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Development of a magnetic nanoparticle-based method for concentrating SARS-CoV-2 in wastewater

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ABSTRACT

Several virus concentration methods have been developed to increase the detection sensitivity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in wastewater, as part of applying wastewater-based epidemiology. Polyethylene glycol (PEG) precipitation method, a method widely used for concentrating viruses in wastewater, has some limitations, such as long processing time. In this study, Pegcision, a PEG-based method using magnetic nanoparticles (MNPs), was applied to detect SARS-CoV-2 in wastewater, with several modifications to increase its sensitivity and throughput. An enveloped virus surrogate, Pseudomonas phage φ6, and a non-enveloped virus surrogate, coliphage MS2, were seeded into wastewater samples and quantified using reverse transcription-quantitative polymerase chain reaction to assess the recovery performance of the Pegcision. Neither increasing MNP concentration nor reducing the reaction time to 10 min affected the recovery, while adding polyacrylic acid as a polyanion improved the detection sensitivity. The performance of the Pegcision was further compared to that of the PEG precipitation method based on the detection of SARS-CoV-2 and surrogate viruses, including indigenous pepper mild mottle virus (PMMoV), in wastewater samples (n = 27). The Pegcision showed recovery of 14.1 ± 6.3 % and 1.4 ± 1.0 % for φ6 and MS2, respectively, while the PEG precipitation method showed recovery of 20.4 ± 20.2 % and 18.4 ± 21.9 % (n = 27 each). Additionally, comparable PMMoV concentrations were observed between the Pegcision (7.9 ± 0.3 log copies/L) and PEG precipitation methods (8.0 ± 0.2 log copies/L) (P > 0.05) (n = 27). SARS-CoV-2 RNA was successfully detected in 11 (41 %) each of 27 wastewater samples using the Pegcision and PEG precipitation methods.
methods. The Pegcision showed comparable performance with the PEG precipitation method for SARS-CoV-2 RNA concentration, suggesting its applicability as a virus concentration method.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19) worldwide, first appeared in December 2019 in Wuhan, China. Several studies have reported that SARS-CoV-2 RNA is present in the stools of infected patients, including asymptomatic patients (Cevik et al., 2021; Chen et al., 2020; Cheung et al., 2020; Xiao et al., 2020). These findings led to the application of wastewater-based epidemiology as a potential tool to monitor COVID-19 incidence in the community by tracking the SARS-CoV-2 fate in the environment, including the wastewater circulation system (Ahmed et al., 2020a; Gonzalez et al., 2020; Kondinskaia and Gurtovenko, 2018; Wang et al., 2009). PEI has a high cationic charge density because every third atom is a potentially adsorptive polyallylamine, of which PEI is a well-known agent that binds nucleic acids because of its insolubility (Ata and Ingham, 1981). Although PEG precipitation is very convenient and inexpensive, it is time-consuming because of overnight incubation in a shaker at 4 °C (Lu et al., 2020).

Magnetic nanoparticles (MNPs) have been used in immunoassays to bind specific target molecules as well as biomagnetic separation (Huang et al., 2019; Wang and Lin, 2020; Zhou and Ramasamy, 2019). Biomagnetic separation gets much attention from various fields as it is highly specific and simple, and the basic resources for performing it are inexpensive (Safařík and Safaříková, 1999). Several modifications to MNPs have been conducted to increase immunoassay sensitivity and effectiveness of biomagnetic separation: an example is polymer coating (Nagaoka et al., 2011; Xie et al., 2009).

