Korean J. Chem. Eng., 34(7), 2086-2091 (2017)
DOI: 10.1007/s11814-017-0099-9

pISSN: 0256-1115
eISSN: 1975-7220

Synthesis of colloidal plasmonic microspheres via spontaneous formation and three-dimensional assembly of metal nanoparticles

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(*Received 7 February 2017 • accepted 4 April 2017*)

Abstract−We report the synthesis of colloidal plasmonic microspheres by taking advantage of emulsions of polydimethylsiloxane (PDMS) in metal precursor solution. Within the emulsion, both the spontaneous formation and three-dimensional (3D) assembly of metal nanoparticles take place at room temperature. The number of the nanoparticles being assembled in the microsphere is controllable according to the concentration of a metal precursor. In addition, owing to the surface charge and porosity of PDMS, positively charged and neutral molecules can be more concentrated in the plasmonic microsphere. We use this plasmonic microsphere for the detection of environmentally and biologically important molecules via surface-enhanced Raman spectroscopy, since 3D assembly of metal nanoparticles in the microsphere is size-comparable to a probed volume of incident light.

Keywords: Plasmonic Microsphere, Metal Nanoparticles, 3D Assembly, Surface-enhanced Raman Spectroscopy (SERS), Polydimethylsiloxane (PDMS)

INTRODUCTION

Three-dimensional (3D) assemblies of metal nanoparticles have received much attention in a wide variety of applications, ranging from catalysis to sensing [1-10], because such 3D assemblies offer a dramatic increase in the number of functional nanoparticles per unit volume compared to their one-dimensional or two-dimensional (2D) counterparts. So far, 3D assemblies have been prepared on 2D substrates by either drying a drop of colloidal nanoparticle solution [7], layer-by-layer stacking of 2D arrays assembled at liquid/air interface [8], trapping metal nanoparticles in a microfluidic channel [9], or accumulating nanoparticles on photoconductive layer by the formation of non-uniform electric field upon the projection of light [10]. However, the preparation of colloidal 3D assembly directly in a solution remains challenging due to thermal fluctuation and free diffusion. In recent years, few studies have reported solution-based 3D assemblies of metal nanoparticles through interactions between molecules attached onto nanoparticles, such as the complementary hybridization of DNA or hydrophobic interaction of alkanethiol [11,12]. However, attachment of DNA onto surface of metal nanoparticle requires delicate control over temperature [11], which may result in low reproducibility. For the assemblies using alkanethiol, only relatively small assemblies (<*ca.* 100 nm) have been prepared [12].

Here we propose a solution-based method to prepare 3D assembly of metal nanoparticles in polydimethylsiloxane (PDMS) microsphere by forming emulsions of liquid PDMS in metal precursor aqueous solution. During emulsification, metal precursor diffuses into PDMS microsphere, and then is reduced by the Si-H groups of PDMS [13-15], which allows spontaneous formation of metal nanoparticles. Eventually, the nanoparticles are three-dimensionally assembled in the microsphere which serves as a template, resulting in the formation of colloidal plasmonic microsphere (Fig. 1(a)). The plasmonic microsphere has key advantage in optical detection via surface-enhanced Raman spectroscopy (SERS). When colloidal metal nanoparticles are randomly dispersed in solution, the number of metal nanoparticles in a probed volume of incident light (*i.e.*, laser) is extremely low (Right in Fig. 1(b)). On the other hand, for the plasmonic microsphere, the number of metal nanoparticles is significantly increased in the probed volume because the nanoparticles are assembled in the microsphere, having size similar to the probed volume (Left in Fig. 1(b)) [16,17]. This contributes to significant amplification of Raman signal of analyte (Fig. 1(b)).

EXPERIMENTAL

1. Chemicals and Materials

All chemicals including, hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 3H_2O$, 99%), silver nitrate ($AgNO_3$, >99.8%), rhodamine 6G (R6G, 99%), fluorescein sodium salt (99%), bromothymol blue (BTB, 95%), copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 99.5%), ethylene diamine (99%), aniline (≥99.5%), and adenine (99%), were purchased from Sigma Aldrich. Sylgard 184 polydimethylsiloxane (PDMS) was purchased from Dow Corning (Midland, USA). All purchased chemicals were used as received without further purification. Ultrapure deionized (DI) water (18.2 Ω, Millipore) was used throughout the experiments. Glassware containing metal ion was

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‡These authors equally contributed this work.
‡This article is dedicated to Prof. Ki-Pung Yoo on the occasion of his retirement from Sogang University.

