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Ultrasound Enhanced Ferrite Plating of Polyacrylate Microspheres for Medical Applications

M. OJIMA, F. SHIRASAKI, Y. KITAMOTO, M. ABE, and S. NAGAHATA*
Department of Physical Electronics, Tokyo Institute of Technology
O-okayama, Meguro-ku, Tokyo, 152-8552, Japan
Fax: 81-3-5734-2199, e-mail: amasanor@pe.titiech.ac.jp
*Fine Chemicals, NIPPON PAINT Co., Ltd.
4-1-15, Minamishinagawa, Shinagawa-ku, Tokyo, 140-8675, Japan
Fax: 81-3-3740-1186, e-mail: s-nagahata@nipponpaint.co.jp

Polyacrylate microspheres, 4.5 μm in diameter, were coated with magnetite layer by ultrasound enhanced ferrite plating at 70°C from an aqueous solution of $FeCl_2$, utilizing $Fe^{2+} \rightarrow Fe^{3+}$ oxidation by $NaNO_2$. Power ultrasound waves (19.5 kHz, 600 W) were applied to the $FeCl_2$ solution (300 ml in volume, pH=6.0). The ferrite coating is of single phase magnetite, which covers the whole surface of the microsphere, while the ferrite coating synthesized previously without sonication covers the surface only in part. The ferrite coating prepared in this study are ~100 nm in grain size, which is reduced from that, i.e. 300~400 nm, of the coating prepared by ultrasound-enhanced ferrite plating in our previous study. This owes to improvement in plating conditions. The present ferrite-encapsulated microspheres exhibit higher efficiency of magnetic separation than the previous ones, as analyzed by turbidimetry. This will improve performance of enzyme immunoassay which has been put to practical use utilizing the partially-ferrite-coated microspheres.
Key words: ferrite plating, magnetite coating, magnetic separation, immunoassay

1. INTRODUCTION

Ferrite plating enables formation of crystalline spinel ferrite films from an aqueous solution at low temperatures below 100°C [1-3]. Ferrite-plated films prepared from aqueous solution exhibit a high biocompatibility, which enables the films to be used in biomedical applications such as biosensors and immunoassays [2].

Polyacrylate microspheres, 4.5 μm in diameter, coated with magnetite by ferrite plating have been applied to magnetic cellular separation [4]. Antibody immobilized on the ferrite coating traps cancer cell by an immunoreaction between the antibody and the antigen existing on the surface of cancer cell [5]. Magnetic separation technique expedites the separation of the particles from aqueous solution, which facilitates high performance assaying. The ferrite-coated microspheres will be used for the separation and extraction of DNA [6,7] and a system which automate the processes [8].

The quality of the magnetite coating on the polyacrylate spheres were improved by applying power ultrasound waves (19.5 kHz, 600 W) to the reaction solution (ultrasound enhanced ferrite plating) [9]. Without sonication the coating covers the surface of the spheres only in part, while the coating formed under sonication encapsulates the surface entirely and uniformly (as will be shown later in Fig. 5).

In this study we further improved the quality of the magnetite coating by synthesizing under improved conditions, in order to prepare ferrite-coated polymer microspheres which enables higher-performance biomagnetic separation. This paper describes the improved ferrite plating experiments and reports SEM observation on the growth process of the magnetite coating. Magnetic properties, efficiency of magnetic separation, and sedimentation rate measured for the ferrite-coated particles are reported.

2. EXPERIMENTAL

Fig. 1 shows the apparatus used for the magnetite coating. Polyacrylate microspheres (4.5 μm in diameter) were dispersed in an aqueous reaction solution (300 ml) of $FeCl_2$, to which an oxidizing solution of $NaNO_2$ was added and power ultrasound waves (19.5 kHz, 600 W) were applied. Mixed aqueous solution was kept at 70°C by using water bath and at pH=6.0 by adding a NH_4 solution. From our previous experiments we changed experimental conditions as follows: (1) shape of vessel containing the mixed aqueous solution is changed from flat-bottomed to round-bottomed; (2) pH buffer (CH_3COONH_4) previously added to the reaction solution is not used; and (3) oxidation-reduction potential (kept to a constant value previously) is not controlled, and an oxidizing reagent solution of $NaNO_2$ is supplied at a

fixed flow rate (2 ml/min). Change (1) will prevent sedimentation of the particles around the corner of the flat-bottom of the vessel, thus facilitating uniform ferrite encapsulation of the particles. Change (2) will facilitate finer grain growth of ferrite as described later.

