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Stimuli-responsive magnetic particles for biomedical applications

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ABSTRACT

In recent years, magnetic nanoparticles have been studied due to their potential applications as magnetic carriers in biomedical area. These materials have been increasingly exploited as efficient delivery vectors, leading to opportunities of use as magnetic resonance imaging (MRI) agents, mediators of hyperthermia cancer treatment and in targeted therapies. Much attention has been also focused on "smart" polymers, which are able to respond to environmental changes, such as changes in the temperature and pH. In this context, this article reviews the state-of-the art in stimuli-responsive magnetic systems for biomedical applications. The paper describes different types of stimuli-sensitive systems, mainly temperature- and pH sensitive polymers, the combination of this characteristic with magnetic properties and, finally, it gives an account of their preparation methods. The article also discusses the main *in vivo* biomedical applications of such materials. A survey of the recent literature on various stimuli-responsive magnetic gels in biomedical applications is also included.

Contents

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1. Introduction

Much effort has been devoted to the synthesis of organic/inorganic nanocomposites (Khan et al., 2009; Colard et al., 2009; Cheung and Bon, 2009; Cao et al., 2010; Nguyen et al., 2010; Sheibat-Othman and Bourgeat-Lami, 2009) in an attempt to exploit new hybrid properties derived from various components. Because of their small size, nanoparticles (NP) offer unique properties and magnetic nanoparticles (MNP) based on iron oxide composites or hybrid materials, essentially in the form of core–shell systems are finding great interest in the field of biomedical applications (Häfeli et al., 1997; Stanciu et al., 2009; Wong et al., 2008; Thakur et al., 2009; Faraji et al., 2010). The study of microscopic structure of magnetic materials started around 1870 (Arshady, 2001) and left to the production of stable colloidal iron oxide (ferromagnetic) particles by McKeehan and Elmore (1934).

In this context, the term "nanobiomagnetism" can be defined as the intersection of nanomagnetism and medicine that focuses on biological systems and/or process (Leslie-Pelechy et al., 2006). Magnetos can act on objects at a distance and this characteristic makes them available as medical tools. There are works in the literature describing the removal of safety pins, bullets and grenade splinters using magnetos (Luborsky et al., 1965; Douglas et al., 2002). The invention of stronger and smaller permanent magnets made possible more delicate applications, such as temporarily fixing prosthesis in dentistry, guiding catheters through the body, and navigating within the brain (Gillies et al., 1994).

The unique size-dependent properties of magnetic nanoparticles lead to a growing study of elaboration of these materials with interesting perspectives in current technology and future applications (Craik, 1995). Nanoscale materials have a special relevance to biomedical applications due to their size compatibility with cells (10–100 μm), viruses (20–450 nm), proteins (5–50 nm) and genes (2 nm wide by 10–100 nm long). Their sizes are small enough to move inside the body without disrupting normal functions and can access spaces inaccessible by other materials. Cells react in the presence of these nanomaterials and these reactions can induce cellular growth or death (Chen et al., 2008). In this sense, the study of the processes involving magnetic nanoparticles is important in order to prepare available systems for *in vivo* applications.

The combination of magnetic nanoparticles with polymers in order to obtain colloidal or composite stable systems had attracted much interest. A polymer shell can act as compatibilizer interacting with the environment and can be supplied with biologically or catalytically active functional sites (Liu et al., 2008). On the other hand, the magnetic properties of the nanoparticles allow the manipulation of the systems (Schmidt, 2007).

Magnetic biomaterials must present additional characteristics in comparison with materials used for other applications. For *in vivo* applications of MNP, three basic prerequisites have to be met: (1) the surface has to be biocompatible, (2) non-toxic, and (3) the particles must be well-dispersed without forming aggregates. Ideally, surface modification should provide good colloidal stability (Liz-Marzán et al., 1999; Yap et al., 2005), biocompatibility and chemical functionality for the potential attachment of biorecognisable ligands. In this sense, it appears reasonable the use of iron oxide nanoparticles as a magnetic core encapsulated in a protective polymeric shell. *In vitro* applications have less strict requirements, but in techniques involving living cells the effect of the materials on the sample must be previously studied (Gillies et al., 1994).

