Size-Selective Material Adsorption Property of Polymeric Nanoparticles with Projection Coronas

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We prepared irregularly shaped nanoparticles with carboxyl groups on the surface of nanoparticles in a "confetti"-like arrangement by dispersion copolymerization in the presence of the functional macromonomers methacryloyl-endcapped poly(methacrylic acid) (PMAA), poly(ethylene glycol) monomethoxymonomethacrylate (PEGm) with styrene (St), and acrylonitrile (AN), in the presence of 2,2′-azobis(isobutyronitrile) (AIBN) in a polar solvent. Concanavalin A (Con A) can be immobilized on the surface of these confetti nanoparticles, which showed a higher efficiency of Con A immobilization than the corresponding spherical nanoparticles (PMAA−PEGm−St nanoparticles and PMAA−St nanoparticles). Moreover, these Con A-immobilized confetti nanoparticles were able to capture HIV-1 virions (100 nm), even in the presence of erythrocytes (10 μm). This size-selective material adsorption property of the confetti nanoparticles is expected to be useful for various separation materials by changing the projection morphologies.

Introduction

The control of the surface properties and shape of polymeric nanoparticles are of key importance for their biocompatibility, which depends strongly on their interactions with proteins, cells, and tissues. The surface modification of nanoparticles using water-soluble and nonionic polymer such as poly(ethylene glycol) (PEG) can create a bioinert surface, and these nanoparticles are used in biomedical applications. PEG is known to be resistant to the biological adsorption of proteins, cells, tissues, etc. PEG has been introduced onto biomaterial surfaces through grafting[1] by simple surface treatments based on primary adsorption[2] or secondary adsorption[3] and also through bulk incorporation via cross-linking[4] or block copolymerization[5] to develop various biomaterials and biointerfaces.[6,7] Recently, the surprising effects of PEG chains were reported. Lazos et al., Albertorio et al., and others reported size-selective protein adsorption by controlling the density, molecular weight, and conformation of PEG chains on the surface of a substrate.[8−11] This size-selective protein adsorption was effective for separating various biomaterials.

On the other hand, polymeric nanoparticles have also been investigated as a separation material. In particular, polymeric nanoparticles with specific functional groups on their surface were applied to various fields as material capture carriers[12] and as drug carriers.[13]

Previously, we prepared polymeric nanoparticles with various functional groups on their surfaces by the dispersion copolymerization of hydrophilic macromonomers such as PEG and poly(methacrylic acid) (PMAA), and hydrophobic monomers such as styrene (St), in a polar solvent.[14,15] The surface of the prepared polymeric nanoparticles was able to immobilize various materials such as proteins, lectins, and metals.[16,17] In addition, we previously succeeded in capturing human immunodeficiency virus type 1 (HIV-1) by using lectin-immobilized nanoparticles.[12] Intravaginal immunization with inactivated HIV-1 captured nanoparticles induced

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HIV-1 specific IgA antibodies in the murine genital tract, whereas a HIV-1 specific IgA response was not detectable when they were immunized with HIV-1 alone.[18]

Recently, polymeric nanoparticles upon which nanoprojections were uniformly distributed over the whole surface were prepared using a one-step preparation method by the dispersion radical copolymerization of St, AN, and PEGm.[19,20] These nanoparticles were named "confetti" nanoparticles because their shape was just like "confetti". The morphology of confetti nanoparticles was successfully controlled by changing the polymerization conditions such as the polymerization period, the feed monomer composition, and solvent.[21]

If a functional group is introduced onto the surface of confetti nanoparticles, a higher material sorbability than spherical nanoparticles of the same size may be achieved because their projections give a larger specific surface area. Furthermore, the material sorbability of confetti nanoparticles was expected to be controllable because the PEG density of the naonparticle surface depended on the nanoparticle shape.

In this paper, we introduced functional groups such as a carboxyl group on the surface of confetti nanoparticles using the dispersion radical polymerization of PMAA macromonomers, PEGm, St, and AN in ethanol/water media. The material adsorption properties of these confetti nanoparticles were compared with spherical nanoparticles of the same size by performing lectin adsorption tests, HIV-1 capture experiments, and hemagglutination. Furthermore, the HIV-1 capture experiment was performed using three kinds of nanoparticles in the presence of erythrocytes and HIV-1 virions. As a result, we observed size-selective material adsorption properties using confetti nanoparticles.

Experimental Section

Materials. Styrene (St, WAKO Pure Chemical Ind. Ltd.) and acrylonitrile (AN, WAKO Pure Chemical Ind. Ltd.), which were used as monomers, were distilled in vacuo just before use. Poly(ethylene glycol) monomethoxymonomethacrylate (PEGm, number-average molecular weight $M_n = 1740$) was donated by Nippon Oil and Fats Co. and was used as received. The synthetic preparation of methacryloyl-endcapped poly(methacrylic acid) (PMAA) has been described previously.[15] 2,2′-Azobis(isobutyronitrile) (AIBN, WAKO Pure Chemical Ind. Ltd.), which was used as a radical initiator, was recrystallized from ethanol before use. A mixture of distilled water and ethanol (WAKO Pure Chemical Ind. Ltd.) was used as the polymerization medium.

Concanavalin A (Con A) was purchased from Vector Laboratories (Burlingame, CA). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (water-soluble carbodiimide, WSC) was purchased from WAKO Pure Chemical Ind. Ltd. Sodium chloride (NaCl), potassium chloride (KCl), potassium dihydrogenphosphate (KH_2PO_4), and disodium hydrogenphosphate (Na_2HPO_4) were purchased from WAKO Pure Ind. Ltd., and were used without further purification. Ninhydrin, hydrindantine, acetic acid, and sodium acetate were purchased from WAKO Pure Ind. Ltd. and were used without further purification.

Fresh erythrocytes were supplied by Mr. Hamada (27 years old, Osaka University) in Venoject II (7 mL). The erythrocytes were washed in phosphate buffer solution (PBS) and centrifuged three times for 5 min at 2000 rpm. The IIIB type of HIV-1 was used in these experiments. The virus was propagated in MOLT-4 cells. The infected cells were cultured in a RPMI-1640 medium (Nikken Biomedical Laboratory, Kyoto, Japan) supplemented with 10% heat-inactivated fetal calf serum and antibiotics (culture medium), and their culture supernatants were centrifuged at 3000 rpm for 10 min, filtered, and stored at −80 °C until just before use. Heat-treated suspensions were examined for infectivity in MT-4 cells as previously described.[18] Colloidal gold-conjugated anti-gp120 monoclonal antibody was purchased from ImmunoDiagnostics, Bedford, MA, and used without further purification.

