Elucidating the effect of different amino-functionalized spherical mesoporous silica characteristics on ribonucleic acid selectivity and adsorption capacity

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**ABSTRACT**

Purifying ribonucleic acid (RNA) obtained from cells is an essential process in gene analysis and is generally performed using a polythymine oligonucleotide column and an organic solvent. However, these procedures are expensive and complicated. In the present study, we discovered that amino silica with a particle size of 50 nm was able to selectively adsorb RNA and investigated how varying the parameters of this material (i.e. mesopore existence, particle size, surface amino content, and pore diameter) affected the amount of RNA it could adsorb and its selective adsorption ability. Particle size and surface amino content were found to be important factors for RNA/deoxyribonucleic acid (DNA) value (RNA adsorption amount/DNA adsorption amount), and the presence of mesopores was found to promote the amount of nucleic acid that could be adsorbed. Mesoporous silica (MPS) with a particle size of 50 nm was synthesized using a 12/1 ratio of tetraethoxysilane/aminopropyltriethoxysilane, which acts as a silica source. The MPS was also found to have a high RNA adsorption capacity (272.6 \(\mu\)g/mg) and RNA selective adsorption ability (RNA/DNA value = 11.1).

**1. Introduction**

Recently, ribonucleic acid (RNA) has been studied to elucidate the function of cells \([1,2]\) and it has been applied in biosensors \([3,4]\) and targeting therapies \([5,6]\). For such studies, the extraction and purification of RNA from cells is required. There are currently two commercially available procedures for purifying and extracting RNA that are very popular; the oligo thymidylic acid (oligo(dT))-modified cellulose column \([7,8]\) and acid guanidinium thiocyanate–phenol–chloroform methods \([8,9]\). The former method, reported by Aviv and Leder \([7]\), is used for the extraction of poly adenyl(A)-rich RNA, namely, messenger RNA (mRNA). The mRNA binds onto the column using the complimentary binding between the poly(A) tail of the mRNA and the oligo(dT) sequence on the column, and it is eluted by a buffer solution of a low ionic strength. This has the advantage of extracting gene-specific mRNA from cells. The latter method is a procedure combining the protein degradation caused by guanidinium thiocyanate and RNA isolation using phenol–chloroform. The method can effectively extract total RNA from cells. However, these methods have some problems, notably they are expensive, have complicated procedures, and can influence residual solvents during RNA applications.

As one of the inorganic materials used in the column carrier for biomolecular adsorption, mesoporous silica (MPS) is a significantly useful reagent material. It has several features that allow improved adsorption
characteristics: (1) it has a high specific surface area and a large pore volume [10,11]; (2) its surface functional groups are easily changed using silica sources containing different functional groups [12,13]; and (3) its pore size, particle size, and particle morphology can be controlled by synthesis condition [14]. For example, Fan et al. investigated lysozyme adsorption on MPS materials that had different particle morphologies (i.e. discrete and stacking rodlike particles and spherical particles with different particle diameters) [15]. They revealed that the discrete rodlike and small spherical MPS materials exhibited extremely rapid (i.e. they required only 10 min to reach equilibrium adsorption) and high capacity (7.3 and 2.3 times, respectively) adsorption of lysozymes compared with the stacking rodlike and large spherical MPS materials. Moreover, Fuertes et al. tuned the pore size of MPS from 3 to 9 nm by adding a swelling agent (1,3,5-trimethylbenzene [TMB] and N, N-dimethylethylacetamide), and they found that its hemoglobin adsorption capacity (2080 mg/g) was greater with this larger pore size [16].

Previously, we focused on silica morphology and surface functional groups, and reported on their biological applications [17–21]. MPS with sheet morphology was found to improve the activity of cytochrome c by up to 178%, which indicated that it is important for sheet morphology to be used in protein carriers [19]. Additionally, we investigated the effect of particle morphology, pore size, and the surface amino chain structure on MPS for the purpose of deoxyribonucleic acid (DNA) adsorption [20,21]. Nucleic acid adsorption on amino silica is occurred by electrostatic interaction between the negative charge of the phosphate groups of nucleic acids and the positive charge of the surface amino group on MPS. The sheet morphology was found to affect the adsorption and desorption periods, while the DNA adsorption capacity was improved by changing the pore size and surface amino structure. These investigations led to MPS being considered an excellent carrier for DNA adsorption.