Nowadays, there is an increasing interest on the synthesis of new biomaterials produced by polymerization of biocompatible monomers. Biocompatibility is the capacity of the material to be very well tolerated by the body or do not interfere with the normal body functions. This characteristic makes these materials available for *in vivo* biomedical applications (Macazaga, 2007). In addition to biocompatibility, materials must be able of being functionalized with one or more molecules, they must retain their magnetic properties for a reasonable period of time in aqueous media with varying pH, must not be cleared too quickly from the blood stream, and, finally, must form stable, non-aggregating dispersions.

As biocompatible polymers, smart materials are largely studied for *in vivo* applications. Stimuli-responsive polymers are macromolecules that change their properties in a desired way responding to a small change in temperature, pH, electric or magnetic field, among others. This kind of polymers had attracted much attention in nanomedicine. Thermoresponsive polymers are the most studied stimuli-responsive polymers due to the fact that this parameter is directly related with the human body (Konak et al., 2007).

2. "Smart" polymers

Response to stimulus is a basic process of living systems (Jeong and Gutowska, 2002). It is well known the human body ability to respond to its environment from the molecular to the macroscopic level (Nelson, 2008). The functions of living cells are regulated by macromolecules that respond to changes in local environment and these biopolymers form the basis around which all major natural processes are controlled (Alarcón et al., 2005). In this way, stimuli response is crucial for maintaining normal function as well as fighting disease. At the molecular level, for example, the body releases insulin to initiate glycogen formation in response to higher glucose levels in the blood. At the macroscopic level, the body responds to external stimuli with a cascade of events, such as when nerve cells transmit signals to the brain in response to a pain-causing stimulus and subsequently cause muscle contraction (Nelson, 2008). These examples have inspired scientists to fabricate "smart" materials that respond to light, pH, temperature, mechanical stress or molecular stimuli. These responses are manifested as dramatic changes in one of the following: shape, surface characteristics, solubility, among others. These polymers are being developed for uses in fields such as in medicine, and some specific examples range from microfluidic devices (Barker et al., 2000) pulsatile drug release systems (Kikuchi and Okano, 2002) bioadhesion mediators (Ista and Lopez, 1998) and motors/actuators (Hoffman et al., 1999). Responsive polymers are also a major focus in emerging nanoscale technologies (Kim et al., 2003a,b). Stimuli-responsive polymers are studied due to the fact that their properties (viscoelasticity, transparency, conductivity, etc.) can be controlled by modifying the structure and organization of the polymer chains (Wong et al., 2008). This characteristic makes these smart polymers important agents for pharmaceutical delivery systems, among other applications. Since the macromolecules may be able to self-organize in aqueous medium, forming predetermined but highly diversified structures owing to the multiple types of interactions, the challenge is to design models of these systems that lead to the possibility of measuring and controlling the number and the strength of multi-stimuli-responsive associations. Table 1 provides some examples of stimuli-responsive polymers. Kinetic and thermodynamic control of the stimuli-sensitive response is crucial in all applications; therefore understanding the structure–property relationship is essential for further development and rational design of new functional smart materials (Jeong and Gutowska, 2002).

Electro-responsive polymers can be used to prepare materials that swell, shrink, or bend in response to an electric field (Filipcsei et al., 2000). These materials have been investigated as a form of hydrogels to have swelling, shrinking or bending behaviour in response to an external field because this property has been applied for bio-related applications such as drug delivery systems, artificial muscle, or biomimetic actuators (Bawa et al., 2009).

Table 1
Stimuli-responsive polymers and their corresponding stimulus.