Preparation of Nanoparticles. *(i) Confetti Nanoparticles (PMAA−PEGm−St−AN).* PMAA macromonomer, PEGm, St, AN, and AIBN (1 mol % of the total monomer concentration) were added to the ethanol/water (4/1 = v/v, 5 mL) mixture. Each polymerization batch was prepared in a glass tube and was repeatedly degassed by freeze−thaw cycles in a vacuum apparatus, sealed off, and then placed in an incubator at 60 °C for 24 h. The resulting solutions were first dialyzed in ethanol using a cellulose dialyzer tube to remove any unreacted monomer. The nanoparticles were then centrifuged and redispersed in water.

(ii) Double Coronas Spherical Nanoparticles (PMAA−PEGm−St Nanoparticles). PMAA macromonomer (0.016 mmol), PEGm (0.023 mmol), St (3.1 mmol), and AIBN (1 mol % of the total monomer concentration) were added to the ethanol/water (4/1 = v/v, 5 mL) mixture. Each polymerization batch was prepared in a glass tube and was repeatedly degassed by freeze−thaw cycles in a vacuum apparatus, sealed off, and then placed in an incubator at 60 °C for 24 h. The resulting solutions were first dialyzed in ethanol using a cellulose dialyzer tube to remove any unreacted monomer. The nanoparticles were then centrifuged and redispersed in water.

(iii) Single Corona Spherical Nanoparticle (PMAA−St Nanoparticles). PMAA macromonomer (0.043 mmol), St (2.8 mmol), and AIBN (1 mol % of the total monomer concentration) were added to the ethanol/water (4/1 = v/v, 5 mL) mixture. Each polymerization batch was prepared in a glass tube and was repeatedly degassed by freeze−thaw cycles in a vacuum apparatus, sealed off, and then placed in an incubator at 60 °C for 24 h. The resulting solutions were first dialyzed in ethanol using a cellulose dialyzer tube to remove any unreacted monomer. The nanoparticles were then centrifuged and redispersed in water.

Immobilization of Con A onto Nanoparticles. The carboxyl group of the nanoparticles (confetti nanoparticles, PMAA−PEGm−St nanoparticles, and PMAA−St nanoparticles; 5 mg) was first activated by WSC (10 wt % in 0.05 M KH_2PO_4) for 20 min. The nanoparticles that were centrifuged were mixed with Con A in phosphate buffer solution (PBS), and the mixture was kept at 4 °C for 24 h. After the reaction, the centrifuged nanoparticles were repeatedly washed. The amount of immobilized Con A was evaluated by means of the ninhydrin method.

Hemagglutination. Con A-immobilized nanoparticles (5 mg/mL) were mixed with the erythrocyte dispersion solution (final erythrocyte concentration: 4 vol %) to a final nanoparticle concentration of 1.25 mg/mL. The mixture solution was incubated for 6 h at room temperature. After 6 h, the Con A activity was evaluated by observing the supernatant.

Capture of HIV-1 Using Con A-Immobilized Nanoparticles. Con A-immobilized nanoparticles (amount of immobilized Con

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Scheme 1. Molecular Structure of the Poly(styrene-*co*-acrylonitrile)-*co*-PMAA Macromonomer-*co*-PEGm

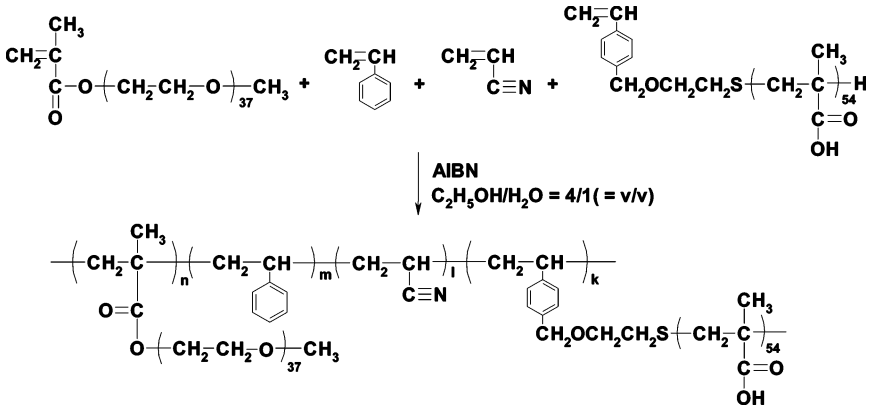


Table 1. Synthesis and Characterization of Poly(styrene-*co*-acrylonitrile)-*co*-PEGm-*co*-PMAA Macromonomer[a]

	PEGm[b]		PMAA[c]		St	AN	d_m[d]	S.D.[e]	C.V.[f]	yield
run	M_n	(mmol)	M_n	(mmol)	(mmol)	(mmol)	(nm)	(nm)	(%)	(%)
1	1740	0.035	4800	3.9×10^{-3}	1.3	2.6	410	148	36.2	38.7
2	1740	0.031	4800	7.8×10^{-3}	1.3	2.6	480	167	34.8	45.9
3	1740	0.027	4800	1.2×10^{-2}	1.3	2.6	550	167	30.3	53.8
4	1740	0.023	4800	1.6×10^{-2}	1.3	2.6	600	135	22.5	44.2

[a] Reaction time, 24 h; temperature, 60 °C; solvent, water/ethanol = 1/4. [b] Run 1 = 0.9 mol % to St and AN; run 2 = 0.8 mol % to St and AN; run 3 = 0.7 mol % to St and AN; run 4 = 0.6 mol % to St and AN. [c] Run 1 = 0.1 mol % to St and AN; run 2 = 0.2 mol % to St and AN; run 3 = 0.3 mol % to St and AN; run 4 = 0.4 mol % to St and AN. [d] Mean diameter: particle diameter was estimated by DLS. [e] S.D. = standard deviation. [f] C.V. = S.D./d_m; coefficient of variation.

A: 35 μg/mg) were mixed with the inactivated HIV-1 suspensions to a final nanoparticle concentration of 1.25 mg/mL. After 6 h of incubation at room temperature, the mixture was centrifuged at 14000 rpm for 10 min. The supernatants were collected, and the residual p24 antigen levels were determined by a p24 antigen capture enzyme-linked immunosorbent assay (ELISA) kit (ZeptoMetrix Corporation, Buffalo, NY).

Capture of HIV-1 Using Con A-Immobilized Nanoparticles in Erythrocyte Dispersion Solution. Con A-immobilized nanoparticles (amount of immobilized Con A: 35 μg/mg) were mixed with the inactivated HIV-1 and erythrocyte suspensions to a final nanoparticle and erythrocyte concentration of 1.25 mg/mL and 4 vol %, respectively. After 6 h of incubation at room temperature, the mixture was centrifuged at 2000 rpm for 5 min to remove the erythrocytes. The supernatants were then collected. The collected supernatant solution was centrifuged at 14000 rpm for 10 min, and the residual p24 antigen levels were determined by a p24 antigen capture enzyme-linked immunosorbent assay (ELISA) kit (ZeptoMetrix Corporation, Buffalo, NY).