Moreover, we adsorbed DNA and RNA on amino-silica particles that had different particle morphologies; they had spherical, polyhedral, sheet, helical, and cauliflower structures (data not shown). Our preliminary results indicated that nonporous spherical silica with a 50 nm particle size (i.e. Stöber silica) had both a high RNA adsorption and a low DNA adsorption capacity; that is, this material had RNA selective adsorption abilities. The other silica morphologies did not exhibit such selectivity. In this study, we focused on using Stöber silica to develop a RNA selective binding material. We investigated how changing the pore existence, particle size, surface amino group content, and pore size affected the Stöber silica’s RNA adsorption capacity and selective adsorption ability, and we aimed to develop an effective RNA extraction and purification material.

2. Experimental

2.1. Materials

Methanol, cetyltrimethylammonium chloride (CTAC), and 1,3,5-TMB were purchased from Wako Pure Chemical Industries, Japan. Tetramethoxysilane (TMOS), tetraethoxysilane (TEOS), and 3-aminopropytriethoxysilane (APTES) were obtained from Shin-Etsu Chemical Co., Japan. Triethanolamine (TEA), DNA from salmon testes, and RNA from torula yeast (Table S1) were purchased from Sigma-Aldrich, St. Louis, MO, USA. A QuantiFluor® dsDNA System and a QuantiFluor® RNA System were bought from Promega, Madison, WI, USA.

2.2. Synthesis of MPS

2.2.1. Stöber silica

Stöber silica spherical particles were synthesized so as to have no pores, using a method previously described [22]. A mixture of deionized water (17.7 g), methanol (175 mL), and ammonia (14.7 M, 7.2 g) was well stirred for 1 h. TEOS (1.46 g) and APTES (0.28 g) were slowly dropped into the mixture under vigorous stirring and then stirred for 3 s. After the mixture had been left to stand overnight, the precipitate was washed with ethanol and acetone and collected by centrifugation.

2.2.2. MCM-41c

Using a method similar to one previously described [23], we prepared four MCM-41c type MPS materials. CTAC (2.5 g) was used as the template of the mesopore, and it was dissolved in ethanol (10 mL) and deionized water (76 mL) at 60°C for 30 min. In order to prepare the MPS materials that would have large pores, namely, MCM-41c 5 nm, 1,3,5-TMB (2.5 g) was added to the solution before the solution was stirred overnight. Subsequently, 2 mL of TEA were added to the solution, and the mixture was again stirred at 60°C for 60 min. Silica sources, using various molecular ratios of TEOS and APTES (TEOS/APTES = 11/2, 12/1, and 25/1), were added dropwise into the solution. After stirring at 50°C for 20 min, the suspension was stirred at room temperature overnight. The solution was used as an organic template by refluxing the particle for 16 h in a mixture of concentrated HCl (2 mL) and ethanol (200 mL).

2.2.3. MCM-41s

Two MCM-41s type MPS materials with different particle sizes (150 and 300 nm) were synthesized by a procedure similar to one previously described by Yano and Fukushima [24]. Sodium hydroxide (1M, 2.28 mL) was added to 800 mL of a mixture containing methanol and deionized water (MeOH/
H₂O = 320 mL/480 mL and 360 mL/440 mL, respectively. TMOS (1.12 g) and APTES (0.29 g) were then added dropwise into the mixture before the solution was stirred for 7 h. After being left to stand overnight, the product was collected and washed. The template was then removed, as described in Section 2.2.2.

2.3. Characterization

The particle size and morphology of the synthesized MPS materials were analyzed by field-emission scanning electron microscopy (SEM) using a S4300 (Hitachi Co., Japan) at an acceleration voltage of 10 kV and transmission electron microscopy (TEM) using a JEM 2010 (JELO, Japan) at an operating voltage of 200 kV, respectively. In order to investigate the surface area, pore volume, and pore size distribution, we used the Brunauer–Emmett–Teller and Barret–Joyner–Halenda methods, and we measured nitrogen adsorption–desorption isotherms using a TriStar 3000 Micromeritics analyzer (Shimadzu Co., Japan). In order to analyze the poro characteristics of the synthesized MPS materials, small-angle X-ray diffractometry (XRD; Rint 2100V/PC, Rigaku Co., Japan) and the zeta (ζ)-potentials of the surfaces of the synthesized MPS were plotted in order to investigate the equilibrium data to Langmuir and Freundlich sorption models [25,26]. The Langmuir model indicates that the adsorbate exists on the surface due to monolayer adsorption. Conversely, the adsorption mechanism of the Freundlich model indicates a heterogeneous system. The Langmuir and Freundlich models are described as Equations (1) and (2), respectively:

