Abstract:
The invention relates to a system comprising magnetic nanoparticles of a metal oxide and a polymer, which in turn contains monomers with different functional groups. Said system can be solid (nanocomposite) or liquid (ferrofluid). The invention also relates to a process for obtaining the system, as well as its use, mainly in biotechnological, veterinary and medical applications, such as for example for the diagnosis and treatment of human diseases.
SYSTEMS CONTAINING MAGNETIC NANOPARTICLES AND POLYMERS, SUCH AS NANOCOMPOSITES AND FERROFLUIDS, AND APPLICATIONS THEREOF

FIELD OF THE ART

The present invention is comprised within the field of new materials, particularly nanoparticle systems with magnetic properties. It is specifically aimed at systems comprising particles of a metal oxide, comprising iron, and an organic polymer, as well a process for obtaining them and their applications in different fields, including biotechnology and particularly biomedicine.

STATE OF THE ART


One of the most important features of these materials is that their properties vary extensively with size [Iglesias, O.; Labarta, A. Phys. Rev. B, 2001, 63, 184416], internal structural disarrangement [Serna, C.J.; Bodker, F.; Morup, S.; Morales, M. P.; Sandiumenge, F.; Veintemillas-Verdaguer, S Solid State Comm. 2001, 118, 437], and aggregation state [Koutani, S.; Gavoille, G.; Gerardin, R.J. Magn. Magn. Matter. 1993, 123, 175]. For example, it is well known in the hyperthermia field that the specific absorption rate (SAR) for a determined alternation frequency and field intensity comes from particles in a very narrow size range.
In magnetic resonance imaging, magnetic nanoparticles work by means of changing the relaxation time in adjacent tissue due to the bipolar magnetic interactions with aqueous protons. The efficiency of a contrast agent in magnetic resonance is measured by relaxivity. Relaxivity is defined as the increase in the proton relaxation rate induced by the contrast agent per concentration unit of the contrast agent. In this case, relaxivity is also related with the particle size and is more homogeneous if the size distribution is narrow.

Another feature determining the magnetic properties of nanoparticles is their shape. For example, one of the terms contributing to the anisotropy energy is anisotropy, such that it is greater in elongated particles than in spherical particles. Therefore, it is desirable to develop methods for producing particles with different shapes, and especially with elongated shapes. As a result, one of the essential requirements for producing magnetic particles optimized as contrast and hyperthermal agents is the control of the size, of the size dispersion and of the shape.

For their use in biomedicine, magnetic nanoparticles must further comply with additional requirements such as water dispersability and biocompatibility.

Methods have been described for producing monodisperse magnetic iron oxide particles with a variable size based on the decomposition of iron coordination compounds in organic solvents in the presence of surfactants consisting of a hydrocarbon chain ending in a polar group. However, these compositions are unstable in aqueous medium. A way of solving this problem consists of the absorption of a second surfactant forming a bilayer around the magnetic nucleus. However, this second surfactant layer is easily desorbed unless it is covalently linked to the first layer. Magnetic nanoparticles coated with stable bilayers are also known [Shen, L.; Stachowiak, A.; Hatton, T.A.; Laibinis, P. E.; Langmuir, 2000, 16, 9907] but they are only stable at a pH greater than 7.4. On the other hand, for their application in biomedicine, a method for preparing nanoparticles which is carried out in aqueous medium is preferable in order to favor subsequent biological functionalization processes. Processes for preparing magnetic nanoparticles in aqueous medium are known [US 4,329,241, Massart]. However, said methods can give rise to aggregation problems and are not very competent in controlling the size and size dispersion.

One of the preferred techniques for increasing the stability of the coating consists of cross-linking polymeric chains [WO03005029, Xu]. However, this methods do not offer the possibility of systematically varying the particle size, they often yield wide size distributions and occasionally, they are not stable in aqueous dispersions.

Another desirable feature for biomedical uses is to prevent the reaction of the immune system against the nanoparticles by means of coatings minimizing said response to achieve higher dwelling times of the nanoparticles in the organism. It is also desirable to anchor to the surface of the particles biologically active molecules allowing a specific localization or a biological functionality. [US 6,514,481, Prasad] describes silica-coated iron oxide nanoparticles to which a peptide is attached by means of a spacer and in [WO 02098364, Perez Manual], the iron oxide nanoparticles are coated with dextran to which peptides and oligonucleotides are anchored.

There is thus a demand for processes for producing biocompatible nanoparticles with a variable size and shape, low size dispersion, which can be stably and homogenously dispersed in physiological media, with coatings which allow them to avoid the attack of the immune system and with functional groups on their surface allowing the anchoring of molecules with a biological functionality. But there is especially a demand for processes which
can simultaneously respond to all these demands. The objective of this invention is to respond to this demand.

**BRIEF DESCRIPTION OF THE INVENTION**

One aspect of the present invention relates to a system comprising magnetic nanoparticles of a metal oxide comprising iron and a polymer (P) in which:

a) the polymer comprises a monomer (I) containing active functional groups which can interact with metal ions by means of Coulomb forces, Van der Walls forces or coordination bonds

b) the molar [Fe]/[monomer (I)] ratio is 0.01 - 10,

c) the nanoparticles have a size dispersion of less than 15% of the average size.

According to one variant, the system is solid (nanocomposite) and according to another variant, the system is liquid (ferrofluid).

Another variant of the system comprises a polymer (P) which, apart from monomer (I), comprises a monomer (II) containing hydrophilic functional groups.

According to another variant, the system comprises a polymer (P) which, apart from monomers (I) and (II) comprises a monomer (III) containing functional groups which can anchor active biological molecules.

A second aspect of the present invention relates to a process for obtaining a system comprising magnetic nanoparticles of a metal oxide comprising iron and a polymer (P) as defined, comprising:

a) mixing

a1) an aqueous solution, optionally mixed with organic solvents, of a polymer (P) comprising a monomer (I) containing active functional groups which can intercat with metal ions by means of coulomb forces, Van der Waals forces or coordination bonds, with

a2) an aqueous solution, optionally mixed with organic solvents, comprising at least one Fe salt

in which the molar [Fe]/[monomer (I)] ratio is 0.01 - 10

b) adding a base in a sufficient amount to reach pH 8 - 14.