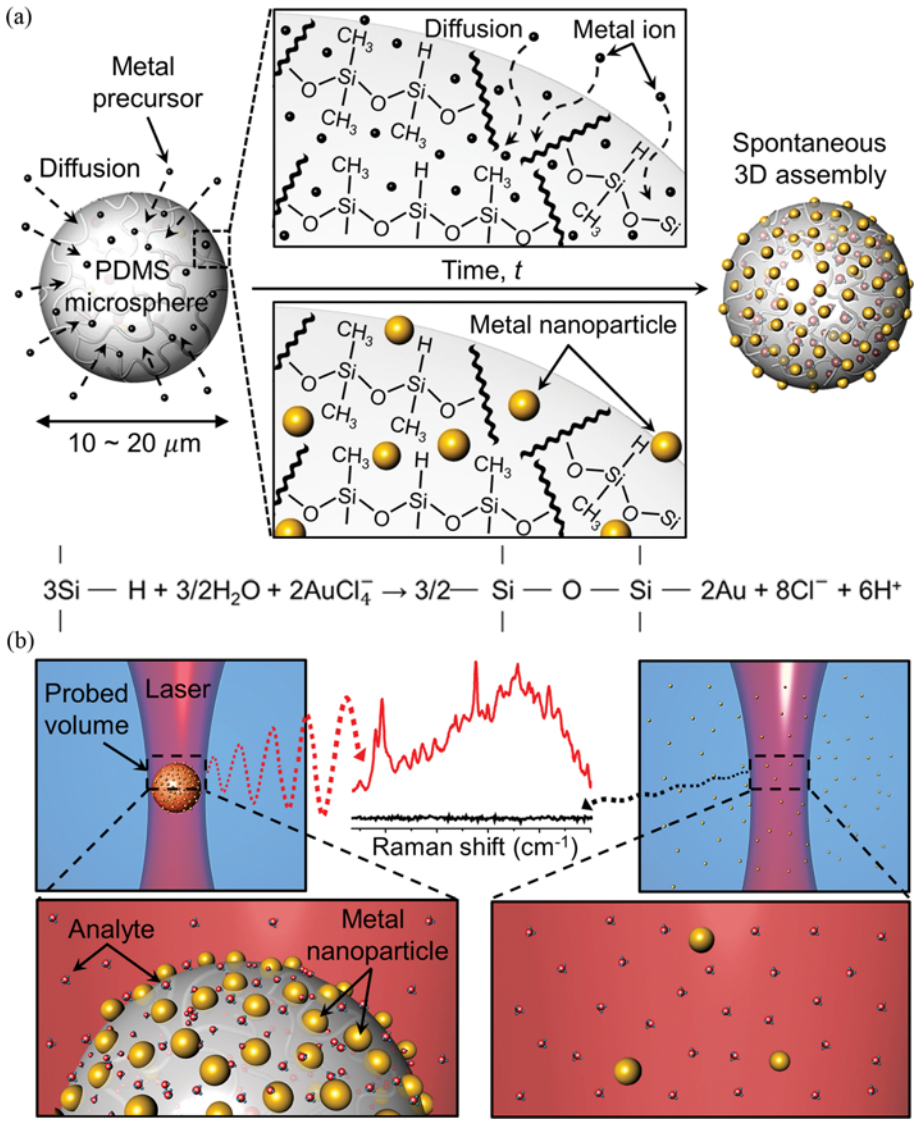

Fig. 1. (a) Schematic illustration of the synthesis of plasmonic microsphere. Metal ions undergo reduction in PDMS microsphere followed by spontaneous formation of 3D assembly of metal nanoparticles. The equation for the reduction of gold precursor by the PDMS structure is shown below. (b) Key advantage of the plasmonic microsphere in molecular detection via SERS. Since the size of the plasmonic microsphere is comparable to the probed volume and metal nanoparticles are three-dimensionally assembled in it, the number of nanoparticle is drastically increased in probed volume.

treated with piranha solution (H_2SO_4 : H_2O_2=7 : 3 v/v, this solution is a very harmful and strong acidic oxidant) for 30 min and rinsed with DI water several times.