Aggregation Behavior of Con A-Immobilized Nanoparticles. Con A-immobilized nanoparticles (amount of immobilized Con A: 35 μg/mg) were mixed with the inactivated HIV-1 and erythrocyte suspensions to a final nanoparticle and erythrocyte concentration of 1.25 mg/mL and 4 vol %, respectively. After mixing, the absorbance of the dispersion solution was measured in a quartz cell with a Jasco V-550 UV/vis spectrophotometer.

Measurements. Fourier transformation infrared (FT-IR) spectra of the nanoparticle surface were recorded by the attenuated total reflection (ATR) method on a Perkin-Elmer Spectrum one FT-IR spectrometer after 16 scans (4 cm^{-1} resolution) over a range of 2500−1000 cm^{-1}.

Scanning electron microscopy (SEM) images were obtained with a JSM-6700F microscope operated at an acceleration voltage of 15.0 kV. The specimens were prepared by the slow evaporation of a drop of approximately diluted solution deposited onto a brass stage, followed by osmium spattering.

Transmission electron microscopy (TEM) images were obtained with an Hitachi H-700H microscope operated at an acceleration voltage of 80 kV. The specimens were prepared by the slow evaporation of a drop of approximately diluted solution deposited onto a collodion-coated copper mesh grid followed by carbon spattering.

Dynamic light scattering (DLS) measurements were performed with a BECKMAN COULTER Laser Diffraction Particle Size Analyzer LS 13-320.

Zeta potential measurements were performed with a Zetasizer 3000 (Malvern Instrument, UK).

UV−vis measurements were performed with a Jasco V-550 UV/vis spectrophotometer (λ = 500 nm).

Results and Discussion

Confetti nanoparticles with carboxyl groups on their surfaces were prepared by the dispersion radical copolymerization of PMAA macromonomer, PEGm, St, and AN in the presence of AIBN in an ethanol/water (v/v = 4/1) mixed solvent at 60 °C for 24 h (Scheme 1).

The in feed monomer ratio was summarized in Table 1. To study the effects of the in feed macromonomer ratio on nanoparticle morphology, the in feed monomer ratio of St and AN was kept constant. The amount of in feed PMAA macromonomer was adjusted over a range of 0.1−0.4 mol % of the hydrophobic monomer (St and AN) concentration. The amount of in feed PEGm was also adjusted over a range of 0.9−0.6 mol % of the hydrophobic monomer (St and AN) concentration.

Figure 1 shows SEM images of the nanoparticles. It was confirmed that projections were formed over the surface of the nanoparticles. Moreover, the nanoparticle size was found to be almost uniform from the results of the dynamic light scattering (DLS) measurements (Table 1). Increasing the in feed PMAA macromonomer composition increased the particle size. This result suggests that a larger core was formed because the solubility of the PMAA macromonomer

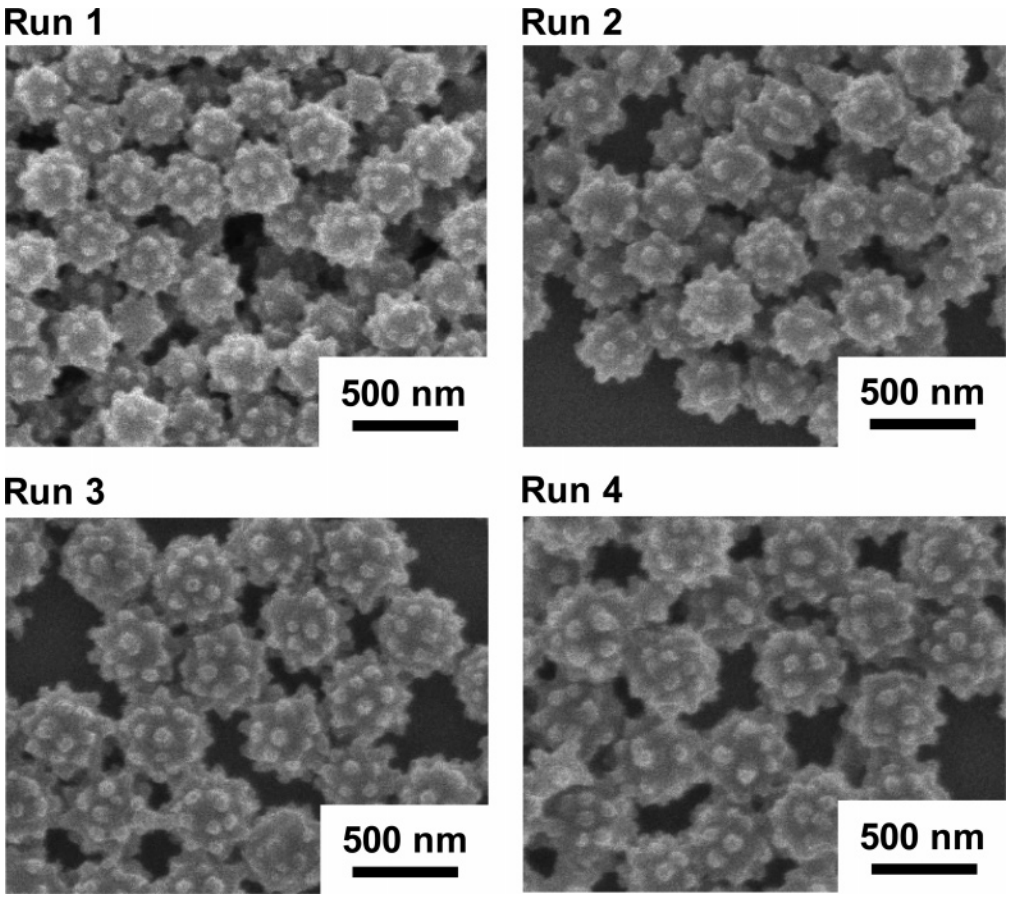


Figure 1. SEM images of nanoparticles composed of poly(styrene-*co*-acrylonitrile)-*co*-PMAA macromonomer-*co*-PEGm (Run 1: PMAA = (St + AN) × 0.1 mol %, PEGm = (St + AN) × 0.9 mol %; Run 2: PMAA = (St + AN) × 0.2 mol %, PEGm = (St + AN) × 0.8 mol %; Run 3: PMAA = (St + AN) × 0.3 mol %, PEGm = (St + AN) × 0.7 mol %; Run 4: PMAA = (St + AN) × 0.4 mol %, PEGm = (St + AN) × 0.6 mol %).

was lower than the PEGm in the mixed solvent (ethanol/water = 4/1 = v/v). We previously reported the same result that increasing the hydrophobic macromonomer composition increased the particle size.[14] The projection size on the surface of the nanoparticles was not changed, even if the size of the nanoparticles was increased. This result is similar to a previous report.[20] Moreover, the projections were not formed on the surface of the nanoparticles when the polymerization was performed in a PMAA macromonomer composition of more than 0.5 mol % (not shown). This result shows that a PEGm of more than 0.5 mol % was necessary to prepare nanoparticles with projection coronas.