Polymer	Abbreviation	Type of stimulus	Reference
Poly(acrylic acid)	PAA	pH	Kratz et al. (2000)
Poly(acetoacetoxyethyl methacrylate)	PAAEM	pH	Pich et al (2004)
Poly[2-(diisopropylamino)ethyl methacrylate]	PDPA	pH	Peng et al. (2010)
Poly(hexyl methacrylate)	PHEMA	pH	Mahajan et al. (2003)
Poly[2-(dimethylamine)ethyl methacrylate)	PDMAEMA	pH	Liu and Urban (2010)
	P4VP	pH	Liu and Urban (2010)
Poly(dimethylsiloxane)	PDMS	Electrical field	Kumar et al. (2007)
Poly[2-(methacryloyloxy)ethyl phosphorylcholine]	PMPC	Electrical field	
Poly(ethylenediamine-co-1,10-bis(chloro-carbonyl)decane)		Electrical field	Okahata et al. (1986)
Poly(*N*-isorpropylacrylamide)	PNIPAam	Temperature	Nakayama et al. (2006)
Poly(*N*-vinylcaprolactam)	PNVCL	Temperature	
	PLA	Temperature	Motornov et al. (2010)
Poly(*N*,*N*-dimethylacrylamide-co-4-phenyl-azophenyl acrylate)		Light	Kim et al. (2009)
Poly(*N*,*N*-dimethyl acrylamide-co-4-phenyl-azophenyl acrylamide)		Light	Kim et al. (2009)
Poly(acrylic acid-graft-vinylidenefluoride)		pH-and ionic strength	Järvinen et al. (1998)

Electro-responsive polymers can transform electrical energy into mechanical energy and also have promising applications in biomechanics, artificial muscle actuation, sensing, energy transduction, sound dampening, chemical separations, and controlled drug delivery, these polymers are an increasingly important class of smart materials (Kim et al., 1999).

Light/Photo-responsive polymers are macromolecules that change their properties when irradiated with light of the appropriate wavelength (Roy et al., 2010). These light-responsive polymers are supplied with photoactive groups such as azobenzene, spirobenzopyran, triphenylmethane or cinnamonyl that can undergo reversible structural changes under UV–vis light by changing their size and shape, or forming ionic or zwitterionic species upon irradiation (Motornov et al., 2010). The changes result from the light-induced structural transformations of specific functional groups presented in the polymer backbone or side chains (Lambeth and Moore, 2007). Light is a particularly attractive source of energy for use in biomedical applications. Its intensity and wavelength can easily be controlled through the use of filters, and photomasks or lasers allow for fabrication of complex features and exposure areas with resolution as small as approximately 1 μm. The majority of light-responsive chemical moieties are responsive in the UV spectral range and this property is useful for *in vitro* applications (Katz and Burdick, 2010). Possible applications of photoresponsive polymers include reversible optical storage, polymer viscosity control, photomechanical transduction and actuation, bioactivity switching of proteins, tissue engineering, and drug delivery (Roy et al., 2010). Several light-sensitive particles have been encapsulated within vesicles that cause a physical change upon light exposure, leading to vesicle disruption. An important aspect of photo-sensitive polymer systems is that using irradiation as a stimulus is a relatively straightforward, non-invasive mechanism to induce responsive behaviour. These types of polymers have been investigated for many years, but there has been a recent expansion in research to create increasingly complex macromolecular architectures. Light-sensitive core-shell nanoparticles of Au and silica were prepared by Liu and Miyoshi (2008). These authors prepared particles with a diameter of approximately 45 nm and a silica shell thickness of within 5 nm using gold nanoparticles as templates with 40 nm in diameter. The light-sensitive molecules of vitamin C (Vc), 5(6)-carboxyfluorescein-*N*-hydroxysuccinimide ester (FLUOS), poly(vinyl alcohol) (PVA), and 2,5-dihydroxy-*p*-benzoquinone (DHBQ) were embedded in silica shells. 3-aminopropyltrimethoxysilane (APS) was used to bind with gold nanoparticle surfaces at different percentages. Silica nanocapsules were then prepared using sodium cyanide to dissolve gold cores. The authors verified that the silica shells became more condense and pore sizes shrunk after light sensitive molecules decomposed following light irradiation because the light-irradiated nanoparticles dissolved more slowly than the non-light-irradiated nanoparticles. TEM micrographs revealed that silica nanocapsules collapsed under high-density electron current.

However, the most important systems, also from a biomedical point of view, are those sensitive to pH or temperature. Some thermoresponsive polymers possess critical temperature close to the physiological value. Moreover, human body presents variations on pH along the gastrointestinal tract, and also in some specific areas like certain tissues (and tumoral areas) or sub-cellular compartments (Aguilar et al., 2007).