A third aspect of the present invention refers to the use of a liquid system as defined previously, comprising magnetic nanoparticles of a metal oxide comprising iron and a polymer (P) as defined, for magnetic refrigeration, magnetic printing, magnetic inks, rotor lubrication, electric
transformers, low noise-level solenoids, switches, magnetorheological fluids, magnetically active fibers, reinforced polymeric composites, sealing in vacuum systems, damping systems, loudspeakers, magnetic sensors, actuators, catalysis, metal recovery and water purification, inductors and antennas in communication technology, magnetic shields and microwave absorption, polymer curing, epoxy resin hardening, contact-free heating and biotechnological, veterinary and medical applications.

**DESCRIPTION OF THE DRAWINGS**

Figure 1 shows a transmission electronic microscopy image of a section with a thickness of 40 nm of a maghemite nanocompound prepared according to Example 1 containing 5% of iron.

Figure 2 shows a transmission electronic microscopy image of a maghemite nanocompound sample prepared according to Example 2 from a [Fe]/[pyhdine] = 0.40 ratio once it has been ground dispersed in acetone and deposited on a grid.

Figure 3 shows SAXS curves of a polymer (PVP) sample and a series of maghemite nanocompounds (S1, S2, S3, S4 and S5) prepared according to Example 2 after pressing them into tablets with a thickness of 0.1 mm.

Figure 4 shows the variation of the particle diameter calculated from SAXS curves by means of adjusting to a Guinier expression, in a series of maghemite compounds prepared according to Example 2.

Figure 5 shows a transmission electronic microscopy image of a maghemite nanocompound sample in the form of a rod prepared according to Example 3 from a polymer of anionic origin containing 27.8% iron, once it has been ground, dispersed in acetone, and deposited on a grid.

Figure 6 shows an electronic microscopy image of a maghemite fluid prepared according to Example 4.

Figure 7 shows the magnetization variation against the field in a series of maghemite compounds prepared according to Example 2. The continuous lines correspond to adjustments to a Langevin expression.

Figure 8 shows the variation of the out-of-phase ac magnetic susceptibility with temperature, for an alternation frequency of 10 Hz, in a series of maghemite nanocompounds prepared according to Example 2.

Figure 9 shows the variation of the out-of-phase ac magnetic susceptibility with temperature, for different alternation frequencies, in a
maghemite nanocompound prepared from an anionic polymer according to Example 3.

Figure 10 shows the variation of magnetization against temperature in the ferrofluid prepared in Example 4, immediately after the preparation and a month after the preparation.

DETAILED DESCRIPTION OF THE INVENTION

The inventors have found a system comprising nanoparticles of a metal oxide, comprising iron and a polymer having a low dispersion of the average particle size, where the shape and the average size of the particles can be selected during the preparation process. Said system can be in the form of a solid (nanocomposite) or a liquid (ferrofluid), being able to be adapted to achieve a good dispersibility in the latter case.

When the system is to be used in biotechnological applications, in veterinary applications and in medicine, it can also be modified to obtain biocompatibility, avoid the attack of the immune system and add functional groups allowing the anchoring of molecules with a biological functionality.

A first aspect of the invention relates to a magnetic nanoparticle system comprising magnetic nanoparticles of a metal oxide, comprising iron, and a polymer (P) donde:

a) the polymer comprises a monomer (I) containing active functional groups which can interact with metal ions by means of Coulomb forces, Van der Waals forces or coordination bonds,

b) the molar [Fe]/[monomer (I)] ratio is 0.01 - 10,

c) the nanoparticles have a size dispersion of less than 15% of the average size.

According to an embodiment of the invention, in the nanoparticle system the metal oxide contains Fe$^{2+}$ and/or Fe$^{3+}$.

A particular embodiment of the invention is the magnetic nanoparticle system in which the metal oxide, apart from Fe, contains a divalent metal, for example, Co$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Gd$^{2+}$, Be$^{2+}$, Mg$^{2+}$, Ca$^{2+}$, Ba$^{2+}$.

A more particular embodiment of the invention is the magnetic nanoparticle system in which the metal oxide comprises maghemite (γ-Fe$_2$O$_3$).

Another particular embodiment of the invention is the magnetic nanoparticle system in which the metal oxide comprises magnetite (Fe$_3$O$_4$).
Another particular embodiment of the invention is the magnetic nanoparticle system in which the metal oxide comprises ferrite MFe$_{2}$O$_{4}$, M being Co$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Gd$^{2+}$, Be$^{2+}$, Mg$^{2+}$, Ca$^{2+}$ or Ba$^{2+}$.

A particular embodiment of the invention is the magnetic nanoparticle system in which the metal oxide is barium ferrite (BaFe$_{2}$O$_{4}$).

Polymer (P) can be an organic polymer or an organic polymer containing inorganic residues such as alkoxy silyl, titanium silyl or others, covalently bound to the polymeric chain (hybrid organic-inorganic polymer).

According to an embodiment of the invention, in the magnetic nanoparticle system polymer (P) is an organic polymer.

According to another embodiment of the invention, in the magnetic nanoparticle system polymer (P) is a hybrid organic-inorganic polymer.

One aspect of the invention comprises a polymer (P) comprising a monomer (I) containing active functional groups which can interact with metal ions by means of Coulomb forces, Van der Waals forces or coordination bonds, for example alcohol, alkoxy, carboxylic, anhydride, phosphate and/or phosphine groups. The functional groups can also be nitrogenated functional groups such as amine, amide, nitrile, azide groups. Other nitrogenated functional groups can be imines and heterocycles such as pyridine, pyrrole, pyrrolidone, pyrimidine, adenine.

Therefore, an embodiment of the invention is the magnetic nanoparticle system in which the monomer (I) contains alcohol, alkoxy, carboxylic, anhydride, phosphate and/or phosphine type functional groups.

Another embodiment of the invention is the magnetic nanoparticle system in which the monomer (I) contains nitrogenated functional groups such as amine, amide, nitrile or azide.

Another embodiment of the invention is the magnetic nanoparticle system in which the monomer (I) contains imines, or heterocycles containing nitrogen such as pyridine, pyrrole, pyrrolidone, pyrimidine, adenine.

A particular embodiment is the magnetic nanoparticle system in which the monomer (I) is a vinyl type monomer. The vinyl monomer is preferably vinylpyridine.

The groups which can interact with metal ions by means of Coulomb forces, Van der Waals forces or coordination bonds comply the function of molding the size and the shape of magnetic particles contained in the system.
during the synthesis thereof. They also comply the function of coating the particles with the organic polymer.

The inventors have discovered that it is possible to control the size of the magnetic nanoparticles of the magnetic nanoparticle system of the invention by varying the molar [Fe]/[monomer] ratio during the preparation method. The greater the ratio, the larger the size. In the magnetic nanoparticle systems of the invention, the molar [Fe]/[monomer] ratio varies between 0.01 and 10, preferably between 0.03 and 2.

