표적지향 약물전달시스템을 위한 Fe_3O_4를 캡슐화한 PCL 나노입자의 제조 및 특성(Ⅱ)

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Characterization and Preparation of PCL encapsulated Fe_3O_4 nanoparticle for targeted drug delivery system(Ⅱ)

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Introduction

Magnetic polymer particles have been of interest since they exhibit wide application in the fields of gene manipulation, immunoassay, cell isolation, enzyme immobilization, targeted delivery of drugs, DNA separation, and so on. (1) Magnetic drug targeting should be safe and effective, i.e. particles capable of carrying the desired and releasing drug at a controlled rate. Since magnetic colloidal particles could in principle be a suitable vehicle their external-field responsiveness. In general, magnetite is well-known materials, properly characterized in many aspects, of which toxicity has been demonstrated to be quite low (LD_{50} in rat: 400mg/kg). (2) Magnetic particles alone, however, are not most suitable as drug vehicles, because of limitations in controlling the amount of drug they carry and the rate at which they can release it. Unlike inorganic magnetic particles, biodegradable polymers can release previously absorbed drugs at a rate determined by their degradation. (3) In addition, nanoparticle encapsulation has been proven useful for reducing the toxicity of certain drugs. Nanoparticles, like other colloidal carriers extended for drug delivery, after intravenous administration, are normally retained mainly by the reticuloendothelial system. (4)

In this study, nano-sized poly å-caprolactone (PCL) particles, widely used as a biodegradable polymeric carrier containing magnetite were prepared employing emulsification-diffusion method and double emulsion method. This study focused on the development of the encapsulation process utilizing ethyl acetate as a dispersed solvent. The Key factors which affected the characteristics of magnetic PCL particles such as their emulsion stability and morphology were investigated. Also magnetic PCL particles such as their shape, size distribution were characterized by SEM, TEM, DLS Magnetic hysteresis of magnetic pcl particles was investigated by VSM (vibrating sample magnetometer).

Experimental

1. Preparation of PCL encapsulated magnetite nanoparticles by emulsification-diffusion

0.5g of PCL and 0.1g of magnetite were dissolved in 10ml of ethyl acetate. The organic phase was added into 20ml of aqueous phase containing stabilizer. After mutual saturation of organic and continuous phase, the mixture was emulsified for 10min with ultrasonic

homogenizer at 50% power. In order to allow for diffusion of ethyl acetate into water, water was subsequently added to o/w emulsion solution under the moderate stirring. After reaction sample was obtained through cleaning procedure that included serum replacement in a stirred filtration cell and three cycles of centrifugation at 12000rpm. The magnetic PCL nanoparticles were dried in a vacuum oven at 35℃ and stored in container.

2. Preparation of PCL encapsulated magnetite nanoparticles by w/o/w double emulsion

Given amounts of the magnetite was dispersed in 2ml of PVA solution (0.5% w/w) and this aqueous solution was emulsified into the PCL solution(5% w/w) dissolved in 10ml of ethyl acetate by probe sonication at 30% power for 4minute. The resulting suspension or w/o emulsion was then emulsified into the external aqueous phase containing PVA (2% w/w) by probe sonication at 15% power for 1minute. Then, the double emulsion was diluted into 100ml of D.D.I water and the system was maintained under the moderate stirring. The final particles were collected by cleaning procedure, centrifugation as above.

Result and Discussion

1. Size & Size distribution

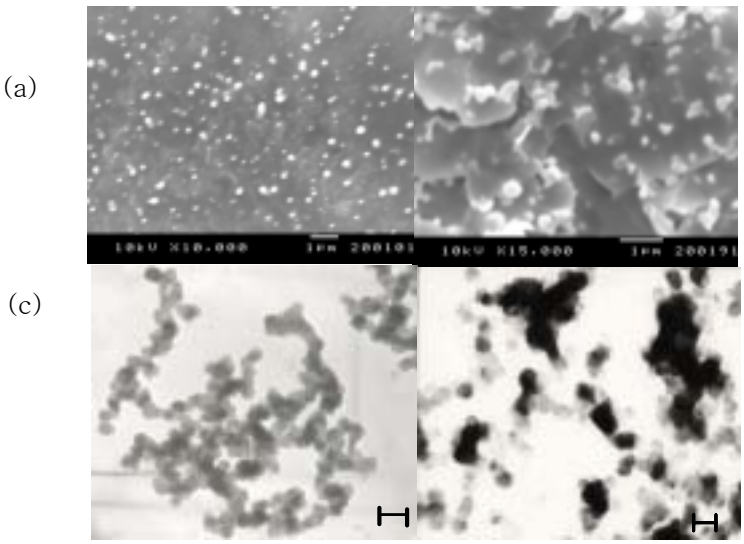


Fig 1. Comparison of magnetic PCL particles prepared by emulsification-diffusion(a,c) and w/o/w double emulsion method (b,d) (bar length = 200nm)

The morphology, average size and size distribution of magnetic PCL particles with magnetite/polymer proportions ranging from 1:5 to 4:5 were determined by SEM, TEM. As observed by figure 1, magnetic PCL particles prepared by the emulsification-diffusion process were smooth, well individualized and homogeneous in size. The results indicated that the average size of the magnetic PCL particles increased in the size range 150 to 170nm with increasing magnetite (not shown figures). Nanoparticles obtained by the double emulsion process had a broader size distribution. They appeared more aggregated and some had a network of spherical particles, upon drying. The average size of magnetic PCL particles ranging from 350 to 370nm could be prepared by increasing the magnetite/polymer ratio (not

shown figures).