2.1. Thermally-responsive polymers

Thermally-responsive polymers undergo a coil-globule transition in aqueous solution at temperature values which is known as lower critical solution temperature (LCST) (Dimitrov et al., 2007).

Aqueous soluble polymers represent a diverse class of polymeric materials ranging from biopolymers to synthetic systems of enormous commercial utility. These materials have acquired increasing importance due to the demand for water-based instead of the traditional solvent-based technological processes. Water, being easily available and environment friendly, will become the solvent of choice for a wide range of products (Chen et al., 2008).

There are many different polymers and systems that show thermoresponsive behaviour. Table 2 presents LCST values for different thermoresponsive polymers.

Table 2
Transition temperature of thermoresponsive polymers (cloud point of 1% aqueous solution) (adapted of Kawaguchi, 1999 and Liu et al., 2009a,b).

Polymer	Transition temperature (°C)
Poly(*N*-ethylacrylamide)	72
Poly(*N*-cyclopropymethacrylamide)	59
Poly(*N*-methyl-*N*-ethylacrylamide)	56
Poly(*N*-acryloylpyrrolidine)	56
Poly(*N*-ethylmethacrylamide)	50
Poly(*N*-cyclopropylacrylamide)	45.5
Poly(*N*-isopropylmethacrylamide)	44
Poly(*N*,*N*-diethylacrylamide)	32
Poly(*N*-isopropylacrylamide)	30.9
Poly(*N*-vinylcaprolactam)	31
Poly(*N*-*n*-propylmethacrylamide)	28
Poly(*N*-methyl-*N*-isopropylacrylamide)	22.3
Poly(*N*-*n*-propylacrylamide)	21.5
Poly(*N*-methyl-*N*-*n*-propylacrylamide)	19.8
Poly(*N*-acryloylpiperidine)	5.5

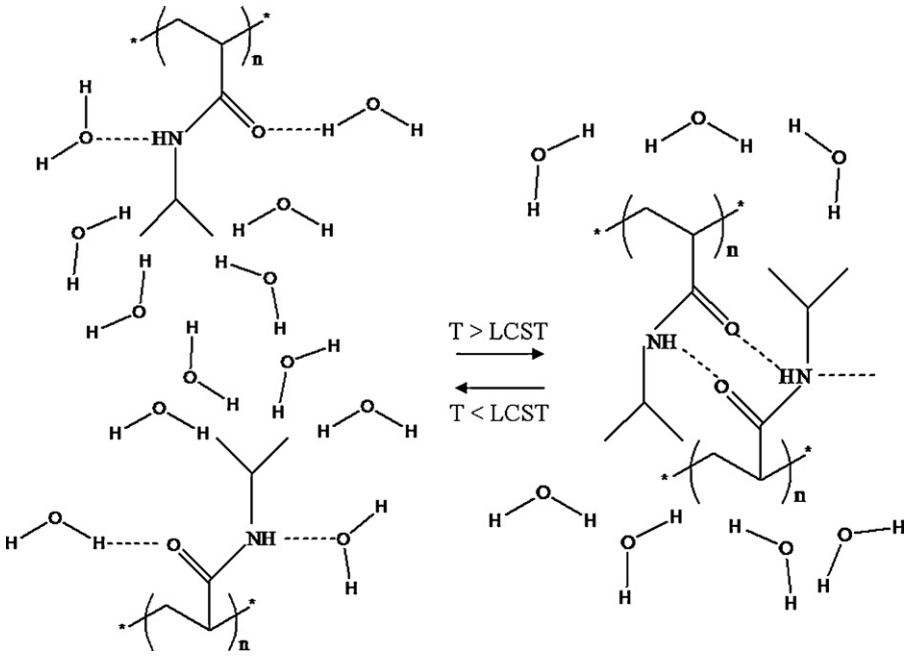

Fig. 1. Illustration of the effect of the temperature on the phase transition of PNIPAam (adapted from Dimitrov et al., 2007).

The materials based on reversible supramacromolecular organization have become increasingly important in recent decades. These systems may offer a number of advantages, including lower processing costs due to their reversible properties (Dimitrov et al., 2007).