In the magnetic nanoparticle systems of the invention, the average size of the nanoparticles of metal oxide comprising iron can be of 1 to 1000 nm, preferably of 1 to 100 nm.


Therefore, a particular embodiment of this invention is formed by the magnetic nanoparticle system in which the particles are spherical and polymer (P) is a polymer obtained by a radical pathway.

Another particular embodiment of this invention is formed by the magnetic nanoparticle system in which the particles are elongated and polymer (P) is obtained by an anionic pathway.

The rod-shaped nanoparticles have an extraordinarily narrow out-of-phase susceptibility peak, as discussed in example 3.2 and shown in Figure 9. This feature makes said particles be especially suitable for uses in which hyperthermia is a property to be exploited, such as for example in certain oncological treatments of infectious diseases.

The nanoparticle system of the invention can be in solid form or in liquid form.

In the present invention, the magnetic nanoparticle system of the invention in solid form is called "nanocomposite" and the magnetic nanoparticle system of the invention in liquid form is called "ferrofluid".
As used in the present invention, the term "nanocomposite" relates to dispersions of nanoparticles of a metal oxide comprising iron, in a solid polymer matrix.

A particular aspect of this invention is formed by the solid magnetic nanoparticle system (nanocomposite).

As used in the present invention, the term "ferrofluid" relates to a stable and homogeneous colloidal suspension of magnetic particles, i.e., with a net magnetic moment, in a carrier liquid. The carrier liquid can be, for example, water or an aqueous solution containing a substance acting as a buffer and other water-soluble substances.

Another particular aspect of this invention is formed by the liquid magnetic nanoparticle system liquid (ferrofluid).

A particular embodiment of the invention is the magnetic nanoparticle system liquid (ferrofluid) comprising water or a biocompatible aqueous solution, preferably a biocompatible aqueous solution containing a substance acting as a buffer and optionally other water-soluble substances.

In the liquid magnetic nanoparticle system of the invention (ferrofluid), it is important that the iron oxide nanoparticles are homogeneously dispersed in the liquid medium and that the dispersion is stable. In particular, for biotechnological applications, in medicine and in veterinary applications, it is interesting that said dispersion is homogeneous and stable in physiological media and that the nanoparticles are biocompatible. A particular embodiment of the invention is thus the magnetic nanoparticle system in which polymer (P), apart from monomer (I), comprises a monomer (II) containing functional hydrophilic groups.

Another particular embodiment of the invention is the magnetic nanoparticle system in which monomer (II) is a vinyl type monomer, such as acrylate, methacrylate, methyl methacrylate, vinylpyrrolidone and derivatives thereof, preferably polyethylene glycol (PEG) methacrylate.

Another particular embodiment of the invention is the magnetic nanoparticle system in which polymer (P), apart from monomers (I) and (II), comprises a monomer (III) containing functional groups which can anchor biologically active molecules. Said groups can be for example -NH₂, -SH, -COOH, and -CONH₂.

Another particular embodiment of the invention is the magnetic nanoparticle system in which monomer (III) is a vinyl type monomer.
Another particular object of the invention is the magnetic nanoparticle system in which the biologically active molecules are anchored to monomer (III) by means of covalent bonds.

As used herein, the term "biologically active molecules" relates to biological molecules or analogs of biological molecules including a functional group with the capacity to accept electronic density belonging, by way of illustration and without limiting the scope of the present invention, to the following list: amino groups, thiol groups, disulfide groups, dialkyl sulfides, epoxy groups, as well as amines and alcohols in platinum. These biomolecules having said functional groups, both in the structure itself of the molecule and due to the effect of the synthetic addition of said group, can be selected from one of the following groups for example:

a) natural biomolecules: single- or double-stranded nucleic acids (DNA or RNA), enzymes, antibodies, membrane proteins, heat shock proteins, chaperonins, other proteins, monosaccharides, polysaccharides, glycoproteins, fatty acids, terpenes, steroids, other molecules of a lipid nature, lipoproteins, hormones, vitamins, metabolites, hydrocarbons, thiols, or macromolecular aggregates formed by proteins and/or nucleic acids or other combinations of the previously mentioned molecules;

b) natural biomolecules obtained by in vitro selection processes: aptamers, ribozymes, aptazymes; and

c) artificial biomolecules: PNA, other analogs of natural nucleic acids, natural and artificial nucleic acid chimers, polymers with the capacity to recognize shapes ("molecular imprinted polymers" or MIPs), artificial antibodies, recombinant antibodies and mini-antibodies.

Another particular embodiment of the invention is the magnetic nanoparticle system in which all the monomers in polymer (P) are vinyl type monomers.

A particular embodiment of the invention is the liquid magnetic nanoparticle system (ferrofluid) comprising:

a) maghemite as a metal oxide,

b) a polymer matrix containing:

i. 4-vinylpyhdine [monomer (I)],

ii. a vinyl monomer functionalized with poly(ethylene glycol) (PEG) [monomer (II)]
iii. a vinyl monomer containing functional groups selected from -NH2, -SH, -COOH, and -CONH2 [monomer (III)]
c) an aqueous solution of phosphate buffer (PBS), maintaining the system at pH 7.4.

A second aspect of the present invention is formed by the process for preparing the magnetic nanoparticle system comprising the following steps:

a) mixing
1. an aqueous solution, optionally mixed with organic solvents, of a polymer (P) comprising a monomer (1) containing active functional groups which can interact with metal ions by means of Coulomb forces, Van der Waals forces or coordination bonds, with
2. an aqueous solution, optionally mixed with organic solvents, comprising at least one Fe salt,
in which the molar [Fe]/[monomer (I)] ratio is 0.01 - 10

b) adding a base in a sufficient amount to reach pH 8 - 14.

A particular embodiment of the invention is the process for preparing magnetic nanoparticles in which solution a2) comprises at least one salt of a divalent metal and a Fe⁺³ salt. The divalent metal salt can be for example a Fe²⁺, Co²⁺, Ni²⁺, Mn²⁺, Gd²⁺, Be²⁺, Mg²⁺, Ca²⁺ and Ba²⁺.

A more particular embodiment of the invention is the process for preparing magnetic nanoparticles in which the divalent metal salt in solution a2) comprises a Fe²⁺Salt.

Another more particular embodiment of the invention is the process for preparing magnetic nanoparticles in which solution a2), the Fe²⁺ salt is FeBr₂ and the Fe⁺³Salt is FeBr₃.

