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Magnetite/Polymer Composite Particles Prepared by Molecular Assembling Followed by In-Situ Magnetite Formation

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Summary: Magnetic nano-particles composed of superparamagnetic iron oxide in the core and hydrophilic polymer in the shell were prepared for the use of MRI contrast agents. Ferric and ferrous ions were added to an aqueous solution of block copolymer, poly(ethylene oxide)-block-poly(glycelol methacrylate-co-acrylic acid), which was prepared by living radical polymerization using iniferter. After iron ions-polymer complex formation, the pH of the solution was raised to 11 to get magnetite nanoparticles which were encapsulated with polymer. Use of the polymer having the molecular weight of ca. 10,000 and the 100 × AAc/GLM of 1–10 and incubation of the mixture of iron ions and water-soluble polymers at a moderate temperature resulted in the formation of favorable composite particles of 10–20 nm in diameter. The saturation magnetization and other instrumental analysis of composite particles suggested that the iron oxide formed in the above-mentioned procedure was magnetite.

Keywords: block copolymer; composite particle; iron ions-polymer complex; living radical polymerization; magnetite

Introduction

Micro- and nano-spheres containing magnetic substances are in the spotlight because of their significant applications, especially in biomedical fields. For example, if affinity latex particles, which immobilize biospecific molecules to catch their counterparts selectively, contain magnetic substances, the particles can be used as an easily collectable bio-reactor, bio-separator and diagnostic reagent, that is, they can be easily recovered from the dispersion without applying high-speed centrifugation after the function was completed.[1,2] Magnetite-containing particles are also used in drug release systems in which magnetic force carries the particles to the target sites and makes them to stay there. Submicron-sized particles are recommendable for these purposes. Magnetic substance-containing particles with smaller sizes have some other applications such as a heat source for hyper-thermia[3] and a contrast enhancer for magnetic resonance imaging (MRI).[4] In the former, the magnetic nanospheres targeted to diseased cells are treated with alternating magnetic force to generate heat, which kills the cells. In the latter, the magnetic nanospheres are injected to the site where they contribute to increase the contrast of the image in MRI.

Above-mentioned composite particles are prepared via several routes which are presented in Fig. 1. In Route 1 which starts from polymer particle and precursor, ferric and ferrous ions have been used as the precursor. The ions were permeated into existing particles and then oxidized in an alkaline solution to form magnetite in-situ.[5,6] In another routes, Route 2 starting from

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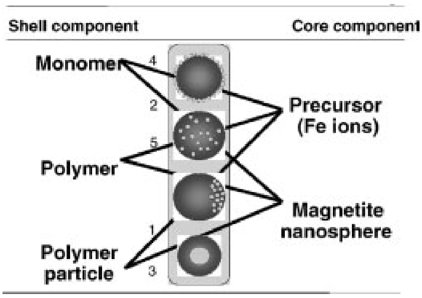


Figure 1.
Starting materials for the preparation of magnetic composite particles.

monomer and magnetic nanoparticles, mini-emulsion polymerization have been used often because mini-emulsion polymerization is the most efficient in preparing composite particles among particle-forming polymerization.[7,8]

A typical example of composite particle formation from combination of polymer particle and magnetite nanoparticle, corresponding to Route 3, is hybridization in dry state and hetero-coagulation in wet state.[9] In contrast to them, the route starting from monomer and ferric ion, corresponding to Route 4, seldom gives good results because polymerization and in-situ magnetite formation hardly take place at a balanced rates.

In this study, polymer solution and ferric /ferrous ion solution were chosen as starting materials. This corresponds to Route 5.[10] To obtain composite particles from these materials, the polymer molecules must have a favorable structure and they must be assembled by some forces. The forces include the changes of temperature, pH, solvent, and salt concentration, and addition of multi-valent ions, biospecific compounds, etc. In this study, ferric/ferrous ions, originally the starting material for magnetite, were used as the force to assemble the polymers dissolving in water because iron ions can interact with polymers having OH and NH_2 via coordination reaction, and with those having COO– via Coulomb force. After assembling reaches the equilibrium, magnetite was formed in-situ from the existing ferric/ferrous ions by adding alkali,[11] and then, the composite might be crosslinked to avoid the destruction of assembly. Suitable conditions for each step were searched. Especially, the effect of structure of polymer molecules on the formation of particle was discussed. The obtained magnetite-containing particles were characterized in terms of size, magnetite content, magnetization and its relaxation mode.

