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Polymeric superparamagnetic carriers as potential drug delivery systems

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Abstract

This work evaluates an experimental set-up to produce superparamagnetic polymeric microcarriers. First, coprecipitation of iron salts in alkaline medium was used to synthesize superparamagnetic particles. Afterward, a emulsification/cross-linking reaction was carried out in order to produce superparamagnetic polymeric microcarriers. The sample characterization was performed by X-ray powder diffraction, laser scattering particle size analysis, optical microscopy, scanning electron microscopy, and vibrating sample magnetometry. The characterization data have demonstrated the feasibility of the presented methods to synthesize magnetic particles and to produce polymeric superparamagnetic carriers. Such systems may be promising for biotechnological purposes, such as magnetic drug targeting.

*Keywords***:** magnetic carriers; superparamagnetism; magnetic drug targeting.

Resumo

O objetivo deste trabalho foi avaliar um protocolo experimental para obtenção de partículas superparamagnéticas poliméricas. Inicialmente o processo de co-precipitação em meio alcalino de sais de ferro foi empregado para a produção das partículas superparamagneticas. Em seguida, uma técnica de emulsificação em conjunto com um processo de ligação cruzada polimérica foram realizados para a produção final de micro-carreadores poliméricos superparamagnéticos. A caracterização dos sistemas foi realizada por difração de Raios X, determinação da granulometria por espalhamento de luz, microscopia ótica, microscopia eletrônica de varredura e magnetometria de amostra vibrante. Os resultados do processo de caracterização demonstraram a viabilidade o método empregado para obtenção de partículas superparamagnéticas poliméricas. Tais sistemas podem ser promissores no tocante a biotecnologia de sistemas nanoestruturados como sistemas terapêuticos magnéticos para carrear fármacos.

Palavras-chave: carreadores magnéticos; superparamagnetismo; vetorização magnética.

Introduction

Aqueous suspensions containing small magnetic particles have been increasingly used in biosciences and biotechnology. When a magnetic field is applied, such systems develop magnetic polarization and magnetophoretic mobility and, because of such unique features, these particles are promising candidates for delivering drugs to specific locations within the body (ALEXIOU et al., 2000; VOLTAIRAS et al., 2002). By means of a selective application of a magnetic field on a desired area, drugs bound to these particles can be successfully carried to their site of action with high accuracy, minimum or no surgical intervention and maximum concentration (VOLTAIRAS et al., 2002). Therefore, regional therapy efficacy could be improved by increasing local drug concentration while systemic drug biodistribution and toxic side effects would be limited (ALEXIOU et al., 2002; VOLTAIRAS et al., 2002). An additional advantage is that magnetic particles have been shown to degrade in vivo. It occurs under the influence of a variety of hydrolytic enzymes, low pH and proteins participating in iron metabolism (WEISSLEDER et al., 1995).

Despite the promising properties, magnetic particles tend to agglomerate and are chemically unstable concerning oxidation in air. In order to overcome these drawbacks, and also increase biocompatibility, particle coating has been suggested (SANGREGORIO et al., 1999). Biodegradable and biocompatible materials such as gelatin have been investigated. The biocompatibility and the degradation to non-toxic and readily excreted products were the main attractive characteristics of gelatin, which suggests its use in the drug delivery field. However, being a soluble polymer, gelatin has to be modified to prepare drug delivery systems. Thus, to obtain a hydrophilic polymer insoluble at 37°C, the chemical formation of crosslinks among the macromolecular chains has been proposed (VANDELLI et al., 2001). The aim of this work was to evaluate an experimental procedure to synthesize magnetite particles and produce polymeric superparamagnetic carriers. The method of coprecipitation of iron salts in alkaline medium was used to synthesize magnetic particles (WANG et al., 1998; KIM et al., 2001). In order to improve particle properties to in vivo use, emulsification/cross-linking reaction (VANDELLI et al., 2001) was attempted in the production of carriers of cross-linked gelatin and superparamagnetic particles.

Materials and Methods

Materials

Ferric chloride hexahydrate, $FeCl_3.6H_2O$ (Synth chemical-Brazil); ferrous sulphate heptahydrate, $FeSO_4.7H_2O$ (Synth chemical-Brazil); sodium hydroxide, NaOH (J.T.Baker chemical-USA); hydrochloric acid, HCl (Vetec chemical-Brazil); chloroform, $CHCl_3$ (Vetec chemical-Brazil), ciclohexane, C_6H_{12} (Vetec chemical-Brazil); terephthaloyl chloride, $C_8H_4Cl_2O_2$ (Sigma chemical-German); ethanol, C_2H_6O (Vetec chemiacal-Brazil); polysorbate 20, $C_{58}H_{114}O_{26}$ (Vetec chemiacal-Brazil); polysorbate 80, $C_{64}H_{124}O_{26}$ (Vetec chemiacal-Brazil); gelatin (Vetec chemiacal-Brazil) and sorbitan triesterate, $C_{60}H_{114}O_8$ (Aldrich chemical-USA) were used as received from manufactures.