2. Preparation of PDMS Microsphere

Liquid PDMS was prepared by mixing of Sylgard 184 (Dow-Corning Corporation, USA) elastomer with curing agent in 10 : 1 (w/w) ratio, followed by degassing in open air at room temperature for about 30 min to remove the air bubbles from PDMS. For reduction of metal ion, 50 ml vials were cleaned in a piranha solution (H_2SO_4/H_2O_2=7 : 3 v/v) for 30 min, followed by washing with DI water and drying in the vacuum oven at 65 °C. 0.5 ml of liquid PDMS was pipetted and added to 10 ml of solution containing trace molecules or metal precursor or both in 50 ml vial, followed by vortex for 10 min.

3. Absorbance Spectrum Measurement

Absorbance spectra of the PDMS microspheres were recorded from ultraviolet-visible spectrophotometer (Agilent). Absorbance spectrum of molecular solution was recorded before PDMS emulsification and after PDMS emulsification followed by centrifugation (12,000 rpm, 10 min) to remove PDMS microspheres.

4. Optical Microscope Imaging

Morphology and size of the PDMS microspheres and plasmonic microspheres were examined by optical microscope (Eclipse Ti-U, Nikon). After emulsification of liquid PDMS in solutions, resulting emulsions were dropped onto a glass substrate and characterized by the microscope.

5. Dark-field Scattering Spectroscopy

PDMS microspheres mixed with metal precursor solution were

characterized by using enhanced dark-field transmission optical microscope (Olympus BX43, Tokyo, Japan) equipped with hyperspectral imaging spectrophotometer (CytoViva Hyperspectral Imaging System (HSI), Auburn, AL, USA). Using this system, scattered images of nanoparticles from the plasmonic microsphere surface were taken using an immersion dark-field condenser with a 20× objective lens and a true-color camera with a white light illumination. Scattering spectra from the surface of the plasmonic microsphere were collected.

6. Surface-enhanced Raman Scattering (SERS) Mapping of the Plasmonic Microsphere

The SERS signal was measured on the plasmonic microsphere through confocal Raman spectroscopy (NTEGRA Spectra, NT-MDT). The distribution of trace molecules inside the microsphere was detected with SERS map imaging. Rhodamine 6G (R6G) was used as Raman probe molecule. SERS signal was obtained from the PDMS microsphere synthesized with 10 μM R6G solution. Then, the SERS spectra were measured at five different spots with same distances from center of the microsphere and the intensity of the spectra was averaged. SERS spectra were collected using 3 mW of 633 nm laser and integration time was 3 s.

7. Raman and SERS Measurement

A commercial Raman spectrometer QE65000 from Ocean Optics Inc. and 785 nm laser module I0785MM0350MS from Innovative Photonic Solution Inc. were used for Raman and SERS measurements. The illumination source was a 785 nm laser operated at power of 250 mW, and an integration time of 5 s was used in the Raman and SERS measurements. Raman measurements were carried out for mixed solution of 1 M of copper sulfate pentahydrate and 2 M of ethylene diamine, aniline and adenine. SERS measurements were conducted with 50 μl of plasmonic microspheres on a glass substrate.