Experimental

1. Materials

Glycelol methacrylate (GLM) was purchased from Nippon Oil and Fats Co. and used without further purification. Acrylic acid (AAc, Wako Pure Chemicals Co.) was distilled at 38.5 °C/10 mmHg before use. PEO2K iniferter ($MeO–(C_2H_4O)–C_2H_4–S–C(=S)–N–Et_2$) was kindly presented by Dr. S. Arimori (Molecular Engineering Institute, Iizuka, Fukuoka). Ferric and ferrous chlorides were extra-pure reagent of Wako Pure Chemicals Co.

2. Preparation of Block Copolymer

Test tubes containing 20 ml of aqueous or aqueous methanol solutions of PEO2K and monomer were set beside a UV lamp (UVL-400HA, Rikougaku Sangyo Co., UV wavelength range: 312–577 nm, wavelength at the maximum: 365 nm). Air in the tube was purged with nitrogen and UV was irradiated at 20 °C under mild stirring with a magnetic stirrer. Polymerization was continued for a scheduled period of time and then the content was dialyzed for three days. Then each sample was dried under vacuum to be obtained as a solid material.

3. Characterization of Polymers

The sample for GPC measurement was prepared as follows: the polymer mentioned above was dissolved in a mixed solvent of methanol/water (80/20 in volume) including 10 mM LiBr. The solution was injected to GPC column (TSKgel α-M) at 40 °C. Monodisperse standard poly(ethylene oxide)s with molecular weights of 1,460, 12,600, and 23,600 (Polymer Laboratories Co.), and 50,000 and 107,000 (Toso Co.) were used for calibration.

Molecular structure of polymers was analyzed by FTIR (FTS-60A, BIORAD Co.), NMR (JEOL, with CD3OD solution) and conductometric titration (AUT-3000/FUT-3040/SBT-1010, Toa Dempa Industry Co.).

4. Formation of Magnetite/Polymer Composite Particles

Standard procedure for preparation of magnetite/polymer composite particle is shown in Fig. 2. The concentration of reagents, time interval for each step, and temperature suitable for preparation of aimed composite particles were searched.

5. Characterization of Composite Particles

The hydrodynamic diameter of composite particle was measured by dynamic light scattering/photon correlation spectroscopy with a particle analyzer PAR-III (Otsuka Electronics Co.) using He-Ne laser of 632.8 nm at a fixed angle 90° with 50 counts. The shape and size of composite particles were observed with a field emission transmission electron microscope (TECNAI, Philips Electron Optics Inc.). The content of magnetite in composite particle was measured by thermal gravimetry using TG-DTA6200 (Seiko Instruments Inc.) at an elevating rate of 10 °C. The composition and structure of iron oxide were determined with an X-ray diffractometer RAD-C CN-2013 (Rigaku Co.) and with a vibrating sample magnetometer (BHV-50, Riken Denshi Co. Ltd). NMR characteristics such as longitudinal and spin-spin relaxations were measured with Philips Gyroscan ACS-NT at high magnetic field and AIRIS II (Hitachi Medico Inc.) at low magnetic field using composite particle dispersion containing 0.1 mM iron.

Results and Discussion

1. Preparation of Block Copolymer

Copolymerization of GLM and AAc using PEO2K iniferter was carried out in water or aqueous methanol at room temperature under nitrogen with the aid of UV irradiation.[12] Polymerization in aqueous methanol enabled us to prepare a series of block copolymers, PEO-block-poly(GLM-co-AAc) with relatively narrow molecular weight distribution. The molecular weights of some polymers are presented in Fig. 3 as a function of conversion. The polymers were abbreviated as AG(x) in which ‘‘x’’ meant the molar percentage of AAc to GLM. The results indicate that the living radical polymerization in Scheme 1 proceeded satisfactorily from PEO2K whose molecular weight was 2,000.[13] The polydispersity, that is, the ratio of weight-average molecular weight to number-average molecular weight, was 1.4 in average so that the

polymer (g)	water (mL)	0.2M $FeCl_2 \cdot 4H_2O/FeCl_3 \cdot 6H_2O$ (mL)	0.5M NaOH (mL)	DVS (mg)	water (mL)
0.0388	26.1	1.5	2.4	5.91	1.0

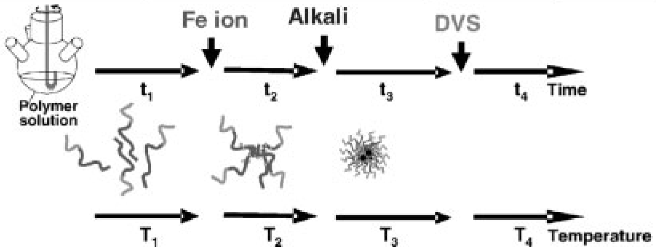


Figure 2.
Representative recipe and Procedure to prepare magnetite/polymer composite particle.