Pegcision is a virus concentration method based on biomagnetic separation using MNP coated with polymers (Fujita and Ohnishi, 2007). Viruses as phospholipid vesicles will bind to MNPs coated with cationic polymers to form complexes, which will be separated from a liquid portion using a magnetic separator. The liquid portion will be discarded, and the complexes in solid form will be recovered as a virus concentrate sample. Many cationic polymers have been used in gene delivery as agents, including polyethyleneimine (PEI), poly-L-lysine, polyvinylamine, and polyallylamine, of which PEI is a well-known agent that binds nucleic acids because of its high positive charge characteristics (Chertok et al., 2010; Kondinskaia and Gurtovenko, 2018; Wang et al., 2009). PEI has a high cationic charge density because every third atom is a potentially protonable amino nitrogen (Kircheis et al., 2001). PEI's high molecular weight has relatively high transfection efficiency, but it is also high in cytotoxicity (Wang et al., 2012). A high transfection efficiency expresses a good DNA or RNA delivery into the cell; therefore, a higher transfection efficiency means a higher amount of DNA or RNA that binds to the agent. A study reported that other cationic polymers that were derived from PEI and linked with diglycylidyl ester (Yu et al., 2015), such as diglycylidyl tartrate, diglycylidyl succinate, diglycylidyl malate, and PEG (Petersen et al., 2002; Zhang et al., 2008) gave higher transfection efficiency. Other studies also reported that adding polyacrylic acid (PAAc), a synthetic polyanion, to DNA and cationic polymer complexes can increase the transfection efficiency (Jiang et al., 2007; Trubetskoy et al., 2003).

The Pegcision method uses dextran magnetite-PEI (DM-PEI) as coated MNPs to increase the binding efficiency of viral complexes in wastewater samples, enabling biomagnetic separation. Adding PAAc to the samples is also considered to improve virus recovery. Pegcision is rapid and simple to conduct. Thus, it is expected to show higher throughput than other concentration methods. However, information about the recovery of SARS-CoV-2 and other viruses in wastewater using this method is unavailable.

Based on this background, this study aims to optimize the protocol of the Pegcision method for concentrating SARS-CoV-2 in wastewater, then evaluate the optimized Pegcision method by comparing its performance with a conventional PEG precipitation method to assess the applicability of this method to detect SARS-CoV-2 and other viruses (both enveloped and non-enveloped) as surrogates in wastewater.

2. Materials and methods

2.1. Wastewater samples

A total of nine grab influent wastewater samples were collected from three lines of two wastewater treatment plants (WWTPs) in Japan (n = 3 each) between September 13 and October 7, 2021. The samples were collected in autoclaved polyethylene bottles, transported to the laboratory on ice, and maintained at 4 °C until further analysis. In addition, 18 grab influent wastewater samples were also collected from another WWTP between March 9 and April 4, 2022. The sample bottles were kept in a freezer, transported to the laboratory frozen, and defrosted in a refrigerator at 4 °C for 1–2 days prior to further analysis.

2.2. Surrogate virus seeding

Pseudomonas phase φ6, a surrogate of enveloped viruses (Aquino De Carvalho et al., 2017), including SARS-CoV-2 and coliphage MS2, a surrogate of non-enveloped viruses (Ye et al., 2016), was used as a whole process control (Haramoto et al., 2018). Pseudomonas syringae (NBRC4084, National Institute of Technology and Evaluation (NITE), Tokyo, Japan) was used as a host strain for Pseudomonas phase φ6 (NBRC 105899, NITE), as described previously (Torii et al., 2021). For MS2 (ATCC 15597-B1), Salmonella typhimurium WG49 was used as a host strain. The initial concentrations of φ6 and MS2 stocks were approximately 10^{10} plaque-forming units (PFU)/mL and 10^{11} PFU/mL, respectively. The φ6 and MS2 stocks were diluted by 20-fold and 200-fold, respectively, before seeding into the samples. The seeded samples were mixed slowly by a rotator (Nichiryo, Koshigaya, Japan) at 30–40 rotations per minute (rpm) for 20–30 min to mimic the real sewage condition.