Methods

Synthesis of superparamagnetic particles

Solutions of ferric chloride and ferrous sulphate were prepared as a source of iron by dissolving the respective chemicals in HCl 0.4 N solution under vigorous stirring using a mechanical stirrer (Nova Ética, Model 103 - Brazil) at 100 mA. As a second step, solutions were combined and a homogenous mixture of $FeCl_3$ (0.1 mol l^{-1}) and $FeSO_4$ (0.05 mol l^{-1}) was formed. An aqueous dispersion of particles was obtained just after adding 10 ml of the mixture of ferrous and ferric salts drop-wise into 250 ml of NaOH 1N under vigorous mechanical stirring, as in a previous step, at room temperature (25°C) (SILVA et al., 2005). For such synthesis, the chemical reaction is expected as follows:

$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O$ (Equation 1)

Production of polymeric superparamagnetic microcarriers

In the following step, 2.4 g of gelatin was dissolved in 20 mL of phosphate buffer (pH 9.8) under vigorous stirring at 60ºC. The magnetic suspension [14.4g/L], previously neutralized, was sonicated for 40 min and 2 mL of this suspension was added into the aqueous gelatin solution. Emulsification was then carried out in 30 mL of chloroform:ciclohexane [1:4 $_{(v/v)}$] containing 5%$_{(w/v)}$ sorbitan triesterate. By adding 40 mL of a 5%$_{(w/v)}$ terephthaloyl chloride solution, interfacial cross-linking reaction took place under vigorous stirring. Microcarriers were separated by centrifugation and several washes.

Sample characterization

The structural properties of the particles were characterized by X-ray powder diffraction (XRPD), which was carried out in an X-ray diffractometer (Shimadzu, XRD-6000 - Japan)

using the *K* α line Cu as a radiation source. The mean diameter of the particles was examined using a laser scattering particle size analyzer (Cilas, 1064L - France). Morphology analysis was conducted by microscopy on a scanning electron microscope (Philips, XL30 - Netherlands) and on an optical microscope (Zeiss – Germany). Magnetization measurements were performed using a homemade vibrating sample magnetometer (VSM).

Results

Magnetic particles were analyzed by XRPD, laser scattering particle size analysis and scanning electron microscopy. According to XRPD patterns**,** magnetite was the dominant phase in the analyzed sample, with a primary scattering peak at around $2\theta=35^{o}$ (CHEN e HU, 2003), as shown in Fig. (1). The width of the peaks in the XRPD data revealed that the coherence length in the crystal lattice was of the order of 5.8nm according to Debye-Scherrer equation ($D=0.9\lambda/\beta\cos\theta$). The size distribution of the magnetic particles was investigated by laser scattering particle size analysis. More than 50% of the particles were found to be in the size range of 0.5-5 μm. 90%, 50% and 10% of the sample was smaller than 6.85μm, 2.48μm and 0,73μm, respectively. Scanning electron micrographs showed that particles are roughly spherical to oval in shape and they showed a size distribution in agreement with the laser scattering particle size data.

Polymeric superparamagnetic microcarriers were analyzed by optical microscopy and vibrating sample magnetometry. The size distribution and morphology of the polymeric superparamagnetic microcarriers were investigated by optical microscopy. As it can be seen in Fig.(2), microcarriers were spherical in shape. Their mean size was found to be 33 ± 10.2 μm. Magnetization measurements on powder samples were made at room temperature using a vibrating sample magnetometer. The results indicated that the microcarriers readily displayed magnetization when subjected to a magnetic field. There was no remanent magnetization (M=0 for H=0), suggesting that the magnetite particles are superparamagnetic. The same properties were found in magnetic particles before polymeric coating.

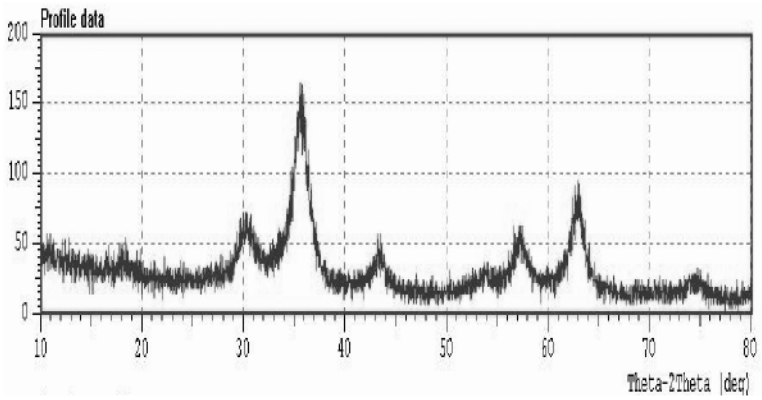

Figure 1 - X-ray diffraction pattern of magnetite (Intensity versus 2θ).