RESULTS AND DISCUSSION

Typically, for the preparation of PDMS microsphere, liquid PDMS

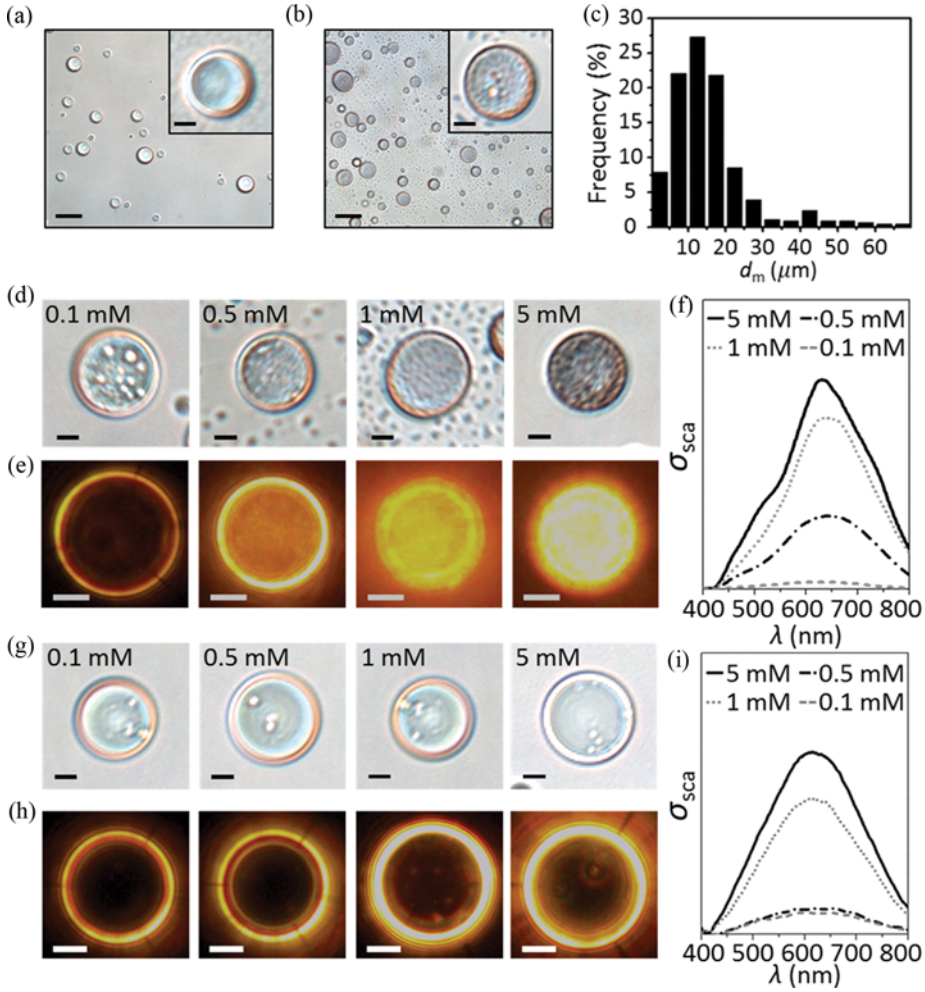

Fig. 2. Optical microscope images of PDMS microsphere in (a) deionized water and (b) $HAuCl_4$ solution. Scale bars are 30 μm and 5 μm (inset). (c) Size distribution of PDMS microspheres obtained from optical microscope image. (d) Optical microscope images, (e) dark-field scattering images, and (f) dark-field scattering spectra of the plasmonic microsphere with different $HAuCl_4$ concentrations. (g) Optical microscope images, (h) dark-field scattering images, and (i) dark-field scattering spectra of the plasmonic microsphere with different $AgNO_3$ concentrations. Scale bars are 5 μm.

was emulsified with deionized (DI) water for 10 min. The mixture immediately became turbid, indicating the formation of emulsion. To characterize the resulting emulsion, an optical microscope image was obtained. The representative image clearly shows spherical PDMS microparticles (Fig. 2(a)). To statistically analyze their sizes, 200 particles in optical images were randomly selected. The average diameter of PDMS microspheres was 16.5 μm (Fig. 2(c)).

Next, liquid PDMS was added to gold precursor (*i.e.*, $HAuCl_4$) solution with vortexing for the synthesis of plasmonic microsphere. The color of mixture gradually changed to violet. After the emulsification for 10 min, the mixture was then sampled for optical microscope measurements. The PDMS microspheres show violet color (Fig. 2(b)), indicating the formation of gold nanoparticles. To examine the formation of gold nanoparticles in PDMS microsphere, optical microscope bright-field images and dark-field scattering images along with scattering spectra were obtained with different $HAuCl_4$ concentrations. The color of microspheres becomes darker with increasing concentration (Fig. 2(d)). The scattering images of the microspheres show that the brightness increases as a function of $HAuCl_4$ concentration (Fig. 2(e)). The corresponding scattering spectra clearly exhibit broad surface plasmon resonance (SPR) band ranging from 500 to 800 nm (Fig. 2(f)). The intensity of scattering spectra increases as $HAuCl_4$ concentration increases, while shift of the SPR band is negligible. Similar transitions were also observed in the microspheres for $AgNO_3$ with increasing the concentration (Fig. 2(g), 2(h), and 2(i)). To further estimate the size of the nanoparticles formed in the plasmonic microspheres, transmission electron microscope (TEM) measurement was used. The sizes of the nanoparticles have quite broad distributions ranging from *ca.* 10 nm to *ca.* 120 nm irrespective of metal precursor concentrations. Overall, these results indicate that the increase of intensity with increasing the metal precursor concentrations could be attributed to increase in density of nanoparticles assembled in the microsphere.

