Magnetite fine particles highly loaded PMMA microspheres for hyperthermia of deep-seated cancer

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Poly(methyl methacrylate) (PMMA) microspheres several tens micrometers in diameter, containing highly dispersed sub-micron sized magnetite (Fe₃O₄) particles, were successfully prepared via an emulsion polymerization process. Both oleic acid and sodium oleate acted as suitable agents for high dispersion of the Fe₃O₄ particles in the starting monomer solution (MMA). Spherical PMMA particles encapsulating 65–72 mass % (30–38 vol %) of the Fe₃O₄ were obtained. The magnetic properties of the Fe₃O₄ loaded PMMA microspheres depended on the amount of Fe₃O₄ present in the resultant microspheres. The saturation magnetization and coercive force of the microspheres were 49–63 emu·g⁻¹ and 162–170 Oe, respectively. The rate of an increase in temperature by heat generated from an agar phantom dispersed with a microsphere sample was 1 K·min⁻¹ under an alternating magnetic field (100 kHz, 300 Oe). This material is expected to be utilized as a thermal seed for hyperthermic treatment of deep-seated cancers in our bodies using a gap-type alternating current field apparatus in combination with a prospective drug delivery system.

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1. Introduction

Ferrimagnetic fine particles such as magnetite (Fe₃O₄) have been attracting our interests since they can be used as a thermal seed of hyperthermia for local heating by their hysteresis loss to destroy deep-seated cancer cells with minimal adverse effects, after being delivered to blood capillaries around the cancer cells and then subjected to an external alternating magnetic field. For the practical use of the magnetic particle as a thermal seed effective for hyperthermia, the scale of the magnetic particle should be nanometer-size to show superparamagnetic properties, since it can generate heat quite efficiently. A previous report¹ demonstrated that magnetic nanoparticles dissipate heat not only from the rotation of the magnetic particles themselves (Brownian relaxation) but also from the rotation of the magnetic moment within the particles (Néel relaxation), and particles around 12 nm in diameter are estimated theoretically to show the maximum heat dissipation by both Brownian and Néel relaxation under the alternating magnetic field. Furthermore, to provide such particles with the ability both to be delivered readily and to block the blood capillaries around cancer tissues, preparation of microspheres 20–30 μm in diameter composed of ferrimagnetic or ferromagnetic particles has been attempted. However, all of the microspheres obtained in the previous studies were prepared finally subjected to a heat treatment at 673–873 K)³–⁴ or hydrothermal treatment.⁵ Therefore, it is favorable to prepare the microspheres at ambient temperature. Recently, polymeric microspheres a few tens micrometers in diameter were reported to be successfully fabricated via emulsion polymerization processes under ambient conditions.⁴⁰ On the other hand, magnetic fine particles-polymer composites such as Fe₃O₄-PDMA⁷–⁹ were prepared under mild conditions, however, their particle sizes (less than 150 nm) is too small to be sealed in blood capillaries. Spires et al.⁶ reported Fe₃O₄-poly(L-lactic acid) microsphere 23–32 μm in diameter prepared by an ultrasonic atomization technique. By abstracting several useful points of such methods shown above, in the present study, encapsulation of Fe₃O₄ fine particles as high content as possible with PMMA a few tens micrometer in diameter without any calcinations, ultrasonic or hydrothermal treatment was attempted. Then the magnetic properties and thermogenic activity of the resultant composite were evaluated whether it can be proposed as an alternative thermal seed for hyperthermia of deep-seated cancers. Although Fe₃O₄ around a dozen nm in diameter should be synthesized before or during the encapsulation with PMMA ideally since such nanoparticles are anticipated to achieve effective heat dissipation as described above, sensitive conditions and environment are required to synthesize it. Thus a commercially available sub-micron sized Fe₃O₄ particle was preliminary used as a starting ferrimagnetic component, and whether this encapsulation technique is beneficial or not was evaluated in the present study.