The resultant confetti nanoparticles were measured by the FT-IR/ATR and zeta potential methods to confirm whether the carboxyl group had been introduced onto the surface of nanoparticles.

Figure 2 shows the FT-IR/ATR spectrum of these confetti nanoparticles. The confetti nanoparticles showed distinct absorption spectra representing the stretching frequency of the carboxyl groups at 1720−1730 cm^{-1}. This shows that the PMAA macromonomer had been successfully introduced into the nanoparticles. The peak strength of the carboxyl groups increased with an increase in the amount of in feed PMAA macromonomer. This result shows that the PMAA macromonomer introduction ratio increased as the amount of in feed PMAA macromonomer increased. Furthermore, the stretching frequency of C≡N derived from AN appeared at 2240 cm^{-1}, showing that AN had also been successfully introduced into the nanoparticles.

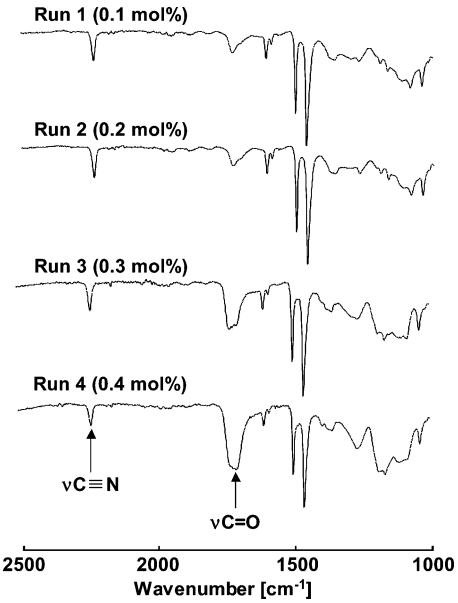


Figure 2. FT-IR/ATR spectra of poly(styrene-*co*-acrylonitrile)-*co*-PMAA macromonomer-*co*-PEGm.

Table 2 shows the results of the zeta potential measurement of the prepared confetti nanoparticles. All of the nanoparticles had a negative charge. This result showed that the carboxyl group had been introduced at the surface of the nanoparticles.

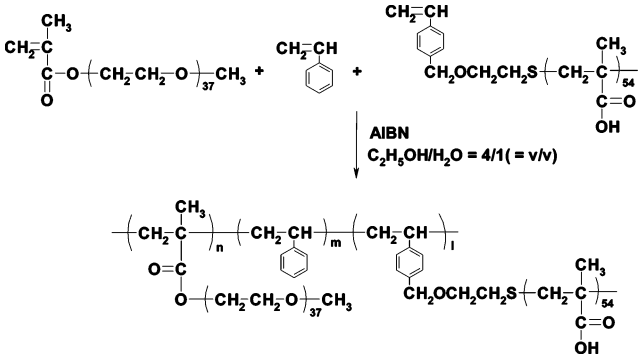
Consequently, it was confirmed that the prepared confetti nanoparticles had carboxyl groups on their surfaces based

Table 2. Zeta Potential of Poly(styrene-*co*-aclyronitrile)-*co*-PMAA Macromonomer-*co*-PEGm[a]

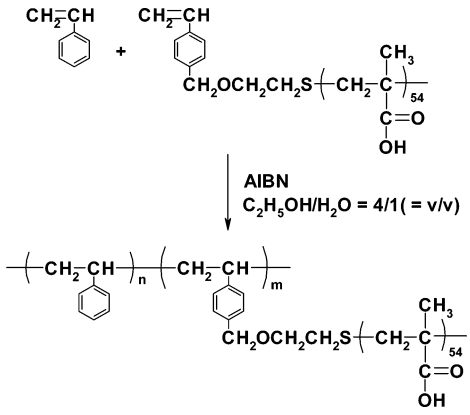
	run 1	run 2	run 3	run 4
zeta potential (mV)	−31.2	−32.3	−29.4	−31.5

[a] The solvent was PBS.

Scheme 2. Molecular Structure of the Polystyrene-*co*-PMAA Macromonomer-*co*-PEGm



Scheme 3. Molecular Structure of the Polystyrene-*co*-PMAA Macromonomer



on the results of the FT-IR/ATR and zeta potential measurements. Confetti nanoparticles with carboxyl groups on their surfaces can absorb various materials such as proteins and peptides. Furthermore, the material adsorption properties of confetti nanoparticles are expected to improve because the specific surface area increases more than a spherical nanoparticle of the same size.

Spherical nanoparticles of the same size (Table 1, Run 4; 410 nm) and same surface composition as the "confetti" nanoparticles were prepared to estimate the difference in their material adsorption properties. Two types of spherical nanoparticles with double corona chains (PMAA macromonomer and PEGm) (Scheme 2) and a single corona (PMAA macromonomer) (Scheme 3) were prepared. Spherical nanoparticles with two coronas were prepared by the dispersion copolymerization of PMAA macromonomer, PEGm, and St in the presence of AIBN in an ethanol/water mixed solvent at 60 °C for 24 h (PMAA−PEGm−St nanoparticles). Spherical nanoparticles with a single corona were also prepared using the PMAA macromonomer and St by a similar method (PMAA−St nanoparticles).[15] Nanoparticles with the same size as the confetti nanoparticles were prepared by optimizing the in feed monomer composition.

Table 3. Amount of Carboxyl Acid Groups on the Surface of Nanoparticles

confetti (mol/g)	PMAA−PEGm−St (mol/g)	PMAA−St (mol/g)
3.60×10^{-4}	3.10×10^{-4}	12.2×10^{-4}

Figure 3 shows SEM images of the resultant nanoparticles. It was confirmed that the prepared nanoparticles had the same size as the confetti nanoparticles based on the SEM images and DLS measurements. Furthermore, it was confirmed that the carboxyl group had been introduced onto the surface of the nanoparticle by both FT-IR/ATR and zeta potential measurements (data not show).