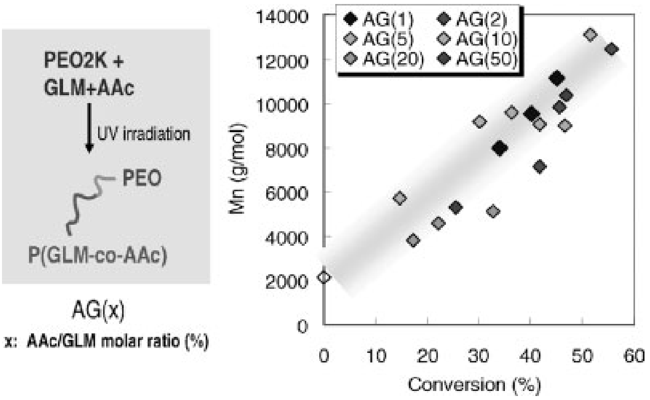


Figure 3.
Conversion vs. number average molecular weight (Mn) of AG(x).

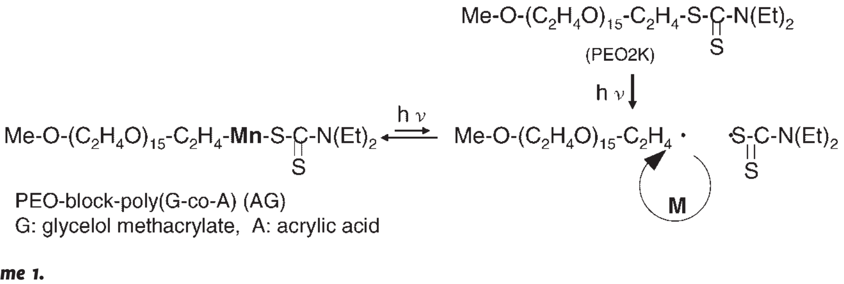
polymers were not so strictly monodisperse, perhaps due to insufficient livingness caused by side reactions of iniferter.

It is worth mentioning that the elution solvent for GPC of our products must be carefully chosen. The best solvent for the copolymer with high × (x ≧ 20) was aqueous solution of 20% acetonitrile and 0.2 M sodium phosphate whereas aqueous methanol with 0.05 M phosphate was available for the copolymers with low AAc contents. Above-mentioned special requirement as to the elution solvent for the copolymer with high AAc content results from the condition that both electrostatic and hydrophobic interactions between polymer molecules and gels in the column should be decreased as possible.

In NMR measurement, the peak size ratio of chemical shift of 3.3–3.5 ppm assigned to methylene group to that of 0.9 ppm assigned to methyl group gave the molar ratio of PEO/PGLM in PEO-block-PGLM. Copolymerizations of GLM and AAc with different monomer ratio resulted in the formation of copolymers having x from 0 to100.

2. Assembling of Block Copolymer and In-Situ Formation of Magnetite

Ferric and ferrous ions have affinity with hydroxyl and carboxyl groups via coordination binding and Coulomb force, respectively.[14] Therefore, addition of ferric and ferrous ions into the aqueous solution of AG(x) caused formation of Fe ion/polymer complex. The complex was water-soluble and gave yellow, transparent aqueous solution. But the component became insoluble when 0.5 M NaOH was added because black magnetite nanoparticles formed. TEM view, for example, in the center–bottom TEM view of Fig. 4, shows that such nanoparticle(s) were surrounded by polymers.



Scheme 1.
Living radical polymerization using PEO-iniferter.

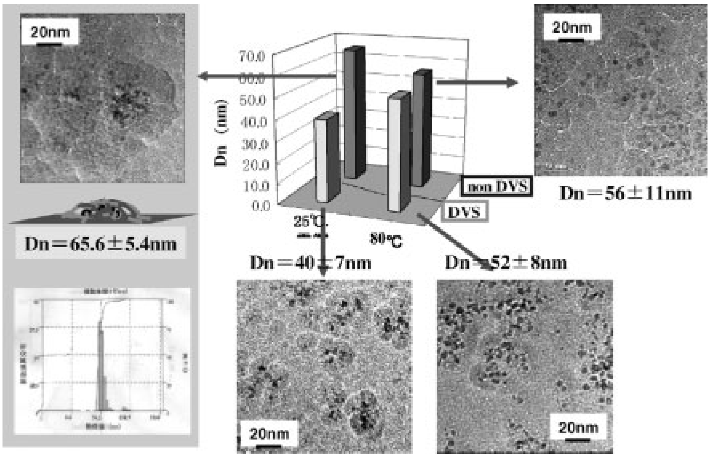


Figure 4.
Effect of temperature for magnetite formation and crosslinking of matrix on morphology of composite particle. Molecular weight of polymer: 12,200, Polymer/magnetite = 167/100 (w/w).