2.3. Synthesis of DM-PEI

DM-PEI was synthesized with two main steps: preparation of aldehyde group-introduced dextran magnetite and preparation of PEI 1,800-introduced dextran-coated magnetite, as stated in Fujita and Ohnishi (2007), with some modifications. A mixed solution of ferrous and ferric ions (molar ratio, 1:2) was prepared by dissolving 0.5 mmol of FeCl₂·4H₂O and 1.0 mmol of FeCl₃·6H₂O in a 5% (w/v) aqueous solution of dextran (FUJIFILM Wako Pure Chemicals, Osaka, Japan; average molecular weight, 32,000–45,000) at 65 °C. Under agitation, the mixed solution was...
supplemented with a 28 % (w/v) ammonia solution, agitated for 30 min, and cooled to room temperature. Centrifugal separation was then performed at 15,000 rpm for 30 min to eliminate the precipitates and the supernatant was neutralized by dialysis. The supernatant fluid was collected as the dextran-magnetite complex.

The PEI 1,800-introduced dextran-coated magnetite was prepared as described below. Sodium periodate was added to an aqueous solution of dextran-coated magnetic particles and stirred at 25 °C for 3 h to obtain aldehyde group introduced dextran-coated magnetic particles, and then an aqueous solution of PEI (molecular weight, 1,800) was added and stirred for 3 h, followed by an addition of sodium borohydride to form a solution of PEI 1,800-introduced dextran-coated magnetite. This solution was subjected to suction filtration under reduced pressure, and the resulting filtrate was subjected to dialysis using ion-exchanged water for two cycles, that consist of four times of 3-h dialysis and one time for 12 h. From this process, DM-PEI particles with a diameter of 107 ± 11 nm were obtained. The DM-PEI solution was stable under room temperature but preferably stored at 4 °C.

2.4. Virus concentration using Pegcision method

For Pegcision, 35-ml wastewater sample was added to a 50-ml tube and seeded with 35-μL diluted stock of φ6 and MS2. Then, 1.8-3.6 mL of cross-linked DM-PEI (6.07-6.5 mg/mL) was added to the sample to obtain a final concentration of 0.25 or 0.5 mg/mL (Table 1). The mixture of the sample and DM-PEI was mixed by gently shaking the tube. Then, 2.04-ml 2.0 M NaCl (Kanto Chemical, Tokyo, Japan) was added to the mixture sample to obtain a final concentration of 0.1 M. The effect of PAAc, an anionic polymer, was assessed by adding 0.96-4.16-ml PAAc (molecular weight, 5,000 and 25,000; 1 % (w/v); FUJIFILM Wako Pure Chemicals) to the sample to obtain final concentrations of 0.021 %, 0.042 %, and 0.084 % (w/v). A 6-ml PEG 8000 (Sigma-Aldrich, St. Louis, MO, USA) with an initial 50 % concentration (w/v) was added to reach a final concentration of 6.4 % (w/v). The mixture with a total volume of approx. 46 mL was incubated for 5 min after gently shaking. Then, it was placed in a magnetic separator for 20 min. The supernatant was carefully removed by pouring off, and the attached portion was recovered by adding 300-μL autoclaved MilliQ water (Merck Millipore, Tokyo, Japan) to obtain 1.0–1.5 mL of the concentrated sample. The virus loss in the supernatant in every 10, 20, 30, and 60 min was also calculated to determine the optimum reaction time in the magnetic separator.

2.5. Virus concentration using the PEG precipitation method

The selected samples were also concentrated using the PEG precipitation method to compare the performance between these two methods. The PEG precipitation method was conducted as described previously (Hata et al., 2021; Torii et al., 2021), with slight modifications by directly adding 4-g PEG 8000 and 2.35-g NaCl into 40-ml seeded sample without centrifugation, to final concentrations of 10 % (w/v) and 1.0 M, respectively. The mixture was incubated overnight at 4 °C, continuously mixing with a magnetic stirrer. Subsequently, the mixture was centrifuged at 10,000 × g for 30 min. The resulting supernatant was discarded, and the pellet was resuspended with 500-μL phosphate-buffered saline to obtain a concentrated sample.

