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ARTICLE

Magnetically Transportable Core-Shell Emulsion Droplets with Antioxidative All-Organic Paramagnetic Liquid Shell

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Magnetically-transportable core-shell emulsion droplets with antioxidative all-organic paramagnetic liquid shell [Nitroxide Radical Liquid (NRL) microcapsules] were demonstrated. We successfully fabricated stable NRL microcapsules with microfluidic devices. The NRL microcapsules are magnetically transportable and are likely to protect the inner phase from oxidants. Consequently, the NRL microcapsules can serve as a flexible antioxidative magnetic carrier for nanoliter cargoes.

Introduction

Transport of substances on small scales using external stimuli is crucial to biochemical assays [1], drug delivery [2], and highly controlled chemical reactions [3]. Controlled transport of nanometer- to micrometer-size cargoes has been achieved using laser light [4], UV light [5], and electric [6], acoustic [7] and magnetic stimuli [8, 9]. Magnetic manipulation strategies are based on the interplay between local magnetic fields originating from, for example, microparticles and external magnetic fields from solenoids or permanent magnets [8]. Thus, transport by means of a magnetic stimulus is superior to others in terms of non-contact control of carriers and reusability.

The shape and size of carriers are also important elements for successful transport. Nanoparticles [10], microparticles [1], single and double emulsion droplets [11], and colloidosomes with nanoparticles [9] are often used as carriers. Among them, the use of core-shell double emulsion droplets as nanoliter volume transporting carriers is promising, because the middle phase of the droplets can act as the shell which has the ability to encapsulate reagents that are soluble in both the inner droplet and outer continuous phases [12]. The fabrication techniques of core-shell double emulsion droplets are well established. Microfluidic devices are one of the most useful instruments to fabricate such monodisperse core-shell structures; glass capillary microfluidic devices enable the fabrication of double, triple, and even higher-order emulsions with complicated interfaces [13]. Recently, the fabrication by using this device and magnetic manipulation of core-shell double emulsion droplets with ferrofluid as the inner or middle phase has been reported [8].

It should be possible to add diverse functions to such liquid microcapsules by dissolving or dispersing appropriate materials in the shell region. For example, some liquid microcapsules may protect the reagents dissolved in the inner phase from reactive chemicals in the outer phase. Thus, the choice of carrier constituents is also crucial to the fabrication of core-shell double emulsion droplets having a function of a magnetically transportable microcapsule. Ferrofluid is a colloidal suspension of magnetic metal-oxide nanoparticles in oil or aqueous solution; under certain conditions the nanoparticles often aggregate together to lead to phase separation from the solvent. Meanwhile, all-organic paramagnetic oils are a more suitable magnetic carrier than metal-oxide nanoparticles, because the pure organic liquid should not show phase separation and is favorable in terms of biocompatibility and lower environmental burden. Despite being organic radicals, pyrrolidine *N*-oxides fully substituted on the adjacent two carbon atoms by methyl and phenyl groups are very stable, because the sterically bulky groups protect the unpaired electron. Previously we synthesized pyrrolidine *N*-oxides that are thermally stable up to about 150 °C in both the air and water [14-18], and observed that liquid crystalline droplets of these pyrrolidine *N*-oxides placed on the surface of hot water were attracted by weak permanent magnets [14, 15]. Other advantages to use pyrrolidine *N*-oxides as the major shell region component in core-shell emulsion droplets are an antioxidative ability to protect cargoes from external oxidative environment [19], transparency, and high solubility of various organic reagents in the organic liquid.

In this paper, we report the fabrication and properties of the first water-in-oil-in-water (W/O/W) double emulsion droplets containing an all-organic paramagnetic oil phase. First, we describe the fabrication of monodisperse W/O/W emulsion droplets containing a racemic pyrrolidine *N*-oxide, 2,2,5-trimethyl-5-phenylpyrrolidine-1-oxyl radical (**1**) (Figure 1a), which is very stable liquid in both the air and water at room temperature [20], as the middle oil phase [Nitroxide Radical Liquid (NRL) microcapsules] using glass capillary microfluidic devices [21]. The phenyl group of compound **1** makes the oil more hydrophobic. Next, we demonstrate the magnetic transport of the NRL microcapsules. Finally, we discuss the antioxidative abilities of the NRL microcapsules to deactivate reactive oxygen species by examining whether the luminol reaction, in which hydrogen peroxide (H_2O_2) acts as a reactive oxygen species to oxidize luminol, occurs in NRL

microcapsules having luminol solution as the inner phase when H_2O_2 is added to the outer aqueous phase.