2. Chemical Structure

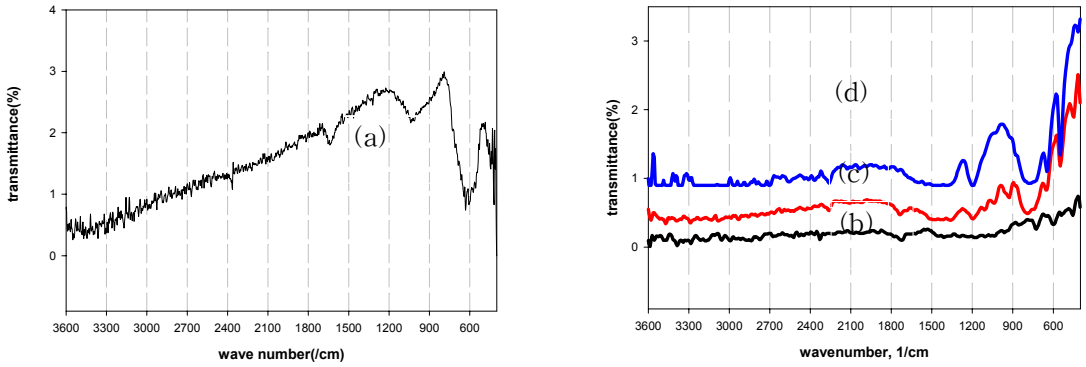


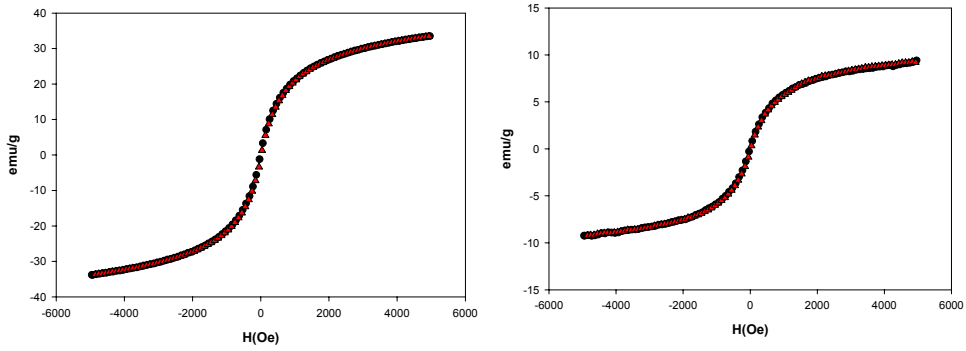
Fig 2. IR spectra of the PCL and magnetic PCL particles
(a) magnetite (b) PCL particles (c)emulsification-diffusion (d)w/o/w emulsion

Figure 2 showed the infrared spectra of the presence of active groups on magnetic PCL particles obtained by two methods. Strong peaks at 1726/cm(carbonyl bond), 2943/cm (carboxyl-hydro bond) were shown on the PCL particles. The most significant band of magnetic pcl particles (570/cm) was identified by comparison with magnetite(570/cm).(5) From the analysis of the FTIR spectra, it was clearly demonstrated that the magnetite materials are well incorporated in the core of PCL nanoparticles.

3. Magnetic properties

A magnetic hysteresis loop of the magnetic PCL particles was given in Fig.3. This displays the magnetization of the sample at 25℃, as the magnetic field of the susceptometer cycles between +6000 and -6000Oe. Under magnetic fields, the curves showed no hysteresis, also the remanence and coercitivity were zero. The saturation magnetization of magnetic pcl particles is smaller than that of bulk magnetite, but composite particles had properties that were close to the paramagnetic behavior.

(a)



(b)

Fig 3. VSM analysis of magnetite(a) and magnetic pcl particles (b)

4. Fe_3O_4 loading efficiency

Table 1. Incorporation loading and efficiency of magnetite loaded pcl nanoparticles

Methods	Fe_3O_4/PCL ratio (w/w)	Mean size (nm)	Fe_3O_4 loading (%)[a]	Entrapment efficiency (%) [b]
Emulsification-diffusion	**4:5**	**167±±0.5**	**3.5**	**7.84**
w/o/w emulsion	**4:5**	**339.5±0.5**	**6.7**	**15.80**

[a] Actual loading is expressed as mg of encapsulated magnetite per mg of microspheres
[b] Entrapment efficiency is the ratio between magnetite actual and theoretical loading percent

Table 1 indicated that magnetite incorporation and final particle size were investigated by comparison of emulsification−diffusion and w/o/w emulsion process. The size of the nanoparticles obtained by two methods increased consistently with increasing Fe_3O_4 content. But the size of the particles did not changed significantly(not shown tables). The entrapment efficiency was different according to methods and maximum magnetite entrapment efficiency were approximately 7.84%, 15.8% of the theoretical loading, respectively. The low entrapment efficiency of particles prepared by emulsification−diffusion could be due to the diffusion of magnetite/PCL solution from the inner oil phase to the external aqueous phase during diffusion step. Nevertheless, ones prepared by w/o/w double emulsion can be seen that the viscosity would increased with increasing the Fe_3O_4/PCL ratio and the high viscosity of the magnetic PCL solution tends to restrict migration of the inner aqueous/magnetite phase to the external water phase. Therefore, the results would be expected to increase the nanoparticle size and more the magnetite to be incorporated into the PCL particles than ones prepared by emulsification diffusion method.

Conclusion

In this study, well shaped spherical magnetic PCL nanoparticles were produced using the emulsification-diffusion method and w/o/w double emulsion process. The particles prepared in our study present some advantages. First, preparation of the particles was very simple. Second, the magnetic particles having suitable magnetic properties could easily and gently separated by using a magnetic field. Third, magnetic PCL particles were applicable in magnetically controlled drug targeting.

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