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# Preparation of Magnetic Gelatin Microspheres for the Targeting of Drugs

**Kang Choon Lee*, Ik Bae Koh and In Joon Oh**

*College of Pharmacy, Chonnam National University, Gwangju 500, Korea*

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**Abstract** □ Magnetically responsive gelatin microspheres for the targeting of drugs have been prepared using a water-in-oil emulsion technique with chemical cross-linking of the protein. The manufacturing variables affecting microsphere size, size distribution and surface characteristics have been examined as well as the magnetic responsiveness *in vitro*.

Sesame oil was utilized for non-aqueous phase and magnetic gelatin microspheres of different size from 1.89 to 14.88$\mu$m in mean diameter could be obtained with variation of HLB values of non-ionic surfactants. The content of magnetite which uniformly distributed throughout the microspheres was 26.7% (w/w). It was possible to control the localization of magnetic gelatin microspheres at specific sites within capillary models by using external magnetic field of under 5K gauss.

**Keywords** □ Magnetic microspheres, Gelatin, Drug targeting, Water-in-oil emulsion, Cancer chemotherapy, Gelatin microspheres.

Major objectives of recent effort on the drug delivery system have been an increased duration of activity, a reduced toxicity, a cell-specific therapy, or an improved stability during storage. Of them, specific delivery of drugs to desired target sites with a minimum of systemic side effects constitutes one of the ongoing challenge of this field (1-3). A good example of the need of drug targeting is in cancer chemotherapy where agents that show activity against cancer cells also have a toxic effect on normal tissue. This is because, due to the normal broad tissue distribution of the drug, it will result in killing off healthy cells as well as cancer cells.

The approaches that are being used differ widely and range from chemical methods such as the use of prodrugs (4), to the use of liposome (5), microspheres (6, 7) and macromolecules (3, 8).

Although a variety of innovative drug delivery systems has been proposed, none of them to date have achieved reasonable levels. To achieve the first-order targeting that involves the restricted distribution of drugs to the capillary beds of a predetermined target site, Widder *et al.* (9) and Morimoto *et al.* (10) proposed a magnetic method of concentrating magnetically responsive drug-bearing albumin microspheres in a required target stite.

The aim of this study is to prepare magnetic responsive gelatin microspheres by water-in-oil emulsion polymerization modified from both methods of Tanaka *et al.* (11) and Scheffel *et al.* (12). Gelatin is known to be biodegradable, less antigenic, and capable of incorporating a wide variety of drug molecules in a basically non-selective way, as well as being regarded as excellent delivery systems for cancer chemotherapy (13, 14).

## EXPERIMENTAL METHODS

### *Materials*

Purified gelatin was obtained from Nakarai Chemicals, Japan, Span 85, Span 80, Span 20, glutaraldehyde solution (25%) were purchased from Tokyo Kasei Chemicals, Japan, cotton seed oil, from Hanawa Chemicals, Japan, and sesame oil, soybean oil and corn oil were supplied by Dong-Bang Oil Company. Water-based magnetic fluid which was a suspension containing 40 percent magnetite fine particles ($Fe_3O_4$, diameter; 10-20 nm, W-40 type) was obtained from Taiho Indus-

* To whom all correspondence should be addressed.

tries, Japan, and ethyl ether and acetone were from Wako Chemical, Japan.

***Instruments***

Stirrer with glass rod; No 7604 (Black & Decker, USA), Emulsifier; Ultra Turrax (Janke & Kunkel, W. Germany), Centrifuge; Minor (MSE, England), Light Microscope (Vicker, England), Scanning Electronic Microscope with Electron Prove Microanalysis; JCM-35C (Jeol, Japan), Ion sputter; JFC-1100 (Jeol, Japan), Atomic Absorption Spectrophotometer; Hitachi 170-30 (Hitachi, Japan), Electromagnet (Cenco, USA), Gauss meter; Type 3251 (Yokogawa, Japan), and Syringe Pump; Model 341 (Sage Instruments, USA).