In the context of thermoresponsive materials, poly(*N*-isopropylacrylamide) PNIPAam is the most studied polymer exhibiting LCST at around 31 °C, as shown in Table 2. PNIPAam has commonly been referred as a smart material (Fernández-Barbero et al., 2009).

The phase's transition of PNIPAam in water is schematically illustrated in Fig. 1. We can observe that the origin of the "smart" behaviour, arises from the entropic gain as water molecules associated with the amide groups are released when the temperature is increased above the critical point.

As shown in Fig. 1, at temperatures below the LCST, water acts as a good solvent for the polymer chains forming hydrogen bonds with the amide oxygen. When the temperature is raised above the LCST, polymer-polymer interactions become dominant expelling water.

In opposition to the LCST behaviour, some thermoresponsive polymers possess an upper critical solution temperature (UCST). These materials collapsed at temperatures below the UCST and swells when heated this temperature (Ohnishi et al., 2006).

Although PNIPAam is a thermoresponsive polymer largely used in the literature, the most of the works describes the synthesis of this polymer for *in vitro* applications. The presence of the amide groups leads to the non biocompatibility of the material and, consequently, limits its use for biomedical applications.

In this sense, another example of thermally-sensitive and biocompatible polymer that has been studied for therapeutic purposes is poly(*N*-vinylcaprolactam) (PNVCL) (Vihola et al., 2002; Vihola et al., 2008; Imaz and Forcada, 2008, 2009; Imaz et al., 2008; Iskakov et al., 2007).

Biocompatible thermoresponsive copolymers are also being developed for application in drug delivery and regenerative medicine. Thermoresponsive copolymers combine two or more comonomers where at least one of these would give a thermoresponsive homopolymer (Liu et al., 2009a,b). They have attracted considerable interest in both the polymer and biomaterial literature due to their thermally triggered contraction and aggregation as well as other potentially useful properties such as reversible gelation. The LCST can be tuned by the incorporation of additional functionality. Several studies have been performed considering the copolymerisation of NIPAam with more hydrophilic comonomers. The LCST of copolymers such as poly(*N*-isopropylacrylamide-*co*-2-hydroxyisopropylacrylamide) (poly(NIPAm-*co*-HIPAam)) or poly(*N*-isopropylacrylamide-*co*-*N*-vinylpirrolidone) (poly(NIPAam-*co*-NVP)), for example, is higher than that of PNIPAam. Thermoresponsive copolymers that do not contain *N*-alkyl substituted polyacrylamides, such as poly(ethylene oxide) PEO-based copolymers and poly(*N*-vinylcaprolactam) PNVCL-based copolymers are also of interest. PNVCL and PEO-based copolymers are important classes of biocompatible non-acrylamide-containing thermoresponsive homopolymers (Verbrugghe et al., 2003).

2.1.1. Thermoresponsive property of poly(N-vinylcaprolactam) (PNVCL)

Poly(*N*-vinylcaprolactam) (PNVCL) is known as biocompatible and suitable for *in vivo* applications (Maeda et al., 2001a,b). PNVCL shows LCST in water close to physiological temperature (35–38 °C), depending on the polymer concentration, molecular weight and the composition in the case of copolymers, which opens perspectives for applications in biochemistry and medicine (Yanul et al., 2001; Shtanko et al., 2003). PNVCL is composed of amide group that render the polymer as a whole hydrophilic (Wallace et al., 2002). It has a repeat unit consisting of a cyclic amide where the amide group nitrogen is directly attached to the hydrophobic polymer backbone. Thus, unlike the thermo-sensitive poly(*N*-alkylacrylamides), it does not produce small amide derivatives upon hydrolysis. This feature, together with its overall low toxicity, high complexing ability and good film forming properties enables to use in many medical applications. Up to now, several studies have reported the phase behaviour of PNVCL in water (Lau and Wu, 1999; Peng and Wu, 2000; Makhaeva et al., 2003; Lozinskii et al., 2006). Applications of PNVCL as a biomedical material either in stabilization of proteases or as a carrier and drug delivery agent, have been previ-