\[ Q_e = \frac{Q_m K_L C_s}{1 + K_L C_s} \]  

\[ Q_e = K_F C_s^{1/n} \]  

where \( Q_e \) (mol/g) is the amount of nucleic acid adsorption obtained by the equilibrium data, \( Q_m \) (mol/g) is the theoretical maximum adsorption capacity, \( K_L \) and \( K_F \) are the constants of the Langmuir and Freundlich isotherm models, respectively, \( C_s \) (mol/L) is the concentration of the nucleic acid in the solution, and \( 1/n \) is the constant related to the sorption intensity.

Moreover, Equations (3) and (4) show the linear forms of Equations (1) and (2), respectively:

\[ \frac{C_s}{Q_e} = \frac{C_s}{Q_m} + \frac{1}{Q_m K_L} \]  

\[ \ln Q_e = \ln K_F + \frac{1}{n} \ln C_s \]  

The Langmuir parameters of \( Q_m \) and \( K_L \) were estimated from the slope and intercept by plotting \( C_s/Q_e \) versus \( C_s \). The linear plot of \( \ln Q_e \) versus \( \ln C_s \) was used to calculate the \( K_F \) and 1/n values of the Freundlich parameters.

2.4. Nucleic acid adsorption on silica

The synthesized materials (1.5 mg) were dispersed into a 10 mM Tris-HCl buffer solution (pH 7.4, 500 μL) using an ultrasonic bath, and then 500 μL of a nucleic acid solution (double stranded DNA [dsDNA] or RNA, 1 mg/mL) were added to the suspension. After stirring for 6 h at room temperature, the supernatant was separated by centrifugation. The amount of nucleic acid adsorbed was calculated from the concentration of the nucleic acid of the supernatant, which was measured by UV–vis spectroscopy at 260 nm.

2.5. Adsorption isotherms and mechanism study

The adsorption isotherms of the dsDNA and RNA on the synthesized MPS were plotted in order to investigate the adsorption kinetics. The MPS was then dispersed in a solution containing an optimal DNA and RNA concentration, and the mixture was then stirred for 6 h. The concentration of nucleic acid in the separated supernatant was measured using the same methods as in Section 2.4, and the supernatant concentration and the amount adsorbed on the MPS materials were calculated.

The adsorption kinetics were evaluated by fitting the equilibrium data to Langmuir and Freundlich sorption models [25,26]. The Langmuir model indicates that the adsorbate exists on the surface due to monolayer adsorption. Conversely, the adsorption mechanism of the Freundlich model indicates a heterogeneous system. The Langmuir and Freundlich models are described as Equations (1) and (2), respectively:

\[ Q_e = \frac{Q_m K_L C_s}{1 + K_L C_s} \]  

\[ Q_e = K_F C_s^{1/n} \]  

described in Section 2.4, and the supernatant concentration and the amount adsorbed on the MPS materials were calculated.

We performed a RNA purification test using MCM-41c 12/1, in order to determine if the materials we developed could be used for an isolation and purification column. A nucleic acid mixture (1 mL) containing dsDNA (150 μg) and RNA (150 μg) was added to a suspension of dispersed MCM-41c 12/1 (1.5 mg) in a Tris-HCl buffer solution (49 mL). After adsorption for 5 min, the supernatant was removed and the precipitation was redispersed into 15 mL of the Tris buffered saline (TBS), which consisted of a 10 mM Tris-HCl buffer solution (pH 7.4) and 1.2 M NaCl. The mixture was then stirred for 2 h before the eluant and solids were separated by centrifugation. The amounts of dsDNA and RNA obtained were determined from the nucleic acid concentration of the supernatant after adsorption and from the eluant.
using the QuantiFluor® dsDNA System and the QuantiFluor® RNA System, respectively.

3. Results and discussion

3.1. Characterization of the synthesized silica materials

We prepared seven silica materials with different structures; we varied whether they contained or lacked mesopores, their particle size, surface amino content, and pore size. These different silica structures were chosen in order to obtain a high RNA adsorption capacity and enhanced RNA selectivity. To begin with, the synthesized silica materials were characterized; Figures 1 and S1 show the SEM and TEM images of the synthesized silica materials. Stöber 11/2 and MCM-41c type silica were found to have particle sizes of approximately 50 nm, while spherical particles of approximately 150 and 300 nm were observed in the images of MCM-41s 11/2 0.15 and 0.3, respectively. Furthermore, the existence of pores was confirmed in all of the silica materials, except for the Stöber 11/2 (Fig. S1).