2. Materials and methods

2.1 Surface modification of Fe₃O₄ particles

Surface modification of commercially available Fe₃O₄ particles (200–500 nm, Wako Pure Chemical Industries Ltd., Japan) was attempted with oleic acid¹¹,¹² (OA; Wako Pure Chemical Industries Ltd., Japan) or sodium oleate (SO; Wako Pure Chemical Industries Ltd., Japan), to provide the surfaces of the Fe₃O₄ with hydrophobicity. Twenty grams of Fe₃O₄ particle were added to 40 cm³ of ethanol containing 4.8 vol % of OA or to 40 cm³ of water containing 4.8 vol % of SO in a zirconia pot, and underwent planetary ball-milling at 300 rpm for 7.2 ks at room tempera-
2.2 Preparation and characterization of PMMA microspheres containing Fe$_3$O$_4$ particles

The surface treated Fe$_3$O$_4$ particles were loaded into PMMA microspheres via an O/W type-emulsion polymerization process. Precisely, appropriate amount of the surface-modified Fe$_3$O$_4$ particles as described above were added to 10 cm$^3$ of methyl methacrylate (MMA; Kanto Kagaku, Japan) monomer solution together with 0.52 g of benzoyl peroxide (BPO; Kanto Kagaku, Japan) and 0.14 g of N,N-dimethyl-p-toluidine (DMPT; Kanto Kagaku, Japan). After the mixture was vigorously stirred for 180 s using a homogenizer, it was introduced into 300 cm$^3$ of 3.5 mass% poly(vinylalchol) (PVA; Mw = 2000, Kanto Kagaku, Japan) aqueous solution, and then the solution was stirred at 400 rpm for 5.4 ks at 310 K. Ar gas was previously bubbled in the PVA aqueous solution for 1.8 ks to remove the dissolved oxygen within the solution. During stirring, MMA oily phase was adsorbed to consist of Fe$_3$O$_4$ and to look spherical shapes several tens of micrometers in diameter, were subjected to a vibrating sample magnetometer (VSM; VSM-5, Toei Industry, Co. Ltd., Japan) under magnetic fields up to 10 kOe at room temperature to evaluate their magnetic properties.

2.3 Magnetic properties and in vitro heat generation of microspheres

Several kinds of the samples obtained, which were confirmed to consist of Fe$_3$O$_4$ and to look spherical shapes several tens micrometers in diameter, were subjected to a vibrating sample magnetometer (VSM; VSM-5, Toei Industry, Co. Ltd., Japan) under magnetic fields up to 10 kOe at room temperature to evaluate their magnetic properties.

Heat generation capacity of the samples, which looked spherical shapes and consisted of Fe$_3$O$_4$, was examined by placing the sample dispersed in an agar phantom, (agar content = 1.0 mass %, sample concentration in the agar solution = 25 mg cm$^{-2}$) in a glass tube under an alternating magnetic field of 100 kHz, 300 Oe. The heat generated from the sample was traced by measuring the change in temperature of the agar phantom as a function of time using a fiber optic temperature sensor (TempSens, Opsens Inc., Canada).

3. Results and discussion

3.1 Structural features of microspheres

Unless otherwise noted, all of the results shown in the following recitation were obtained under the ‘standard conditions’: the concentration of PVA solution of 3.5 mass % and the stirring rate of 400 rpm during the encapsulation process of the surface-treated Fe$_3$O$_4$ particles into PMMA.

Figure 1 shows SEM images of Fe$_3$O$_4$ particles before and after the surface modification with OA. Sub-micron sized particles were aggregated in the field of the visions, and no considerable change in size and morphology was observed. Figure 2 shows FT-IR spectra of the Fe$_3$O$_4$ particles before and after being subjected to the surface treatment with OA. A strong bands attributed to Fe–O bonds were confirmed at 584 cm$^{-1}$ both in the spectra of Figs. 2(a) and 2(b). On the other hand, characteristic adsorption bands related to the vibration of C–H bonds of the alkyl chains were newly found at 2954, 2927 and 2852 cm$^{-1}$. Moreover, a band related to the vibration of C=C bond of the olefin was detected at 1637 cm$^{-1}$. However, it is difficult to be assigned confidently because the spectrum of the sample before the surface treatment [Fig. 2(a)] also has such a band. In any case, the Fe$_3$O$_4$ particles were proved to be successfully subjected to the surface modification with OA from the FT-IR spectra. For the sample shown in Fig. 4(a), the mass fraction of the Fe$_3$O$_4$ used as a starting material, and caused the Fe$_3$O$_4$ to be reduced.