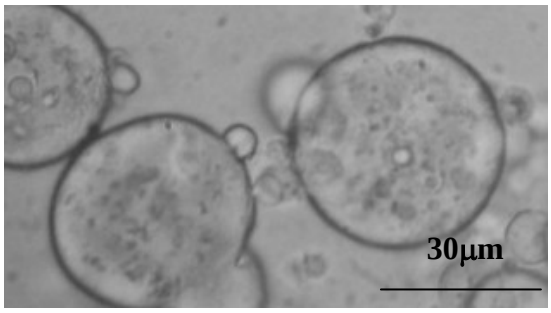

Figure 2: Optical microscopy of polymeric superparamagnetic microcarriers.

Discussion

Magnetic particles have been largely produced by means of coprecipitation technique. Although the reported method is based on coprecipitation protocol, it differs from the traditional Massart one (MASSART, 1981) in iron salt concentration, type of iron salts, nature and concentration of the agent inducing precipitation. In the presented protocol, precipitation was carried out using solutions of $FeCl_3$ (0.1 mol l^{-1}), $FeSO_4$ (0.05 mol l^{-1}) and NaOH (1 mol l^{-1}). In Massart's procedure, magnetic particles were obtained using $FeCl_3$ (1 mol l^{-1}), $FeCl_2$ (2 mol l^{-1}) and NH_4OH (0.7 mol l^{-1}). Such facts are relevant since the structure, dispersity and morphology of the particles depend on the temperature of the process, concentration of the original solutions, pH value of the medium, nature of the agent inducing precipitation and the presence of surfactant (VOOGT et al., 1998)**.**

As presented above, samples consisted of nearly spherical particles with diameters of the order of 10^3nm. Therefore, there should be a liquid magnetization in the absence of external

field. One possible mechanism for this unique form of superparamagnetism is the independent thermal fluctuation of small ferrimagnetic domains inside the particles. The XRPD data supported this picture. The width of the peaks in the XRPD data revealed that the coherence length in the crystal lattice was of the order of of 5.8nm. The boundaries of these small crystallites, with linear dimensions of the order of a few nanometers, may contain lattice defects that impede the propagation of the magnetic order. Similar phenomena has been recently observed in Fe_3O_4 thin films (MARGULIES et al., 1997; VOOGT et al., 1998), where stacking defects in the cotion sub-lattices induced superparamagnetism by breaking up the thin films in a large number of uncorrelated small ferrimagnetic domains with dimensions of the order of a few nanometers.

Two factors may contribute to the formation of micrometric particles consisting of the assembly of superparamagnetic nanometric crystallites. First, sulphate ions are strong flocculating agents (MASSART, 1981), inducing agglomeration of crystallites. Second, no surfactant was used in the coprecipation process, favoring the formation of large particles. Particles in the size range of 0.5-5μm, as produced by the present method, are particularly desired for deep organ target (GOODWIN et al., 1999; ALEXIOU et al., 2000).

In order to develop polymeric superparamagnetic carriers, a second experimental step, based on emulsification/cross-linking reaction, was carried out. According to optical microscopy, carriers of cross-linked gelatin and superparamagnetic particles were successfully produced. In fact, not only magnetic particles, but also drugs may be successfully incorporated into such system. As they readily displayed magnetization when subjected to a magnetic field, polymeric superparamagnetic carriers may target the drugs to their site of action by means of a magnetic field. Additional advantages can be pointed out. First, agglomeration and oxidation may be prevented. Second, magnetic particle incorporation into gelatin systems may also play an important role in increasing particle biocompatibility, since gelatin is regarded as a well-tolerated and biodegraded compound (VANDELLI et al., 2001). In spite of the improved properties, the size range of these carriers is not rather eligible for intravenous use (BURY e BOYMOND, 1990). In fact, for intravenous application, smaller carriers are regarded as safer once embolism is prevented (HAFELI e PAUER, 1999). However, the systems produced by this technique can be largely used by oral route, and future application can be considered.

Conclusions

Characterization data have demonstrated the feasibility of the presented method to synthesize magnetite particles. Their properties and size range were found suitable for deep organ drug targeting. Such magnetic particles were successfully incorporated into gelatin systems. Although the size range of these polymeric superparamagnetic carriers is not rather eligible for intravenous use (BURY e BOYMOND, 1990), such approach is useful for intravenous use and it is likely to prevent particle agglomeration and oxidation. Furthermore, it may improve particle biocompatibility. Currently, we have been investigating ways of modifying the experimental set-up in order to reduce carrier size and produce intravenous biocompatible polymeric magnetic carriers.

Acknowledgments

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