![Figure 1. SEM images of the synthesized silica materials; (a) Stöber, (b) MCM-41c 25/1, (c) MCM-41c 12/1, (d) MCM-41c 11/2, (e) MCM-41c 12/1 5 nm, (f) MCM-41s 11/2 0.15, and (g) MCM-41s 0.3. Scale bar: 250 nm.](image-url)
MCM-41c 12/1 5 nm expanded more than the other MPS materials (Fig. S1 (d)). In order to investigate the pore diameter, surface area, and pore volume, we measured the nitrogen adsorption–desorption isotherms, which are shown in Figure 2. From Figure 2 (a), we can see that all of the MPS materials displayed type-IV curves, thereby confirming the existence of the mesopores, and they all had hysteresis loops. In the pore size distributions (Figure 2(b)), similar pore size peaks of less than 3 nm were observed for all of the MPS materials, except MCM-41c 12/1 5 nm, whose pore diameter was 5.3 nm. These parameters are listed in Table 1. The pore volumes decreased from 0.72 to 0.24 cm$^3$/g as the particle size increased from 50 to 300 nm. The MPS materials that had different amino content, namely MCM-41c 11/2, 12/1, and 25/1, had surface areas of 323, 733, and 804 m$^2$/g, respectively; that is, the decrease in their surface area correlated with the increase in the aminopropyl groups. The surface area of MCM-41c 12/1 5 nm (719 m$^2$/g) was similar to that of MCM-41c 12/1 (733 m$^2$/g), but their pore volumes were a little different (2.20 and 1.47 cm$^3$/g, respectively). Small-angle XRD were performed in order to confirm the pore structure (Fig. S2). Generally, MCM-41 type silica has a 2D-hexagonal pore structure, which displays (1 0 0), (1 1 0), and (2 0 0) XRD patterns [24,27]. For the different surface amino content in MCM-41c type silica, there were broad peaks at $2\theta = 2.1$, while MCM-41s 11/2 0.15 and 0.3 had no

![Figure 2.](image)

**Figure 2.** (a) Nitrogen (N$_2$) adsorption–desorption isotherms and (b) pore size distributions of the synthesized MPS materials.

| Sample          | Particle size$^a$ (nm) | Pore size$^b$ (nm) | Surface area$^b$ (m$^2$/g) | Pore volume$^b$ (cm$^3$/g) | NH$_2$$^c$ (μmol/mg) | ζ-Potential$^d$ (mV) |
|-----------------|------------------------|--------------------|-----------------------------|-----------------------------|----------------------|----------------------|
| Stöber 11/2    | 49.9                   | -                  | 87.5                        | -                           | 1.45                 | 17.3                 |
| MCM-41c 25/1   | 45.0                   | 2.9                | 804                         | 1.29                        | 1.40                 | 6.5                  |
| MCM-41c 12/1   | 46.1                   | 2.7                | 733                         | 1.47                        | 1.40                 | 19.2                 |
| MCM-41c 11/2   | 52.4                   | 2.7                | 323                         | 0.72                        | 2.33                 | 44.1                 |
| MCM-41c 12/1 5 nm | 46.7                   | 5.3                | 719                         | 2.20                        | 1.40                 | 17.7                 |
| MCM-41s 11/2 0.15 | 150.2                   | <2.0              | 620                         | 0.45                        | 2.00                 | 32.0                 |
| MCM-41s 11/2 0.3 | 301.4                   | <2.0              | 425                         | 0.24                        | 1.32                 | 38.6                 |

$^a$Particle size was obtained directly from SEM images by measuring the diameters of 50 particles.

$^b$The Brunauer–Emmett–Teller surface area, pore volume, and pore size distribution were obtained through nitrogen adsorption–desorption isotherms by the Barrett–Joyner–Halenda method.

$^c$Amino functionalization amount was estimated from thermogravimetry (TG).

$^d$ζ-Potential of the amino-silica surface was determined by electrophoresis light analysis using ELS-Z.
peaks. This means the lowering of the ordered mesoporous structure, which occurs due to the repulsion between the cationic surfactants containing CTAB and the positively charged aminopropyl groups containing APTES in the silica source [28,29].