Figure 4 shows OM images of microspheres with and without an introduction of the surface-treated Fe$_3$O$_4$ particles using OA. For the sample shown in Fig. 4(a), the mass fraction of the Fe$_3$O$_4$ fed into the mixture of MMA, the polymerization agents and the Fe$_3$O$_4$ was 35 mass %. Spherical and fully opaque micro-
spheres smaller than 100 micrometers in diameter were optically observed as shown in Fig. 4(a), while spherical but transparent microspheres [Fig. 4(b)] which were smaller than those shown in Fig. 4(a) were observed. This suggests that a high dispersion of the Fe3O4 particles in PMMA microspheres and a homogeneous formation of the PMMA microspheres containing the Fe3O4 particles several tens micrometers in diameter were successfully achieved. Mean particle sizes estimated by manual measurements of the microspheres’ largeness taken in the OM images (average ± standard deviation calculated using random 20 microspheres in Fig. 4) were 49 ± 14μm and 72 ± 16μm for Fe3O4-free and -present microspheres, respectively. In addition, the crystalline phase in the sample was maintained as Fe3O4 even after the emulsion polymerization process [See Fig. 3(c)].

Figure 5 shows OM images of microspheres in which the fed amount of the surface-treated Fe3O4 was 52 mass%. The sizes of the microspheres clearly increased (125 ± 27μm) in comparison to those where the fed amount of the Fe3O4 was 35 [Fig. 4(a)] and 0 mass % [Fig. 4(b)]. On the other hand, curiously, the shape of the obtained microspheres where the fed amount of the magnetite was 68 mass % was very strange because it looked like a crescent moon (data not shown). The reason why the resultant composite was formed in such a shape under this condition is now being considered. By the way, the sizes, shapes and opacity of the microspheres obtained were quite similar, even when the concentration of PVA was changed to 4.0 mass % and the stirring rate to 600 rpm, to those prepared under the conditions of 3.5 mass % PVA at 400 rpm during encapsulating process of the Fe3O4 into PMMA, so further experiments were performed under the ‘standard conditions’ early mentioned in this section. However, since the size of the microspheres obtained in this study was somewhat larger than that which was aimed at, an alternative strategy should be considered to reduce the size of the microspheres to the intended one, such as making an attempt to increase the ratio of BPO to DMPT when they are mixed with MMA for activating nucleation more and rapid completion of the polymerization,9) or to raise the temperature of O/W emulsion for inhibiting viscosity increase of polymerizing MMA.

Figure 6 shows TG–DTA curves of microspheres in which the fed amount of the surface-treated Fe3O4 was 35 mass%. Between 500 and 700 K, a distinct decrease in weight and a sharp exothermic peak were seen, around which it seemed that the polymeric components burned out. Tilting of the exothermic peak to
the right side was assumed to be reflected by a delay for the rate of temperature increase against the heat generation from the sample detected. By reference to the result obtained in this measurement, an estimation of Fe$_3$O$_4$ content in the PMMA microspheres was carried out for 4 kinds of samples. The amount of the surface-treated Fe$_3$O$_4$ particles present in the PMMA microspheres corresponding to the fed amount of them, estimated by TG-DTA analysis, was summarized on Table 1. For all of the samples, the percentage of the residual weight of the samples heated at 1237 K was determined as the loaded amount, in spite that Fe$_3$O$_4$ must have been oxidized at such a high temperature, because no weight change was seen except for the significant weight loss between 500 and 700 K. For almost all the samples, the loaded amount of the Fe$_3$O$_4$ was larger than the fed amount and the former increased with increasing the latter but its increase rate was unproportional to a linear function. This may be attributed to an incomplete recovery of MMA, which was not polymerized very well, from the resultant O/W emulsion. Henceforth, these samples were then subjected to further experiments, and they are noted as OA-65, OA-72 SO-65 and SO-67 shown in the right hand side on Table 1.