***Preparation of Magnetic Gelatin Microspheres***

A mixed aqueous solution containing 600mg of gelatin in a volume of 3.6ml of water and 0.6ml of magnetic fluids was emulsified into 100 ml of vegetable oil (sesame oil) containing 10 per cent (v/v) non-ionic surfactant (Span 80) with stirring at 2500 rpm for 15 minutes at 75℃. The resultant emulsion was homogenized again with an Ultra Turrax at 20000 rpm for 5 minutes at the same temperature.

The obtained emulsion was cooled in an ice bath to induce complete gelation of microspheres and then treated with 1 ml of glutaraldehyde solution (5.0%) for 20 minutes to harden the matrix followed by a successive washing with acetone and ether and vacuum-drying. The final product was in the form of discrete spherical units and recovered as a free-flowing powder.

***Microscopic Observation and Particle Size Analysis***

Surface charateristics and particle size analysis were done with a scanning electron microscope. About 1 mg of magnetic gelatin microspheres was placed on an electron microscope stub and coated with gold. Pictures of the microspheres were taken in various values of magnification by random scanning of the stub at 15KeV. The diameter of the microspheres were manually measured from the resultant photomicrographs, about 500 particles were counted per batch. Particle size distributions were calculated according to a method by Allen (31).

***Assay and Electron Prove Microanalysis of Magnetite***

Magnetic gelatin microspheres were dissolved into the 2 ml of 2N sodium hydroxide and then dissolved the magnetite with 3ml of hydrochloric acid. After dilution with distilled water, the content of iron was assayed by atomic absorption spectrophotometer at 248nm.

Distribution of magnetite in the magnetic gelatin microspheres was detected by electron prove microanalysis with the same procedure of scanning electron microscope described above except the condition of 25KeV and X-ray emission spectrum of 1.936Å.

***Measurement of the Magnetic Responsiveness of the Microspheres***

The magnetic responsiveness of the magnetic gelatin microspheres was observed by using a constant flow apparatus modified from the Morimoto's (15) as shown in Fig.1. A 0.2 per cent polysorbate solution(Tween 80) in isotonic phosphate buffer(pH 7.4) was used as the suspension medium.

The values of magnetic strength developed in the capillary tubes (1.0mm in diameter, 18cm in length) were adjusted from 0.5K to 20K gauss through electromagnet pole (15mm in diameter) and the flow rates were adjusted to 1.0cm/sec and 10.0cm/sec, respectively. The magnetic gelatin microspheres suspended in the suspension medium were injected into the introduction chamber. The microspheres retained in the capillary tube

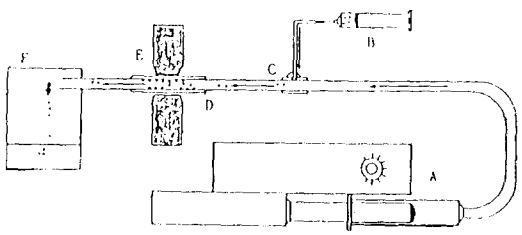


**Fig.1. A constant-flow apparatus used to measure the magnetic responsiveness of magnetic gelatin microspheres.**
A: Syringe pump, B: Microsyringe, C: Introduction chamber, D: Capillary glass tube, E: Electromagnet, F: Container.

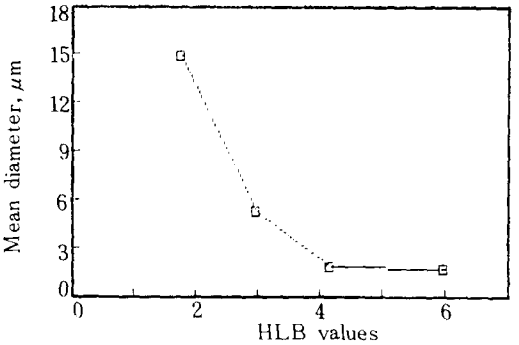


**Fig.2. Effect of HLB values on the mean diameter of magnetic gelatin microspheres.**

were collected and assayed for iron. Each retained amount of magnetite was calculated as a per cent of magnetites relative to the initial amount injected.

