to obtain an optical image of the sample without any interference with the Raman measurement using NIR laser light. A commercial Raman microprobe spectrometer (Ramascope, Renishaw Ltd.) was specially modified for NIR laser light. The excitation light source is a continuous-wave, single-frequency Ti:sapphire ring laser (Titan-Cw, Schwartz Electrooptics) tuned to 780 nm in the TEM_{00} mode. The pump source for the Ti:sapphire ring laser is the 532-nm line (pumping power, 5 W) of a solid-state visible continuous-wave laser (Millenia, Spectra-Physics Lasers). Two objective lenses are used to expand the laser beam. The expanded beam, which passes through an optical band-pass filter, is reflected by the front holographic notch filter (HNF) and then reflected by the dielectric-multilayer-coated beam splitter (Tokyo Instruments, Inc). Then the beam passes through the beam splitter located in the optical microscope (BH-2, Olympus Optical Co. Ltd.) and is focused onto the sample using the objective lens. This lens is specially adjusted for NIR light and mounted on an optical microscope. Light from a halogen lamp passes through the NIR-blocking filter and is then focused through the same objective lens to illuminate the sample. The sample is fixed on an automatic three-dimensional stage under the objective lens. The same objective lens is used to collect scattered light reflected from the sample at 180° with respect to the incident laser light. The scattered light is separated into two paths by the dielectric-multilayer-coated beam splitter. One path delivers the NIR light (less than 3% of the total) to a charge-coupled device (CCD) camera (DXC-151A, Sony) and HNF (Kaiser Optical Systems, Inc.) to record an optical image of the trapped particle in which an image of the focused spot of the laser beam is superimposed. The other path delivers the rest of NIR light (over 97%) to another CCD camera and two HNFs with a polychromator for the Raman spectrum measurement. After passing through these HNFs, which remove the Rayleigh scattering component, the scattered light is focused onto the entrance slit of the polychromator. The 0.25-m single polychromator is fitted with a 1200-mm^{-1} grating and is used to disperse the scattered light, which is then focused onto the CCD camera (Wright Instruments Ltd.), which contains a Peltier-cooled slow-scan 384 × 576 CCD chip (EEV Ltd.) maintained at 200 K. The two-slit confocal arrangement is used for Raman measurement.[17] Optical images and Raman spectra of samples obtained using the CCD cameras are recorded by personal computers.

Figure 1B is a schematic of the optical arrangement of the NIR laser Raman trapping system for a single droplet in the solution between the glass plates. The NIR laser beam was focused with the objective lens through the top of the glass plate to trap the toluene droplet. Toluene was selected as the solvent for droplets because it has a much larger refractive index N_D (N_D = 1.497 at 293 K) than water (N_D = 1.333 at 293 K).[18] The higher N_D leads to a larger optical radiation force in water. The toluene

(17) Ajito, K.; Morita, M. *Mol. Cryst. Liq. Cryst.* **1998**, *314*, 191−196.

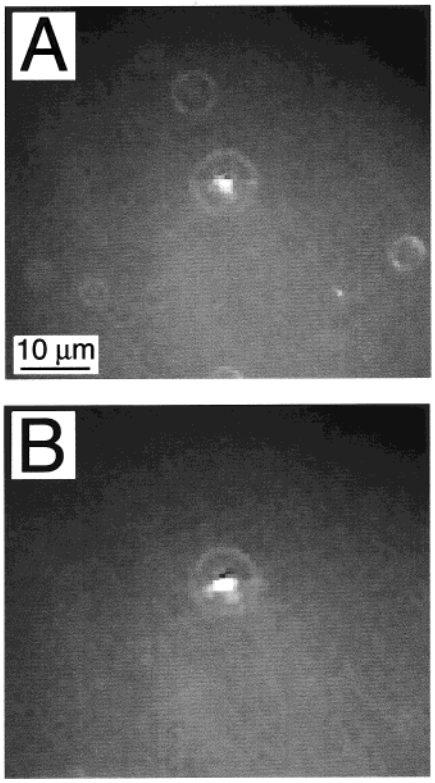

Figure 2. Images of a single subpicoliter toluene droplet (A) just trapped and (B) 3 min after image (A) was obtained.

