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Magnetic Nanoparticles for Magneto-Resonance Imaging and Targeted Drug Delivery

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In this paper are presented our research about the magnetic nanoparticles with potential applications in malignant tumors diagnostic: cobalt nanoparticles, cobalt-nickel nanoparticles and mixed oxide particles, i.e. magnetite nanoparticles. Cobalt and nickel-cobalt nanoparticles have been prepared by co-reducing the corresponding salts. The reduction methods yield a dispersion of nanocrystals in liquids and need a ligand shell or a capping layer to prevent aggregation. The particles of magnetite were prepared by boiling in reflux of a mixture formed by γ-Fe_2O_3 and Fe(II) salt. The surfaces of magnetite nanoparticles were encapsulated by polymer, polyvinylpyrrolidone (PVP), and a saccharide (used for transportation the magnetic nanocomposite to malignant cells) [22]. TEM examination shows the average sizes of NiCo particles, 5-10nm and the average sizes of Co particles 2-5 nm. Magnetic measurements of nanoparticles were performed at room temperature using Vibrating Sample Magnetometer. Co, NiCo and magnetite nanocomposite exhibit a coercive field of 10-100 Oe, correlated with the sizes of nanoparticles. The magnetic properties of nanoparticles and core-shell nanocomposites have good quality for biomedical applications, for enhance the signal from magnetic resonance imaging examinations, as diagnostic tool of cancer tumors and targeting treatment of diseases.

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Keywords: soft chemistry, nanoparticle, magnetic diagnostic agent, magnetic field.

1. Introduction

Magnetic nanoparticles provide unprecedented levels of new functionality for nanomedicine. Such particles commonly consist of magnetic elements such as iron, nickel and cobalt and their chemical compounds. After the modification of surface for to provide both biocompatibility and functionality, the magnetic nanoparticles can be manipulate with external field gradients and the applications can be opened up in guided transport/delivery of drugs and genes, as well as immobilization and separation of magnetically tagged biological entities. Magnetic nanoparticles also resonantly respond to an alternating or time-varying magnetic field. The dynamic relaxation of the nanoparticles, when subject to an alternating magnetic field can be used for therapeutics (hyperthermia), imaging (magnetic particle imaging) or diagnostics (biosensing). The exploitation of Néel relaxation of superparamagnetic [1] particles is an effective way to heat up the nanoparticles and the surrounding tissue by transferring energy to them from the external magnetic field. In this way, localized heat can be delivered to targeted sites such as tumors for the cancer therapy called hyperthermia [2]. Such heating can be combined with chemotherapy or radiation for a mild increase in tissue temperature and thereby increase the effectiveness of the chemo-radiation

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treatment while minimizing dose [3]. These are a few of the many possibilities that magnetic nanoparticles offer as imaging, diagnostic and therapeutic tools in nanomedicine [4, 5, 6].

The size, shape and composition of magnetic nanoparticles being trialed as biochemical probes depend of their intended application, as well as the method of fabrication.

A possible method to create new magnetic nanoparticles with a very narrow size distribution is the preparation of self-assembled magnetic nanoparticles of cobalt, cobalt –nickel, or these particles encapsulated in a organic shell material [7, 8]. The cobalt or cobalt-nickel nanoparticles which form the magnetic dipoles also produce a collective magnetic state. Many biomedical applications, as diagnostic and targeting treatment, require the use of iron oxide particles (usually γFe_2O_3 or Fe_3O_4) with diameters ranging from 2 nm to 100 nm. They exhibit super-paramagnetic or ferromagnetic behavior, magnetizing strongly under an applied field, but retaining no permanent magnetism once the field is removed. In biomedical application magnetic nanoparticles placed at the side of the solution beaker induces a magnetic moment in each of the freely floating beads and sets up a field gradient across the solution [9].

The goals of our work are: 1) the study of soft chemical preparation routes for Co, NiCo nanoparticles, iron oxide particles and magnetic nanocomposite; 2) show the magnetic properties in correlation with microstructural properties of nanoparticles, which establish the capability of magnetic nanoparticles as diagnostic tools in cancer, as well as smart treatment agents; 3) synthesis of magnetic core-shell nanocomposite: *magnetite- (PVP)-2 Deoxy- D-glucose*[22] which replace radioactive ^{18}F *D-glucose* as contrast agents for a unradioctive imaging method (MRI) of malignant cells.