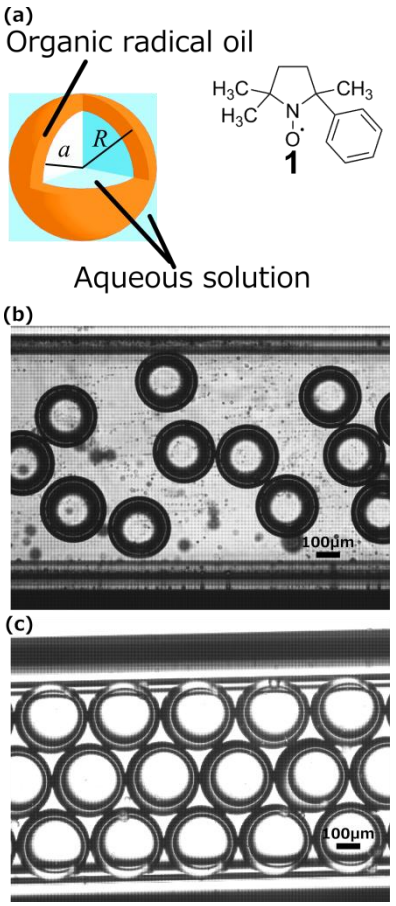

Figure 1. Structure of W/O/W emulsion droplets. (a) Schematic of a W/O/W emulsion with an organic radical oil shell consisting of compound **1**. The W/O/W emulsion has inner and outer aqueous phases and middle organic radical oil phase. (b)(c) Bright-field microphotographs of the mono-dispersed W/O/W emulsion droplets. Double emulsion droplets (b) with paramagnetic liquid **1** as the middle phase (R = 135 μm, a = 110 μm), and (c) with PDMS oil as the middle phase (R = 155 μm, a = 120 μm).

Results and discussion

To examine whether compound **1** works as an antioxidant to deactivate reactive oxygen species, e.g., hydroxyl radical (•OH) and superoxide (O_2^-), generated from H_2O_2 and thereby inhibit the luminol reaction, we carried out electrochemical studies using cyclic voltammetry (CV) in an acetonitrile solution (1 mM) of **1**. The obtained voltammogram exhibited a reversible half-wave oxidation potential ($E_{1/2}^{ox}$) of +0.404 V (vs. Ag/Ag^+, E_{pc} = +0.369 V, E_{pa} = +0.439 V, $E_{pa} - E_{pc}$ = 0.070 mV) in the presence of tetrabutylammonium perchlorate (100 mM). Thus, **1** is likely to have the ability to deactivate •OH with $E_{1/2}^{ox}$ of + 1.58 V (vs. Ag/Ag^+).

Next, we prepared NRL microcapsules with a middle phase consisting of **1** using a microfluidic device (See Fig. S1) [13]. Surfactants were added to the outer and middle phases to stabilize the structure of the emulsion. 87–89% hydrolyzed poly(vinyl alcohol) (PVA) was added to the outer aqueous phase (10 wt%) to stabilize the NRL microcapsules as a surfactant and to make the fabrication of the emulsion easier by making the solution viscous. Dow Corning 749 Fluid was added to the middle phase (3 mg/mL) as a surfactant [22]. The inner phase was filled with deionized water in this case. The resultant NRL microcapsules consisted of an inner aqueous droplet encapsulated within a nitroxide radical drop immersed in the PVA aqueous solution. We used microfluidic device **I** in which the orifice diameters are 110 and 380 μm for the inlet and outlet capillaries, respectively (Fig. S1). The inner and outer diameters of the droplets ($2a$ and $2R$) and shell thickness of obtained NRL microcapsules (R-a) were several hundreds and several tens of μm, respectively (Figure 1). The resultant W/O/W emulsion droplets were stable for over a week. For comparison, we fabricated diamagnetic emulsion droplets containing poly(dimethylsiloxane) (PDMS) oil phase as the middle phase (PDMS microcapsules). Dow Corning 749 Fluid was used as a surfactant in the oil phase of the PDMS microcapsules.