Next, in order to confirm the amino functionalization of the silica surfaces, FT-IR and TG were performed. It is believed that there were hydroxyl and aminopropyl groups on the silica surface because TEOS and APTES were used as silica precursors. From the FT-IR spectra of all of the amino-functionalized silica materials (Fig. S3), C–H stretching was found at 2920 and 2850 cm\(^{-1}\), and N–H vibration peaks were found at 1625 and 1515 cm\(^{-1}\), which indicates that the aminopropyl groups on the silica surface, particularly the peak at 1625 cm\(^{-1}\), had slightly increased due to the greater amount of APTES. In addition, the large loss of intensity at 1080 cm\(^{-1}\) was due to the Si–O–Si vibration. The amounts of the surface amino groups on the amino silicas were estimated from the weight loss measured by the TG-DTA shown in Fig. S4 and tabulated in Table 1. The calculation range was from 250°C to 450°C, based on the exothermal peaks of the DTA (data not shown). As expected, the amount of amino groups on MCM-41c decreased from 2.33 to 0.74 \(\mu\)mol/mg as the APTES amount decreased from 11/2 to 25/1 of the TEOS/APTES ratios. In the MPS materials with different particle sizes (50, 150, and 300 nm), a reduction in the amino groups from 2.33 to 1.32 \(\mu\)mol/mg was observed as the particle size increased. However, MCM-41c 12/1 5 nm (which had a particle size of 50 nm) had a similar amino content (1.40 \(\mu\)mol/mg) to that of MCM-41c 12/1 (particle size of 50 nm). It is important to discuss about the density of amino group. However, it is difficult to estimate amino density on the silica surface because we considered that amino groups were located on the surface and incorporated inside silica. The main mechanism governing the nucleic acid adsorption on the silica materials was the electrostatic interaction between the amino group of amino silica and the phosphate group of the nucleic acids. Therefore, it is important to investigate the \(\zeta\)-potential of the silica surface as well as the measurement of surface amino content. The potential has a similar tendency to the surface amino content, in that the potential can be increased (from -6.5 to 44.1 mV) by increasing the APTES content (from MCM-41c 25/1 to MCM-41c 11/2) while having a similar surface potential (19.2 and 17.7 mV) and different pore sizes (MCM-41c 12/1 and MCM-41c 12/1 5 nm, respectively). However, MPS materials with different particle sizes did not exhibit this tendency and maintained their high positive potentials (44.1, 32.0, and 38.6 mV for MCM-41c 11/2, MCM-41s 11/2 0.15, and MCM-41s 11/2 0.3, respectively).

### 3.2. Adsorption of DNA and RNA on MPS materials

DNA and RNA were adsorbed on prepared silica materials in order to investigate their nucleic acid adsorption capacity. Silica particles were dispersed in DNA or RNA solutions, and then the amount of nucleic acid adsorbed was calculated from the nucleic acid concentration of the supernatant after centrifugation. In this section, nucleic acid adsorptions were performed with each nucleic acid. We described what effect the pore existence, particle size, surface amino content, and pore size of silica had on the amount of nucleic acid adsorbed as well as on the RNA/DNA value (the ratio of RNA and DNA adsorption amount).

#### 3.2.1. Presence or absence of mesopores

First, silica both with and without mesopores, namely MCM-41c 11/2 and Stöber 11/2, were used for the nucleic acid adsorption tests, and the results are shown in Figure 3(a). These silica materials were all synthesized as silica precursors with similar TEOS/APTES ratios, and they all had similar particle sizes (i.e. approximately 50 nm). The amounts of DNA and RNA adsorbed on the Stöber 11/2 were 13.7 and 63.2 \(\mu\)g/mg, respectively, which indicates that the Stöber 11/2 had a RNA adsorption ability (RNA/DNA value = 4.6). Surprisingly, the MCM-41c 11/2 with mesopores retained its RNA adsorption ability (RNA/DNA value = 4.6) and was of the same efficacy as the Stöber 11/2, though it adsorbed approximately four times more DNA and RNA (54.1 and 249.2 \(\mu\)g/mg, respectively). In order to compare the adsorption amounts and the surface areas measured by the nitrogen adsorption–desorption isotherms, we calculated the adsorption amount per specific surface area (Figure 4(a)). Both MCM-41c 11/2 and Stöber silica 11/2 had similar capacities for adsorbing nucleic acid (167.5 and 159.3 \(\mu\)g/m\(^2\) for DNA, respectively, and 771.5 and 734.9 \(\mu\)g/m\(^2\) for RNA, respectively). From these results, we determined that the increase in the surface area caused by the existence of the approximately 3 nm mesopores gave rise to greater nucleic adsorption amounts; furthermore, we found that the existence of the mesopores on silica does not affect RNA/DNA value. DNA has two main chain with 2 nm of size, and the size of RNA is smaller than that of DNA. These nucleic acids well used the pore for adsorption; therefore, we considered that RNA/DNA value of MCM-41c 11/2 indicates the same value of Stöber 11/2.