droplets were prepared by vigorously stirring a mixture of 10 μL of toluene (reagent grade) and 1 mL of deionized water (Milli-Q, Millipore Ltd.). The concentration of *p*-nonylphenol in the water was determinded using a gas chromatograph/mass spectrometer (QP5050, Shimadzu Corp.). The saturated *p*-nonylphenol aqueous solution, ~0.019 wt % at 293 K, was made by a mixing *p*-nonylphenol (reagent grade) with deionoized water. About 100 μL of aqueous solution containing toluene droplets was transferred and put onto a glass plate using a glass microsyringe. A glass microsyringe was also used to add ~5 μL of the *p*-nonylphenol aqueous solution.

RESULTS AND DISCUSSION

Figure 2A shows a typical image of subpicoliter droplets in water obtained using the optical microscope in the system. White light, from which the NIR component was eliminated by the optical filter, was used to illuminate the droplets. The image shows that the droplets prepared in water were separated from one another. The trapped single toluene droplet near the center of the image is ~10 μm in diameter, which corresponds to a volume of ~0.52 pL when the droplet is spherical. The bright area in the center of the trapped droplet is the focal spot of the NIR laser beam visualized using the CCD camera and a HNF. The intensity of the raw NIR scattered light from the focal spot is much higher than that of the white light reflected from the droplets; however, the HNF reduced the intensity of the NIR scattering light to only a factor of 10^{-4}. The result was that the focused spot could be clearly observed in an image of the laser-trapped droplet. The focused spot is ~1 μm in diameter at a power of 120 mW. This very small focused spot, provided by using an objective lens with high magnification of 100× and a large numerical aperture (NA = 0.8), allows a very large optical radiation force with which to three-dimensionally trap a droplet. Image B in Figure 2 shows a laser-trapped toluene droplet obtained 3 min after image A. The area of image B was exactly the same as image A's. Image B indicates the trapped droplet in water was very stable for a long time; however, untrapped droplets very probably moved to the water's surface and evaporated.

Figure 3A shows a closeup image of the trapped droplet. Image A was recorded just after the *p*-nonylphenol aqueous solution was