2.6. RNA extraction

RNA extraction was conducted from 140 μL of the concentrated sample using a QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer’s protocol. For the Pegcision method, several additional steps were conducted. Briefly, 560-μL Buffer AVL (viral lysis buffer) was added to 140-μL concentrated sample from the Pegcision method and incubated for 10 min. Then, the mixture was put in a magnetic separator for 10 min, and the supernatant was recovered to continue to the next step of RNA extraction to obtain a total of 60-μL viral RNA. Another condition was also applied by adding PAAc in RNA extraction (before adding Buffer AVL) to see any synergistic or even antagonistic recovery effect. A 3-μL PAAc was added to 140-μL concentrated sample in a 1.5-ml tube, followed by 10-min incubation. The mixture was then subjected to a magnetic separator for 10 min, and the supernatant was discarded, followed by adding 560-μL Buffer AVL to the solid portion. Similarly, as described previously, the mixture was put in a magnetic separator for 10 min, and the supernatant was used for further RNA extraction processes.

2.7. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

A 30-μL viral RNA was proceeded to RT to obtain 60-μL cDNA using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer’s protocol. CDC-N1 and CDC-N2 assays (Centers for Disease Control and Prevention, 2020), where both probes were labeled with FAM reporter dye, were combined and used as a qPCR assay for targeting SARS-CoV-2 RNA to increase the detection in the sample. In addition to φ6 (Gendron et al., 2010) and MS2 (Friedman et al., 2011), pepper mild mottle virus (PMMoV), an indigenous and the most abundant virus in wastewater (Kitajima et al., 2018), was determined using RT-qPCR (Haramoto et al., 2013; Zhang et al., 2006). In qPCR, 2.5-μL cDNA was mixed with 22.5-μL qPCR mixture containing 12.5-μL probe qPCR Mix with UNG (Takara Bio, Kusatsu, Japan), 0.1-μL each forward and reverse primers (100 μM), 0.05-μL probe (100 μM), and PCR-grade water for the remaining volume. qPCR thermal conditions were conducted as follows: initial incubation at 25 °C for 10 min and denaturation at 95 °C for 30 s, followed by 45 cycles of denaturation at 95 °C for 5 s and primer annealing and extension reaction at 60 °C for 30 s (CDC-N1N2 and φ6) or at 60 °C for 60 s for PMMoV. For MS2, primer annealing and extension reaction was conducted at 56 °C for 60 s.

The SARS-CoV-2 Detection RT-qPCR Kit for Wastewater (Takara Bio) was used to detect SARS-CoV-2, φ6, and PMMoV in the wastewater samples that had been collected between March and April 2022: the CDC-N1N2 assay using Cy5-labeled probes was used for SARS-CoV-2 RNA detection, whereas φ6 and PMMoV RNA were detected in a duplex RT-qPCR using HEX- and FAM-labeled probes, respectively. Better SARS-CoV-2 RNA detections were observed using this one-step RT-qPCR kit than two-step RT-qPCR described above, while comparable performance of φ6 and PMMoV was observed (data not shown). In the one-step RT-qPCR, 25.0 μL of an RT-qPCR mixture containing 5.0 μL of the RNA extract, 2.5 μL of a mixture of primers and probe, 12.5 μL of one-step RT-qPCR mix, and 5.0 μL of RNase-free water. Thermal conditions of qPCR were as follows: at 25 °C for 10 min, at 52 °C for 5 min, 95 °C for 10 s, and 45 cycles of at 95 °C for 5 s and at 60 °C for 30 s.

Five or six of gBlocks (Integrated DNA Technologies, Coralville, IA, USA) or Positive Control DNA included in the one-step RT-qPCR kit were used with 10-fold serial dilutions (concentration ranged from 5.0 × 10^9 to 5 × 10^8 copies/reaction) to obtain a standard curve. Negative control was also included in every qPCR run to confirm no contamination in the reagents. All samples, including standards and negative control, were performed in duplicate. Threshold cycle (Ct) values above 40 were counted as negative.