The effect of temperature for Fe ions-polymer hybridization on the morphology of composite particles was examined in the temperature range of 25 to 80 °C. The effect of crosslinking of composite particle was also examined using divinylsulfone (DVS) which linked OH groups on adjacent polymer chains. The results in Fig. 4 show that high temperature and DVS made the polymer phase compact and the contour sharp. The size of magnetite became larger and the size distribution became narrower at high temperature. The conclusive procedure for the preparation of magnetic composite particle is presented in Fig. 5.

3. Effect of Polymer Architecture on the Structure of Composite Particle

The procedure shown in Fig. 5 was applied to a series of polymers with small modification in terms of temperature. Fig. 6 shows the effect of molecular weight of polymer on the structure of composite particles such as size and morphology. When low molecular weight polymer was used, clear contour of composite particle was not observed, perhaps, due to weak assembling force of the polymer, as in the left picture of Fig. 6. Instead, when high molecular weight polymer was used, loose aggregate resulted. This was attributed to the bridging effect by long extended polymer chains. Therefore, suitable molecular size must be chosen for the preparation of composite particles.

Next, the effect of AAc content in block copolymer on the structure of composite particle was studied using block copolymers with AAc/GLM ratio of 0 to 50%. The results were summarized in Fig. 7, according to which, the size of composite particles increased first with increasing AAc/GLM lead in low AAc/GLM range. This was attributed to the increase in the intra-chain repulsion in the particle containing AAc/GLM up to 5%. But the use of higher AAc/GLM polymer caused a decrease in the size

Figure 5.
Time and temperture for preparation of magnetite/polymer composite particle.

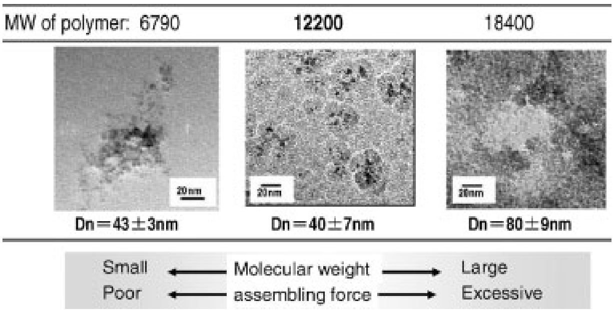


Figure 6.
Effect of molecular weight of polymer on structure of composite particle. The polymer used: AG(0) (= PEO-PGLM including no AAc).

of microgels, the number of polymer chains consisting of particle, and the number of magnetite in the composite particle. These seemed to result from the increasing inter-chain repulsive force.

Finally the effect of polymer architecture on the formation of composite particle was summarized in Fig. 8, in which open circles indicated the polymer architecture to be able to give stable composite particles and the solid circles to fail the composite formation. Judging from the total results, AG(10) seems to have moderate interaction with iron ions, be attracted by magnetite moderately, and then give a tight shell.

4. Effect of Polymer/Magnetite Ratio on the Composition and Structure of Composite Particle

Composite particles having different compositions, polymer/magnetite = 167/100 and 251/100 (w/w), were prepared according to the procedure presented in Fig. 5. X-ray diffraction measurement revealed that the iron oxide was superparamagnetic and composed of magnetite with a small amount of maghemite. The analysis of iron oxide in composite particle by thermal gravimetry (TG) revealed that the polymer/iron oxide ratio in the composite was almost the same as that of the charged reagents in all of the cases examined. TG curves of