The surfaces of the ferrite coatings were observed by a field emission type scanning electron microscope (FE-SEM). The crystallographic and magnetic properties of the ferrite coated particles were investigated at room temperature using a CuKα X-ray diffractometer (XRD), a Co^{57}-Mössbauer spectrometer, and a vibrating sample magnetometer.

Efficiency of magnetic separation and also rate of sedimentation from water were measured by turbidimetry, measuring time dependence of absorption of light (λ=660 nm) transmitted through the water containing the microspheres. Into a 2 ml of distilled water contained in a glass cell a 2 mg of microspheres was dispersed by ultrasound sonication. Immediately after sonication a pair of magnets was placed to sandwich the glass cell (applied magnetic field is ~40 Oe) and the transmitted light intensity was measured as a function of time. In a similar way but without putting the magnets the sedimentation rate was evaluated. The light intensity increases with time because turbidity of water decreases as the microspheres are coagulated by magnetic field and/or sedimented by gravity.

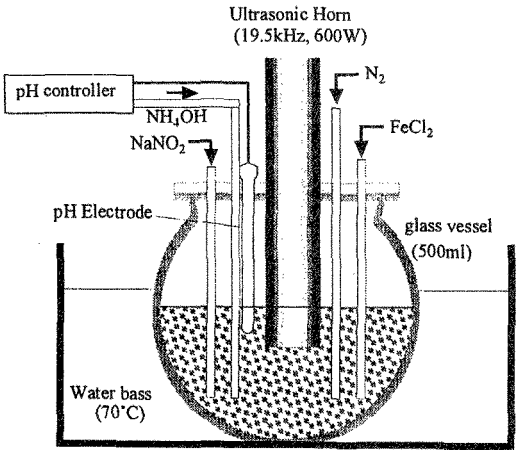


Fig. 1 Improved experimental apparatus used for ultrasound-enhanced ferrite plating of polyacrylate microspheres.

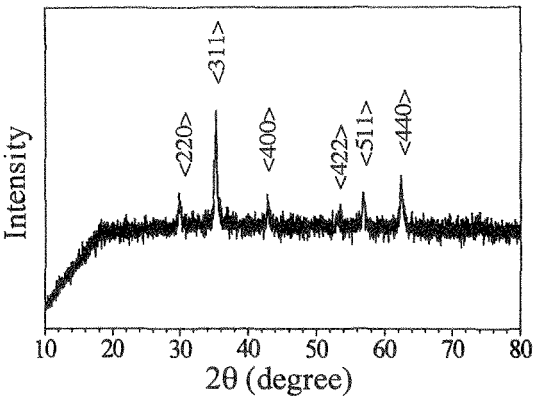
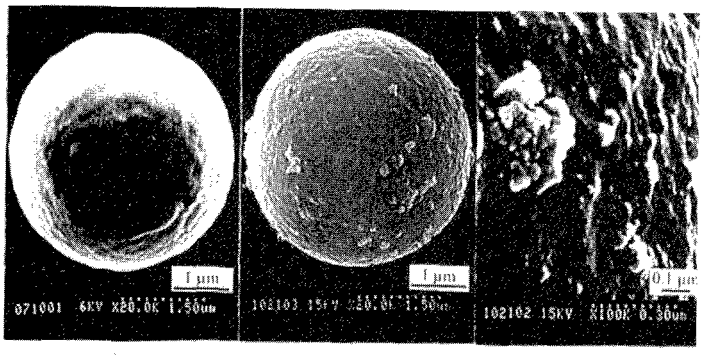
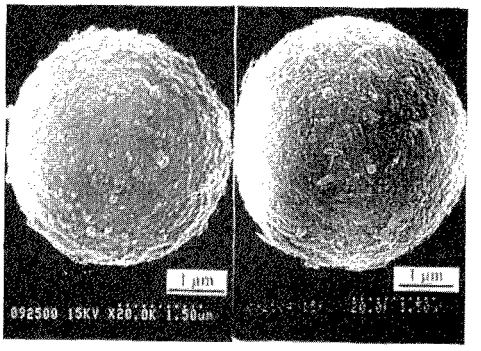


Fig. 2 X-ray diffraction diagram for polyacrylate microsphere plated for 4 min under sonication.