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which solution a2) further comprises a monovalent bromide, for example KBr, RbBr, NaBr, CsBr, (CH₃)₄NBr, (CH₃CH₂)₄NBr).

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which solution a2), the Fe²⁺ salt is FeCl₂ and the Fe⁺³ salt is FeCl₃.

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which solution a2) further comprises a monovalent chloride, for example KCl, RbCl, NaCl, CsCl, (CH₃)₄NCl, (CH₃CH₂)₄NCl).
Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which solutions a1) and a2) are mixed in a molar [Fe]/[monomer \((I)\)] ratio of 0.01 to 10.

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which solutions a1) and a2) are mixed in a molar [Fe]/[monomer \((I)\)] ratio of 0.03 to 2.

Apart from discovering that the molar [Fe]/[monomer] ratio affects the size of the nanoparticles of metal oxide comprising iron, the inventors also discovered that the size of said nanoparticles can be varied by means of using different molar ratios of Fe\(^{+2}\) and Fe\(^{+3}\) in solution a2).

Therefore, a particular embodiment of the invention is the process for preparing magnetic nanoparticles in which the average size of the nanoparticles of metal oxide comprising iron is regulated by varying the molar ratio of Fe\(^{+2}\) and Fe\(^{+3}\) in solution a2), by means of varying the proportion of the dissolved salts of both cations.

A third discovery of the inventors is that the average size of the nanoparticles of metal comprising iron can be regulated by varying the molar ratio between the base added in b) and the iron contained in a). Therefore, another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which the average size of the nanoparticles of metal oxide comprising iron is regulated by varying the molar ratio between the base added in b) and the iron contained in a).

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which in step b) the base is added until reaching a pH of 12.5 to 13.

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which the polymer (P) used in a1) comprises a monomer \((I)\) containing active functional groups which can interact with metal ions by means of Coulomb forces, Van der Waals forces or coordination bonds, for example alcohol, alkoxide, carboxyl, anhydride, phosphate, and/or phosphine.

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which polymer (P) used in a1) comprises a monomer \((I)\) containing nitrogenated functional groups, such as amine, amide, nitrile, azide groups.
Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which polymer (P) used in a 1) comprises a monomer (I) containing imines or heterocycles such as pyridine, pyrrole, pyrrolidone, pyridine, adenine.

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which polymer (P) used in a 1) comprises a vinyl type monomer (I).

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which polymer (P) used in a 1) comprises vinylpyridine as a vinyl monomer.

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which polymer (P) is obtained by a radical pathway.

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which polymer (P) is obtained by an anionic pathway.

The process can include a polymer (P) preparation step. Therefore, another particular object of the invention is the process for preparing magnetic nanoparticles in which polymer (P) is prepared by means of a process previos to step a).

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which polymer (P) is a copolymer and is prepared by simultaneous or successive copolymerization of a monomer (I) with a monomer (II) containing hydrophilic groups and optionally with a monomer (III) containing functional groups which can anchor biologically active molecules.

Monomers (II) and (III) are as described previously in this application.

The copolymerization of the polymer P can also be carried out after preparing the magnetic nanoparticles of the invention. Thus, a particular embodiment of the invention is the process for preparing magnetic nanoparticles in which, after step b), the process comprises a step c) comprising the copolymerization of polymer (P) with a monomer (II) containing hydrophilic groups and optionally with a monomer (III) containing functional groups which can anchor biologically active molecules, and when the copolymerization is carried out with the two monomers (II) and (III), said copolymerization is carried out successively or simultaneously.
Another particular embodiment of the invention is the process for preparing magnetic nanoparticles which comprises subjecting the product of step b) to a solid-liquid phase separation to obtain a solid system (nanocomposite) comprising magnetic nanoparticles containing a metal oxide core and a polymer (P).

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles comprising subjecting the product of the additional step c) to a solid-liquid phase separation to obtain a solid system (nanocomposite) comprising magnetic nanoparticles of a metal oxide comprising iron and an organic polymer (P).

An additional step to the process for preparing magnetic nanoparticles of the invention comprises dispersing the solid product (nanocomposite) in a suitable liquid medium to obtain a liquid system (ferrofluid). In a particular embodiment, the liquid is water or a biocompatible aqueous solution, preferably the aqueous solution acting as a buffer.

Thus, a particular embodiment of the invention is the process for preparing magnetic nanoparticles in which, after steps b) or c), the solid product (nanocomposite) is dispersed in a suitable liquid medium to obtain a liquid system (ferrofluid).

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which the solid product (nanocomposite) is dispersed in a biocompatible aqueous solution.

Another particular embodiment of the invention is the process for preparing magnetic nanoparticles in which the solid product (nanocomposite) is dispersed in an aqueous solution comprising a substance acting as a buffer.

A particularity of liquid nanoparticle systems (ferrofluids) is that the size of the particles is not modified in relation to the size of the particles in the solid system (nanocomposite) and aggregates are not observed, as shown in Figure 6.

Another aspect of the invention is the use of the ferrofluid of the invention and of the nanoparticles it comprises in industrial applications belonging, for example, to the following group: magnetic refrigeration, magnetic printing, magnetic inks, rotor lubrication, sealing in vacuum systems, damping systems, loudspeakers, magnetic sensors, actuators, catalysis, metal recovery and water purification, inductors and antennas in
communication technology, magnetic shields and microwave absorption, biotechnological, veterinary and medical applications [Jech T.J., Odenbach S. Ferrofluids: Magnetically Controllable Fluids and Their Applications, Springer, Berlin, 2002; Goldman A.J. Handbook of Modern Ferromagnetic Materials, Kluwer Academic Publishers, Norwell, 2002]. The industrial applications based on the magnetothermal properties of the magnetic nanoparticles include but are not limited to the hyperthermal use of magnetic nanoparticles in curing polymers, hardening epoxy resins, contact-free heating and biomedical applications.

As mentioned previously, the nanoparticles of the ferrofluid of the invention can anchor biologically active molecules which opens up the biotechnological field of the applications thereof in any of the specific areas, for example, food and agriculture, environment, chemical synthesis by means of enzymes, veterinary applications and medicine. A particular embodiment of the invention is formed by the use of the ferrofluid of the invention in the field of diagnosis and therapeutics of human and animal diseases.