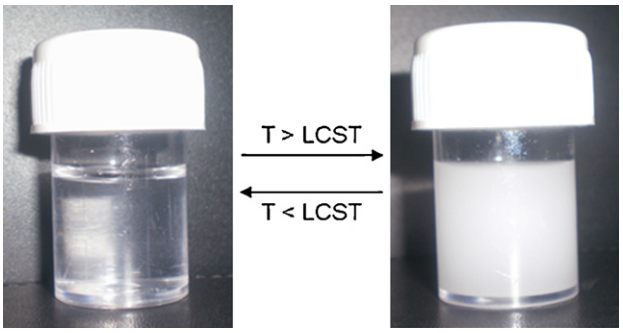

Fig. 2. Effect of the temperature in the appearance of an aqueous solution (5 g/L) of PNVCL.

ously published in the literature (Schmidt, 2007; Kawaguchi, 1999; Müller-Schulte and Schimitz-Rode, 2006). The first work with this monomer had been published in 1968. In this work, Solomon et al. (1968) studied the kinetic of the bulk polymerization of NVCL. In 1969, Solomon et al. (1969) studied the kinetic of the polymerization of NVCL in toluene. Nowadays, it can be observed an increasing interest in the thermoresponsive behaviour of PNVCL, which originates largely from its biocompatibility and low toxicity (Konak et al., 2007; Chee et al., 2006). Vihola et al. (2005) studied the citotoxicity of PNVCL of various molecular weights and the results revealed that the polymers with molecular weights ranging from 3.30×10^5 g mol^{-1} to 1.50×10^6 g mol^{-1} were well tolerated. However, for biomedical *in vivo* applications, the synthesis of PNVCL with lower molecular weight is interesting (Inoue et al., 1997). In Fig. 2 we can observe the variation in the appearance of a 5 g/L aqueous solution of PNVCL with the increase of the temperature at values above the LCST.

Below the transition temperature, the PNVCL aqueous solution is transparent while the increase of the temperature above the LCST is accompanied by the higher turbididity (Fig. 2).

2.2. pH-sensitive polymers

2.2.1. The pH-sensitive property

pH-sensitive polymers are materials that vary their dimensions with the changes in the pH of the surrounding media. These materials will swell or collapse depending on the pH of their environment. This behaviour is attributed to the presence of certain functional groups in the polymer chains. There are two kinds of pH sensitive materials: one which have acidic group (–COOH, –SO_3H) and swell in basic pH (Fig. 3), for example poly(acrylic acid) and another one which have basic groups (–NH_2) and swell in acidic pH, as chitosan. The mechanism of response is same for both, just the stimuli vary.

Microgel particles from crosslinked acid segments, for example, dispersed in water are sensitive to changes in pH of the aqueous suspension. Increasing the pH causes changes (increase) in the ionization degree of the ionizable polymer. The osmotic pressure due to the increased ionization will result in swelling of the particles. The resulting response can be read out as changes of the solution turbidity. Thus, this responsive behaviour can be described as a sequence of steps: (i) ionization of the polymer chains; (ii) swelling of the microgel - changes in the material properties; and finally (iii) changing the turbidity of the dispersion–the response. Obviously, all changes in the microgel particles were related and resulted in changes of a range of properties and change in turbidity is just an example. Examples of pH-responsive polymers are macromolecules with acidic or basic functional groups (carboxylic, phosphoric, or amino functional groups). Changes in pH result in a shift of chemical equilibrium and in a change of the ionization degree of the polymer chains (Rühe et al., 2004).

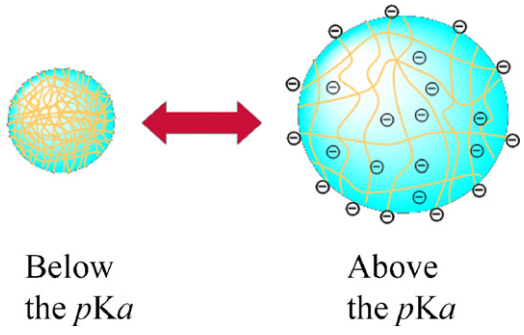

Fig. 3. Schematic illustration of the pH-responsive behaviour (below and above the *p*K*a*) of a particulate system constituted by a polymer with carboxilic acid segments.