Fabrication of NRL microcapsules using microfluidic device enables the precise size control of the double emulsions. The size of the inner and outer droplets, and thus the thickness of the NRL shell, can be tuned by changing the size of the capillary orifice and/or the flow rate of each phase. For example, we measured the dependence of the thickness of the NRL shell by using microfluidic device **II** in which the orifice diameters are 110 and 500 μm for the inlet and outlet capillaries, respectively. NRL shells and inner droplets become thinner and larger, respectively, as the flow rate of the middle phase decreases (Figure 2). Moreover, since the orifice diameter of the outlet capillary of the device **II** is larger than that of the device **I**, the outer droplet size is larger than that made by the device **I**.

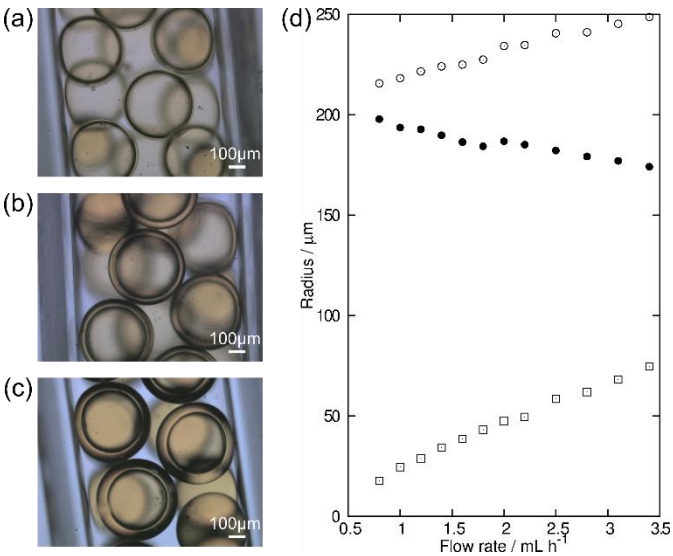

Figure 2. Dependence of the size of the NRL microcapsules on the flow rate of middle phase. The flow rates of the inner and outer aqueous phases are kept constant at 1.1 and 11 mL h^{-1}, respectively. Bright-field microphotographs of the NRL microcapsules fabricated at the flow rate of the middle phase of (a) 0.8, (b) 2.0, and (c) 3.4 mL h^{-1}. (d) Plots of the diameter for inner radius a (closed circle), outer radius R (open circle), and the thickness of NRL shell R-a (open square) as a function of the flow rate of middle phase.

To confirm whether the NRL microcapsules can function as a magnetically controllable carrier, we have examined how individual droplets in water behave under the influence of a weak permanent magnet. When a rod-like rare-earth magnet

(maximum 0.5 T, 6 mmϕ × 20 mm) was approached to the NRL microcapsules, the droplets in the continuous phase were clearly attracted by both the N and S poles of the magnet (See Movie S1). In contrast, the diamagnetic PDMS microcapsules did not move under the influence of the same magnet. These behaviours were fully reproducible, indicating that the NRL microcapsules were magnetically controllable owing to the paramagnetic nature of oil **1**.

We measured the temperature dependence of the magnetic susceptibility of **1**. As expected, **1** exhibited positive magnetic susceptibility (9.3×10^{-4} emu mol^{-1} at 293 K). Meanwhile, water, which the inner phase of the NRL microcapsules consists of, is known to have negative magnetic susceptibility (-7.19×10^{-7} emu cm^{-3} at 293 K) [23]. Volumes of outer and inner droplets made by the device **I** are 10.3 and 5.58 nL, respectively. Therefore, the magnetic susceptibilities of the inner droplet and the NRL shell are estimated to be -3.4×10^{-12} and 2.4×10^{-11} emu at 293 K, respectively. As a result, the whole droplet has positive magnetic susceptibility (2.0×10^{-11} emu). This is why the NRL microcapsules are attracted by a magnet, and in each NRL microcapsule, the inner water droplet goes to the opposite side of the magnet as shown in Movie S1.