To indirectly visualize the distribution of metal nanoparticle in plasmonic microsphere, Raman signal of rhodamine 6G (R6G) in plasmonic microsphere was measured by SERS. A 633 nm laser illuminates the microsphere and focuses on the middle of the microsphere in parallel to z-axis (Fig. 3(a)). The Raman map shows a clear image that is consistent with the shape of the microsphere (Fig. 3(b)), showing existence of nanoparticles over the entire microsphere. SERS spectra were collected with different distance from the center of the microsphere (Fig. 3(c)). The intensity of characteristic Raman transitions of R6G (1,545 cm^{-1}) were also obtained with different distance from the center (Fig. 3(d)). The Raman intensity is higher at the surface of the microsphere and gradually decreases as the distance from the center decreases. Based on the measurement, we postulated that the number of nanoparticles and R6G molecules increases near the surface of microsphere due to diffusion limitation of molecules in the microsphere.

Since PDMS has negative surface charge [18,19], the plasmonic microspheres can be used for molecular detection of positively charged molecules. First, zeta potential was measured for PDMS microspheres at different pH. The microspheres are neutral at acidic condition while negatively charged at neutral and basic conditions (Fig. 4(a)). Positively charged molecule such as R6G was introduced to the solution during formation of PDMS microsphere. R6G was selected for visualization and optical analysis. Interestingly, PDMS microspheres exhibit red color, which is different from transparent PDMS microspheres formed with DI water (Fig. 4(b)).

To further investigate the observation, change in absorbance of the solution before and after emulsification with removal of microspheres was measured by UV-vis absorbance spectroscopy (Fig. 4(c)). Noticeable color change in the solution from pink to transparent in the photographs and significant decrease in the intensity at maximum absorbance peak of R6G (526 nm) are obviously observed (Fig. 4(d)). For quantitative analysis, maximum possible number of R6G molecules that can be adsorbed onto the surface of PDMS microsphere and the number of the R6G molecules decreased in the solution were calculated. The number of R6G molecules concentrated in PDMS microsphere from the experimental result (2.35×10^{17} molecules) (Table S1) is two-times greater than the calculated maximum number of molecules possible to be adsorbed onto the surface of the microsphere (9.28×10^{16} molecules) (Table S2). Such discrepancy could possibly be attributed by the diffusion of the molecules within the PDMS microsphere due to their porosity. We tested negatively charged (*i.e.*, fluorescein disodium) and

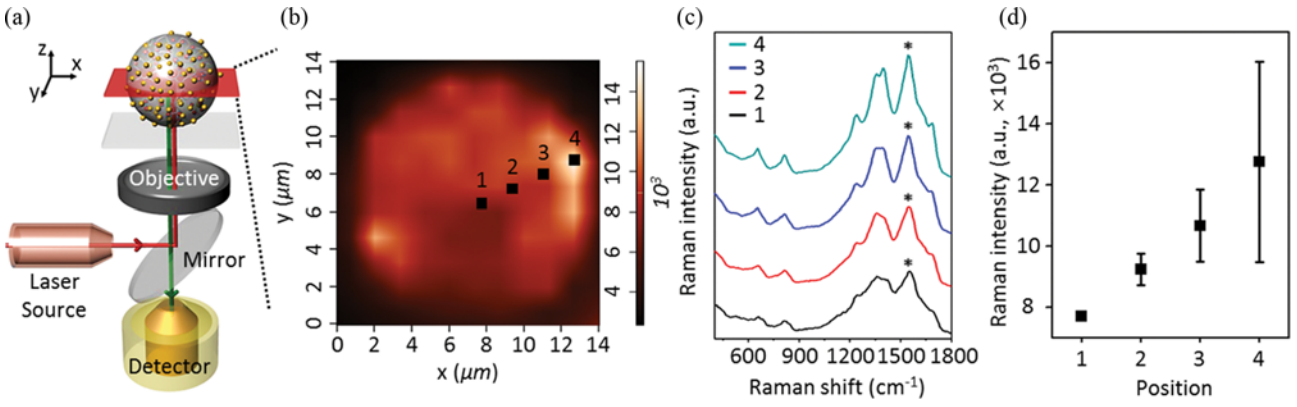

Fig. 3. (a) Schematic illustration of SERS measurement set-up for indirect visualization of distribution of nanoparticles in plasmonic microsphere prepared with 1 mM of $HAuCl_4$. (b) Surface-enhanced Raman mapping of cross-section of the microsphere. (c) SERS spectra and (d) the maximum peak intensity (1,545 cm^{-1}) of R6G according to the positions in the microsphere. The illumination source was a 633 nm laser operated at power of 3 mW and an integration time of 3 s were employed in the SERS measurements.