#### 3.2.2. Particle size

Second, in order to investigate the main factor behind the RNA adsorption ability, we focused on the particle size of the MPS materials. In our preliminary experiments, nucleic acid adsorption was performed on MPS materials that had different morphologies –
spherical, polyhedral, sheet, helical, and cauliflower (data not shown). The spherical MPS that had particles of 50 nm displayed RNA adsorption ability (RNA/DNA value = approximately 4); however, the other MPS materials did not (RNA/DNA value of less than 2). Therefore, we estimated that the particle size of the MPS materials governs the RNA adsorption ability. MCM-41c 11/2, MCM-41s 11/2 0.15, and MCM-41s 11/2 0.3 have particle sizes of 50, 150, and 300 nm, respectively, and all have similar TEOS/APTES ratios. The result of nucleic acid adsorption on these MPS materials is shown in Figure 3(b). The amount of RNA adsorbed decreased as the particle size increased (249.2, 119.8, and 93.2 μg/mg for 50, 150, and 300 nm particles, respectively); however, there was no big difference between the DNA adsorption amount of these three (54.1, 49.9, and 40.0 μg/mg, respectively). These results suggest that particle size is a factor in RNA/DNA value (4.6, 2.4, and 2.3, respectively) because the
3.2.3. Surface amino functionalization amount

Next, we investigated the nucleic acid adsorption capacity of MCM-41c using different amounts of amino groups. As described above, the adsorption of the nucleic acid occurred due to the electrostatic interaction between the positively charged surface of an MPS and the negatively charged DNA. The positive charge of the MPS was caused by the aminopropyl group in APTES; therefore, it was also important to investigate the TEOS/APTES ratios in order to understand the RNA selective adsorption ability of MCM-41c. Three MCM-41c materials were synthesized, with 11/2, 12/1, and 25/1 TEOS/APTES ratios, and were named MCM-41c 11/2, 12/1, and 25/1, respectively. As shown in Figure 3(c), the lower amount of DNA adsorbed (from 54.1 to 3.3 μg/mg) on the MPS materials was due to fewer amino groups. However, MCM-41c 12/1 had the highest RNA capacity (272.6 μg/mg) and RNA selectivity (RNA/DNA value = 11.1). From this result, we can determine that MCM-41c 12/1 was the best material for RNA selective adsorption. In addition to the adsorption amount as a function of the mass of the MPS, we calculated the adsorption amount as a function of the specific surface area of the MPS materials, because the three had different specific surface areas; the results of this are shown in Figure 4(b). In contrast to the nucleic acid adsorption capacities as a function of mass, we observed different amounts as a function of the MPS specific surface area. MCM-41c 11/2 and 12/1 had the highest DNA and RNA adsorption amounts as a function of mass, respectively. Conversely, for the adsorption amounts as a function of the specific surface area, the sample that had the highest DNA and RNA adsorption was MCM-41c 11/2 (167.5 and 771.5 μg/m2, respectively). Moreover, although the amount of DNA adsorbed increased with more surface amino groups, the RNA/DNA value greatly decreased from 28.2 (MCM-41c 25/1) to 4.6 (MCM-41c 11/2). As the plot of the amount of nucleic acid adsorbed as a function of the specific surface area versus the surface amino group content shows (Figure 4(c)), these adsorption amounts correlate with the surface amino content; this indicates that the amount of nucleic acid adsorbed increased as the surface amino group content increased. The adsorption capacity of the RNA increased more than that of the DNA. From these results, we can determine that surface amino content of MPS is one of the factors for nucleic adsorption capacity and RNA adsorption ability. We considered that adsorption point between amino groups and phosphate groups was increased by the increasing of the amino amount on the surface.