2. Magnetism of nanoparticles and biological applications

The magnetic behavior of materials is a function of size and dimensionality. For the bulk materials the microstructure determines the magnetic (hard and soft) behavior. For the nano-particles the size of domain wall-widths (nanostructures), lateral confinement (shape and size) and inter-particle exchange effects are dominated [10].

Considering only dipolar interactions between magnetic particles, the spin-flip barrier for a small magnetic object [11] is a product of the square of the saturation magnetization, and its volume. Thus, for small volumes the magnetic reversal energy is small enough that the moment becomes unstable, or thermally activated.

As a first approximation of this characteristic size, one can set the simple magnetization reversal energy equal to the thermal energy, i.e., at room temperature, and for typical ferromagnets obtain a characteristic length 5–10 nm, below which ferromagnetic behavior gives way to superparamagnetism (Fig. 1(a)). In real materials, changes in magnetization direction occur via activation over an energy barrier and associated with each type of energy barrier is a different physical mechanism and a characteristic length. These fundamental lengths are the crystalline anisotropy length, the magnetostatic length and the applied field length. In principle, if multiple barriers N are present, for a given time, the one with the shortest characteristic length determines the material's properties [12].

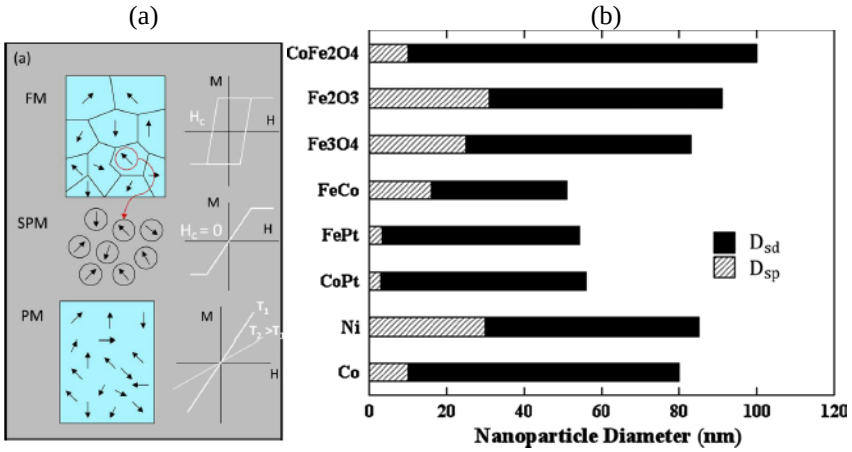

Fig. 1. (a) Materials show a wide range of magnetic behavior. The non-interacting spins in paramagnetic materials (bottom) characterized by a linear susceptibility that is inversely dependent on the temperature (Curie law). The ferromagnetic materials (top), characterized by exchange interaction, hysteretic behavior and a finite coercivity, H_C . If reduce the size of the ferromagnetic material to ultimately reach a size where

It is also important to consider what is the critical size that determines whether it is favorable to be uniformly magnetized (single domain), or to break into multiple domains. These series of magnetic "phases" as a function of size is shown in (Fig. 1(b)). For diameters of particles, $D<D_{sp}$, they exhibit superparamagnetism; for $D> D_{sd}$, they split into multiple domains to minimize their overall energy and in between, $D_{sp} <D< D_{sd}$, they are ferromagnetic *and* single domain. Critical sizes for the observation of superparamagnetism is D_{sp} and for single-domain is D_{sd} [13].

For *in vivo* applications, it is important that magnetic nanoparticles have the ability to overcome the main biological barriers that prevent them from reaching their targets [14]. Examples of such barriers include the protective exclusion by the blood-brain barrier [15] or the vascular endothelium; the typically higher osmotic pressure in cancer lesions causing the outward flow of any therapeutic agents and the clearance from circulation by the reticulo-endothelial system (RES). Like macroscopic biomaterials [16], host and material response is a concern for nanoparticles and their surfaces.