### 3.2 Magnetic properties and in vitro heat generation of microspheres

Figure 7 shows magnetic hysteresis curves of the PMMA microspheres including the surface-treated magnetite particles with OA [Fig. 7(a)] and SO [Fig. 7(b)], together with untreated bare Fe$_3$O$_4$ particles only, after being subjected to VSM. From Figs. 7(a) and 7(b), the saturation magnetization (Ms) and the coercive force (Hc) were determined for the 4 kinds of samples and summarized on Table 2. Ms were found to increase with increasing the loaded amount of the Fe$_3$O$_4$ in the sample microspheres (49–63 emu·g$^{-1}$), whereas Hc was almost the same independent of the Fe$_3$O$_4$ content under the conditions adopted in this study (162–170 Oe). The values of Ms are reasonable since they are corresponding to the content of the Fe$_3$O$_4$ present in the sample microspheres in comparison to that of the untreated bare Fe$_3$O$_4$ particles (85 emu·g$^{-1}$). The results agree very much with the theory that Ms strongly depends on the density of a magnetic material per unit volume. However, the values of Hc were much larger for the samples with surface-treated Fe$_3$O$_4$ particles than that without ones (137 Oe), in spite that Hc depends on the particle size of a magnetic material. This phenomenon found in this study is illustrated by an assumption that the particle sizes of the Fe$_3$O$_4$ decreased to a certain degree after the ball-milling process although it could not be realized from the SEM image (Fig. 1). To clarify the change in crystallite size of the Fe$_3$O$_4$, half maximum full-widths (HMFW) of the largest XRD peak for the samples shown in Figs. 3(a)–3(c). As a result, it was found that the HMFW values of the peaks were almost the same among the samples. The crystallite sizes estimated by Scherrer’s equation for the samples, profiled in Figs. 3(a)–3(c), were 43, 37 and 36 nm, respectively. It is very difficult to evaluate whether the effect of the crystallite size on the Hc value is considered to be negligible or not only from this data, because Hc for 36 nm is about 50 Oe larger than that for 65 nm. On the other hand, amorphous phase(s) might have been removed from the surface of the bare Fe$_3$O$_4$ through the process of the ball-milling, which can affect the Hc values of the samples, because a weak signal around

![Fig. 6. TG (solid)-DTA (dotted) curves of microspheres with surface-treated Fe$_3$O$_4$ particles with OA (Fed amount of Fe$_3$O$_4$: 35 mass %).](image)

**Table 1.** Fed and loaded amount of surface-treated Fe$_3$O$_4$ into resultant microspheres. The latter was estimated by TG analysis

| Agent | Fed amount of Fe$_3$O$_4$ | Loaded amount of Fe$_3$O$_4$ | Sample notation |
|-------|--------------------------|-------------------------------|-----------------|
|       | gram % |                 | mass % | vol % |          |       |
| OA    | 5      | 35               | 65     | 30    | OA-65   |
|       | 10     | 52               | 72     | 38    | OA-72   |
| SO    | 5      | 35               | 65     | 30    | SO-65   |
|       | 10     | 52               | 67     | 31    | SO-67   |

*aUntreated bare Fe$_3$O$_4$.

![Fig. 7. Magnetic hysteresis curves of PMMA microspheres including surface-treated Fe$_3$O$_4$ particles with (a) OA and (b) SO, shown together with bare Fe$_3$O$_4$.](image)

**Table 2.** Saturation magnetization and coercive force of PMMA microspheres including surface-treated Fe$_3$O$_4$ particles

| Sample | Ms/emu·g$^{-1}$ | Hc/Oe |
|--------|----------------|-------|
| OA-65  | 53             | 162   |
| OA-72  | 63             | 165   |
| SO-65  | 49             | 166   |
| SO-67  | 56             | 170   |
| Bare*  | 85             | 137   |
$2\theta = 30^\circ$ which has not been identified yet in detail can be seen in Fig. 3(a). Thus, these assumptions should be well confirmed by further experiments, such as Mössbauer spectroscopic investigation,\textsuperscript{14} in the future.

Figure 8 shows time-dependent temperature curves of the agar phantoms in which the sample microspheres were dispersed. As mentioned before, an evaluation of heat generation was focused on the 4 kinds of samples which had been confirmed to look spherical shapes several tens micrometers in diameter. The temperature detected increased with increasing time, and the rate of an increase in temperature was almost 1 K·min$^{-1}$ for each sample. This result suggests that temperature around the sample may get to 316 K and therefore cancer cells are destroyed within 600 s after applying the magnetic field. As a result, the increase rates of temperature corresponded to areas surrounded by magnetic hysteresis curves for the samples up to 300 Oe. As a result, the areas of the magnetic hysteresis loops were almost the same for the 4 samples, which indicates that heat generated by hysteresis loss was similar among the samples. The amount of heat generated (P) was calculated to be 7 W·g$^{-1}$ by an assignment of frequency applied (100 kHz) and the area of the obtained hysteresis loop to the formula shown in a previous report.\textsuperscript{3} Although P value for the samples obtained in this study is relatively low compared to those for the microspheres reported previously, it can be acceptable as a thermal seed. However, the reason why their areas were not so different in spite that they had different content of Fe$_3$O$_4$ is now under consideration. Although now we have no data about the heat generation of the PMMA microspheres without Fe$_3$O$_4$ and even of the agar phantom, the amount of the heat generation of them might be negligible, since the change in temperature of a simple PMMA microsphere reported elsewhere\textsuperscript{5} was less than 1 K within 600 s under the same alternating magnetic field.\textsuperscript{3}\textsuperscript{1} However the heat generation behavior of them should be verified near the future.