3.2.4. Pore size

Lastly, we investigated how the pore diameter affects the adsorption of nucleic acids. We previously reported that the pore diameter of MPS affects the protein and DNA adsorption capacities [18,21]. The diameter of the DNA double strand is approximately 2 nm [30], which may be related to the low DNA adsorption capacities observed in the present study. We prepared MCM-41c 12/1 with pores that were approximately twice as large as those of MCM-41c 12/1, and named it MCM-41c 12/1 5 nm. These MPS materials had similar surface areas and surface amino contents (Table 1). Figure 3(d) and 4(d) indicates the adsorption amount as a function of the mass and specific surface area, respectively. As expected, the DNA adsorbed on MCM-41c 12/1 5 nm increased by about double (51.0 μg/mg and 70.9 μg/m2). In contrast, these MPS materials exhibited similar RNA adsorption capacities, namely 272.6 and 258.4 μg/mg, respectively. The RNA adsorption amount as a function of the specific surface area was similar to that found for the mass (371.9 and 359.4 μg/m2 for MCM-41c 12/1 and MCM-41c 12/1 5 nm, respectively). These results suggested that 2.7 nm was an essential pore size for obtaining a low DNA adsorption capacity while retaining a high RNA adsorption capacity and RNA/DNA value. DNA was easily introduced inside larger pore because the diameter of double strand for DNA is 2 nm. On the other hand, RNA has single chain and we considered that RNA was sufficiently adsorbed inside 3 nm of pore.

To summarize this section on DNA and RNA adsorption on MPS materials (Fig. S5), in order to obtain a high RNA adsorption capacity, it is important to have mesopores (with a diameter of approximately 3 nm). RNA/DNA value is also affected by the particle size and surface amino content on an MPS. MCM-41c 12/1, which was an MPS with particles of 50 nm and was synthesized with 12/1 of TEOS/APTES ratio, was the best material for RNA adsorption ability and high capacity.

3.3. Adsorption isotherms and mechanisms

In order to identify the nucleic acid adsorption mechanisms of MPS surfaces, we investigated the DNA and RNA adsorption isotherms on MCM-41c 12/1 and fitted them to the Langmuir and Freundlich models. These adsorption isotherms are shown in Figure 5, and the data of the constants and the correlation coefficients ($R^2$ value) obtained from the fitting to the adsorption isotherm models are listed in Table 2. High amounts of DNA and RNA were adsorbed onto the particles at low concentrations, and these isotherms reached adsorption equilibrium at approximately 20 and 300 μg/mg of adsorption, respectively. As for the correlation coefficient that would indicate how well the results fit the
adsorption model, the $R^2$ values of the Langmuir model (0.9903 and 0.9801 for DNA and RNA, respectively) more closely approached one than those of the Freundlich model (0.8333 and 0.6536). This result indicated that Langmuir adsorption model was observed on the surface for both the DNA and RNA. By fitting the adsorption isotherms to the Langmuir adsorption model, we were able to calculate the maximum adsorption amounts; the maximum adsorption capacity of DNA and RNA were 21.6 and 340.7 $\mu$g/mg, respectively. The DNA adsorption capacity was similar to that discussed in Section 3.2.3, but the maximum amount for RNA had increased. That is, the RNA/DNA value of MCM-41c 12/1 had increased to 15.7.

Additionally, in order to identify the loading of the nucleic acids into the pores, we measured the $N_2$ adsorption–desorption isotherms of MCM-41c 12/1 after the DNA and RNA adsorptions had been done. After these adsorption, the amount of $N_2$ adsorbed (Fig. S6 (a)) decreased from the amount found for MCM-41c 12/1 before adsorption, and the curve of the sample-adsorbed RNA (RNA/MCM-41c 12/1) was significantly lower than that of the adsorbed DNA (DNA/MCM-41c 12/1). For a pore diameter of approximately 2.7 nm (Fig. S6 (b)), the curve of DNA/MCM-41c 12/1 showed that there was a high pore volume; however, the peak of RNA/MCM-41c 12/1 disappeared at this pore diameter. Moreover, the surface area and pore volume calculated from these isotherms showed that they had decreased to 530 m$^2$/g and 1.27 cm$^3$/g for DNA and 152 m$^2$/g and 0.74 cm$^3$/g (standardized by the silica) for RNA (Table S2). From these results, we determined that DNA finds it difficult to enter pores that have a width of 2.7 nm and are adsorbed on the surface of the sphere. RNA adsorption, however, occurred in the pores of the MPS.