It is necessary to quantify the amount of carboxyl groups present on the surface of the nanoparticle to estimate the material adsorption properties of the confetti nanoparticles and spherical nanoparticles. The carboxyl groups on the surface of the nanoparticles was quantified by potentiometric titration. The potentiometric titration was performed by adding 0.005 N NaOH into each nanoparticle (10 mg) dispersion solution.

Table 3 shows the amount of carboxyl groups measured by potentiometric titration. The confetti nanoparticles and PMAA−PEGm−St nanoparticles had about 3.0×10^{-4} mol/g carboxyl groups. On the other hand, the PMAA−St nanoparticles had 12.0×10^{-4} mol/g carboxyl groups, which was about 3 times more than the other two kinds of nanoparticles.

Based on these results, the material adsorption properties of each type of nanoparticle were evaluated. The material adsorption experiments were performed by immobilizing concanavalin A (Con A) on the surface of the nanoparticles.

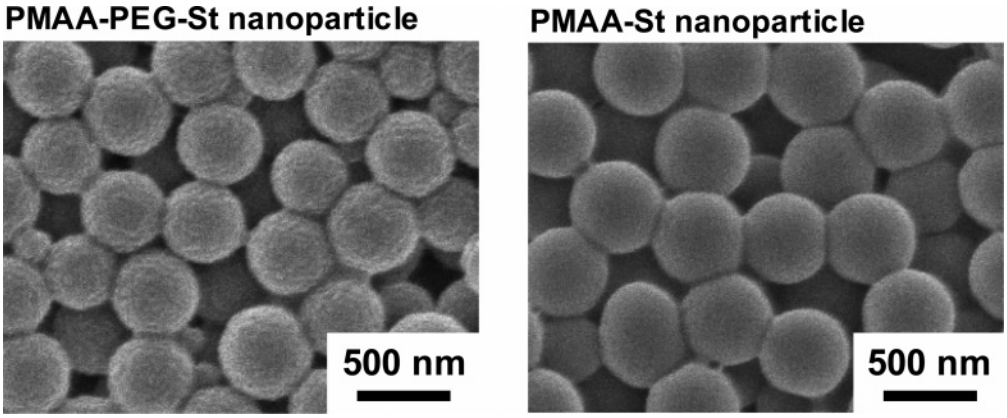


Figure 3. SEM images of nanoparticles composed of polystyrene-*co*-PMAA macromonomer-*co*-PEGm and polystyrene-*co*-PMAA macromonomer.

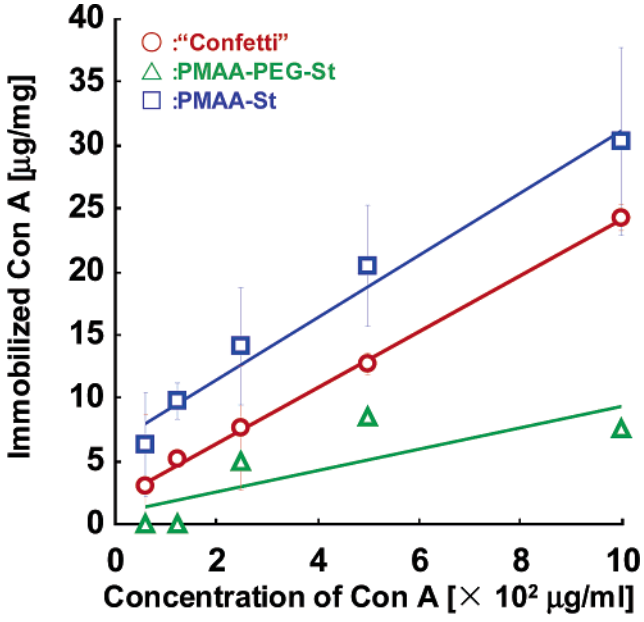


Figure 4. Effects of the concentration of the reaction solution on the amount of Con A immobilized onto the nanoparticles (○, confetti; Δ, PMAA−PEGm−St; □, PMAA−St).

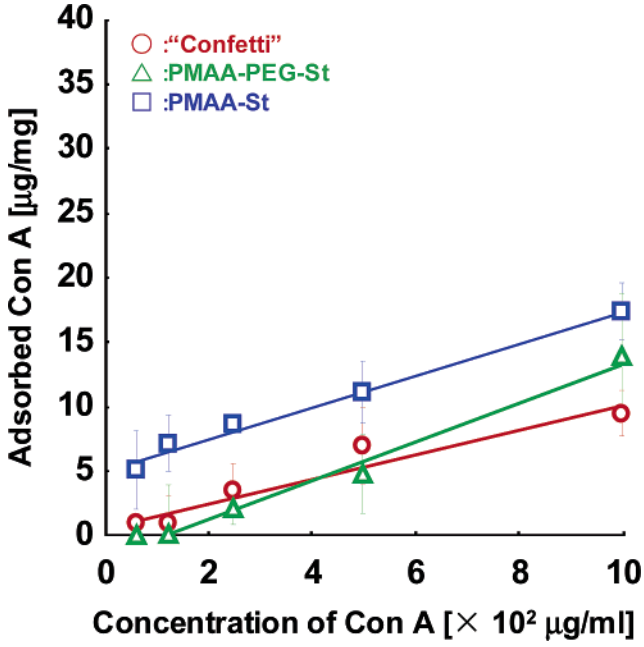


Figure 5. Effects of the concentration of the reaction solution on the amount of Con A immobilized onto the nanoparticles (○, confetti; Δ, PMAA−PEGm−St; □, PMAA−St).

Con A was immobilized by combining with carboxyl groups on the surface of the nanoparticles, which were activated using WSC. Moreover, the physical adsorption experiment was performed by a similar method that did not use WSC.

Figure 4 shows the amount of Con A immobilized onto the surface of the nanoparticles by covalent bonding. It was confirmed that the amount of immobilized Con A on the surfaces of all nanoparticles increased with an increase in the Con A concentration. The PMAA−St nanoparticles with the most carboxyl groups were able to immobilize the most Con A on their surfaces. On the other hand, the amount of Con A immobilized on the surface of the confetti nanoparticles decreased slightly as compared to that on the PMAA−St nanoparticles. It was thought that the amount of carboxyl groups on the surface of the confetti nanoparticles was less than that on the PMAA−St nanoparticles. It is also believed that the physical adsorption was inhibited by the PEG chain on the surface of the confetti nanoparticle. The amount of Con A immobilized on the PMAA−PEGm−St nanoparticles also decreased following the inhibition of the PEG chains. Nevertheless, the amount of immobilized Con A was obviously decreased as compared with the confetti nanoparticles, regardless of the amount of carboxyl groups. It is believed that the amount of immobilized Con A was controlled by the change in the specific surface area, surface composition, and shape of the nanoparticles.