Next, to examine whether the NRL microcapsule works as an antioxidant to protect the reagents dissolved in the inner phase, we have performed chemiluminescent assays for the microcapsules by observing the chemiluminescent light produced when luminol reaction occurs in the inner phase. Because this assay is based on the catalytic oxidation of luminol with H_2O_2, we have fabricated W/O/W emulsion droplets with an inner phase containing luminol and Cu(II) ions (See Experimental section). In the NRL microcapsule with luminol, a basic aqueous buffer solution (pH = 10) was used in the inner phase of the emulsion to accelerate the luminol reaction, while a neutral aqueous buffer solution (pH = 7) was used in the outer phase to avoid luminol reaction. Note that compound **1** and PDMS were both stable in the high pH environment. As soon as PVA aqueous solution containing an aqueous H_2O_2 solution (30 wt%) was added to the outer phases of the NRL microcapsules and PDMS microcapsules to induce luminol reaction, we started detecting chemiluminescent light via a photomultiplier (Figure 3a). In the PDMS microcapsules, chemiluminescent light was detected up to several hundred seconds after the addition of the H_2O_2 solution, while no luminescence was observed in the NRL microcapsules (Figure 3b). These results indicate that the nitroxide radical **1** inhibited the chemiluminescent luminol reaction by acting as an antioxidant. However, the validity of this simple argument should be checked more carefully, because nitroxide radicals can also act as a fluorescence quencher.

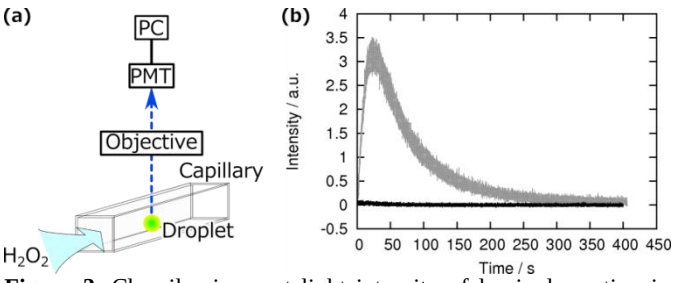

Figure 3. Chemiluminescent light intensity of luminol reaction in droplets. (a) Experimental setup. (b) Time-dependent chemiluminescent light intensity of luminol reaction in NRL microcapsules (black line) and in PDMS microcapsules (grey line).

To shed light on the reason for the inhibition of chemiluminescence in the case with compound **1**, we observed the fluorescence after breaking the emulsion by giving a strong impact. As soon as the NRL microcapsules were vigorously stirred in a H_2O_2 solution (pH 10), fluorescent light was detected. Accordingly, H_2O_2 should not be able to penetrate through the nitroxide radical shell region to leave luminol intact in the NRL microcapsule. However, the above-mentioned magnet attracted the NRL microcapsules after adding H_2O_2 solution to them. It suggests that the NRL not simply reduces H_2O_2 but indirectly degrade it with the metal catalyst while preserving the NRL.[19]

Here, we discuss the mechanism of the inhibition of luminol reaction in NRL. In luminol reaction, one •OH and one superoxide (O_2^-) are thought to be needed for the oxidation of one luminol molecule [24]. One NRL molecule (RNO•) is known to react directly with a •OH [19].

$$RNO\bullet + \bullet OH \rightarrow RN^+{=}O + OH^- \quad (1)$$

Furthermore, the oxoammonium cation ($RN^+{=}O$) can deactivate O_2^- and yield RNO• again [19].

$$RN^+{=}O + O_2^- \rightarrow RNO\bullet + O_2 \quad (2)$$

If the inhibition of the luminol reaction occurs by this mechanism, the NRL should not change after the reaction at all. In fact, the NRL microcapsules even after luminol reaction are attracted by the same rare-earth magnet. We can conclude that these two reactions occur in the NRL microcapsules and luminol reaction is inhibited.

Experimental

General

Unless otherwise noted, solvents and reagents were reagent grade and used without further purification. Phase transition temperatures were determined by differential scanning calorimetry (DSC) (SHIMADZU DSC-50). DSC analysis was performed at a scanning rate of 5°C min^{-1}.

Microfluidics

Unless otherwise noted, the outer and inner phases were a 10 wt% poly(vinyl alcohol) aqueous solution (PVA; Mw: 13000-23000 g mol^{-1}, 87-89% hydrolyzed). For the NRL and PDMS microcapsules, the middle phase was compound **1** and PDMS with Dow Corning 749 Fluid as a surfactant. For the chemiluminescent intensity measurements, luminol (9.1×10^{-2} wt%) and $CuSO_4$ (2.6×10^{-2} wt%) were dissolved in the inner phase (carbonate-bicarbonate buffer solution at pH 10) and outer phase (phosphate buffer solution at pH 7).

