MAGNETIC PARTICLES FOR APPLICATION IN BIOMEDICINE

M. Timko, M. Koneracká, N. Tomašovičová, P. Kopčanský, V. Závišová

Institute of Experimental Physics SAS, 47 Watsonova, 043 53 Košice, Slovakia (timko@saske.sk)

Introduction. Magnetic drug delivery by particulate carriers is a very efficient method of delivering a drug to a localized disease site. Very high concentrations of chemotherapeutic, inflammatory or radiological agents can be achieved near the target site, such as a tumor, without any toxic effects to normal surrounding tissue or to the whole body. In magnetic targeting, a drug or therapeutic radioisotope is bound to a magnetic compound, injected into a patient's blood stream, and then stopped with a powerful magnetic field in the target area. Depending on the type of drug, it is then slowly released from the magnetic carriers (e.g., release of chemotherapeutic drugs from magnetic microspheres or biodegradable polymers) or confers a local effect (e.g., irradiation from radioactive microspheres; hyperthermia with magnetic nanoparticles). It is thus possible to replace large amounts of freely circulating drug with much lower amounts of drug targeted magnetically to localized disease sites, reaching effective and up to several-fold increased localized drug levels [1]. One of the prerequisites for success of the application of drug targeting for the treatment of localized diseases is the development of an effective method to transport the drug to the target site in the organism. Biocompatible ferromagnetic particles have been used effectively as potential drug carriers since 1970. The targeting of drug-bearing magnetic particles to a specific part of the body has been achieved by several directions. One solution is based on the use of a biocompatible polymers such as Polyvinyl alcohol (PVA), dextran, polyethyleneglycol or cyclodextrin [2]. The coating acts to shield the magnetic particle from the surrounding environment and can also be functionalized by attaching carboxyl groups, biotin, avidin and so on. The second way consists in encapsulation of both a magnetic material and a drug in various special matrix materials like albumin, polysaccharide or liposomes [3]. In the next direction, a drug can be grafted directly on the surface of a magnetic carrier without using polymeric binders or encapsulating materials to obtain a magnetite-drug complex.

Indometacin (previously spelt indomethacin in the UK) is a type of medicine called a non-steroidal anti-inflammatory drug (NSAID). All the medicines in the NSAID group reduce inflammation caused by the body's own immune system and are effective painkillers. Indometacin works by blocking the action of an enzyme in the body called cyclo-oxygenase. Cyclo-oxygenase is involved in the production of various compounds in the body, some of which are known as prostaglandins.

In this work we give a survey of the preparation and characterization of magnetic carriers for two different modes for coupling biologically active substances to magnetic particles, e.g., covalently bound proteins and enzymes to freshly prepared magnetite in the presence of carbodiimide (CDI) and entrapment in biodegradable Poly D,L-lactide polymer (PLA), respectively.

1. Experimental methods. Magnetite particles (Fe_3O_4) were prepared by co-precipitating ferric and ferrous salts in an alkaline solution followed by wash-

ing in hot water. 27.8 g of $FeSO_4.7H_2O$ and 54 g of $FeCl_3$. $6H_2O$ were each dissolved in 100 ml double distilled water and thoroughly mixed and added to 75 ml 8M NH_4OH, with continuous stirring at room temperature. Impurity ions such as chlorides and sulphates were removed by washing with copious amounts of hot distilled water. The amount of magnetic particles in a given volume of the ferrofluid was estimated by thermogravimetry and by magnetic measurements of magnetization curves (VSM magnetometer). The particle size distribution (10 nm) was determined by electron microscopy and magnetic measurements by Chantrell *et al.* [4] procedure.

The clinically important proteins and enzymes bovine serum albumin (BSA), dispase, glucose oxidase (GOD), chymotrypsin, streptokinase and dispase were immobilized onto magnetic particles using CDI. The coupling reactions were carried out under different conditions to determine the optimum conditions for immobilisation of proteins i.e., change of the pH of the reaction mixture and proportion of magnetic particles to proteins.

The polymer magnetic nanospheres were prepared according to a modified nanoprecipitation method. The starting procedure was as follows. PLA polymer (38 mg) and specified quantity of drug IND were accurately weighed and dissolved in the mixed organic solvent of acetone (10 ml) (miscible with water) and chloroform (2.5 ml). Then the organic phase was added dropwise into the aqueous phase containing of 0.5 ml the magnetic fluid with the concentration Fe_3O_4 = 1 mg/ml, 10 ml Pluronic (1.25 mg/ml) and 10 ml of phosphate buffer of pH =7.4 and stirred magnetically at room temperature until complete evaporation of the organic solvent had taken place. Drug free polymer magnetic nanospheres were prepared according to the same procedure omitting the drug. To investigate the influence of various formulation parameters on the drug incorporation efficiency the parameters as pH of reaction mixture, volume of water, volume of stock polymer solution and concentration of magnetic particles were followed.

2. Results and discussion. The obtained results regarding to immobilization of several clinically important proteins and enzymes bovine serum albumin (BSA), dispase, glucose oxidase (GOD), chymotrypsin and streptokinase to fine magnetic particles (Fe_3O_4) using carbodi-imide (CDI) as a coupling agent have been reported in our previous works [5]–[6]. In Figs. 1 and 2 the electron

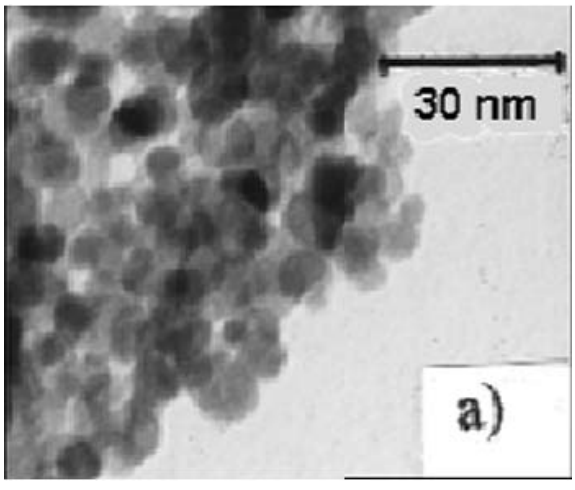

Fig. 1. Electron micrograph of pure magnetic particles.