Figure 5 shows the amount of Con A adsorbed onto the surface of nanoparticles by physical adsorption. PMAA−St nanoparticles were able to adsorb the most Con A on their surface. The physical adsorption of Con A onto the surface of the PMAA−PEGm−St nanoparticles was prevented by the PEG chains on the surface of the nanoparticles. However, the amount of physically adsorbed Con A was drastically increased when the concentration of Con A increased over 250 μg/mL unexpectedly.

On the other hand, it was confirmed that the confetti nanoparticles were the most inhibited by physical adsorption, even if the concentration of Con A increased above 250 μg/mL. The difference between the PMAA−PEG−St nanoparticles and the confetti nanoparticles was due to the amount of PEG chain, which increased upon increasing the specific surface area and the high mobility of PEG chains on the convex surface. However, the detailed reasons are not clear because a determination of the PEG chains on the surface of the nanoparticles was extremely difficult.

Figure 6 shows the amount of Con A immobilized per carboxyl group based on Figures 4 and 5. It was confirmed that the amount of Con A immobilized onto the surface of the confetti nanoparticles was remarkably increased as compared with the other two kinds of nanoparticles. This result showed that confetti nanoparticles can efficiently immobilize Con A in spite of their few carboxyl groups. Therefore, confetti nanoparticles are valuable materials that can prevent nonspecific adsorption such as that by proteins and also efficiently immobilize them by covalent bonding.

The hemagglutination of Con A-immobilized nanoparticles was performed to confirm Con A activity. It is generally known that erythrocytes with mannose on their surfaces interact with Con A and then precipitate. Hemagglutination was performed such that the Con A-immobilized nanoparticles were mixed with an erythrocyte dispersion solution to a final erythrocyte concentration of 4 vol %. After incubation for 6 h at room temperature, the Con A activity was evaluated by observing the supernatant. Moreover, the hemagglutination was performed using nanoparticles with different amounts of immobilized Con A.

Figure 7 shows the results of the hamagglutination. The activity was estimated by checking the supernatant solution after the reaction. If an aggregate was formed by the interaction between the nanoparticles and erythrocytes, then the supernatant becomes transparent because the nanoparticles precipitated with the erythrocytes. If the nanoparticles did not interact with the erythrocytes, then the supernatant

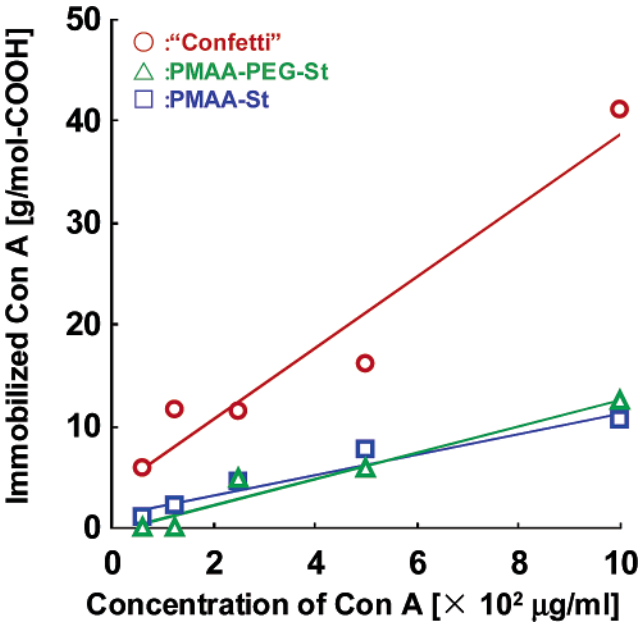


Figure 6. Effects of the concentration of the reaction solution on the amount of Con A immobilized onto the nanoparticles (per unit carboxyl group) (○, confetti; Δ, PMAA−PEGm−St; □, PMAA−St).

becomes cloudy because the nanoparticles are suspended in the supernatant.

In the case of using PMAA−St nanoparticles, the supernatant becomes transparent with amounts of immobilized Con A higher than 10 μg/mg. On the other hand, we confirmed that the supernatant of the confetti nanoparticles and PMAA−PEGm−St nanoparticles was not transparent up to 30 μg/mg of Con A because the PEG chains present on the surface of the nanoparticles prevented the interaction between the nanoparticles and the erythrocytes. Furthermore, the confetti nanoparticles did not interact with the erythrocytes more than the PMAA−PEGm−St nanoparticles. It was concluded that the confetti nanoparticles and the PMAA−PEGm−St nanoparticles still had Con A activity, but interacted poorly with the erythrocytes because of the PEG chains on their surface.

The Con A-immobilized nanoparticles can interact with materials with a mannose moiety. HIV-1 is known as an enveloped virus with a diameter of 100 nm and expresses gp120 and gp41 antigens on its envelope.[22] Since gp120 is a heavily mannosilated glycoprotein, it interacts strongly with some lectins.[23] In particular, Con A has a high affinity for gp120. Therefore, gp120 and virions should be effectively captured by Con A when it is ideally immobilized on the surface of the nanoparticles. The capture experiment for HIV-1 was performed by interacting the three kinds of Con A-immobilized nanoparticles and the HIV-1 dispersion solution. In previous studies, we reported that Con A-immobilized PMAA−St nanoparticles could capture HIV-1.[12] The prepared confetti nanoparticles efficiently captured HIV-1 because the specific surface area of the confetti nanoparticles was larger than that of the PMAA−St nanoparticles. The HIV-1 capture experiment was performed by incubating the HIV-1 dispersion solution mixed with the nanoparticles dispersion solution for 6 h at room temperature. The nanoparticles were precipitated by centrifugation after 6 h, and the p24 of the HIV-1 present in the supernatant was determined by Enzyme-Linked ImmunoSorbent Assay (ELISA).

The amount of p24 in the supernatant was measured by ELISA (Figure 8). The Con A-immobilized confetti nanoparticles, PMAA−PEGm−St nanoparticles and PMAA−St nanoparticles (amount of immobilized Con A: 35 μg/mg) were able to capture p24 at 72%, 62%, and 88%, respectively. The results for the PMAA−St nanoparticles were almost identical to the previous report.[12] The confetti nanoparticles and the PMAA−PEGm-St nanoparticles increased the p24 level because their PEG chains prevented the physical adsorption onto the surface of the nanoparticles. It was confirmed that the amount of p24 captured by the confetti nanoparticles was slightly higher than that of PMAA−PEGm−St nanoparticles. The explanation for this discrepancy is that the surface area of the confetti nanoparticles was larger than that of the PMAA−PEGm−St nanoparticles. As a consequence, we prepared three kinds of nanoparticles that were able to capture the p24 of HIV-1 using surface-immobilized Con A. The PMAA−St nanoparticles were able to capture the most p24 of HIV-1. Remarkably, it was