## RESULTS

Four kinds of vegetable oils, *e.g.*, sesame oil, cotton seed oil, soybean oil and corn oil were used to prepare the magnetic gelatin microspheres on the standard preparative conditions. Of them relatively narrow particle size distribution, fewer aggregation and more spherical particles appeared in the microspheres obtained with sesame oil.

Smaller particles with broad size distibution than those of sesame oil were obtained with cotton seed oil. Magnetic gelatin microspheres with corn oil and soygbean oil appeared to get porous, more aggregated and erratic nature with poor reproducibilities.

Fig.2 shows the mean diameters of magnetic gelatin microspheres prepared with sesame oil on the same concentration of non-ionic surfactants, sorbitan esters (Span series) having different HLB values.

The HLB values had a relatively large effect on the mean diameter of magnetic gelatin microspheres with the larger HLB values producing the smaller particle size. As the HLB values of non-ionic surfactants were 1.8, 3.0, 4.2 or 6.0, then the mean diameter ($\mu$m $\pm$S.D) decreased to 14.88 $\pm$ 3.27, 5.40 $\pm$1.99, 2.06 $\pm$0.94 and 1.89 $\pm$1.19, respectively.

But a decrease in HLB values is accompanied by a broader size distribution of magnetic gelatin microspheres prepared with sesame oil as shown in Fig.3 and also by having more spherical, dense and clean surface nature as shown in Fig.4.

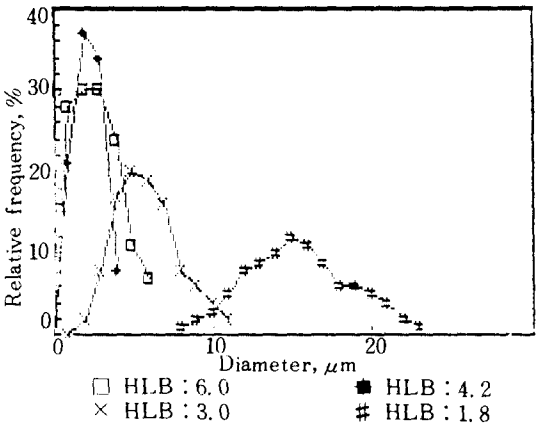


**Fig.3. Effect of HLB values on the size distribution of magnetic gelatin microspheres.**

Using the preparation method of magnetic gelatin microspheres described in experimental method we examined the effect of glutaraldehyde concentration on mean diameter and size distribution of magnetic gelatin microspheres.

In this study the glutaraldehyde solution was added just after ice-cooling the Ultra Turrax emulsified product.

The results in Fig.5 show that glutaraldehyde concentrations less than 5 per cent had no effect on the mean diameter and size distribution of magnetic gelatin microspheres but as a consequence of increasing the concentration of cross-linking agent in the order of 5, 12, 25 per cent there was a corresponding increase in the mean diameter from 2.06 to 2.63 to 6.01 $\mu$m on the sesame oil magnetic gelatin microspheres and also broadness in the size distribution. In addition, with

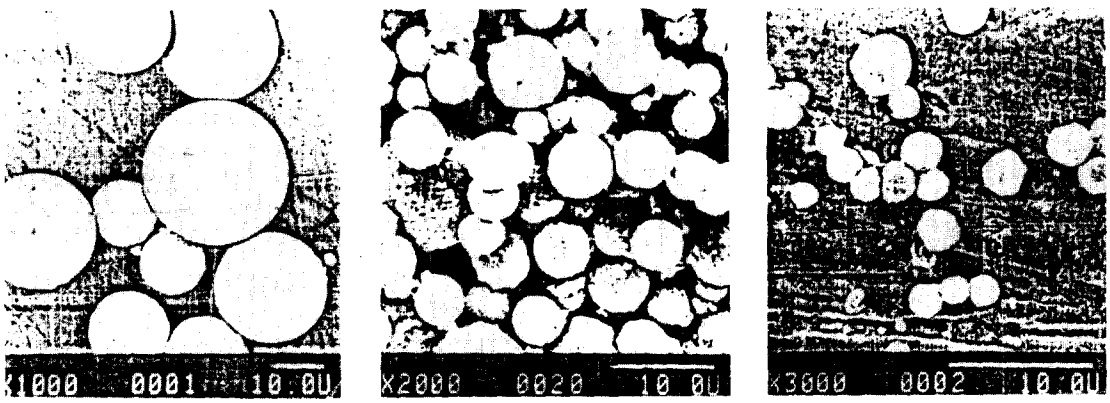

**Fig.4. Scanning electron microscopic appearance of magnetic gelatin microspheres.**
Left: HLB 1.8, Center: HLB 3.0, Right: HLB 4.2.