In this sense, the use of this ferrofluid with the nanoparticles in diagnosis and clinical treatment involves a very significant progress in these fields because, for example, a small amount of magnetic nanoparticles can be resuspended in large volumes of sample to be analyzed and later recovered by means of applying an external magnetic field. It is thus possible to purify and/or pre-concentrate very small diluted amounts of a target biological molecule which is specifically hybridized with an organic biomolecule acting immobilized on said nanoparticles, whereby the detection limit is reduced to a great extent and the possibilities of a correct clinical diagnosis are exponentially improved.

This type of systems allows determining the presence of specific biological material of interest in situations in which an early detection thereof can be critical, to prevent the harmful effects entailed by the existence of the species or strains of organisms having said characteristic sequences. This fact has great application in human and veterinary biomedicine, including in the following aspects: i) detection of viral, bacterial, fungal or protozoan type pathogens; ii) characterization of mutations or genetic polymorphisms (SNPs) in said agents which can make them resistant to drugs or facilitate vaccine escape; iii) characterization of mutations or SNPs in human or animal genes related to diseases or prone to them; iv) detection of human disease markers
as specific tumors. This detection potential also has important applications in food and environmental control in aspects including the following: i) detection of specific microorganisms, pathogens or contaminants; ii) detection of the presence of genetically modified organisms (GMOs) or transgenic organisms, it being possible to quantify if their presence is above the allowed limits.

On the other hand, these ferrofluids can also be used in human therapy when it is necessary to destroy cells in patients, for example, cancer cells, immune system cells in autoimmune processes, pathogenic microorganisms, etc. Nanoparticles can also have biomolecules, an antibody for example, anchored thereto, which by specifically recognizing a specific tumor marker, a breast cancer marker for example, which allows carrying the nanoparticle to these target cells, which target cells would transfer said nanoparticle to their inside, in which place the target cell could be destroyed thanks to the hyperthermia property.

**EMBODIMENTS**

**Example 1.** Preparation of a maghemite-poly(4-vinylpyridine) nanocompound/nanocomposite of the invention

1.1. Synthesis of poly(4-vinylpyridine) by a radical pathway.

Approximately 2 grams of the 98% 4-vinylpyridine monomer previously treated in a molecular sieve were weighed. At the same time a dry schlenk type flask was prepared and immersed in an oil bath at 60°C. The monomer and 15-20 ml of tetrahydrofuran (THF) were introduced in this flask. The mouth of the flask was sealed with a septum and 2 or 3 vacuum-argon cycles were carried out. When the flask is under an argon atmosphere, 2% AIBN was added. It was allowed to react with stirring for 24 hours. Methanol drops were added for the purpose of stopping the reaction.

In order to purify the polymer obtained, it was precipitated by adding cold hexane to the solution and it was subsequently plate-filtered.

1.2. Preparation of a nanocompound of maghemite-poly(4-vinylpyridine).

For the preparation of an Fe-polymer compound, 0.2 grams of radical poly(4-vinylpyridine) were first dissolved in 5 ml of a 50% mixture of water and acetone and 0.1 ml of a solution containing 0.1 1 moles/L of FeBr₂, 0.89 moles/L of FeBr₃ and 0.5 moles/L of RbBr were added. It was evaporated to dryness, first at room temperature and then in an oven at 50°C.

The previously obtained Fe-polymer compound was then immersed in 5 ml of 1 M NaOH for 1 hour. It was filtered and washed with water until the
pH of the washing water decreases to 7. It was dried, first at room
temperature and then in an oven at 60°C. A nanocomposite was obtained
which according to images obtained by high resolution transmission
electronic microscopy (HRTEM) (Figure 1) contains dispersed spherical iron
oxide nanoparticles with an average size of 4.0 nm and a standard deviation
of ±0.4 nm, approximately 10% of its average size, in a solid polymer matrix.

The electronic diffraction analysis of said nanocomposite shows that
said nanoparticles have a spinel structure and can therefore consist of
magnemite or magnetite. The analysis of the nanoparticles by means of
spectroscopy of the energy loss of electrons shows that said particles consist
of magnemite (data not shown). The analysis of the nanocompound by
titration with K₂Cr₂O₇ indicates the absence of Fe²⁺ ions, which definitively
discards the presence of magnetite in the nanocompound.

Example 2.- Preparation of a series of magnemite-poly(4-vinylpyhdine)
nanocompounds/nanocomposites containing spherical nanoparticles with an
average diameter which can vary between 1.5 nm and 15 nm.

5 type 1 polymer solutions were prepared by means of dissolving 0.4 g
of radical poly(vinylpyhdine) respectively in 10 ml of a 50% mixture of water
and acetone. Amounts of 0.15, 0.88, 1.76, 2.64, 3.52, 6.60 ml respectively of
a solution containing 0.40 moles/L of FeBr₂, 0.60 moles/L of FeBr₃ and 0.5
moles/L of RbBr were added. It was evaporated to dryness, first at room
temperature and then in an oven at 50°C.

Each of the Fe-polymer compounds was immersed in 40 ml of 1 M NaOH
respectively for 1 hour. It was filtered and washed with water until the pH of
the washing water decreases to 7. It was dried, first at room temperature and
then in an oven at 60°C. Six nanocompounds, called S₁, S₂, S₃, S₄, and
S₅, respectively, were obtained. A study of the size distribution of sample S₄
from images obtained by high resolution transmission electronic microscope
(HRTEM) (Figure 2) indicates that the particles are spherical with an average
size of 6 nm and a standard deviation of ±0.7 nm. The electron diffraction
analysis shows that said particles have a spinel structure and can therefore
consist of magnemite or magnetite. The analysis of the particles by titration
with K₂Cr₂O₇ indicates the absence of Fe²⁺, which definitively discards the
presence of magnetite in the nanocompound. The analysis of
nanocompounds S₁, S₂, S₃, S₄, S₅ by small-angle X-ray scattering (SAXS)
(Figure 3) indicates that the particles are spherical and have an average size
of 1.6 nm, 2.5 nm, 3.5 nm, 5.2 nm, 15 nm respectively. It was observed that the variation of the size of the particles with the [Fe]/[pyridine] ratio used in the preparation follows a virtually linear trend (Figure 4).

**Example 3.** Preparation of a maghemite poly(4-vinylpyhydine) nanocompound/nanocomposite containing rod-shaped nanoparticles.