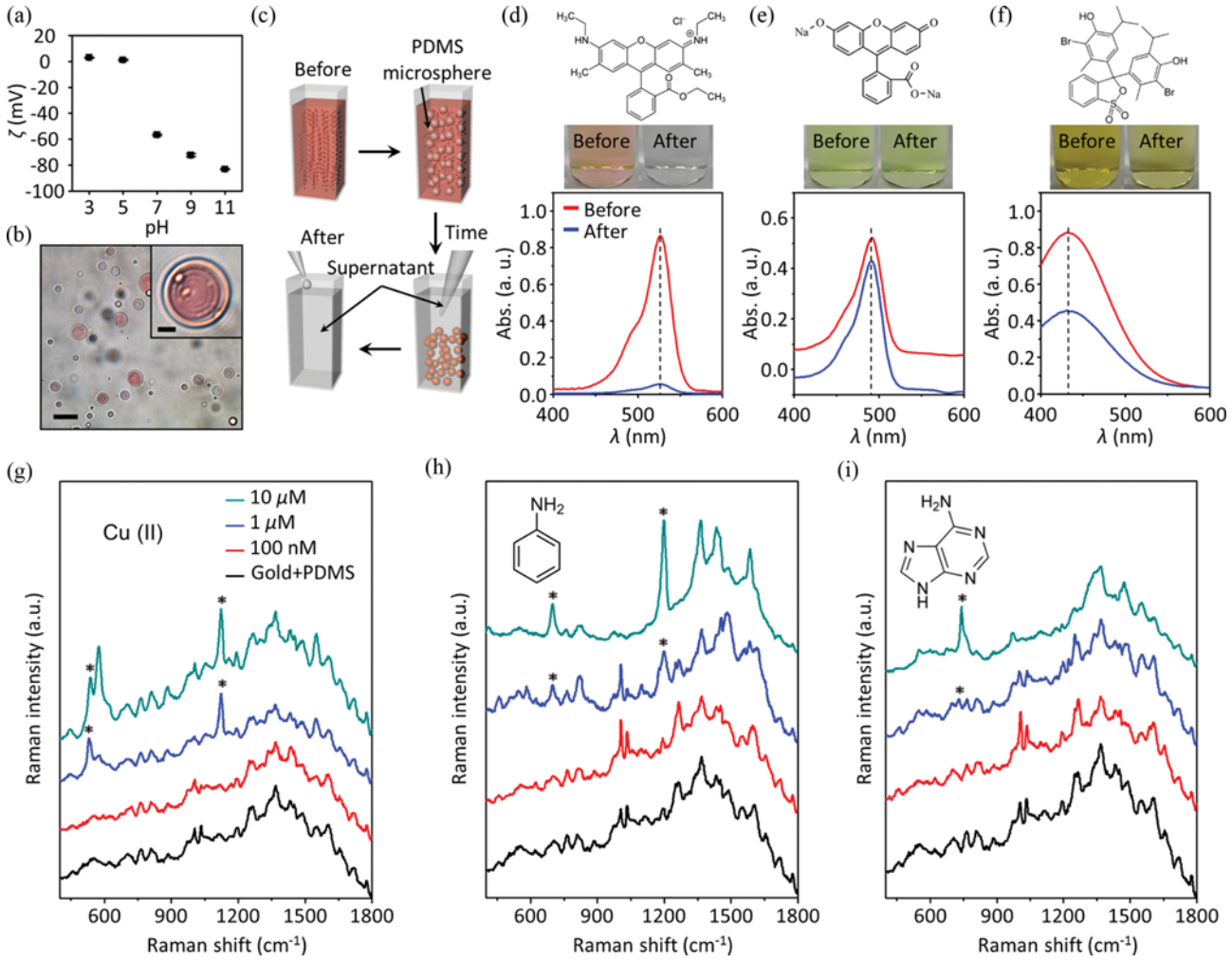

Fig. 4. (a) Zeta potential of PDMS microspheres with different pH. (b) Optical microscope image of PDMS microspheres formed with rhodamine 6G (R6G) solution. Scale bars are 30 μm and 5 μm (inset). (c) Schematic illustration of the experiment with differently charged molecules. Photographs and UV-vis absorbance spectra of the solutions before and after emulsification with removal of microspheres for three different molecules; (d) R6G (positively charged), (e) fluorescein disodium (negatively charged), and (f) bromothymol blue (neutral). SERS spectra of the microsphere with 1 mM of $HAuCl_4$ and different concentrations of (g) copper ion, (h) aniline, and (i) adenine, respectively. All SERS spectra were collected using 250 mW of 785 nm laser and integration time was 5 s.

