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Short communication

Rapid and mild purification method for nanoparticles from a dispersed solution using a monolithic silica disk

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ABSTRACT

A rapid and mild purification method for nanoparticles using the commercially available monolithic silica disk, MonoSpin®, was developed. The nanoparticles were purified from a dispersed solution by filtration with the aid of centrifugation at 2290 × *g* for 2 min. The purification conditions were rapid, mild, and simple compared with those of the conventional purification methods such as ultracentrifugation, dialysis, size exclusion chromatography, and ultrafiltration. The method was shown to be applicable for the purification of various nanoparticles, regardless of their size (from 21 to 100 nm), composition material (silica, polyethylene glycol, and pegylated liposome), and encapsulated molecule (rhodamine 110 and doxorubicin). It was shown that this method is applicable to the purification of a wide range of nanoparticles in many different fields.

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1. Introduction

In recent years, nanoparticles, which are defined as particles of size 1–100 nm, have received a great deal of attention in many fields, as the properties of nanoparticles show many differences from, and advantages over, those of bulk materials [1–3]. New functional nanoparticles are continually being developed for applications in fields such as diagnostics, therapeutics, catalysis, tissue engineering, and imaging [4–6]. Encapsulating functional molecules within nanoparticles is an effective method to add functionality to nanoparticles [7–9]. Moreover, encapsulation also improves the stability and usability of functional molecules [10,11]. In general, the functional molecule-containing nanoparticles do not exist as independent nanoparticles, but are coexist with other molecules. For instance, some of the non-encapsulated functional molecules remain in the preparation solution of the nanoparticles. Even after the non-encapsulated molecules have been removed by purification, there is the possibility that free functional molecules can leach from the nanoparticles into the nanoparticle medium. The presence of free molecules can change the properties of the nanoparticle and may present health or environmental risks. Therefore, a method for the easy and prompt removal of free functional molecules from the dispersed nanoparticle solution is required [12].

Although many purification methods, such as dialysis, ultrafiltration, size exclusion chromatography (SEC), and ultracentrifugation, have been developed and widely used for the purification of nanoparticles [13–15], these are labor-intensive and time-consuming procedures. Hence, there is a demand for a more rapid and mild purification method.

We developed a simultaneous separation method for nanoparticles and small molecules by HPLC using a silica monolith column [16]. However, this method is not ideal because of its low applicable sample volume (less than few tens of microliters) and long analytical time (about 40 min/sample). Nowadays, not only separation columns but also monolith disks (filter diameter: 4 mm, length 1.5 mm) are commercially available; an example of such a disk is MonoSpin® [17,18]. The bimodal structures of such disks and columns are different. The disks have larger micrometer-sized flow-through pores for the penetration of eluents at a low pressure (5 μm for disk and 2 μm for column) and smaller nanometer-sized mesopores for increased surface area (10 nm for disk and 18 nm for column). These structural differences do not appear to have a significant effect on the separation of nanoparticles and small molecules by the disk.

In this study, we used a MonoSpin® disc for the purification of silica, polyethylene glycol (PEG) and pegylated liposome nanoparticles, and evaluated our system as a tool for nanoparticle pretreatment by investigating the recovery ratios for nanoparticles and small molecules.

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2. Experimental methods

2.1. Chemicals

Pentaerythritol tetra (aminopropyl) polyoxyethylene (SUNBRIGHT PTE-050PA; Mn, 5328 g/mol) was purchased from NOF Corporation (Tokyo, Japan). Doxil® was purchased from Janssen Pharmaceutical K.K. (Tokyo, Japan). L-Arginine, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), dichloromethane, acryloyl chloride, triethylamine, ammonium persulfate (APS), hydrochloric acid, methanol, diethyl ether, acetic acid and acetonitrile were purchased from Wako Pure Chemical Industries (Osaka, Japan). Tetraethyl orthosilicate (TEOS), doxorubicin hydrochloride and cyclohexane were obtained from Tokyo Chemical Industry (Tokyo, Japan). Rhodamine 110 (rhodamine) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Water was purified with a Milli-Q apparatus (Millipore, Bedford, MA, USA).

2.2. Preparation of nanoparticles containing rhodamine

Silica nanoparticles containing rhodamine were prepared by means of a reported method [16,19,20]. Arginine (10 mg) and rhodamin 110 (1 μg) were dissolved in 10 mL of deionized water, and then 0.45 mL of cyclohexane and 0.55 mL of TEOS were added to the solution. The mixture was stirred at 60 °C for 20 h using a Teflon-coated magnetic stirring bar at 300 rpm. The size of the nanoparticles increased when the amount of TEOS in the reaction solution was increased.