A significant benefit of nanoparticles is that they can be injected and circulate for extended periods of time within the vasculature providing access to areas of the body not otherwise accessible. After injection, magnetic nanoparticles circulating within the blood, transfer to the interstitial fluid (extravasations), then to the lymph (drainage) and eventually return to the blood via lymphatic vessels through chains of lymph nodes [17]. The rapid delivery of nanoparticles in the blood to the interstitium of the tissues is also based on diffusion and the sizes of the pores of the capillary; hence, the endothelial cells lining their walls regulate the permeability of the agents. The majority of tissues (muscle, skin, lung and connecting tissue) have continuous capillaries and particles/molecules larger than 8–10 nm cannot diffuse into these tissues. Foreign bodies, such as macromolecules or nanoparticles, less than 50 nm in diameter are filtered and excreted from the kidneys (renal filtering), which have fenestrated capillaries. Larger particles, diameter 200 nm, are cleared in the liver and spleen. In other words, blood filtration by the reticulo-endothelial system (RES) establishes a lower (50 nm) and upper (200 nm) bound for nanoparticle circulation. Note that because of its role in blood-filtration, the kidney can be readily, and passively, targeted; i.e., if magnetic nanoparticles, including the functionalized molecules on their surface, are below a critical size they will be automatically delivered to the kidney.

Rapid clearance of nanoparticles from circulation can substantially reduce their biomedical functionality. Active clearance of nanoparticles is mainly due to their recognition by macrophages of the mononuclear phagocyte system [18]. Progress in reducing the rapid clearance and enhancing the circulation time has been achieved by developing coatings of high-density polymers.

3. Experimental

3.1 Preparation of magnetic nanoparticles

The technique of microemulsion acting as “nanoreactor” inside which salt reduction and particle growth occurs, has allowed to obtain monodisperse particles which may display a define shape. For the dispersion and to prevent aggregation in other reducing methods are used typical ligands or capping agents like: sodium citrate, polymers, long chain thiols or amines [19, 20, 21].

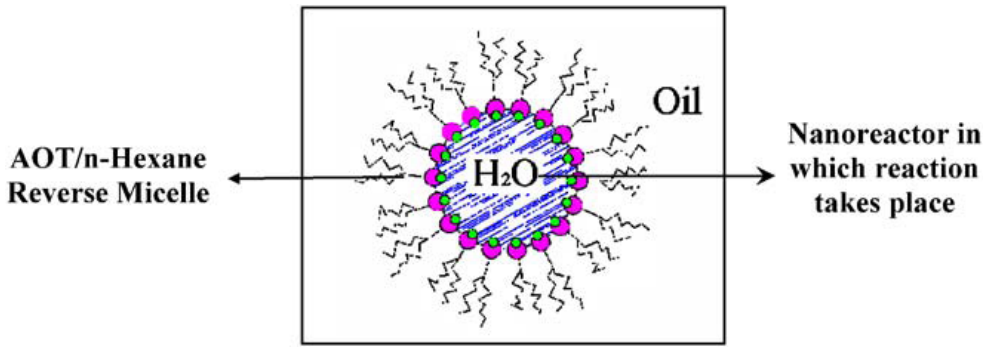

Fig. 2. Structure of reverse micelles formed by dissolving AOT, a surfactant, in n-hexane. The inner core of the reverse micelle is hydrophilic and can dissolve water-soluble compounds. The size of these inner aqueous droplets can be modulated by controlling the parameter Wo (Wo ¼ [water]/[surfactant]).

In our work, the magnetic particles have been obtained by reducing or co-reducing of the metallic salts using microemulsion technique and dispersion in a capping agent. The preparation of cobalt nanoparticles has made using cobalt nitrate hexahydrate, $Co(NO_3)_2.6\ H_2O$ in concentration 0.01M – 0.02 M dissolved in 10 ml of sodium bis (2-ethylphenyl) sulfosuccinat / toluene solution. By addition of 5 ml from 10 M sodium borohydride aqueous solution results a stable black colloid at room temperature. The particles of cobalt-nickel alloy with the composition $Co_{0.9}Ni_{0.1}$ have been obtained by boiling in reflux of an ethylene glycol solution of cobalt and nickel acetates. A mixture of 2 g of $Co(CH_3COO)_2.4\ H_2O$ and $Ni(CH_3COO)_2.4\ H_2O$ (in 9Co:1Ni molar ratio) dissolved in 10 ml of ethylene glycol, and refluxed at 130^0C with continuous stirring. After 15 hours of boiling, 2g of sodium citrate was added at the hot solution and the reaction was kept up 2 hours at the same temperature. At the end of the reaction, the particles were precipitated by adding 20 ml water and isolated by centrifugation. The black particles of alloy were washed, with water several times and dried. Combinations of myristic acid (MA), oleic acid (OA) were used for coating magnetic nanoparticles in order to be dispersed in water.