3.1. Synthesis of poly (4-vinylpyhydine) by an anionic pathway.

Approximately 2 grams of the 98% 4-vinylpyhydine monomer previously treated in a 4Å type molecular sieve were weighed. At the same time, a dry schlenk type flask was prepared and immersed in a bath at -78°C consisting of a mixture of isopropanol and liquid nitrogen. The monomer and 15-20 ml of distilled tetrahydrofuran (THF) were introduced in this flask. The mouth of the flask was sealed with a septum and 2 or 3 vacuum-argon cycles were carried out. When the flask is under an argon atmosphere, 5% BuLi was added. The start of the reaction gives rise to reddish orange color of the solution due to the fact that the carboanion is colored. It was allowed to react with stirring for 24 hours. Methanol drops were added for the purpose of stopping the reaction.

In order to purify the polymer obtained, it was precipitated by adding cold hexane to the solution and it was subsequently plate-filtered.

3.2. Preparation of a rod-shaped of maghemite-poly(4-vinylpyhydine) nanocomposite.

A type 1 polymer solution was prepared by means of dissolving 0.3 g of anionic poly(4-vinylpyhydine) in 5 ml of a 50% mixture of water and acetone. 0.506 ml of a solution containing 0.5 moles/L of FeBr₂, 1 mol/L of FeBr₃ and 0.5 moles/L of RbBr were respectively added. It was evaporated to dryness, first at room temperature and then in an oven at 50°C. The Fe-polymer compound obtained was immersed in 20 mL of 1 M NaOH for 1 hour. It was filtered and washed with water until the pH of the washing water decrease to 7. It was dried, first at room temperature and then in an oven at 60°C. The examination of the sample by high resolution transmission electronic microscopy (HRTEM) (Figure 5) indicates that the particles are rod-shaped with an average length of 60 nm and a thickness of 6 nm. The electron diffraction analysis shows that said particle have a spinel structure and can therefore consist of maghemite or magnetite. The analysis of the particles by titration with K₂Cr₂O₇ indicates the absence of Fe²⁺ ions, which definitively discards the presence of magnetite in the nanocompound.
The analysis by means of small angle neutron scattering (SANS) of a dispersion of the nanocompound in a solution containing 0.1 mol/L of HNO\textsubscript{3} in a 40% mixture of D\textsubscript{2}O and H\textsubscript{2}O, which cancels the dispersion of the polymer, gives an I(Q) curve corresponding only to the scattering of the particles consists of a line with gradient -2. This result can be interpreted considering a planar particle shape or by means of associating the elongated particles in planar structures in accordance with the observations carried out by HRTEM.

Furthermore, measurements were carried out of the variation of the alternating (ac) susceptibility against the temperature for different field alternance frequencies (Figure 9) in a nanocompound prepared according to this example containing rod-shaped particles. It was observed that the out-of-phase susceptibility peak is extraordinarily narrow. This quality makes said particles be very suitable for hyperthermal uses, i.e. for heating cells, tissue or non-biological media if they are industrial applications by means of an alternating magnetic field, given that the magnetocaloric effect for a certain field frequency and intensity are generated in a very narrow susceptibility range.

**Example 4.** Preparation of a stable maghemite ferrofluid of the invention at pH=7.4 by means of copolymerization with polyethylene glycol methacrylate.

10 milligrams of maghemite-poly(4-vinylpyhdine) nanocomposite obtained previously according to Example 1 were dissolved in 1 ml of 0.1 M HNO\textsubscript{3}. It was resuspended in 1 ml of phosphate buffer solution (PBS) at pH 7.4, a turbid dispersion being generated.

10 mg of maghemite-poly(4-vinylpyhdine) nanocomposite obtained previously according to the Example 1 were dissolved in 1 ml of 0.1 M HNO\textsubscript{3}. A solution formed by 1 ml of ferrofluid at pH 2.4 and 3 ml of poly (ethylene glycol) (PEG) functionalized with a methacrylate group with a concentration of 7.5 mg/mL was added. It was resuspended in 1 ml of phosphate buffer solution (PBS) at pH 7.4, a turbid dispersion being originated.

10 mg of nanocomposite of maghemite-poly(4-vinylpyhdine) were dissolved in 1 ml of 0.1 M HN03. A solution formed by 1 ml of ferrofluid at pH 2.4 and 3 ml of poly (ethylene glycol) (PEG) functionalized with a methacrylate group with a concentration of 7.5 mg/mL was added. Said dispersion was incubated at 40°C for 8 hours to achieve the copolymerization
of PEG methacrylate with the polyvinylpyridine coating the nanoparticles. After this incubation process, 300 µl of this mixture were resuspended in 1 ml of phosphate buffer solution (PBS) at pH 7.4 to adjust the acidity to a physiological pH, thus generating the ferrofluid of the invention, which is transparent, stable at physiological pH, biocompatible and can be biologically functionalized.

The obtained dispersion is purified by means of magnetic separation and subsequent re-dispersion in phosphate buffer solution (PBS) at pH 7.4. The transmission electronic microscopy images of the ferrofluid show that the size of the nanoparticles is not modified with respect to the starting nanocompound and large aggregates are not observed (Figure 6).

**Example 5.** Controlled variation of the magnetic properties in nanocomposites with different particle sizes.

Measurements of the variation of magnetization against the applied field in nanocomposites S1-S5 of Example 2 were carried out. A regular increase was observed in the magnetization curves obtained in nanocompounds with an increasing particle size (Figure 7). The saturation magnetization of the different nanocomposites calculated by means of extrapolating the linear part of the curve at 0 field shows a variation from virtually 0 emu/g until 50 emu/g, close to the saturation magnetization of macroscopic maghemite (76 emu/g), or in other word, in virtually the entire superparamagnetic range.

Furthermore, measurements were carried out of the variation of the alternating (ac) susceptibility against the temperature for a field alternation frequency of 10 Hz (Figure 8) in nanocomposites S1-S5. The blocking temperature of the different nanocompounds, calculated as the susceptibility temperature in the maximum out-of-phase susceptibility, shows a variation from less than 1.8 K to 300 K, or in other words, in virtually the entire superparamagnetic range. Therefore, starting from the basis that the particles are superparamagnetic at room temperature to prevent aggregation, the method allows, within the widest range possible, synthesizing nanoparticles with a maximum magnetocaloric performance for a certain frequency.