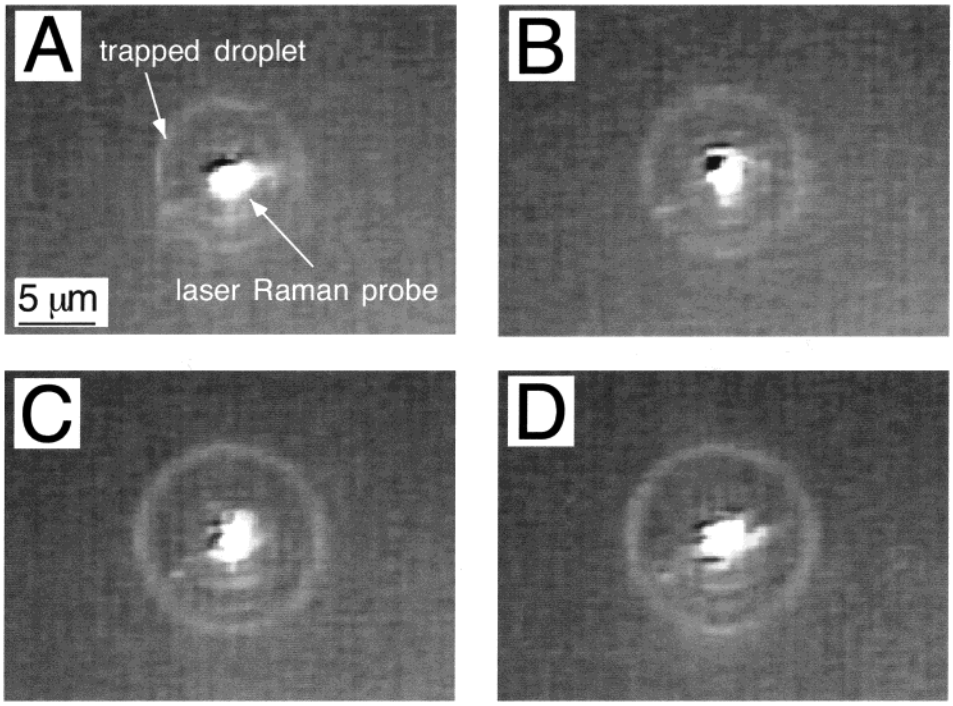

Figure 3. Time-dependent images of a single subpicoliter toluene droplet and the focal spot of the NIR laser beam during trapping in water: (A) 0, (B) 1, (C) 2, and (D) 3 min after adding the *p*-nonylphenol aqueous solution.

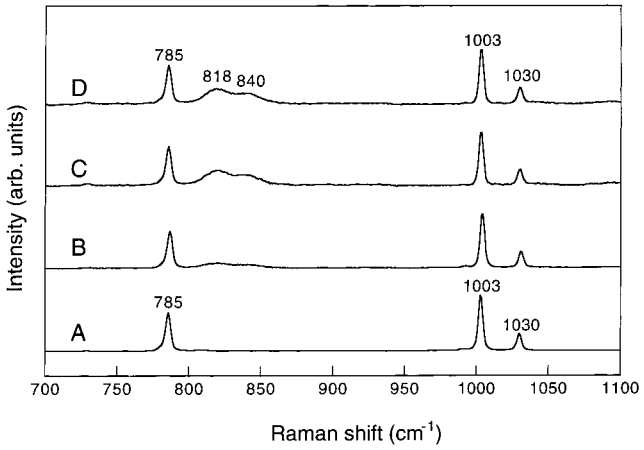

Figure 4. Time-dependent NIR Raman spectra of a single subpicoliter toluene droplet laser-trapped in water: (A) 0, (B) 1, (C) 2, and (D) 3 min after adding the *p*-nonylphenol aqueous solution. The spectra correspond to images A−D in Figure 3.

added to the solution in the cell. The Raman spectrum of this trapped droplet is spectrum A in Figure 4. The exposure time for this spectrum was 3 s. Raman scattered light around the focal spot of the laser beam was eliminated by the confocal arrangement. The intensites of peaks in the spectrum indicate the amount of molecules in the focal spot of the laser beam. The sharp peaks at 785, 1003, and 1030 cm^{-1} are attributed to the modes of the phenyl group in toluene.[19] This indicates that the trapped droplet contains only toluene. Images B, C, and D in Figure 3 are time-dependent images of this measurement obtained 1, 2, and 3 min after adding the *p*-nonylphenol aqueous solution. Images B−D have exactly the same area as image A. The spectra in Figure 4 were normalized by the peaks at 1003 cm^{-1}. The normalization using the toluene peaks enables us to easily monitor the change in intensities *p*-nonylphenol peaks in the Raman spectra. Spectra B, C, and D in Figure 4 correspond to images B, C, and D in Figure 3. The peaks at 818 and 840 cm^{-1} in spectra B−D in Figure 4 are attributed to the doublet of the ring-breathing mode of *p*-nonylphenol. These peaks have been observed for a large number of para-substituted benzenes.[20] The size of the trapped droplet was determined by the average of five measurements of the diameter of the brighter circle in each optical image in Figure 3. The trapped droplet gradually increased in 2 min from 10.0 ± 0.1 to 13.0 ± 0.1 μm in diameter, which corresponds to an increase in volume from about 0.52 to 1.15 pL, and saturated after that. Furthermore, the peaks of *p*-nonylphenol at 818 and 840 cm^{-1} in Figure 4 increased relative to the peaks of toluene at 1003 cm^{-1} for 2 min and then saturated. The concentrations of *p*-nonylphenol in the toluene droplet in spectra B, C, and D in Figure 4 were about 4.3, 39.0, and 39.0 mol % respectively, as calculated from the ratio of the peak intensities of *p*-nonylphenol and toluene. A comparison between the images in Figure 3 and the Raman spectra in Figure 4 indicates that the size of the trapped droplet was increased due to the extraction of *p*-nonylphenol from water. The same experiments were done 10 times for droplets of various sizes.

