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Synthesis of monodisperse ferrite nanoparticles coated with polyacrylic acid (PAA)

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Ferrite nanoparticles are used in the field of biotechnology and clinical test agent, because of their magnetism. Ferrite particles now in practical use don't disperse in themselves, and they easily aggregate and exist as a mass of about 50-200 nm in water solution. However, especially in appricational use for higher density and sensitivity, monodisperse ferrite nanoparticles are needed. We found out that ferrite nanoparticles (FP) are monodispersed by slow oxidation process of Fe^{2+} in the presence of polyacrylic acid (PAA). The size of ferrite nanoparticles coated with polyacrylic acid (FP-PAA) was estimated to be ~10 nm in mean size in transmission electron microscopy (TEM) observation, and the dispersiblity of FP-PAA was evaluated by dynamic laser scattering (DLS) measurements. The dispersibility of FP-PAA would be originated from electric repulsive force due to negative charge of carboxyl groups in the surface of particles.

Key words: ferrite nanoparticle, polyacrylic acid, monodisperse

1. INTRODUCTION

There are many methods to synthesize ferrite nanoparticle. Chemical coprecipitation, reverse micelle, and sol-gel methods are more commonly used to synthesize ferrite nanoparticle in liquid phase. Recently, progress of synthesis method in liquid phase enabled us to regulate properties of ferrite nanoparticles such as size, shape and crystallinity like hydrothermal crystallization method [1]. On the other hand, global attention for ferrite nanoparticles has been growing in the field of biotechnology and nanotechnology. In biotechnology, ferrite nanoparticles have been expected to be a candidate for a new devise as a substrate of drug delivery system, affinity chromatography, and other biological detection systems [2-4], because of their properties such as shortening of T1, T2 and T2* proton relaxation time in aqueous solution [5], large surface area per volume attributed to nanosize, and difference from their magnetic properties of bulk sample [6]. Several ferrite nanoparticles coated with a dispersing agent which has a specificity against organs have been studied as a contrast agent of MRI [7-11], and some of them are now in clinical stage [12]. These particles used in practical stage are ordinarily stabilized with dextran derivative. Though the size of the ferrite particle is about 6-15 nm, these particle covered with dextran derivative often aggregate in size between 50 and 200 nm. However, in these applications which require higher density and sensitivity, these aggregated nanoparticles are unuseful. Especially, their size is required to be under 100 nm in total diameter in solution in case of using in vivo sensing. Therefore, the dispersibility and size control of the nanoparticles are indispensable for biological applications.

Recently, we found out that certain specific proteins are adsorbed on the surface of ferrite particles synthesized by chemical coprecipitation method in ammonium chloride solution containing cell extract [13, 15]. In addition, we proposed that adsorption of specific proteins is due to a direct binding of their carboxyl group to ferrite [14, 15]. This means that surface condition of ferrite nanoparticle can be changed by molecules which have carboxyl groups. Based on the results, we considered that unconnected carboxyl groups in the surface of particle induces negative charge by immobilization of organic materials, and that aggregation of ferrite nanoparticles in themselves is suppressed by its electric repulsive force. In this study,

we select polyacrylic acid (M.W. 2,000) which contains about 26 carboxyl groups per a molecule. The ferrite nanoparticles coated by polyacrylic acid are expected to have superior dispersibility to nontreated ferrite nanoparticles because of their surface negative charge derived from their unconnected carboxyl groups. The dispersibility and crystallinity of crystal component of FP-PAA are shown, and the origin of the high dispersibility of FP-PAA was discussed below.

2. EXPERIMENTAL

Ferrite nanoparticles coated with polyacrylic acid (FP-PAA) were prepared by the following process. 0.09 g of PAA (M.W. 2000) was added into 50 ml of $FeCl_2$ (0.1 M) aqueous solution. 0.7 M KOH 11 ml was added into this solution, and $Fe(OH)_2$ precipitation was formed. 50 ml of 0.05% H_2O_2 solution was added at the rate of 33 ml/h for the purpose of oxidation of Fe^{2+} after that. All the processes performed at about 283 K under purging an N_2 gas. The synthesized sample was recovered by magnetic separation, washed and dialyzed five times for 6 h with distilled water, and filtrated by using 220 nm pore membrane.