W/O/W double emulsion droplets were fabricated using glass microcapillary devices[25]. The outer radius, *R*, of the double emulsions varied from 350 to 500 μm, while the inner radius, *a*, varied from 260 to 380 μm. These values were controlled by the size of the capillaries used and the flow rates of the different phases.

Chemiluminescent intensity measurements

Chemiluminescent intensity measurements were carried out on a microscope (BX-50: Olympus). The light intensity was detected with a photomultiplier (H10721-210: Hamamatsu

Photonics) and the time dependence of the light intensity was recorded using an oscilloscope (LT-344 500MHz: Iwatsu-Lecroy).

Magnetic transportation of the microcapsules

We examined the behavior of compound **1** under the action of a weak permanent magnet. NRL microcapsules were prepared in a square capillary of 1 mm inner diameter. A rod-like rare-earth magnet (maximum 0.5 T, 6 mm Ø × 20 mm) with a non-uniform magnetic field was moved towards the radical and PDMS microcapsules at room temperature.

Conclusions

We successfully fabricated stable NRL microcapsules consisting of monodisperse core-shell W/O/W emulsion droplets using a microfluidic device. The NRL microcapsules are magnetically transportable and are likely to protect the inner phase from outer oxidants. Consequently, these NRL microcapsules can serve as a flexible antioxidative magnetic carrier for on-demand cargo transport systems and droplet-based sensors. Furthermore, this study can provide a useful reaction field; NRL microcapsules containing enantiomeric pure nitroxide radical oil may act as a nanoliter-scale chiral reactor, because nitroxide radicals are able to act as an oxidation catalyst [19].

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Notes and references

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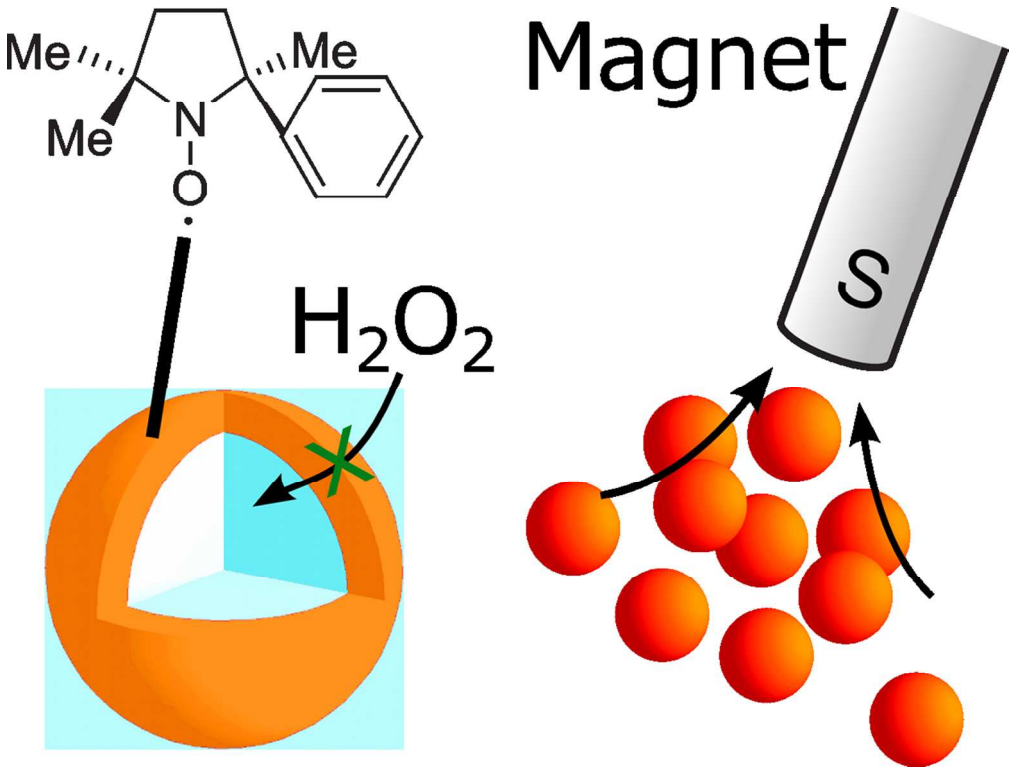

Nitroxide radical liquid microcapsules as an all-organic flexible antioxidative magnetic carrier for nanoliter cargoes have been successfully fabricated.