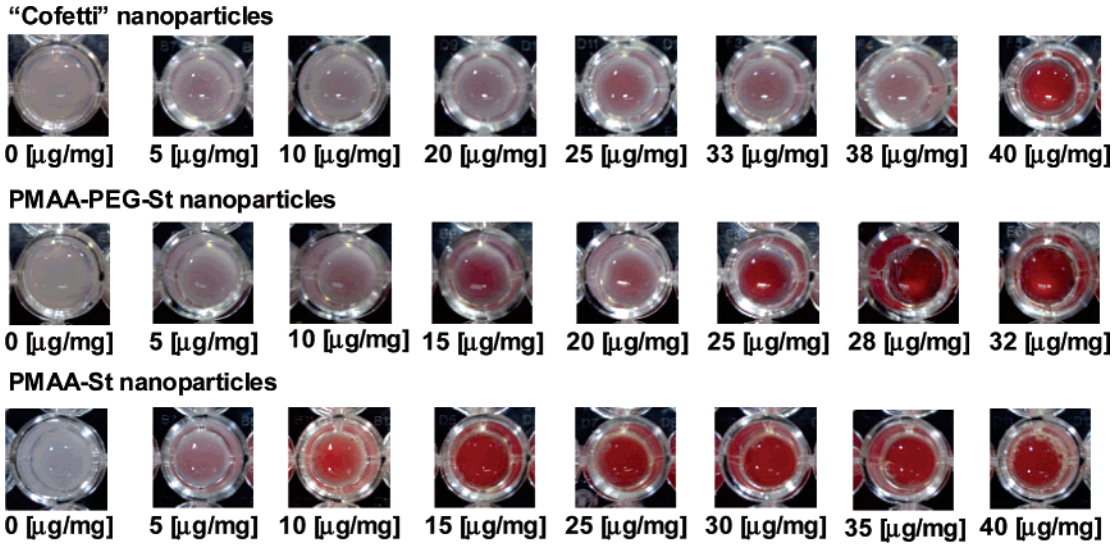


Figure 7. Activity estimation of Con A on confetti nanoparticles, PMAA−PEGm−St nanoparticles, and PMAA−St nanoparticles.

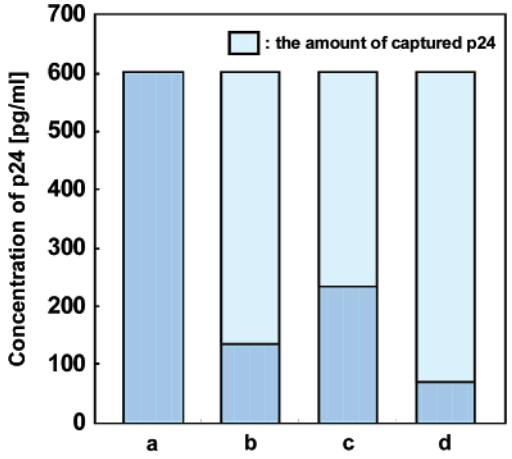


Figure 8. HIV-1 capture ratio of Con A-immobilized nanoparticle: (a) supernatant; (b) confetti nanoparticles; (c) PMAA−PEGm−St nanoparticles; (d) PMAA−St nanoparticles. Capture ratio: (b) 72%, (c) 62%, and (d) 88%.

observed that the confetti nanoparticles were able to capture an amount of p24 similar to the PMAA−St nanoparticles in spite of the PEG chains on the surface of the nanoparticles. From the results of Figures 7 and 8, it is clear that the confetti nanoparticles interacted easily with the p24 of HIV-1, but did not interact well with erythrocytes.

Moreover, the captured HIV-1 was confirmed in greater detail by using a colloidal gold conjugated anti-bp120 monoclonal antibody (immunostaining method). The immunostaining methods were performed by mixing a colloidal gold (30 nm) conjugated anti-gp120 monoclonal antibody solution (100 μL) and the HIV-1 captured nanoparticles dispersion solution (5 mg/mL) (100 μL). Figure 9 shows the TEM images of each nanoparticle after immunostaining. The gold colloids were absorbed onto the surface of the nanoparticles. This result shows that the gp120 of HIV-1 was present on the surface of the nanoparticles, and HIV-1 was captured by all nanoparticles.

Similar to the above results, the confetti nanoparticles were interactive with nanometer-scaled materials such as Con A and HIV-1, but not with micrometer-scaled materials such as erythrocytes. This size-selective material adsorption property can be exploited to separate materials such that the nanoparticles capture only HIV-1 from blood. Consequently, the HIV-1 capture experiment was performed under the conditions of erythrocytes coexisting with the HIV-1 nanoparticles. HIV-1 capture experiment was performed by mixing the erythrocyte dispersion solution (final erythrocyte concentration: 4 vol %), HIV-1 dispersion solution, and Con A-immobilized nanoparticle dispersion solution (amount of immobilized Con A: 35 μg/mg) at room temperature for 6 h. After 6 h, the amount of p24 in the supernatant was determined by ELISA.

The amount of p24 captured by each nanoparticle is shown in Figure 10. The three kinds of nanoparticles (confetti nanoparticles, PMAA−PEGm−St, and PMAA−St) were able to capture p24 at 84%, 46%, and 77%, respectively. Here, the supernatant solution of the PMAA−PEGm−St and PMAA−St nanoparticles became almost transparent after the nanoparticles captured the p24 (Figure 11, green line and blue line). This shows that the nanoparticles were able to interact with the erythrocytes and then precipitate them. On

(22) The heterogeneity and pathogenicity of HIV. In *HIV Molecular Organization, Pathology and Treatment*; Morrow, W. G. W., Haigwood, N. L., Eds.; Amsterdam: Elsevier, 1993; p 29.
(23) Lifson, J.; Coutrė, S.; Huang, E.; Engleman, E. *J. Exp. Med.* **1986**, *164*, 2101−2106.