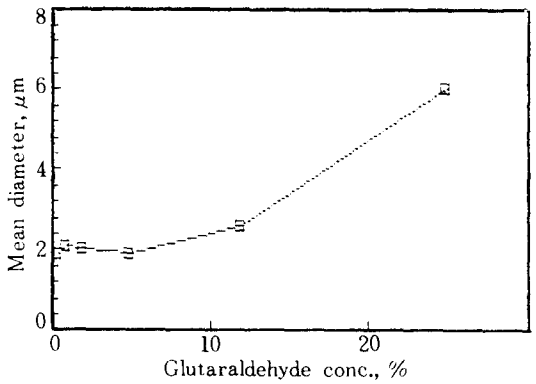


**Fig.5. Effect of the concentration of glutaraldehyde solution on the mean diameter of magnetic gelatin microspheres.**

25 per cent glutaraldehyde there was a some agglomeration of individual gelatin droplets and the resultant microspheres. Reaction time of glutaraldehyde with gelatin had no effect on both mean diameter and size distribution.

The trapping yield of magnetite into the magnetic gelatin microspheres varied according to the vegetable oil used. Magnetite trapped on the gelatin microsphere of sesame oil and cotton seed oil were 26.7% and 24.2% of the microspheres, but on the contrary, in cases of soybean oil and corn oil remained below 9.8% and 12.4%, respectively. But the reproducibilities were good in all cases.

The HLB of non-ionic surfactants had no detectable effect on the trapping yield of magnetite.

The presence and distribution of magnetite into the magnetic gelatin microspheres were observed. The left picture of Fig.6 is a scanning electron micrograph and the right is an electron prove microanalysis micrograph of the same magnetic

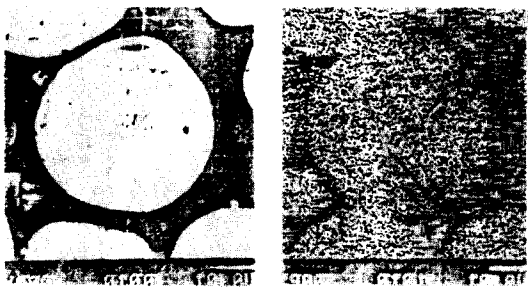

**Fig.6. Magnetite in the magnetic gelatin microspheres detected by electron prove microanalysis.**
Left: Scanning electron micrograph. Right: Electron prove microanalysis micrograph.

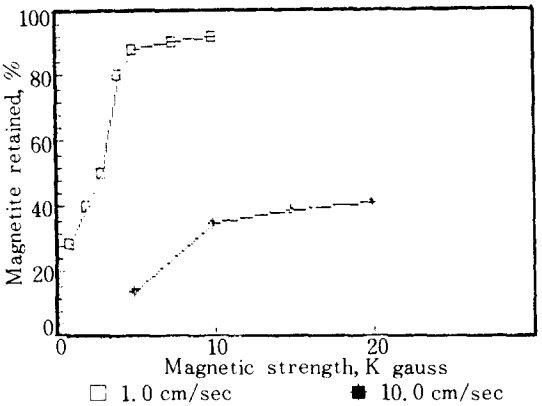


**Fig.7. Retention of magnetic gelatin microspheres as a function of the strength of external magnetic field in different flow rates.**

gelatin microspheres. No localization of magnetite appeared as white stipple in the figure was observed.