pH-sensitive hydrogels are particularly useful, for example, in the delivery of drugs or peptides to a specific site in the gastrointestinal tract or in response to small changes in the pH of blood stream or tissues in a pathological situation, such as a clot or cancer (Alvarez-Lorenzo and Concheiro, 2002). The range of physiological pH is from 1.2 to 7.4, and different body part may have a special pH surroundings. For example, the stomach possesses pH 1.2 while the pH of the intestine is 7.4. Moreover, it is known that the extracellular pH of tumours (6.8–6.9) is more acidic than both tumour intracellular pH (7.2) and normal extracellular tissues (7.4) (Yan et al., 2007). In this sense, pH sensitive polymers seem to have high potential application value as drug delivery agents (Liu et al., 2009a,b). Moreover, it is found that incorporation of a small amount of pH-sensitive ionisable groups, such as carboxyl and amino groups, into thermoresponsive polymers can offer a combination of different stimuli-responsive properties.

2.3. Combination of temperature and pH-responsive properties

There is growing interest in obtaining materials whose aqueous solutions and swelling properties can abruptly and reversibly change in response to simultaneous pH and temperature changes. The pH- and temperature-sensitive polymer sequences with their dual function are an example of systems that can respond to a combination of external stimuli. The combination of temperature and pH-sensitive properties in the same system has been target of greatest scientific and technological interest, primarily because these variables can be changed in typical biological and chemical systems. By substituting either weakly ionisable cationic or anionic pendant groups onto a polymer backbone, the polymer can be tuned to respond to either an increase or decrease in the pH. The ability of the polymer to respond both to temperature and pH offers an additional control over the polymer phase behaviour (Dimitrov et al., 2007). Moreover, the presence of groups from pH-responsive segments modifies the hydrophilicity/hydrophobicity balance, leading to LCST changes. For example, the LCSTs of copolymers increase rapidly with increasing acrylic acid comonomer contents at all pH ranges due to its high hydrophibicity (Khan, 2008).

First of all, it is important to determine the extent and to clarify the mechanism of the combined influence of pH and temperature on the properties of aqueous copolymers solutions and hydrogels. As it is well known, parameters such as LCST, can be modulated by the incorporation of different amounts of charged monomers. Below the LCST both temperature and pH-responsive segments are hydrophilic. Above the LCST, the thermoresponsive polymer chains will become hydrophobic and thus the system can easily form aggregates, micelles or particles with thermosensitive core and pH-sensitive shells, depending on the parameters of the synthesis and the kind of material obtained. Fig. 4 illustrates the behaviour of a temperature and pH-responsive system. Recently, *N*-isopropylacrylamide has been copolymerised with different

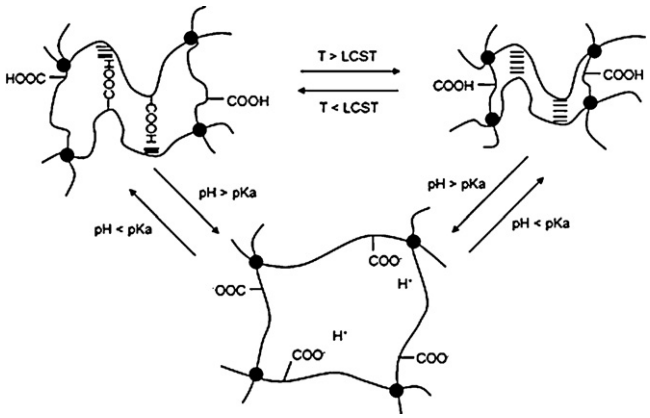

Fig. 4. Schematic illustration phase transition of thermo-sensitive copolymer with acrylic segments.