**Example 6.** Magnetocaloric performance of rod-shaped nanocompounds.

A nanocompound according to this invention containing 28% of rod-shaped particles and 62% of spherical particles is obtained starting from a
commercial poly(4-vinylpyridine) supplied by Aldrich and according to the process described in Example 1, but using 1 ml of the FeBr$_2$/FeBr$_3$/RbBr solution instead of the amount specified in the example. It was calculated from the images obtained by HRTEM that the rod-shaped particles have an average length of 18.4 nm and an average thickness of 2.7 nm and that the spherical particles have an average diameter of 6.2 nm. The magnetocaloric performance of this nanocompound in an aqueous suspension based on the relative temperature increase was measured in the presence of an alternating magnetic field with an intensity of and an alternation frequency of 144 Hz. A SAR performance= 144 w/g is obtained. The magnetocaloric performance of a nanocompound prepared according to this invention starting from a radical polymer containing spherical maghemite nanoparticles with an average size of 7.5 nm was measured in the same conditions. A SAR yield = 8 w/g was obtained.

Example 7. Stability of the magnetic ferrofluids in physiological media.

The variation of the magnetization against the temperature with cooling at 0 field and with cooling with a 25 Gauss field (ZFC-FC) was measured in the ferrofluid prepared in Example 4, immediately after the preparation and a month after the preparation. The results show that the curves are perfectly superimposed (Figure 10), indicating that during this time, the average particle size has not been modified and aggregates have not been formed.
CLAIMS

1.- A magnetic nanoparticle system comprising magnetic nanoparticles of a metal oxide comprising iron and a polymer (P) wherein:

a) the polymer comprises a monomer (I) containing active functional groups which can interact with metal ions by means of Coulomb forces, Van der Waals forces or coordination bonds,

b) the molar \([\text{Fe }]/[\text{monomer (I)}]\) ratio is 0.01 - 10.

c) the nanoparticles have a size dispersion of less than 15% of the average size.

2.- A magnetic nanoparticle system according to claim 1, characterized in that the metal oxide comprises \(\text{Fe}^{2+}\) and/or \(\text{Fe}^{3+}\).

3.- A magnetic nanoparticle system according to claim 2, characterized in that the metal oxide further comprises a metal selected from \(\text{Co}^{2+}, \text{Ni}^{2+}, \text{Mn}^{2+}, \text{Gd}^{2+}, \text{Be}^{2+}, \text{Mg}^{2+}\text{Ca}^{2+}, \text{Ba}^{2+}\).

4.- A magnetic nanoparticle system according to claim 1, characterized in that the metal oxide comprises maghemite \((\gamma\text{-Fe}_2\text{O}_3)\).

5.- A magnetic nanoparticle system according to claim 1, characterized in that the metal oxide comprises magnetite \((\text{Fe}_3\text{O}_4)\).

6.- A magnetic nanoparticle system according to claim 3, characterized in that the metal oxide comprises \(\text{MFe}_2\text{O}_4\) (ferrite), wherein M is \(\text{Co}^{2+}, \text{Ni}^{2+}, \text{Mn}^{2+}, \text{Gd}^{2+}, \text{Be}^{2+}, \text{Mg}^{2+}\text{Ca}^{2+}\text{Ba}^{2+}\).

7.- A magnetic nanoparticle system according to claim 6, characterized in that the ferrite is barium ferrite \((\text{BaFe}_3\text{O}_4)\).

8.- A magnetic nanoparticle system according to claim 1, in which the polymer is organic.

9.- A magnetic nanoparticle system according to claim 1, in which the polymer is a hybrid organic-inorganic polymer.

10.- A magnetic nanoparticle system according to claims 1 to 9, characterized in that the monomer (I) contains functional groups selected from alcohol, alkoxide, carboxyl, anhydride, phosphate and/or phosphine.

11.- A magnetic nanoparticle system according to claims 1 to 9, characterized in that the monomer (I) contains nitrogenated functional groups.
12.- A magnetic nanoparticle system according to claim 11, characterized in that the nitrogenated functional groups are selected from amine, amide, nitrile, azide.

13.- A magnetic nanoparticle system according to claim 11, characterized in that the nitrogenated functional groups are selected from imine and heterocycles such as pyridine, pyrrole, pyrrolidone, pyrimidine, adenine.

14.- A magnetic nanoparticle system according to claims 1 to 13, characterized in that the monomer (I) is a vinyl type monomer.

15.- A magnetic nanoparticle system according to claims 1-14, characterized in that the particles are spherical and polymer (P) is obtained by a radical pathway.

16.- A magnetic nanoparticle system according to claims 1-14, characterized in that the particles are elongated and polymer (P) is obtained by an anionic pathway.

17.- A magnetic nanoparticle system according to claim 14, characterized in that the vinyl type monomer is vinylpyridine.

18.- A magnetic nanoparticle system according to claims 1 to 17, characterized in that the molar [Fe]/monomer (I) ratio is 0.03 - 2.

19.- A magnetic nanoparticle system according to claims 1 to 18, characterized in that the system is solid (nanocomposite).

20.- A magnetic nanoparticle system according to claims 1 to 18, characterized in that the system is liquid (ferrofluid).

21.- A magnetic nanoparticle system according to claim 20, characterized by comprising water or a biocompatible aqueous solution.

22.- A magnetic nanoparticle system according to claim 21, characterized in that the aqueous solution comprises a substance acting as a buffer.

23.- A magnetic nanoparticle system according to claim 20, characterized in that polymer (P) further comprises a monomer (II) containing hydrophilic functional groups.

24.- A magnetic nanoparticle system according to claim 23, characterized in that the monomer (II) is a vinyl type monomer.

25.- A magnetic nanoparticle system according to claim 24, characterized in that the vinyl monomer is selected from acrylate, methacrylate, methyl methacrylate, vinylpyrrolidone or derivatives thereof.
26.- A magnetic nanoparticle system according to claim 25, characterized in that the vinyl monomer is polyethylene glycol (PEG) methacrylate.

27.- A magnetic nanoparticle system according to claims 23 to 26, characterized in that polymer (P) further comprises a monomer (III) containing functional groups which can anchor biologically active molecules.

28.- A magnetic nanoparticle system according to claim 27, characterized in that the biologically active molecules are anchored to monomer (III) by means of covalent bonds.

29.- A magnetic nanoparticle system according to claim 28, characterized in that functional groups of monomer (III) are selected from -NH₂; -SH, -COOH, and -CONH₂.

30.- A magnetic nanoparticle system according to claim 27, characterized in that the monomer (III) is a vinyl type monomer.

31.- A magnetic nanoparticle system according to claim 27, characterized in that polymer (P) comprises two types of monomers.

32.- A liquid magnetic nanoparticle system (ferrofluid) comprising magnetic nanoparticles of a metal oxide and an organic polymer characterized by comprising:

a) maghemite as a metal oxide,
b) a polymer matrix containing:
   • 4-vinylpyridine [monomer (I)],
   • a vinyl monomer functionalized with poly(ethylene glycol) (PEG) [monomer (II)]
   • a vinyl monomer containing functional groups selected from -NH₂, -SH, -COOH, and -CONH₂ [monomer (III)]
   • an aqueous solution of phosphate buffer (PBS), maintaining the system at pH 7.4.