(a) 0 sec (b) 20 sec (c) 20 sec

Fig. 3 FE-SEM observation of polyacrylate microspheres, original ((a)), ferrite-plated for 20 sec under sonication ((b) and (c)).



(a) 40 sec (b) 120 sec

Fig. 4 FE-SEM observation of early stage (40 and 120 sec) of ultrasound enhanced ferrite plating on polyacrylate microspheres.

3. RESULT AND DISCUSSION

X-ray analysis revealed that all the coating prepared were crystallized in single phase with spinel structure, giving scattering peaks similar to those reported for standard powder samples of Fe_3O_4, as a typical example is shown in Fig. 2. Mössbauer analysis also revealed that the coating is of single phase; the spectra are composed of Zeeman-split lines ascribed to slightly cation-vacant magnetite as will be described elsewhere [10].

Fig.3 shows SEM surface images of the microspheres, original and plated for 20 sec. The initial nucleation islands of ferrites are 10~20 nm in size. The grains grows in number and in size, up to ~100 nm when plated for 40 sec as shown in Fig. 4 (a). When plated for 2 min, continuous ferrite coating, having grains ~50 nm in average size, covers the whole surface (Fig. 4 (b)). Fig. 5 compares the microspheres plated for 20 min in this study with those plated in previous studies for 20~25 min. Without sonication some part of the surface of the surface remains uncoated ((c)). With sonication in the previous study the surface is completely encapsulated by ferrite coating which is, though, bigger in size i.e. 300~400 nm ((b)). Therefore, the improved conditions (use of round-bottomed vessel and no use of pH buffer) in this study reduce the grain size, improving the quality of the ferrite coating.

Fig. 6 shows plating time dependence of saturation magnetization of the ferrite-coated microspheres and thickness of the ferrite coating calculated assuming that the coating has magnetization and density equal to those of bulk sample of Fe_3O_4. The magnetization and thickness increase with time. Experience has shown that magnetic separation using the 4.5μm-sized particles is best performed when the thickness of the magnetite coating is ~50 nm, or magnetite occupies ~20 % in total weight fraction of the encapsulated particles. This optimum thickness is obtained when plated for ~2 min, as shown in Fig. 6.

Fig. 7 shows the results of the sedimentation rate analyses performed on particles plated for various lengths of time and on those uncoated and coated in the previous study for comparison. The rate becomes higher with the increase of plating time, because the weight of the magnetite coating and therefore the average density of the whole coated particles become large. The particles plated for 2 min to have ~20 % in weight fraction exhibited a sedimentation rate roughly the same to those having the same weight fraction prepared in the previous study without sonication. However, Fig. 7 shows that the particles plated for 2 min in this study have a higher efficiency of magnetic separation than the particles prepared in the previous study; the normalized turbidity for the latter remains at ~0.2 even after the lapse of 180 sec, while that of the former decreases down to ~0.02 at the same lapse of time.

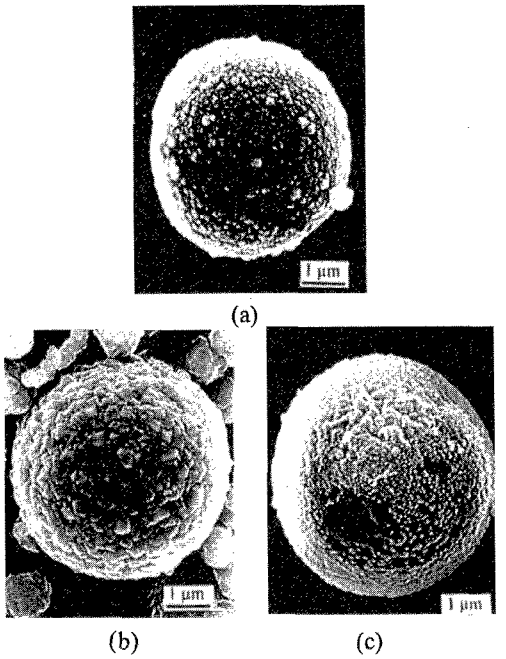


Fig. 5 FE-SEM images of polyacrylate microspheres ferrite-plated for 25 min under sonication in this study (a), for 25 min in our previous study (b), and plated for 20 min without sonication (c) [4].