Table 1 Example of conditions of reagents in Pegcision method.

| Component | Volume added (mL) | Initial concentration | Desired concentration |
|-----------|------------------|-----------------------|----------------------|
| Sample    | 35.0             | –                     | –                    |
| DM-PEI    | 1.8              | 6.5 mg/mL             | 0.25 mg/mL           |
| NaCl      | 2.04             | 2 M                   | 0.1 M                |
| PAAc 25,000 | 1.96           | 1 % (w/v)             | 0.042 % (w/v)        |
| PEG 8000  | 6.0              | 50 % (w/v)            | 6.4 % (w/v)          |
2.8 Statistical analysis

A paired t-test was used to compare the mean recovery of targeted viruses using the PEG precipitation and the Pegcision method using Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA) with a significant value set at 0.05. $P < 0.05$ was considered significant.

3. Results and discussion

3.1 Optimization of DM-PEI concentration

SARS-CoV-2 detection in wastewater using different DM-PEI concentrations (0.25 and 0.5 mg/mL) was assessed to establish the optimum condition. The result showed no detection of indigenous SARS-CoV-2 at either DM-PEI concentrations. Thus, q6 recovery was used to determine the optimum DM-PEI concentration. Based on q6 recovery, comparable result was observed (paired t-test, $P > 0.05$): 9.5 %–11.5 % and 10.9 %–11.3 % (n = 4 each) using DM-PEI concentrations of 0.25 and 0.5 mg/mL, respectively. Since DM-PEI with a 0.5-mg/mL concentration was more turbid because more MNPs were present, it became more difficult to get the concentrated sample. Therefore, DM-PEI with a 0.25-mg/mL concentration was used for further Pegcision method.

3.2 Optimization of PAAc conditions

Adding PAAc with molecular weights of 5,000 and 25,000 (0.042 % (w/v)) was assessed to determine which condition is better to detect SARS-CoV-2 in wastewater samples. As shown in Table 2, since adding PAAc 25,000 showed better detection of indigenous SARS-CoV-2 than PAAc 5,000, PAAc 25,000 was used for Pegcision protocol. Additional PAAc treatment in RNA extraction was also assessed under three conditions: PAAc 5,000, PAAc 25,000, and without adding PAAc. Concentrated samples that used PAAc 25,000 were subjected to a further modified RNA extraction step when additional PAAc was added. As shown in Table 3, adding PAAc in RNA extraction step decreased the sensitivity in the detection of SARS-CoV-2 RNA, suggesting that there are some antagonistic effects of adding PAAc in both virus concentration and RNA extraction steps.

Other experiments were also conducted to determine the optimum PAAc concentration. As shown in Table 4, adding PAAc 25,000 with a concentration of 0.042 % gave the best result for detecting SARS-CoV-2 RNA in the samples (100 % (16/16) positive wells of eight samples in qPCR). A previous study (Tripathi et al., 2016) reported that a higher amount or concentration of PAAc did not affect the release pattern of DNA or RNA from the complexes, which is different from what was observed in this study. After confirming the best combination, adding PAAc 25,000 with a concentration of 0.042 % in the virus concentration step was selected as the optimum condition for the Pegcision method.

Several other polyanions beside PAAc can be used in this method as long as it consists of at least one acid structure from the group of carboxylic acid, phosphoric acid, sulfuric acid, and boric acid, such as poly(carboxymethylstirene), hyaluronic acid, carboxymethyl cellulose, carboxymethyl dextran, polyphosphoric acid, and polystyrylboric acid (Fujita and Ohnishi, 2007). Further studies are needed to assess the applicability of these polyanions in the Pegcision method.