We observed transmission electron microscopy (TEM) images to investigate the particle size of the sample directly. This observation was carried out by using H-7500 (Hitachi) at 20 k magnification and accelerating voltage of 100.0 kV.

To determine the crystal component of FP-PAA and investigate its crystallinity, we carried out powder x-ray diffraction (XRD) using a Fe $K\alpha$ radiation at room temperature. The powder sample was dried by vacuum dryer, and ground with agate mortar. The XRD patterns were collected with a RINT2000 (Rigaku) spectrometer at 30 kV, 20 mA.

Magnetic field, H, dependences of magnetization, M, of the dried sample were measured at room temperature by a vibrating sample magnetometer (VSM) (custom model, Riken Denshi). Magnetic field was scanned in the order of $0 \rightarrow 2 \rightarrow -2 \rightarrow 2$ T.

The dispersibility of the ferrite nanoparticles in aqueous solution was probed by using dynamic laser scattering (DLS) method. DLS is equipment of particle sizer in solution based on the Doppler effect of scattered light. The mean particle size in aqueous solution was determined by using a FPER-1000 laser scattering system (Otsuka electronics).

The amount of carboxyl groups immobilized on the surface of FP-PAA was investigated by the titration of electroconductivity. It was determined by titrating sample with 0.01N HCl, AUT-501 (TOA-DKK). The amount of PAA immobilized on ferrite nanoparticles was estimated by thermogravimetriy-mass spectrometory (TG-MASS) measurements. The sample was same as XRD and VSM. The measurement was carried out under the following conditions: increasing rate of temperature 5 K/min, flowing rate of air 100 ml/min.

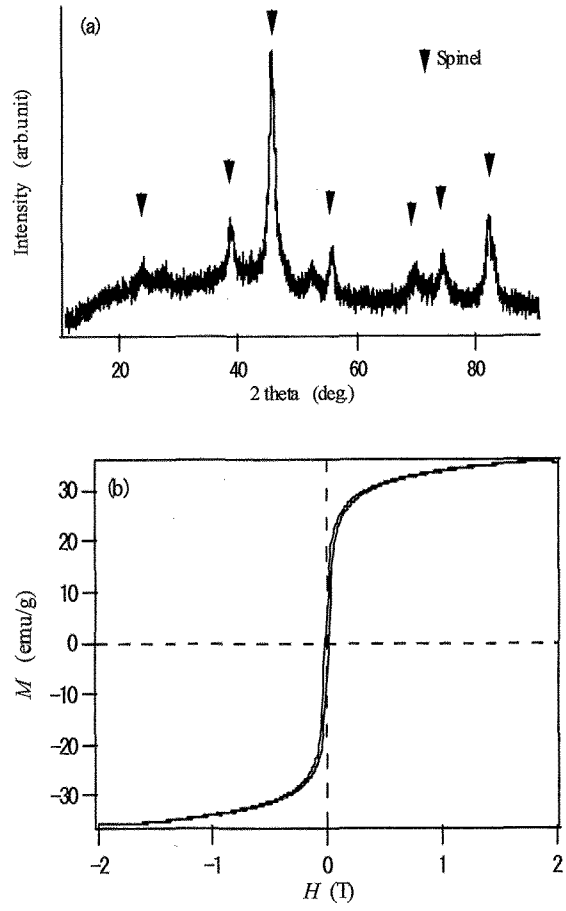

Fig. 1(a) XRD patterns, (b) magnetization per gram vs. magnetic field curve of FP-PAA dried sample at room temperature. The arrow marks in the panel of (a) indicate the peaks of spinel-type structure.

To confirm surface charge of FP-PAA, ζ-potential measurement was carried out in 50 mM HEPES buffer at pH 7.0, room temperature by using ELS-6000 (Otsuka electronics).