3.1 Synthesis of magnetite nano-composite.

The particles of magnetite were prepared by boiling in reflux of a mixture formed by γ-Fe_2O_3 and Fe(II) salt . An aqueous solution of γ- Fe_2O_3 and FeC_2O_4 (2 Fe_2O_3: 1 FeC_2O_4 molar ratio) was boiled, 100^oC, in reflux for two hours with vigorous stirring. After this time, a new quantity of deionized water (150 ml) was added and the boiling was proceeded up to 2 hours. After the cooling the solution, the particles were filtered, washed with water and dried in air.
From the last type of magnetic particles of magnetite we prepared a core-shell nanocomposite: magnetite-PVP-saccharide. The magnetite particles, after colloidal stabilization by surfactants, are dispersed in to aqueous solution of 0.8 mM polyvinylpyrrolidone (PVP) and 5mM 2-Deoxy-D-

glucose. The aqueous solution is refluxed at 70 - 80^{o}C temperature, for 12 h. The synthesis of magnetite nanocomposite is described elsewhere [22].

4. Results and disscution

Magnetic measurements of magnetic metal and oxides nanoparticles were performed at room temperature using Vibrating Sample Magnetometer (VSM) Lake Shore 7300.
Magnetic NiCo nanoparticles (Figure 3) have soft ferromagnetic behavior, with high saturation magnetization: 60 emu/g at relative high magnetic field (H_S) of 3000 Oe; the coercivity (H_c) is 10 Oe. Figure 4 shows the hysteresis loop of Co nanoparticles. This sample has small ferromagnetic behavior at room temperature: saturation magnetization of 0.6 emu/g, saturation magnetic field (H_S) of 4500 Oe and the coercivity (H_c) is 50 Oe. Magnetic behavior of Co particles sample suggest that cobalt particles are covered with cobalt oxide. Co oxide is antiferromagnetic at room temperature.

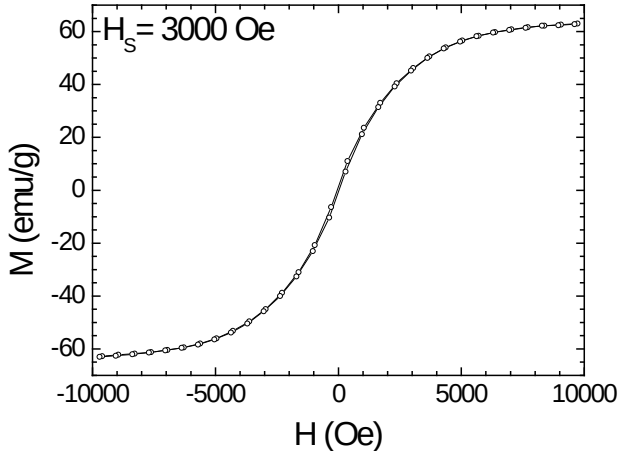

Fig.3. VSM hysteresis loop of NiCo nanoparticles, measured at room temperature.

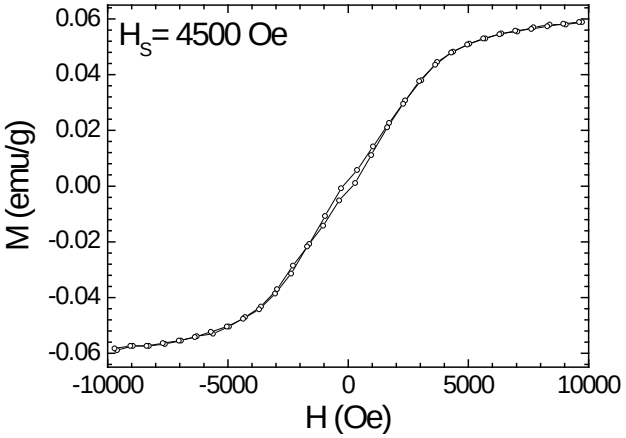

Fig.4. VSM hysteresis loop of Co nanoparticles, measured at room temperature.