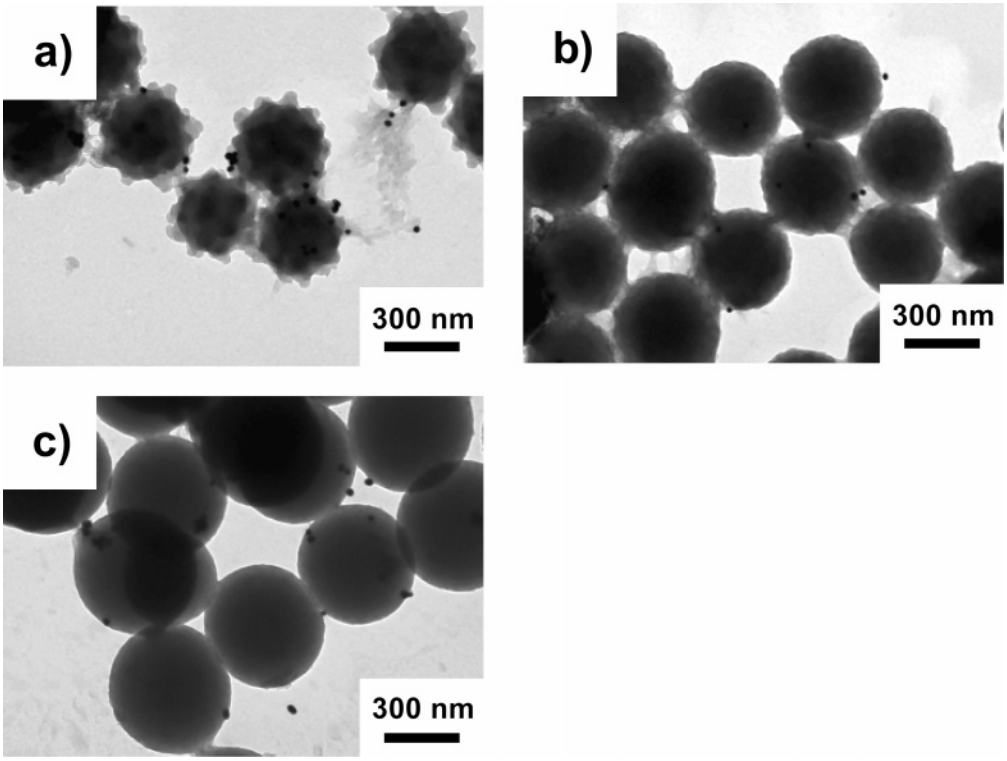


Figure 9. TEM images of HIV-1 captured nanoparticles after immunostaining with a colloidal gold-conjugated anti-gp120 monoclonal antibody (a) confetti nanoparticles, (b) PMAA−PEGm−St nanoparticles, and (c) PMAA−St nanoparticles) (amount of Con A: 35 μg/mg).

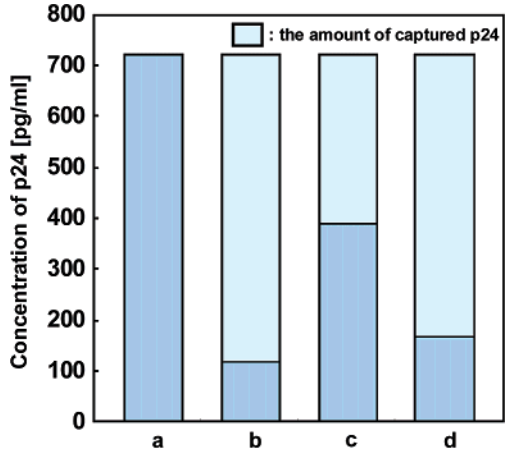


Figure 10. HIV-1 capture ratio of Con A-immobilized nanoparticles: (a) supernatant; (b) confetti nanoparticles; (c) PMAA−PEGm−St nanoparticles; (d) PMAA−St nanoparticles. The medium containing the HIV-1 was mixed with an erythrocyte dispersion solution (16 vol %) to a final concentration of 4 vol % (amount of Con A: 35 μg/mg).

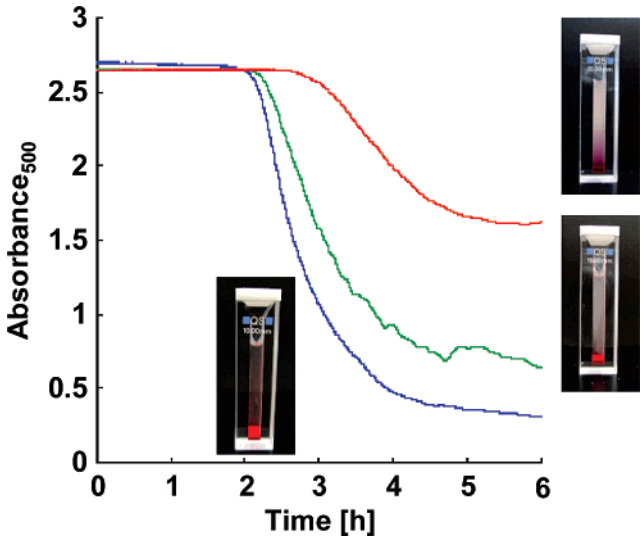


Figure 11. Time dependence of the absorbance of nanoparticle, erythrocytes, and HIV-1 mixed dispersion solution (red, confetti nanoparticles; green, PMAA−PEGm−St nanoparticle; blue, PMAA−St nanoparticle) (amount of Con A: 35 μg/mg).

the other hand, the supernatant solution of the confetti nanoparticles remained cloudy after capturing the HIV-1 (Figure 11, red line). This shows that the confetti nanoparticle does not interact with erythrocytes under these conditions. From the results of the ELISA measurements, the confetti nanoparticles were able to capture HIV-1. Consequently, the confetti nanoparticles were able to capture HIV-1 without interacting with the erythrocytes.

The HIV-1 capture ratio of the confetti nanoparticles was also slightly increased in the presence of erythrocytes. This result shows that HIV-1 precipitated by slightly interacting with the erythrocyte. On the other hand, the HIV-1 capture ratio of the other nanoparticles (PMAA−PEGm−St and PMAA−St nanoparticles) was slightly decreased in the presence of erythrocytes. This result indicates that most of the nanoparticles were precipitated before interacting with the HIV-1. Consequently, it was demonstrated that the confetti nanoparticles were able to capture HIV-1 without interacting with the erythrocytes, and the capture ratio of the PMAA−PEGm−St nanoparticles and PMAA−St nanoparticles were reduced by interaction with the erythrocytes. As a result, the confetti nanoparticle is an excellent material that can selectively separate only HIV-1 when erythrocytes are present.

Thus, the size-selective material adsorption property of confetti nanoparticles is very unique in biological materials and is expected to be useful in the field of bioseparation, isolation, and concentration to analyze rare biological materials.

Conclusion

"Confetti" nanoparticles with carboxyl groups on their surfaces were prepared by the dispersion copolymerization of PMAA macromonomer, PEGm, St, and AN in the presence of AIBN in an ethanol/water mixed solvent at 60 °C for 24 h. The prepared confetti nanoparticles had numerous carboxyl groups on their surfaces. The confetti nanoparticles were able to immobilize Con A more efficiently than spherical nanoparticles of the same size. Moreover, the confetti nanoparticle was able to capture HIV-1 in the presence of erythrocytes. The surface morphology control of nanoparticles is a novel concept for bioseparation and is expected to have applications in the field of rare biomaterial collection.

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