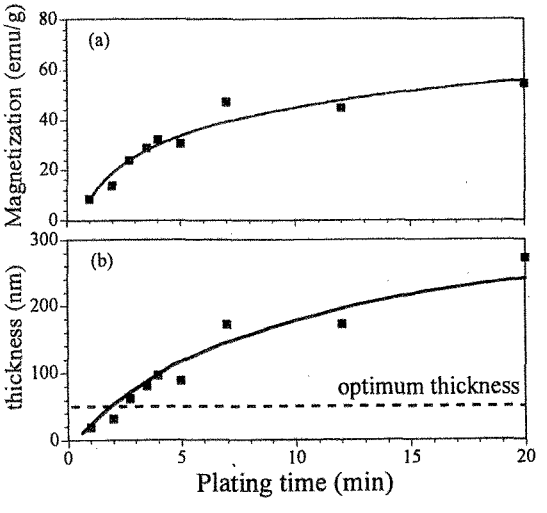


Fig. 6 Saturation magnetization (a) and thickness of ferrite coating calculated from it (b), plotted as a function of plating time. Optimum thickness for biomagnetic separation is indicates.

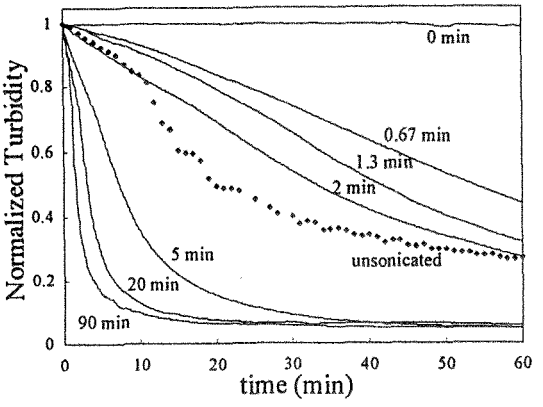


Fig. 7 Turbidimetric analysis of sedimentation rate from water for microspheres ferrite-coated for various lengths of time.

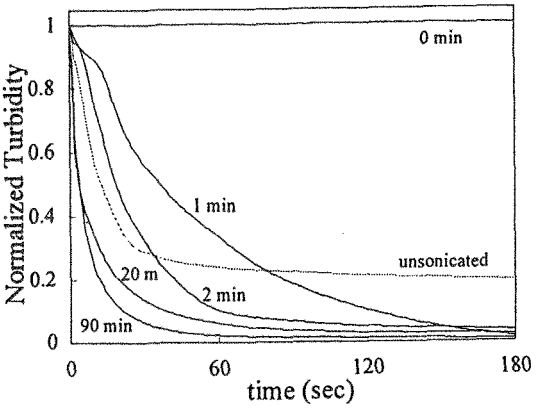


Fig. 8 Turbidimetric analysis of efficiency of magnetic separation from water under external magnetic field of 40 Oe for microspheres ferrite-coated for various lengths of time.

4. CONCLUSION

By improving conditions of the ultrasound enhanced ferrite plating, polyacrylate microspheres, ~4.5μm in diameter, are successfully coated uniformly with magnetite layer. Compared to those prepared under sonication in the previous study [9], the grain size is reduced from 300~400 nm to ~100 nm. The surfaces of the present particles are completely covered with the magnetite coating, while surfaces of those plated without sonication (which have been used for magnetic cellular separation [4]) are coated only in part (c.f. Fig. 5).The smaller grain give wider area of the ferrite surface on which the greater number of antibody can be immobilized, which will increase the efficiency of biomagnetic separation. The complete encapsulation of the polyacrylate surface with ferrite also improves efficiency of biomagnetic separation. This is because (1) partially-coated particles have magnetization widely dispersed in magnitude, and those having weaker magnetization exhibit slower response to magnetic field, thus reducing magnetic separation efficiency; and (2) partially covered particles have two materials, ferrite and polyacrylate, on the surface, which reduces the specificity of the immunoreaction; spurious reactions occur on the uncoated polyacrylate surface in addition to the specific antibody-antigen immunoreaction.

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