3. RESULTS AND DISCUSSION

The XRD pattern of the FP-PAA is shown in Fig. 1(a). The detectable peaks showed only peaks for the spinel-type structure and could be indexed by space group $Fd\bar{3}m$. The peaks appeared in the pattern were somewhat broad but relatively sharp, indicating that spinel-type ferrite was almost formed with no by-products in the presence of PAA. The lattice constant was estimated to be a = 0.838 nm based on XRD patterns, which showed that between magnetite, Fe_3O_4 (a = 0.839 nm [16]) and maghemite, γ-Fe_2O_3 (a = 0.835 nm [17]). These results suggested that ferrite in FP-PAA is a solid solution composed of magnetite and maghemite.

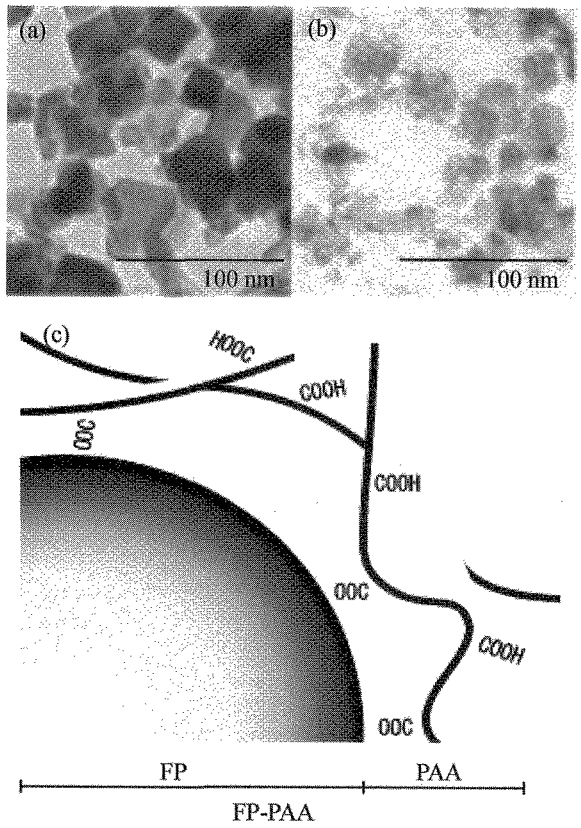

Fig. 2 TEM images of (a) FP, (b) FP-PAA, and (c) a PAA coating model of FP-PAA

Fig. 1(b) shows magnetic field, H, dependences of magnetization, M, of FP-PAA at room temperature. This shows a similar nonlinearity and small hysteresis to those of magnetite and maghemite, and in addition, considering that the ferrite in FP-PAA is spinel-type structure as described above, it was supposed that it is ferrimagnetism as reported in magnetite and maghemite. The magnetization saturates as scanning magnetic field to $H = 2$ or -2 T, and the saturation magnetization, M_S, was estimated to be 35.4 emu/g at $H = 2$ T. The value of M_S is small as compared to bulk magnetite or maghemite. This difference indicates that crystal component of FP-PAA contains amorphous state. Even though *Ms* value of FP-PAA is small against bulk ferrite, it is no problem for use in the application field, because it shows enough magnetization at room temperature.

Fig. 2 shows TEM images of (a) FP, (b) FP-PAA, and (c) a PAA coating model in the surface of FP. As shown in Fig. 2(a), the ferrite particles synthesized in the absence of PAA are remarkably laminating, and the size of particles were about 30 nm in diameter. However, in the presence of PAA, the particles was monolayer, not laminating. This is often observed when sample have superior dispersibility. The size of crystal component that is ferrite particle of FP-PAA, is estimated to be ~10 nm in average diameter from the image in Fig. 2(b). These results show that PAA were immobilized on the ferrite surface and the dispersibility of ferrite was enhanced. However, the distribution state of this ferrite nanoparticles in aqueous solution was not able to be known from TEM and XRD. Therefore, in order to evaluate the dispersibility of the ferrite, dynamic laser scattering (DLS) analysis of the FP-PAA and FP in the distilled water was carried out at room temperature. The results of weight conversion analysis are shown in Fig. 3. The size at main peak of (a) FP and (b) FP-PAA was estimated to be 139.1 nm and 13.7 nm, respectively. It is clear that the size distribution of FP-PAA is much narrower than that of FP as a control synthesized without PAA, and aggregation of ferrite particles is remarkably suppressed. Considering that the ferrite surface was coated with PAA, and coordinated by H_2O molecules in the solution, it can be judeged to be almost same as the size (~10 nm) obtained by TEM observation (see Fig. 2(b)). These strongly indicate that FP-PAA is almost monodisperse in the aqueous solution. The PAA would be possibly immobilized on the surface of ferrite via carboxyl groups of that as discussed in the previous paper [2], but, it is supposed that all the carboxyl groups of PAA molecule is not binding because the