33.- A process for preparing the magnetic nanoparticle system of the invention described in claims 1 to 32 comprising the following steps:

a 1) an aqueous solution, optionally mixed with organic solvents, of a polymer (P) comprising a monomer (I) containing active functional groups which can intercat with metal ions by means of coulomb forces, Van der Waals forces or coordination bonds, with
a2) an aqueous solution, optionally mixed with organic solvents, comprising at least one Fe salt in which the molar [Fe]/[monomer (I)] ratio is 0.01 - 10

b) adding a base in a sufficient amount to reach pH 8 - 14

34.- A process according to claim 33, characterized in that solution a2) comprises at least one divalent metal salt and a Fe$^{3+}$ salt.

35.- A process according to claim 34, characterized in that the divalent metal salt is at least one of the group formed by a Fe$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Gd$^{2+}$, Be$^{2+}$, Mg$^{2+}$, Ca$^{2+}$, and Ba$^{2+}$ salt.

36.- A process according to claim 35, characterized in that the divalent metal salt is a Fe$^{2+}$ salt.

37.- A process according to claim 36, characterized in that solution a2) contains FeBr$_2$ and FeBr$_3$.

38.- A process according to claim 37, characterized in that solution a2) contains a monovalent bromide.

39.- A process according to claim 38, characterized in that the monovalent bromide is selected from the group formed by: KBr, RbBr, NaBr, CsBr, (CH$_3$)$_4$NBr, (CH$_3$CH$_2$)$_4$NBr.

40.- A process according to claims 36 to 39, characterized in that the average size of the nanoparticles of metal oxide comprising iron is regulated by varying the molar Fe$^{2+}$/Fe$^{3+}$ ratio.

41.- A process according to claims 33 to 41, characterized in that the average size of the nanoparticles of metal oxide comprising iron is regulated by varying the molar ratio between the base added in b) and the iron contained in a).

42.- A process according to claims 33 to 41, characterized in that solutions a1) and a2) are mixed in a molar [Fe]/[monomer (I)] ratio of 0.03 to 2.

43.- A process according to claims 33 to 42, characterized in that in step b), the base is added until reaching pH 12.5 - 13.

44.- A process according to claims 33 to 43, characterized in that polymer (P) used in a1) comprises a monomer (I) containing functional groups selected from alcohol, alkoxide, carboxyl, anhydride, phosphate and/or phosphine.
45.- A process according to claims 33 to 43, characterized in that polymer (P) comprises a monomer (I) containing nitrogenated functional groups.

46.- A process according to claims 33 to 43, characterized in that polymer (P) comprises a monomer (I) containing imines or heterocycles such as pyridine, pyrrole, pyrrolidone, pyrimidine, adenine.

47.- A process according to claims 33 to 46, characterized in that monomer (I) is a vinyl type monomer.

48.- A process according to claim 47, characterized in that the vinyl type monomer is vinylpyridine.

49.- A process according to claims 33-48, characterized in that polymer (P) is obtained by a radical pathway.

50.- A process according to claim 33-48, characterized in that polymer (P) is obtained by an anionic pathway.

51.- A process according to claims 33 to 50, characterized in that it further comprises the preparation of polymer P in a step prior to step a).

52.- A process according to claim 51, characterized in that polymer P is a copolymer and is prepared by the simultaneous or successive copolymerization of a monomer (I) with a monomer (II) containing hydrophilic groups and optionally with a monomer (III) containing functional groups which can anchor biologically active molecules.

53.- A process according to claims 33 to 50, characterized in that it further comprises a step c), after step b), comprising the copolymerization of polymer P with a monomer (II) containing hydrophilic groups and optionally with a monomer (III) containing functional groups which can anchor biologically active molecules and when the copolymerization is carried out with the two monomers (II) and (III), said copolymerization is carried out successively or simultaneously.

54.- A process according to claims 33 to 52, characterized in that it further comprises subjecting the product of step b) to a solid-liquid phase separation to obtain a solid system (nanocomposite) comprising magnetic nanoparticles containing a metal oxide core and a polymer (P).

55.- A process according to claim 53, characterized in that it further comprises subjecting the product of the additional step c) to a solid-liquid phase separation to obtain a solid system (nanocomposite) comprising magnetic nanoparticles of a metal oxide comprising iron and a polymer (P).
56.- A process according to claims 54 and 55, characterized in that it further comprises dispersing the solid product (nanocomposite) in a suitable liquid medium to obtain a liquid system (ferrofluid).

57.- A process according to claim 56, characterized in that the liquid is water or a biocompatible aqueous solution.

58.- A process according to claim 57, characterized in that the aqueous solution comprises a substance acting as a buffer.

59.- Use of the liquid system (ferrofluid) according to any of claims 20 to 32 for magnetic refrigeration, magnetic printing, magnetic inks, rotor lubrication, electric transformers, low noise-level solenoids, switchers, magnetorheological fluids, magnetically active fibers, reinforced polymeric composites, sealing in vacuum systems, damping systems, loudspeakers, magnetic sensors, actuators, catalysis, metal recovery and water purification, inductors and antennas in communication technology, magnetic shields and microwave absorption, polymer curing, epoxy resin hardening, contact-free heating and biotechnological, veterinary and medical applications.

60.- Use of the liquid system (ferrofluid) according to claim 59, characterized in that the medical application includes the diagnosis and treatment of human diseases.
Fig. 3
Fig. 4
Fig. 6A
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C08K3/22 C08F16/00 C08F22/00 C08F26/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C08K C08F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C

See patent family annex

Date of the actual completion of the international search

23 October 2007

Date of mailing of the international search report

06/11/2007

Name and mailing address of the ISA/

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Authorized officer

Schütte, Maya
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<td>TAKAFUJI ET AL: &quot;surface charge controlled magnetic nanoparticles with grafting of poly(4-vinylpyridine)&quot; JOURNAL OF NANOSCIENCE AND NANOTECHNOLOGY, AMERICAN SCIENTIFIC PUBLISHERS, US, vol. 5, no. 3, 2005, pages 390-393, XP009091203 ISSN: 1533-4880 abstract; figure 1</td>
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International application No
PCT/EP2007/058312

Form PCT/ISA/210 (patent family annex) (April 2005)