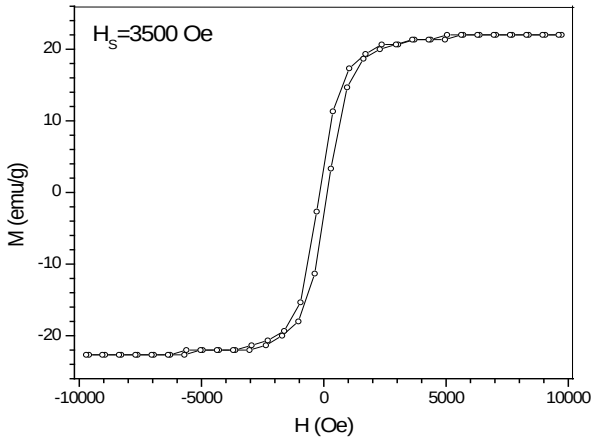

Fig.5. VSM hysteresis loop of iron oxide particles assemblies, measured at room temperature

Figure 5 shows the hysteresis loop of magnetite nanoparticles assemblies. This sample has ferromagnetic behavior at room temperature: saturation magnetization of 20 emu/g , saturation magnetic field (H_S) of 3500 Oe, the coercivity (H_c) of 100 Oe and squareness ratio of 0.145. Magnetic behavior of Fe_3O_4 nanoparticles suggest that assemblies of magnetic multi-domains are formed.

Magnetic nanoparticles were characterized for microstructural properties by Transmission Electron Microscopy (TEM). TEM studies were performed using a JEOL 200 CX at 200kV. To obtain specimens for microstructural characterization, dispersions of nanoparticles in water (sols) were dropped onto carbon coated TEM Cu grids or glass, or Si-substrates.

Figure 6 shows TEM image of NiCo nanoparticles, with average sizes of particles from 5-10 nm. The particles are spheroidal assemblied, because of magnetic interaction forces.

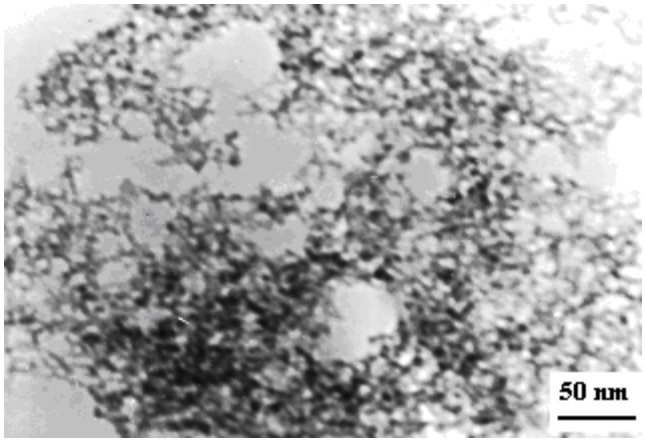

Fig. 6 TEM image of sample NiCo nanoparticles, the average size of 5-10 nm

Figure 7 shows TEM image of Co nanoparticles, with average sizes of particles from 2-5 nm. The particles are spheroidal assemblied, as consequence of magnetic interaction forces, the coercivity of Co particles is relative bigger than the coercivity of NiCo particles .

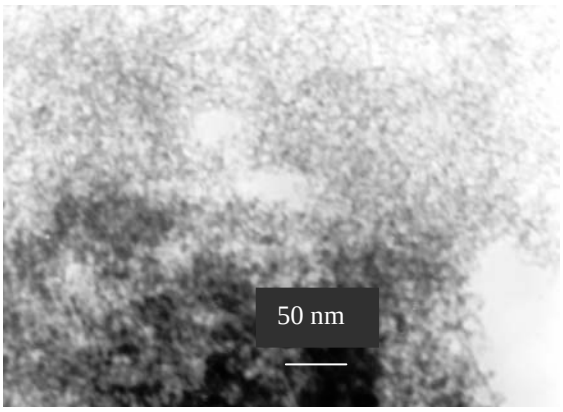

Fig. 7 TEM image of Co nanoparticles, the average size of 2-5 nm

Figure 8 shows SEM image of magnetite particle aggregates, with average sizes of particles from 100-200 nm. The particles are spheroidal assemblies, as consequence of magnetic interaction forces, the coercivity of magnetite particles is relative bigger, 140 Oe.