The magnetic responsiveness of magnetic gelatin microspheres under varying linear flow velocities and strength of external magnetic fields is shown in Fig.7. A glass tubing with an inner diameter of 1.0mm and laminar flow rate of 1.0cm/sec and 10cm/sec were selected as models for capillary and middle blood vessels.

The retention of magnetic gelatin microspheres was directrly dependent on the strengh of applied external magnetic field to 4K gauss at the 1.0cm/sec of flow velocity and about 90 per cent up of magnetic gelatin microspheres injected were retained over 5K gauss. However, progressively less amounts of magnetic gelatin microspheres were retained as velocity was increased to 10cm/sec on which the retention yield could not exceed 40 per cent even with 20K gauss.

## DISCUSSION

For the preparation of gelatin microspheres, complex and simple coacervation techniques (16, 36) are generally used and have been extensively studied but both coacervation ones are inadequate to prepare the gelatin microspheres containing magnetite.

Water-in-oil emulsion polymerization has been employed for the gelatin micropellet for sustained release dosage form (11) and albumin microspheres (12, 17). In this study their modification for the magnetic gelatin microspheres was performed.

Four kinds of non-aqueous phases were tried to

demonstrate if other phases could be utilized for gelatin microsphere preparation instead of cotton seed oil which has been widely used for the preparation of albumin microspheres by emulsion polymerization and Gallo *et al.* (18) recommended on the qualitative studies.

Of them studied, magnetic gelatin microspheres prepared with sesame oil appeared to be resonable on the mean particle size, size distribution, surface characteristics and other properties including trapping yield of magnetite.

It is well documented that the HLB values affect the droplet size of emulsions, and therefore, the resultant microsphere size (19). Generally no surfacants system is employed in the preparation of gelatin microspsheres by coacervation method but seldom used to improve the dispersability of the final product in water and to act as a solubilizing agent for the sparlingly soluble drugs (20). Yoshioka *et al.* (21) also used no surfactants to prepare gelatin microspheres by modified emulsifying method with vortex mixer, but only used non-ionic surfactants to reduce the particle size with ultrasonification, which is considered to effect more on the size reduction than surfactant (22).

Although the HLB effect on the mean diameter was found in this study, it was in the limit range of 1.89-14.88 $\mu$m.

But this range is enough to provide different size range of microspheres with or without magnetite which has been studied for the potential uses for drug trageting.

For example. microsphers above 12 $\mu$m could be adapted in the recently developed chemoembolization technique (33) for cancer therapy by intra-arterial delivery.

For the active drug targeting by intra-venous delivery, particle size should be at least under 3 $\mu$m, whereas microspheres greater than 3 $\mu$m lead to their use for the passive drug targeting by simple alteration in size of microspheres; microspheres between 3 and 12mm will become entrapped within the capillary networks of the lung, liver and spleen and micropsheres above 12 $\mu$m leads to their mechanical filtration by the lungs (23).

Another effect of HLB appeared on the size distribution and rounding of the surface of magnetic gelatin microspheres.

A decrease in HLB values of non-ionic surfactant is accompanied by broader in size distribution and more spherical microspheres. Therefore, non-ionic surfactants having HLB of 4.2 is recommended to get magnetic gelatin microspheres under 3 $\mu$m. From this condition the size distribution was narrow enough not to employ size-sorting process inevitable on general procedures for the preparation of magnetic and non-magnetic albumin microspheres. But to get larger microspheres with lower HLB, size-sorting process with membrane having desired pore size may be recommended.

Formaldehyde and glutaraldehyde are the widely used cross-linking agents for gelatin and of them the latter has been found to be better than the former (24).

Their applications are differ from selection of agent, concentration, and reaction time to reaction steps in the procedure, *e.g.*, pre-microsphere or post-microsphere treatment. Therefore, although no data appeared yet for the gelatin microspheres, the effect on the mean diameter or size distribution reported for the albumin microspheres differ from one another (15, 25, 26).

This work showed that at the concentration below 5 per cent no detectable effect on the mean diameter or size distibution of the microspheres was found but at above 5 per cent there appeared to be a direct relationship beween mean diameter of microspheres and gluaraldehyde concentration.