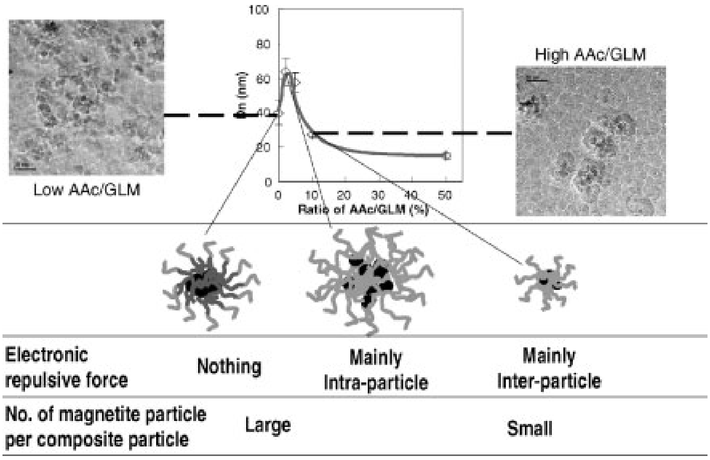


Figure 7.
Effect of AAc content in polymer on composite particle formation.

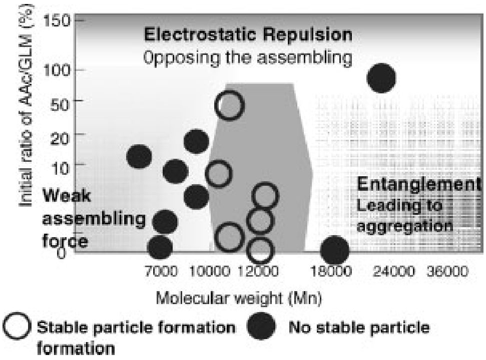


Figure 8.
Polymers favorable for composite particle formation.

polymers started to descend gradually from 260 °C and it continued up to 450 °C. In contrast, TG curves of composite particles descended sharply at 260–270 °C to the final values. The complete weight loss of polymer in composite particle in narrow temperature range was attributed to the acceleration of polymer decomposition by catalytic iron oxide.

When composite particles were prepared by higher polymer/magnetite ratio (251/100) at 80 °C, each composite particle included one magnetite nanoparticle although this was not the case for AG(50), the affinity of which with iron ions was too strong to prevent aggregation of composite.

5. Magnetic Properties and Morphology of Composite Particles

The saturation magnetization depended on the composition of polymer as presented in Fig. 9. The highest saturation magnetization (Ms) was recorded by the composite particles prepared by using AG(10) whose Ms was over 50 emu/g iron oxide. The amount of AG(10) relative to iron oxide affected the morphology of composite particle, FE- TEM revealed that composite particles containing less amount of polymer had dense polymer phase. Such a structure was expected to demonstrate low relaxation of magnetic resonance.

6. Assessment of Composite Particle as MRI Contrast Enhancer

Signal intensity for magnetic resonance imaging (MRI) depends on proton density, which is proportional to transverse magnetization and to longitudinal relaxation before radiofrequency irradiation. Therefore, the ratio of transverse relaxation rate (R2) to longitudinal one (R1) is the key parameter for the performance of MRI contrast enhancer.

$$R1 = (1/T1post - 1/T1pre)/C \qquad (1)$$

$$R2 = (1/T2post - 1/T2pre)/C \qquad (2)$$

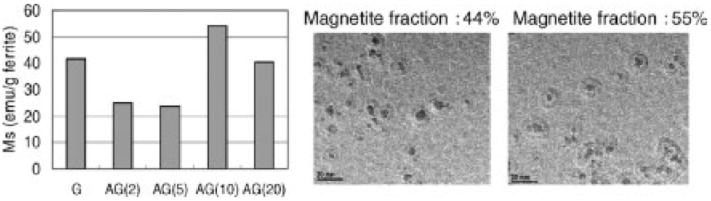


Figure 9.
Saturation magnetization of different composite particles and morphologies of AG(10) particles.

where Tn (n = 1 or 2) is time constant for longitudinal or transverse relaxation and "pre" and "post" are solvent and 0.1 mM solution, respectively. C is concentration.

The characteristic of MRI contrast enhancer should have a large R2/R1 The R2/R1 of AG(10) incubated at 80°C was 8 and 28 at 0.3 tesra and 1.5 tesra, respectively, which were almost the same with those of commercial ones.

Conclusion

Magnetic nano-particles composed of magnetite in the core and hydrophilic polymer in the shell were prepared by assembling polymer molecules in the aqueous medium followed by in-situ magnetite formation and crosslinking. Poly(ethyleneglycol)-block-poly(glycelol methacrylate-co-acrylic acid) with a molecular weight around 10,000 and acrylic acid content of 1–10% gave the most stable and uniform composite particles. Composite particles containing a single magnetite nanoparticle were obtained under a selected condition.

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