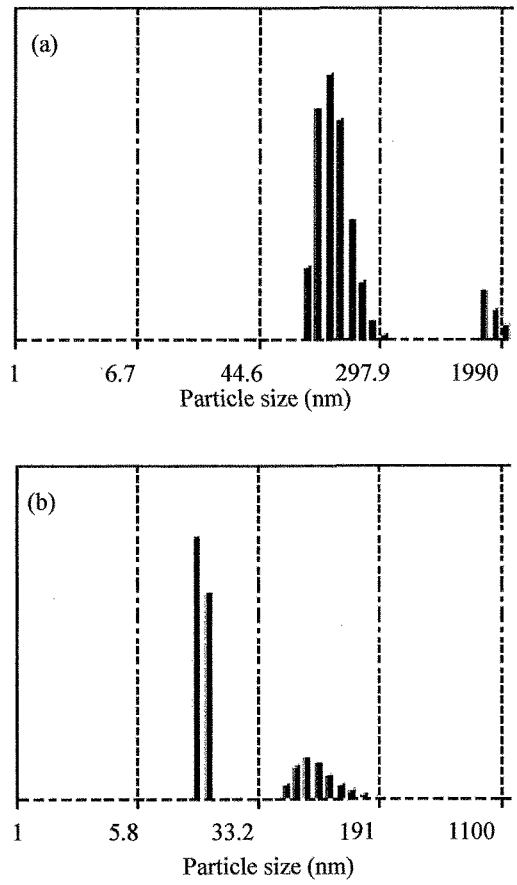

Fig. 3 Dynamic laser scattering analysis. Particle size distribution estimated by weight conversion analysis of (a) FP-PAA and (b) FP in distilled water solution.

dispersibility of FP-PAA was remarkably enhanced by coating of PAA to FP. The higher dispersibility of FP-PAA must be realized for the presence of unconnected carboxyl groups of PAA in the surface.

The amount of carboxyl groups of PAA unconnected to ferrite nanoparticles in the surface was determined to be about 0.4-1.2 μmol/mg FP-PAA by titration of electric conductivity. In addition, the amount of total carboxyl groups of PAA were determined to be about 0.8-1.6 μmol/mg FP-PAA by TG-MASS measurements. From these results, it was estimated that ~25 % of all the carboxyl groups is unconnected at least. Therefore, it suggests that the high dispersibility of FP-PAA is attributed to electric repulsive force among particles which is caused by negative charge of outer carboxyl groups of PAA unconnected to ferrite nanoparticles as shown in Fig. 2(c). This is also supported by comparing potential in the surface, namely ζ-potential between FP-PAA and FP. The potential of FP-PAA and FP in 50 mM HEPES buffer at pH 7.0, 298 K was -32.2 mV and -13.1 mV, respectively. It is clear that unconnected carboxyl group of PAA increases negative charge in the surface of particles and enhances electric repulsive force among particles.

4. CONCLUSION

A coating ferrite nanoparticles with polyacrylic acid was conducted, which was effective to disperse ferrite nanoparticles. It was considered that this high dispersibility is due to electric repulsive force originated from carboxyl group of PAA immobilized on ferrite particles.

The magnetization of the ferrite is also large enough at room temperature, and therefore it was found that FP-PAA is advantage against conventional ferrite nanoparticles in the biological and medical applications. In addition, the surface carboxyl groups in the FP-PAA can be also considered to maintain their dispersibility in neutral buffer conditions as compared to nontreated ferrite nanoparticles. Also, it is useful to immobilize additional chemical and biological compounds such as medical agent and protein like enzyme, RNA and DNA. The development of higher dispersible FP-PAA should yield more advancement for sensitivity and function in applications as affinity chromatography, biological detection sensor chip, and contrast agent of MRI.

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