Addition of high concentration of gluaraldehyde may lead very rapid and excessive cross-linking of protein in a few seconds to make up of larger microspheres or massive aggregates and resultant broad size distribution.

Slow dropping of 5 per cent glutaraldehyde solution while rapid strirring, then slowing down the rpm of stirrer are recommended. The reaction time of cross-linking made no effect on the size and size distribution of magnetic gelatin microspheres but from the qualitative study greatly influenced on the resistance to the water on which later will be studied in this laboratory.

It is known that the power and time of emulsification will affect the resultant microsphere size (22). This was not seen clearly in this study and therefore failed to control the mean diameter and size distibution of magnetic gelatin microspheres by the variation of mechanical conditions.

But from this condition, the gelatin microspheres less than 3$\mu$m can be obtained without sonification step inevitable in general emulsifying procedure to get smaller gelatin or albumin microspheres (18, 21). Sonification generally causes denaturation and aggregation of the gelatin microspheres leading to wide size range because the heat generated during the process is not easy to dissipate (34) and also procedure consisting of sonification has many problems to scale up the process.

Although several apporaches including alternation in surface charge (27), coating of the sur-

face with either a bioadhesive and specific cell or tissue antibodies (28) have been studied to achieve the active first-order targeting with natu ral or synthetic polymer microcpheres, this magnetic microspheres technique has been increasingly studied because the surface of magnetic mircrospheres also can be modifi d with specific cell or tissue antibodies to enhance the first-order targeting efficiency (35) and because of its application for cell sorting (29).

And recently in spite of the successful use of antibody as a drug carrier, it cannot overcome the problem of first-order targeting by itself because the antibodies will interact with the corresponding antigens in the circulating blood before they gain access to the tumor lesion and the bulk of the macromolecular conjugation will be incorporated into the reticuloendothelial system by both nonimmunological and immunological mechanisms. So antibody approach could be used only as a means for second-order targeting which means the selective direction of drug to tumor cells vs. normal cells (32). This implies that the first-order targeting problem is more essential to achieve the effective second- or third-order targeting.

The prerequisites for the first-order targeting by magnetic microspheres are the trapping of magnetite in the gelatin microspheres and magnetic resposiveness of the microspheres.

Generally 20 per cent (w/w) of magnetite has been recommended for magnetic albumin microspheres (9) and gelatin magnetic microspheres from this procedure were enough to meet above recommended value by 26.7%.

The localization of magnetite in the microspheres which appeared in the magnetic albumin microspheres (9) did not happen in this magnetic gelatin microspheres which showed magnetite uniformly distributed throughout the spheres. This may be due to the fact that the magnetite is more homogeneously suspended in the viscouse gelatin solution than in the albumin solution. This phenomenon can be also explained by the good reproducibility of magnetite trapping yield.

Magnetite, one of ferromagnetic materials is readily magnetized in a magnetic field, therefore the magnetic gelatin microspheres prepared in this work were also magnetized in an external magnetic field. The mass of magnetic gelatin microspheres retained was dependent on the strengh of magnetic field and also on the flow velocity.

From the fact that average diameters of capillary and average blood flow velocities are 0.005-0.01 mm and 0.05-0.1cm/sec, respectively (30), the Fig.7 represents that the localization of magnetic gelatin microspheres on the capillaries of specific site in *in vivo* condition could be controlled by external magnetic field of under 5K gauss.

The procedure for preparing magnetic gelatin microspheres could be different according to the properties of intended products but from this primary study the possibilities of the preparation of new magnetic gelatin microspheres by water-in-oil emulsion polymerization and of localization of magnetic gelatin microspheres on the specific site with external magnetic field were confirmed.

In addition, this process could minimize the degradation of drugs to be entrapped in contrast with the case of albumin microspheres in which heating is indispensable and also the size of microspheres can be controlled by the variation of HLB values of non-ionic surfactants in the limit range.

This study has also shown that magnetic gelatin microspheres can be produced without recourse to either size-sorting or sonification.

These considerations will be further discussed in a following paper on drug incorporation and drug release.

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