neutral (*i.e.*, bromothymol blue) molecules for control. Negligible changes are observed for negatively charged fluorescein disodium (Fig. 4(e)). Neutral bromothymol blue solution shows a slight change in absorbance spectra (Fig. 4(f)), showing that the neutral molecules can to some extent be concentrated in the microsphere. On the basis of these experimental results, it is understandable that positively charged and neutral molecules can be concentrated in the PDMS microspheres due to negative surface charge and porosity of PDMS.

To realize the feasibility of the plasmonic microsphere in molecular detection, SERS measurement of copper ion (Cu^{2+}), aniline, and adenine, which are environmentally and biologically important positively charged and neutral molecules [20,21], was performed. SERS spectra from the microsphere with different concentrations of Cu^{2+} were collected (Fig. 4(g)). Intrinsic Raman transitions (882 and 1,121 cm^{-1}) are detectable above 1 μM concentration, which is more than 10 times lower than the EPA limit of Cu^{2+} in drinking water (*ca.* 20 μM) [22]. Raman peaks of aniline are observed at 699 cm^{-1} and 1,198 cm^{-1} down to 1 μM concentration (Fig. 4(h)). 1 μM of adenine was also detectable at 740 cm^{-1} (Fig. 4(i)).

CONCLUSIONS

We have demonstrated that metal nanoparticles are formed and three-dimensionally assembled in colloidal PDMS microsphere via emulsification of liquid PDMS in metal precursor solution. The formation and assembly of metal nanoparticles in PDMS microsphere are confirmed by scattering images and spectra, and SERS measurement. The nanoparticles are distributed throughout the entire microsphere. The number of the metal nanoparticles assembled in PDMS microsphere is controlled by changing the concentration of a metal precursor. We additionally observed that the PDMS microsphere allows the concentration of positively charged and neutral molecules due to their negative surface charge and porosity. The

plasmonic microspheres are successfully applied to molecular detection via SERS, showing the advantage of 3D assembly of metal nanoparticles and concentration of analyte molecules in the PDMS microsphere, which is size-comparable to a probed volume of incident light.

ACKNOWLEDGEMENTS

This research was supported by the International Research and Development Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, and Future Planning (MSIP) (no. 2016K1A3A1A32913356)

SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

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Supporting Information

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(*Received 7 February 2017 • accepted 4 April 2017*)

Calculation of the Number of Adsorbed Molecules

In order to determine the maximum number of molecules which can be possibly adsorbed on the PDMS microspheres, the following expression is used:

$$N_{ads}=A_{sur}/A_{mol}$$

where N_{ads} is the number of adsorbed molecules onto the PDMS microparticles, A_{sur} is the total surface area of the PDMS microparticles, A_{mol} is the surface area of the adsorbing molecule, R6G. In order to simplify the model, we assume that R6G molecules are densely attached in single-layered. A_{sur} can be obtained by product of the number of microparticles and the surface area of single microparticle. The mean diameter of the microparticle was obtained from the optical microscope images. As the long-axis length of an R6G molecule is *ca.* 1.4 nm, we assume that the surface area occupied by R6G molecule is *ca.* 1.96 nm^2/molecule.

Table S1. Number of molecules absorbed by the PDMS microspheres calculated from experimental results. 0.5 ml of liquid PDMS and 10 ml of 50 μM R6G solution was used in calculation. The number of molecules is calculated on the basis on lambert-beer equation (y=ax+b, a=0.054, b=0.15)

	Maximum absorbance intensity (a.u.)	Number of molecules/10 ml
Before emulsification	2.96	3.11×10^{17}
After emulsification	0.83	7.60×10^{16}

Table S2. Theoretically calculated maximum number of molecules possible to be adsorbed on the PDMS microspheres. R6G molecule was used in the calculation

Diameter (m)	Volume per particle (m^3)	Number of particles/ 0.5 ml	Surface area of particles (m^2)	Number of adsorbed molecules
16.5	2.35×10^{15}	2.13×10^{8}	1.82×10^{-1}	9.28×10^{16}