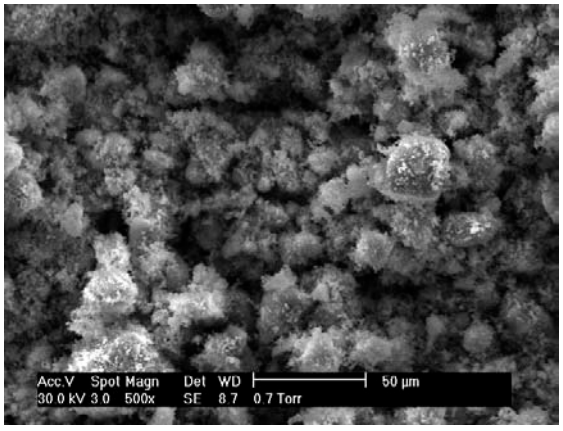

Fig.8 SEM image of Fe_3O_4 particles assemblies, the average sizes of 100-200 nm

Figure 9 shows our model of „magnetite-biocompatibil polymer (PVP)-saccharide nanocomposite". Superparamagnetic iron oxide **,** magnetite, is strong enhancers of proton relaxation. Polyvinylpyrrolidone (PVP) enhances the blood circulation time and stabilizes the colloidal solution. Saccharide: 2-Deoxy-D-glucose is a glucose molecule which has the 2-hydroxyl group replaced by hydrogen, so that it cannot undergo further glycolysis. Glucose hexokinase traps this substance in most cells so that it makes a good marker for tissue glucose use and hexokinase activity. Many cancers have elevated glucose uptake and hexokinase levels. 2 deoxy-D-glucose is used as „vehicle" to target the malignant cells.

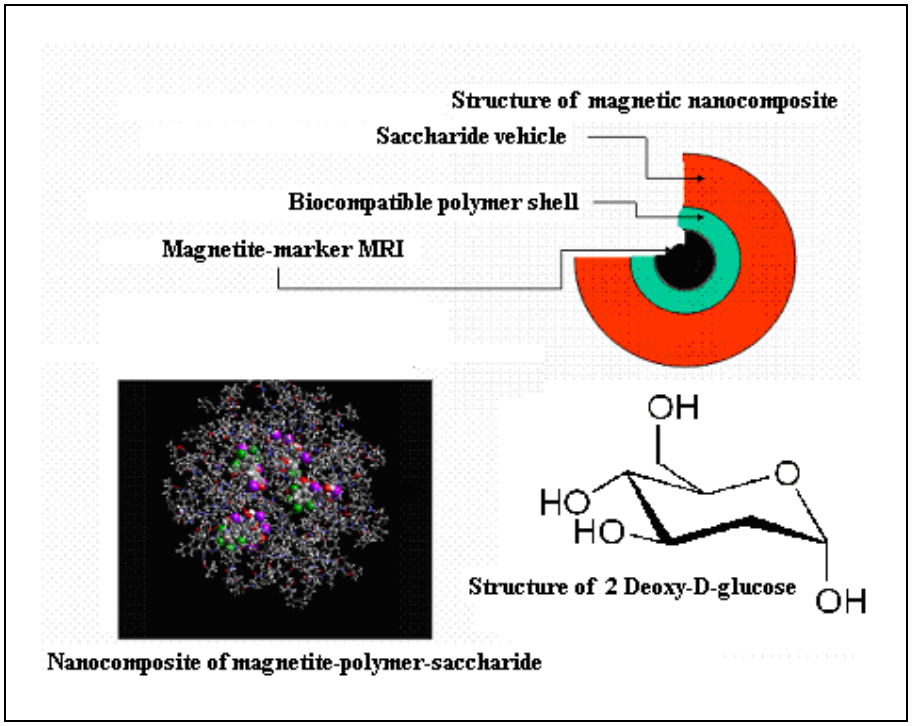

Fig. 9 Model of magnetite- polymer (PVP)-saccharide nanocomposite

Figure 10 shows TEM image of magnetite-polymer (PVP)-2 Deoxy-D-glucose nanocomposite. The sizes of nanoparticles are 2-10nm.