### Table 2
Detection of SARS-CoV-2 RNA with different molecular weights of PAAc.

| Performance characteristic | Without PAAc | PAAc 5,000 | PAAc 25,000 |
|----------------------------|-------------|-----------|-------------|
| No. of positive wells/no. of tested wells (% positive) | 0/4 (0) | 1/4 (25) | 4/4 (100) |
| Ct value                   | Not available | 37.0 | 36.3–37.6 |
| Concentration (log copies/L) | <3.1 | 5.6 | 5.3–5.8 |

### Table 3
Detection of SARS-CoV-2 RNA with addition of PAAc in RNA extraction process.

| Performance characteristic | Without PAAc | PAAc 5,000 | PAAc 25,000 |
|----------------------------|-------------|-----------|-------------|
| No. of positive wells/no. of tested wells (% positive) | 4/4 (100) | 2/4 (50) | 0/4 (0) |
| Ct value                   | 36.3–37.6 | 35.7 | Not available |
| Concentration (log copies/L) | 5.3–5.8 | 5.9 | <3.1 |

### Table 4
Detection of SARS-CoV-2 RNA with different concentrations of PAAc.

| Performance characteristic | 0.021 % | 0.042 % | 0.084 % |
|----------------------------|---------|---------|---------|
| No. of positive wells/no. of tested wells (% positive) | 15/16 (94) | 16/16 (100) | 13/16 (81) |
| Ct value                   | 33.5–37.0 | 32.7–36.6 | 35.5–38.4 |
| Concentration (log copies/L) | 5.1 ± 0.4 | 5.3 ± 0.4 | 4.7 ± 0.3 |

3.3 Viral loss and optimum reaction time

Because of the low concentration of indigenous SARS-CoV-2 in wastewater, q6 and MS2 along with PMMoV concentration in discarded liquid portions were calculated in several timeframes to optimize reaction time between viral complexes and magnet in the magnetic separator. As shown in Fig. 1, the complexes have already attached to the magnet within the first 10–20 min. Moreover, the viral concentrations in the liquid portion (counted as virus loss) were insignificant (<1 %), compared to those recovered in the solid portion, shown as a horizontal straight line in Fig. 1. It is very beneficial and practical since using this method reduces virus concentration step time consumption (up to only 30 min).

3.4 Detection of SARS-CoV-2 RNA in wastewater

Tables 5 and 6 show the results of SARS-CoV-2 RNA detection in influent wastewater samples using the Pegcision and PEG precipitation methods. Using the two-step RT-qPCR, the Pegcision method successfully detected SARS-CoV-2 RNA in four (44 %) of the nine wastewater samples, while the PEG precipitation method detected it in five samples (56 %). In addition, by using the one-step RT-qPCR, the Pegcision method could detect SARS-CoV-2 RNA in eight (44 %) of the 18 wastewater samples, while the PEG precipitation method detected it in six samples (33 %). Moreover, the result of qualitative detection of SARS-CoV-2 RNA by the Pegcision method and the PEG precipitation method...
matched in 14 (78%) of the 18 samples (5 positive and 9 negative results) (Table 6). This means that the Pegcision method has comparable performance with that of the PEG precipitation method, and this suggests its applicability. Additionally, the Pegcision method requires lesser time to process the samples (no need for overnight incubation) than the PEG precipitation method. Several rapid concentration methods usually take <30 min of processing time: simple centrifugation (Kaya et al., 2022; Zheng et al., 2022) and automated direct filtration (Gonzalez et al., 2020; Juel et al., 2021; Kevill et al., 2022) have successfully detected SARS-CoV-2 RNA in wastewater. However, many of such concentration methods require solid and liquid phase separation using a centrifuge, where high initial cost is needed to conduct those methods. The Pegcision method itself doesn’t need a centrifuge, as a whole wastewater sample can be directly subjected to biomagnetic separation step.