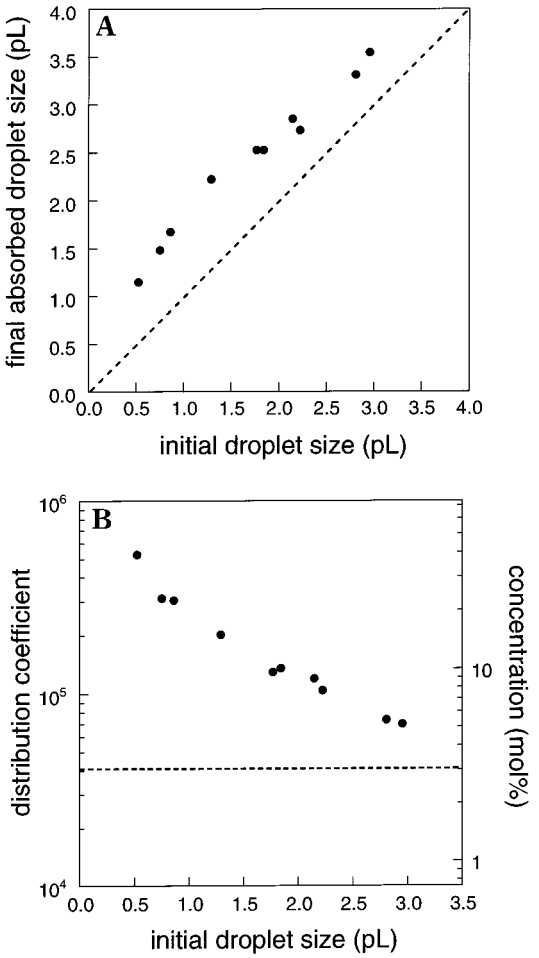

Figure 5. Initial droplet-size dependence of (A) the final absorbed droplet size droplet size and (B) the concentration and the distribution coefficient of *p*-nonylphenol in single subpicoliter and picoliter toluene droplets. The dashed lines indicate the point at which initial droplet size is equal to the final absorbed droplet size in (A) and the distribution coefficient of *p*-nonylphenol in the bulk solution in (B).

Figure 5A shows the final absorbed droplet size against the size of the initial droplet. The dashed line indicates the point at which initial droplet size was equal to the final absorbed droplet size. The results indicate that the sizes of all droplets, which ranged from about 0.5 to 3.0 pL, were increased (by about 0.5−0.7 pL) due to the extraction of *p*-nonylphenol from water. Figure 5B shows the concentration of *p*-nonylphenol (the axis on the right) against the initial droplet size. The concentration of each droplet was determined by the ratio of the intensity for *p*-nonylphenol and toluene in the Raman spectrum. The point indicating the smallest initial droplet size (the upper left) corresponds to spectrum D in Figure 4. We also determined the distribution coefficient of a single droplet. In bulk solution, the distribution coefficient is constant under the partition law.[21] The *p*-nonylphenol concentration (7.40×10^{-5} wt %) in the aqueous solution around the trapped droplet remained almost constant during the liquid−liquid extraction because of the very small volume of the trapped droplet in comparison with the volume of