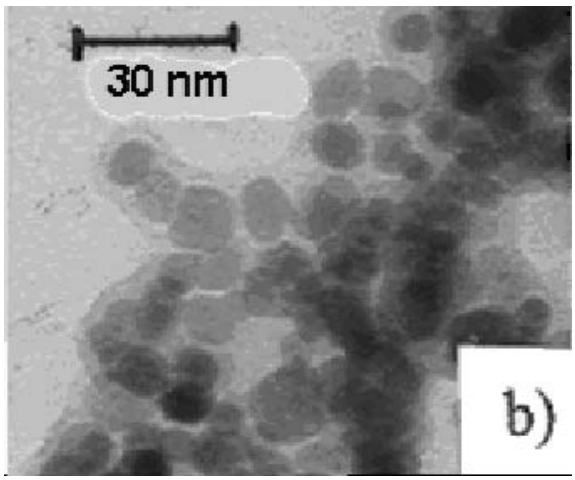

Fig. 2. Electron micrograph of protein bound to magnetic particles.

micrograph of pure magnetite particles and protein immobilized onto magnetite particles are shown, respectively. It is clear that proteins are layered over the magnetic particles.

The extent of coating of BSA onto the magnetic particles was determined by estimating the residual protein in the supernatant and washings. The estimation of protein content by a Bradford's method showed that the extent of coating onto the particles increased as the pH of the reaction mixture was decreased. This is in agreement with the mechanism of the coupling reaction. Also, as expected, with a higher ratio between the magnetic particles and protein, the percent coating attained was higher. The optimum conditions for the binding of BSA to the magnetic particles were observed at a ratio of magnetite : BSA : CDI of 2 : 1 : 2 at room temperature and pH = 6.3, respectively.

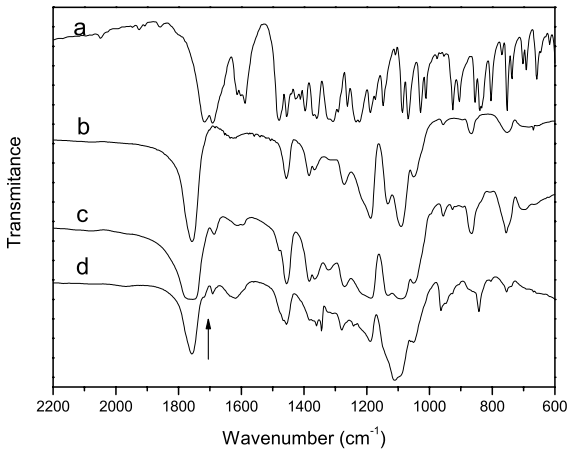

Fig. 3. Infrared spectrum of pure indometacin (a), PLA (b), encapsulated indometacin in PLA (c) and magnetic nanoparticles with indometacin encapsulated by PLA (d). The arrow shows position of $C = O$ streching band of indometacin. The spectra are shifted verticaly for clarity.

Another manner to create immobilize one of magnetically guided drugs is based on the use of a stabilizing agent such as dextran, PVA, pluronic etc., whereby a polymeric coat with a drug is formed on the surface of magnetic particles. In our work we used as a stabilizing agent pluronic, then a polymer shell was formed from biodegradable polymer PLA (Poly D,L - lactic acid). With the aim to analyze the prepared nanospheres, infrared spectra of materials were obtained, as shown in Fig. 3.

The measurements were performed by the KBr pellet method in the range from 4000 to 400 cm^{-1}. In this method, a solid sample is finally pulverized with pure, dry KBr; the mixture is pressed in a hydraulic press to form a transparent pellet, and the spectrum of the pellet is measured. Fig. 3 shows typical spectra of pure indometacin (a), PLA (b), prepared polymeric nanospheres with indometacin (c) and magnetic particles with indometacin incorporated in PLA in the range from 2200 to 600 cm^{-1}. The spectrum of indometacin displays a characteristic absorption doublet at 1695 and 1715 cm^{-1} ($C = O$ streching) and PLA displays a characteristic absorption band at 1758 cm^{-1} ($C = O$ stretching). As observed, the characteristic absorption band of PLA and indometacin observed at 1758 cm^{-1} and 1695 cm^{-1}, respectively, appears in the spectrum of the composite of PLA spheres with indometacin. Note, however, that a single PLA band at 1758 cm^{-1} is broaden suggesting a slight contribution of the 1715 cm^{-1} band of indometacin. The presence of characteristic bands of PLA and indometacin in the infrared spectra of the obtained complex magnetite-PLA-indometacin confirmed a successful encapsulation of indometacin and magnetic particles into PLA.

3. Conclusion. In case of a direct coupling method the present findings clearly show that it is possible to bind proteins (such as Bovine Serum Albumine, Dispase, Chymotrypsine and Streptokinase) onto magnetic particles in the presence of CDI without the aid of a primary coating of freshly prepared magnetic particles. On the other hand, a poorly water soluble drug Indometacine (an antiimflammatory agent) was successfully encapsulated in PLA magnetic nanospheres by a spontaneous emulsification solvent diffusion method.

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