The low detection of SARS-CoV-2 RNA in wastewater samples for both methods during the September 2021 sampling period might be caused by decreasing daily cases or infected patients in the city where WWTPs are located, compared with the peak at the end of August 2021. During that sampling period, 3.3–4.1 daily cases per 100,000 populations were reported in prefectures where WWTPs were located. As shown in Fig. 2, a different situation was observed as more positive samples were detected during the March 2022 sampling period (4.3–56.5 COVID-19 daily cases per 100,000 populations) since the new Omicron variant was emerging in early 2022. This study used whole raw samples in the PEG precipitation method because several studies reported that SARS-CoV-2 was present mostly in solid portions of wastewater (DAoust et al., 2021; Kitamura et al., 2021; Torii et al., 2022).

### 3.5. Recovery of other targeted viruses

As a concentration method, the Pegcision method should efficiently recover other types of viruses, including both enveloped and non-enveloped viruses. Since φ6 is an enveloped virus and MS2 is a non-enveloped virus, the recoveries of these two viruses are quite important to assess this method’s applicability in recovering other novel viruses in the future. As shown in Fig. 3, average φ6 recovery using the Pegcision method in wastewater samples was 14.1 ± 6.3% (n = 27), while for the PEG precipitation, it was 20.4 ± 20.2% (n = 27). This result was quite different from previous studies (Torii et al., 2021, 2022) that showed φ6 recovery of less than −1.0 log (10%) using the PEG precipitation method, followed by RNA extraction using QIAamp Viral RNA Mini Kit. The difference between these studies is the raw sample condition. In this study, raw samples were processed without particle separation, while the previous studies did particle separation by centrifugation before being processed to the PEG precipitation method. Thus, similarly to SARS-CoV-2, φ6 was also considered present in wastewater by attachment to the suspended solid or solid portion. There was insignificant difference in φ6 recovery between Pegcision and PEG precipitation methods (n = 27; paired t-test, P > 0.05). PMMoV RNA concentrations using the Pegcision method were 7.9 ± 0.3 log copies/L (n = 27), while those by PEG precipitation were 8.0 ± 0.2 log copies/L (n = 27), showing that insignificant difference was also observed in PMMoV concentrations between the two virus concentration methods (n = 27; paired t-test, P > 0.05).

The PEG precipitation method showed significantly higher recovery of MS2 (18.4 ± 21.9%; n = 27) than the Pegcision method (1.4 ± 1.0%;
n = 27) (paired t-test, P < 0.05). Since non-enveloped viruses are not surrounded by the outer layer of envelop, which consist of phospholipids, it is plausible that the MNPs could not bind to the viruses effectively. But as described in the previous reaction time result, the virus loss of MS2 in the discarded liquid portion was very low compared with its overall recovery. It means that there is a possibility that MS2 still binds tightly in MNP-polymer complexes and cannot be recovered. Contrastly, PMMoV, as a non-enveloped virus, gave comparable concentration in the samples between the Pegcision and PEG precipitation methods. An explanation is the abundance enveloped virus, which is quite different, can lead to unknown binding mechanisms with MNPs. Therefore, it is recommended to test for another non-enveloped virus to see its applicability as a virus concentration method.

4. Conclusion

In this study, the Pegcision method was optimized based on DM-PEI concentrations, PAAc conditions, and reaction time in a magnetic separator. The best protocol was established using DM-PEI with a concentration of 0.25 mg/mL, PAAc 25,000 with a concentration of 0.042 % (w/v), and a reaction time of 10 min in the magnetic separator. SARS-CoV-2 RNA was successfully detected in 11 of 27 influent wastewater samples using each the Pegcision and PEG precipitation methods. As for other targeted viruses, the Pegcision has comparable performance in φ6 and PMMoV recovery in wastewater, but not in MS2 recovery, suggesting other non-enveloped virus recovery experiments to see this method application for non-enveloped viruses. This study showed that the Pegcision has the potential to serve as a virus concentration method since this method is less time-consuming than the PEG precipitation method. Further improvements are needed to optimize the virus recovery by this method.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ayame Kitano, Xiaomao Xie, Hiroshi Saitoh, and Noriyuki Ohnishi are employees of the JNC Corporation.

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