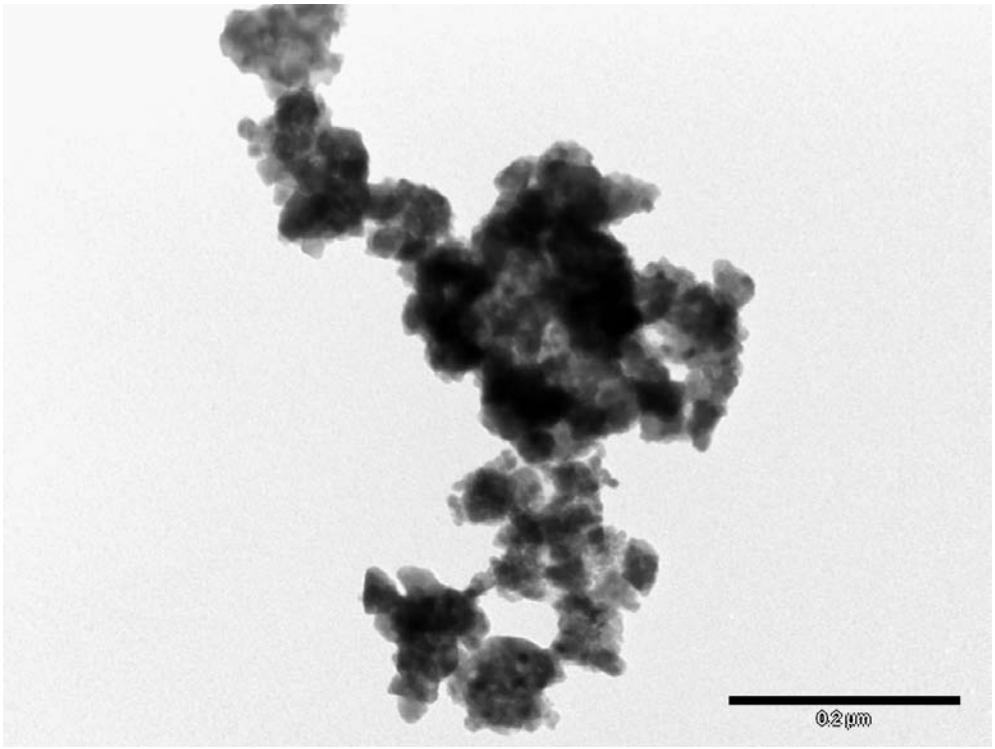

Fig. 10 TEM image of magnetite-polymer (PVP)-2 Deoxy-D-glucose nanocomposite

5. Conclusions

All biomedical applications of magnetic nanoparticles arise from the combination of their magnetic properties with biological relationships and phenomena. Naturally, the convergence of these two areas is most pronounced at the surface of the magnetic nanoparticle where it interfaces with its biological environment. By manipulating the nanoparticle surface it is possible to induce a wide range of biological responses, and the importance of the surface functionalization of the magnetic nanoparticles, especially for *in vivo* biomedical applications.

Soft chemical methods are versatile techniques that can be used to prepare and organise any type of magnetic particles. Althouth NiCo nanoparticles and Co nanoparticles show the magnetic properties, the metallic particles of NiCo and Co have not good biocompatibility.

Magnetite core-shell nanocomposite shows the magnetic properties and answers to magnetic field strengths required to manipulate nanoparticles have no deleterious impact on biological tissue. The spheroidal form of magnetic nanoparticles improve the removal in biological fluids for drug delivery and targeted detection of tumors. The results obtained have demonstrated that the microemulsion is a superior method over other bulk precipitation methods to synthesize magnetic polymeric nanocomposite with a good control over iron oxide amount and magnetic properties. Saturation magnetization values of magnetite depend on the temperature and surface characteristics of the nanoparticles.

The synthesis and properties of the new magnetic core-shell nanocomposite: *magnetite-(PVP)-2 Deoxy- D-glucose* [22] achieved one's end to find a biomedical imaging method unradioctive for diagnostic of malignant cells.

Table 1 shows the comparison between 2 imaging methods for malignant tumor diagnostic, Positron Emission Tomography, PET, which used high energy γ-rays radiation and Magnetic Particle Imaging, MPI.

Table 1

	Radiation Used	Spatial Resolution	Temporal Resolution	Sensitivity	Quality of contrast agent used	Comments
Positron Emission Tomography (PET)	high energy γ-rays	1-2 mm	10 sec to minutes	10^{-11}-10^{-12} Mole/L	Nanograms	Sensitive, Quantitative, Needs cyclotron
Magnetic Particle Imaging (MPI)	Radiowaves	200-500 μm	Seconds to minutes	10^{-11}-10^{-12} Mole/L	Nanograms	Good sensitivity, Quantitative, Fast, Good resolution, No tissue contrast.

Of consequence to this work we have future directions to study possible changes in magnetic behavior upon injection and interactions with cells such as specific binding and endocytosis.

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