### 3.4. Purification study using MCM-41c 12/1

We purified RNA from a mixture containing DNA and RNA using MCM-41c 12/1, which we had found to...
have a high RNA adsorption capacity and RNA/DNA value. We suspected that the nucleic acid adsorption occurred in a similar manner to the electrostatic interactions between the surface amino groups on silica and the phosphate groups of nucleic acids, because DNA and RNA have a similar sugar–phosphate backbone. In this section, we investigated the possibility that competitive adsorption of DNA and RNA occurs on amino-silica materials, and looked how they could be used for RNA purification.

Before the RNA purification, we eluted the RNA using a Tris-HCl buffer containing NaCl. The increase in the salt concentration allowed some of the biomolecules to be adsorbed through electrostatic interactions, which eluted the adsorbents, because a high salt concentration decreases the interaction between a molecule and the surface [31]. The RNA adsorption and elution amounts are shown in Fig. S7. All of the adsorbed RNA was eluted by the buffer containing 150 mM NaCl, which indicates that the RNA was easily eluted from surface of amino silica by a solution comprising 10 mM Tris-HCl buffer and 150 mM NaCl. The RNA purification test was carried out as follows: (1) adsorption of the nucleic acids on MCM-41c 12/1 in the DNA and RNA mixture, and (2) elution by the buffer solution containing 1.2 M NaCl. The NaCl concentration was selected so as to elute enough a large enough amount of DNA and RNA. Figure 6 shows the results of the RNA purification, and “Mixture,” “Adsorption,” and “Elution” in this figure highlight the DNA and RNA content in the mixture before adsorption, the adsorption amount, and the elution amount, respectively. The RNA/DNA value of “Mixture” is 1, because there is an equal amount of DNA and RNA in the mixture. During the nucleic acid adsorption step, the DNA was mostly not adsorbed by the adsorbent (6.2 μg/mg). In contrast, the amount of RNA adsorbed was 95.2 μg/mg, and the RNA selectivity remained at 15.4, which is similar the value obtained by the maximum adsorption rate (RNA/DNA value = 15.7, as described in Section 3.3). The elution amount of the DNA and RNA in the 1.2 M NaCl buffer solution was 4.2 and 30.7 μg/mg, respectively. Moreover, in the agarose gel electrophoresis of free RNA and adsorbed RNA on silica, similar bands were observed, which indicates that the RNA did not degrade due to adsorption and elution on the silica surface (Fig. S8). From these results, we have determined that MCM-41c 12/1 can adsorb RNA from a mixture containing both DNA and RNA without a decrease in its RNA selectivity, and as such we suggest that MCM-41c 12/1 is a suitable material for RNA purification.

4. Conclusion

We synthesized amino-silica materials that had different particle morphologies, such as pore existence, particle size, surface amino group content, and pore diameter. We then investigated the effect of these characteristics on the materials’ RNA adsorption capacity and RNA adsorption ability. When DNA and RNA were adsorbed on these silicas, we found that MCM-41c, synthesized using a 12/1 TEOS/APTES ratio and having a particle size of 50 nm, was the best material for high RNA adsorption (272.6 μg/mg) and RNA/DNA value (11.1). Moreover, we managed to purify the RNA using MCM-41c 12/1. This silica material could adsorb RNA at a 15.7 RNA/DNA value from a mixture containing both DNA and RNA, and the RNA on the silica was easily eluted by a Tris-HCl buffer solution containing 1.2 M NaCl.

In this study, we determined the following about RNA adsorption capacity and selectivity: (1) Having mesopores on a silica material leads to an increased adsorption of both DNA and RNA, because this increases their specific surface area, and expanding the pore diameter further increases the amount of DNA that can be adsorbed; (2) since particle size greatly affects the RNA adsorption capacity, it is essential to control the particle size in order to enable
RNA selective adsorption; (3) Increasing the surface amino group content is related to increasing the nucleic acid adsorption capacities as a function of the specific surface area. Therefore, the specific surface area and surface amino content needs to be carefully set; (4) DNA and RNA on amino-MPS materials experience Langmuir adsorption model. In addition, DNA finds it difficult to enter mesopores and as such is mainly adsorbed on the surface of the particles, whereas RNA adsorption can occur inside the mesopores of an MPS.

Nevertheless, it is still unclear exactly how the particle size of silica and the density of amino group on the surface affect RNA selectivity. In our current work, we are looking to clarify the details of this mechanism by using amino-silica nanoparticles without mesopore.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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