2.3. Preparation of nanoparticles containing doxorubicin

The preparation scheme of 4-armed PEG is shown in our previous papers [21,22]. We mixed a solution of 800 μL of 100 mg/mL PEG-Ac, 250 μL of 1 mg/mL doxorubicin, 950 μL of water, 250 μL of 20 mg/mL APS, and 250 μL of 0.1 M TEMED in 1 M Tris/HCl buffer in a tube and then stirring the mixture for 20 min using a vortex mixing device (VORTEX-GENIE 2, Scientific Industries, Inc., Bohemia, NY, USA). After the mixing the tube of the dispersed solution of nanoparticles was left for 30 min, 2.5 mL of water was added to the dispersion.

2.4. Filtration of the dispersed solutions of the silica nanoparticles by MonoSpin® C18

A MonoSpin® C18 (GL Sciences, Tokyo, Japan) silica disc was preactivated with acetonitrile and water as per the procedure described in the product documentation. A sample solution (0.5 mL) was loaded onto the activated MonoSpin®, which was centrifuged at 2290 × *g* for 2 min using a 7780 II model centrifuge (Kubota Corporation, Tokyo, Japan). After the collection of the first filtrate, pure water (0.4 mL) was placed on the top of MonoSpin®, which was then centrifuged for 2 min to obtain the second fraction. After the collection of the second fraction, the third and fourth fractions were obtained by the same procedure, but with acetonitrile as the eluent. The maximum applicable sample volume of the MonoSpin® is 800 μL.

2.5. Filtration of the dispersed solutions of the nanoparticles containing doxorubicin by MonoSpin® CBA

A MonoSpin® CBA (GL Sciences, Tokyo, Japan), a monolith disk modified with carboxylate groups, was preactivated with phosphate buffered saline as per the procedure described in the product documentation. A sample solution (0.5 mL) was loaded onto the activated MonoSpin®, which was centrifuged at 2290 × *g* for 2 min using a centrifuge.

2.6. HPLC analysis

HPLC (Hitachi, Tokyo, Japan) analysis was performed with two L-2160U LaChrom Ultra pumps, an L-2200U LaChrom auto sampler, an L-2455U LaChrom diode array detector, an L-2485U LaChrom fluorescence detector, and an HPLC system organizer. Monolithic silica columns (250 mm × 3 mm, GL Sciences, Tokyo, Japan) were used. The columns used were unmodified for Doxil® analysis and modified by octadecyl for silica and PEG nanoparticles analyses. Condition for silica nanoparticle analysis; mobile phase A was water/acetonitrile = 95/5, and mobile phase B was acetonitrile. The gradient elution program of the mobile phases was as follows: 95% (A) from 0 to 25 min and then 95–0% (A) from 25 to 40 min. The flow rates were 0.05 mL/min for 0–25 min and 0.5 mL/min after 25 min. Condition for nanoparticles containing doxorubicin analysis; mobile phase A was water containing mixture of 200 mM formic acid and ammonium formate (pH 3.6)/acetonitrile = 95/5, and mobile phase B was acetonitrile. The gradient elution program of the mobile phases was as follows: 80–35% (A) from 0 to 30 min. The flow rate was 0.5 mL/min. The injection volume was 10 μL, and a diode array detector and a fluorescence detector (Ex. 480 nm, Em. 520 nm for rhodamine and Ex. 480 nm, Em. 575 nm for doxorubicin, respectively) were used for detection. All samples were filtered with a Millex-LG syringe filter (pore size 0.2 μm, Millipore) before analysis.

2.7. Particle size measurement

A Nanotrac Wave dynamic light scattering (DLS) instrument (Microtrac BEL Corp., Osaka, Japan) was used to measure the diameters of the nanoparticles. The measurement was performed as the same procedure that was described in our previous paper [16].

3. Results

Non-encapsulated (free form) rhodamine exists in the preparation solution of rhodamine-containing nanoparticles before purification. Therefore, this solution was chosen as a typical model. Fig. 1 depicts chromatograms of the dispersed solution before and after filtration through the MonoSpin® disk. The nanoparticles and rhodamine were eluted at 18 and 28 min, respectively. The large rhodamine peak detected in the chromatogram of the solution before filtration shows that a large quantity of free rhodamine remains in the dispersed solution. Although the intensity of the rhodamine peak in the dispersed solution dramatically decreased by filtration, the peak for the nanoparticle did not significantly change. Furthermore, the concentration and shape of the nanoparticles did not change by filtration, as seen by comparing the transmission electron microscope (TEM) images of the solutions before and after purification (Fig. 1a). Thus, most of the free rhodamine was effectively removed by the MonoSpin® treatment. The recovery ratios were calculated by comparing the intensity of each peak before filtration to that after filtration. The recovery ratios of the nanoparticle and rhodamine were 100% and 2.8%, respectively.

The second fraction was collected after the addition of water (400 μL) to the top layer of the MonoSpin® disk, followed by centrifugation. No nanoparticles or rhodamine were detected in the second fraction. The retained rhodamine was eluted from the MonoSpin® disk with acetonitrile. The presence of a large amount of rhodamine was observed in this third fraction, and no nanoparticles were present. A fourth fraction was collected after further addition of acetonitrile, and a small amount of rhodamine was

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