(18) Riddick, J. A., Bunger, W. B., Sakano, T. K., Eds. *Organic Solvents, Physical Properties and Methods of Purification*, 4th ed.; John Wiley & Sons: New York, 1986.
(19) Wilmshurst, J. K.; Bernstein, H. J. *Can. J. Chem.* **1957**, *35*, 911−925.
(20) Siamwiza, M. N.; Lord, R. C., Chen, M. C.; Takamatsu, T.; Harada, I.; Matsuura, H.; Shimanouch, T. *Biochemistry* **1975**, *14*, 4870−4876.
(21) Glasstone, S.; Lewis, D. *Elements of Physical Chemistry*, 2nd ed.; Van Nostrand/Maruzen: Tokyo, 1972; Chapter 11.

the solution in the cell ($\sim 1 \times 10^{-4}$ L). The distribution coefficient in this measurement, defined as the ratio of *p*-nonylphenol concentration in the trapped toluene droplet to that in the water around the droplet, was 5.27×10^5. The distribution coefficient is shown by the axis on the left. The broken line represents the distribution coefficient ($\sim 4.09 \times 10^4$) of *p*-nonylphenol in water and toluene bulk layers. According to the law of partition, the distribution coefficient should be constant and have good correspondence with the dash line in Figure 5B; however, the distribution coefficient of the single subpicoliter droplet is much larger than that of bulk solution and decreases with increasing initial droplet volume. This phenomenon is caused by the nonuniformity of the concentration of *p*-nonylphenol in the toluene droplet. The focal point of the laser beam during trapping of a lighter toluene particle in water was located near the bottom of the particle, because the focal point indicates the equilibrium position between buoyancy and optical radiation pressure. The larger distribution coefficient of the single subpicoliter droplet indicates that the quantity of the absorbed *p*-nonylphenol near the bottom of the trapped droplet (i.e., near the surface of the tapped droplet) is very large and the diffusion of *p*-nonylphenol shortens with decreasing droplet size. The ratio of the surface area to the volume of a subpicoliter droplet is much larger than that of solution in a conventional container, such as a separating funnel, which strongly affects the local distribution coefficient of the droplet.

CONCLUSION

This paper described the first observation of liquid−liquid extraction in a subpicoliter droplet measured using a new version of the NIR laser Raman trapping system. We trapped a single subpicoliter toluene microdroplet in water and obtained the time-dependent optical images and Raman spectra of a droplet containing *p*-nonylphenol during liquid−liquid extraction. The images showed the size of the droplet gradually increased after *p*-nonylphenol aqueous solution was added to the water. The Raman peak intensities of *p*-nonylphenol in the trapped toluene droplet also increased. The Raman spectra indicated that the trapped droplet extracted *p*-nonylphenol from the water. A comparison between the optical images and the Raman spectra of the trapped droplet suggested that the extraction very probably increases the droplet volume; i.e., *p*-nonylphenol penetrates the droplet surface. Furthermore, the distribution coefficient in the subpicoliter droplet was much higher than that in bulk solution. The result shows that the NIR laser Raman trapping system is useful for investigating molecules in subpicoliter droplets.

The system makes it possible to manipulate and analyze individual microparticles.The advantage of the system is the use of NIR laser light instead of visible laser light, which prevents photochemical damages to organic and biological samples and reduces fluorescence background interference with a Raman spectrum of those samples. The system could be used to capture single biological cells, such as red/white blood cells, sperm cells, or a yeast, and determine their chemical compositions. We foresee even higher levels of resolution, which will make it possible to identify molecules in a submicrometer-sized particle, such as a organelle inside a protozoan, a chromosome inside a cell nucleus, and a synaptosome in a neuron cell. We believe that this technique will be widely used by biological scientists soon.

ACKNOWLEDGMENT

The authors thank Dr. H. Sato, Dr. S. Friberg, and Dr. Y. Iwasaki (NTT) for their helpful discussions, Mr. D. Steenken (Kurdyla and Associates) for English consultation, and Dr. H. Takayanagi (NTT) for his encouragement.

Received for review March 14, 2000. Accepted July 13, 2000.

AC0002994