It is also important to investigate in vitro colloidal behavior of the samples obtained, such as colloidal stability and zeta potential in saline, because the microspheres should maintain their characteristics even through the blood capillaries in our bodies. In vitro properties will be clarified near the future and be reported whether the microspheres obtained in this study can be put into practical uses. In addition, if this encapsulation technique can be applied directly to superparamagnetic nanoparticles, in vitro results may be very useful for future in vivo experiments, since the local environment such as a viscous medium surrounding the highly dispersed particle will not disturb heat dissipation from the magnetic particles.

4. Conclusion

PMMA microspheres several tens micrometers in diameter were polymerized with 65–72 mass % (30–38 vol %) of Fe$_3$O$_4$ particles via an emulsion polymerization process, when the Fe$_3$O$_4$ particles pre-treated with oleic acid or sodium olate were dispersed in MMA. The saturation magnetization and coercive force of the resultant microspheres were 49–63 emu·g$^{-1}$ and 162–170 Oe, respectively. The rate of an increase in temperature by heat generated from an agar phantom dispersed with a microsphere sample was 1 K·min$^{-1}$ under an alternating magnetic field (100 kHz, 300 Oe). This material is expected to be utilized as a thermal seed for hyperthermia of deep-seated cancers using a gap-type alternating current field apparatus in combination with a prospective drug delivery system, in so far as the size of the microspheres is made a little smaller than those obtained in the present study.

References

1) B. Jeyadevan, J. Ceram. Soc. Japan, 118, 391–401 (2010).
2) M. Kawashita, M. Tanaka, T. Kokubo, Y. Inoue, T. Yao, S. Hamada and T. Shinjo, Biomaterials, 26, 2231–2238 (2005).
3) M. Kawashita, S. Domi, Y. Saito, M. Aoki, Y. Ebisawa, T. Kokubo, T. Saito, M. Takano, N. Araki and M. Hiraoka, J. Mater. Sci.: Mater. Med., 19, 1897–1903 (2008).
4) J. Zhao, H. Sekikawa, T. Kawai and H. Unuma, J. Ceram. Soc. Japan, 117, 344–347 (2009).
5) T. Miyazaki, A. Miyaoa, E. Ishida, Z. Li, M. Kawashita and M. Hiraoka, Mater. Sci. Eng., C, 32, 692–696 (2012).
6) T. Kawai, H. Sekikawa and H. Unuma, J. Ceram. Soc. Japan, 117, 340–343 (2009).
7) G. K. Raghuraman and R. Dhamodharan, J. Nanosci. Nanotechnol., 6, 2018–2024 (2006).
8) F. Lan, K.-X. Liu, W. Jiang, X.-B. Zeng, Y. Wu and Z.-W. Gu, Nanotechnol., 22, 225604 (2011).
9) R. Y. Hong, B. Feng, X. Cai, G. Liu, H. Z. Li, J. Ding, Y. Zheng and D. G. Wei, J. Appl. Polym. Sci., 112, 89–98 (2009).
10) K. M. Spies, J. S. Forsythe, K. Suzuki and J. D. Cashion, J. Magn. Magn. Mater., 311, 97–100 (2007).
11) T. Banert and U. A. Peuker, J. Mater. Sci., 41, 3051–3056 (2006).
12) B. J. Park, M. K. Hong and H. J. Choi, Colloid Polym. Sci., 287, 501–504 (2009).
13) Z. Li, M. Kawashita, N. Araki, M. Mitsumori, M. Hiraoka and M. Doi, Mater. Sci. Eng., C, 30, 990–996 (2010).
14) Z. Li, M. Kawashita, N. Araki, M. Mitsumori, M. Hiraoka and M. Doi, Biomed. Mater., 5, 065010 (2010).

Fig. 8. Time-dependent temperature curves of agar phantoms in which sample microspheres were dispersed. The interior Fe$_3$O$_4$ particles were surface-treated with (a) OA and (b) SO.