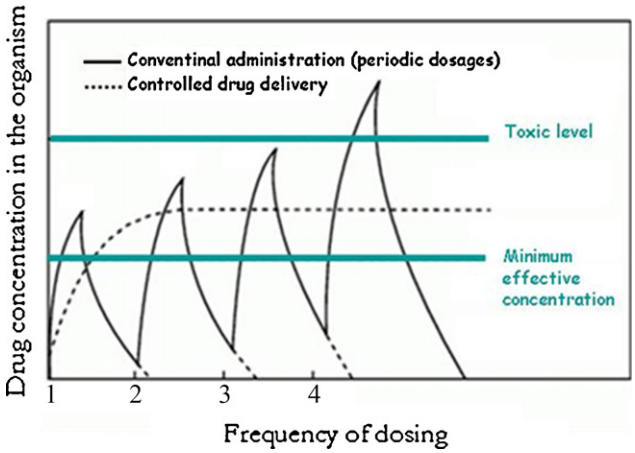

Fig. 5. A schematically illustration of drug administration routes.

monomers in order to modify the LCST of aqueous copolymers solutions as well their sensitivity (enthalpy factor and transition width). When monomers, which originate thermoresponsive polymers, are polymerised with hydrophilic co-monomers, the LCST of the resulting polymer will shift to higher temperatures while copolymerization with hydrophobic co-monomers will lead to more hydrophobic polymers and thus the LCST will increase. Therefore, copolymerization with monomers containing ionisable groups is an interesting approach because it makes possible to adjust the thermosensitivity for temperature values close to the human body temperature.

The structure conformations change with the variation of both temperature and pH. Most of the polymers that respond to more than one stimulus, in particular to temperature and pH, are prepared by random copolymerisations. It is important to know why the hydrophobic effects or hydrogen bonding are the main factors responsible for the LCST behaviour of the thermoresponsive-based random copolymers. In this sense, Salgado-Rodríguez et al. (2004) synthesized a series of random NIPAam-based copolymers of similar composition, which could be split into two groups. One group had a controlled number of units with a free acid group while the other group was methoxy-protected. These experiments were performed in order to clarify the effect of possible hydrogen bonding on the LCST behaviour. These authors observed that in case of the most studied system, poly(NIPAam-*co*-AA), the carboxylic groups can form hydrogen bonds with the amide groups in the PNIPAam structure and these bounds lead to additional polymer-polymer interaction, making phase separation by heating a less endothermic process than in pure PNIPAam since fewer sites will be available for the water to bind to the NIPAam units. However, this is true if all acid units are ionized, which will depend on the *pK*a constant of the acid. Thus, we can observe that the hydrogen-bonding interactions represent an important role in these systems and as a result two types of pH transitions are expected. In the first pH range when the majority of the units are not ionized (low pH); the LCST will decrease with an increasing number of acid units. A second pH range may also be observed when the majority of the units are ionized (high pH), so that, the LCST will increase with an increasing number of acid units.

Bulmus et al. (2000) synthesized copolymers of PNIPAam and Acrylic acid (AA) with low molecular weight. The copolymers displayed both temperature and pH-sensitivity over a wide and useful range of pH and temperatures. The copolymers were bound to a genetically engineered Streptavidin (SAv) at a site specifically designed to resemble the biotin-binding site of the natural SAv. The authors found that: (1) the decrease of the pH, which causes the polymer collapse results in blocking biotin binding as well; (2) the increase of the pH leads to the swelling of the polymer and it allows biotin binding, and (3) lowering the pH once again causes the collapse of the polymer and results in partial ejection of the biotin. These actions are relevant to the study of this system as separation agents, biosensors, diagnostic and targeted delivery of drugs or chemical agents. The effects of pH and temperature on the biotin binding and its release can be useful in a variety of separations and diagnostic applications and may also be useful for pH-triggered drug delivery at specific sites in human body where the pH is significantly below 7.0 such as the stomach, vagina, salivary glands and within intracellular vesicles such as endosomes and lysosomes.

2.4. Stimuli-responsive polymers in nanobiomedicine as controlled drug delivery systems

Nanomedicine is defined as the application of nanobiotechnology to medicine. Nanobiotechnology is also making important contributions to personalized medicine through refinement of various technologies used for diagnostics and therapeutics as well as interaction among these. Polymeric nanomaterials and devices provide unique opportunities to advance medicine, especially in controlled drug delivery strategies.

It is known that the drug concentration levels in the blood plasma depend on the quantity of drug released from the device because drug absorption is determined by its solubility in tissues and availability of local blood flow in tissue. Moreover, there are three general regions or sites where drugs act in the body, i.e., they may act away from cells, in the circulation or in tissues spaces and within the cells. The major routes that are used to deliver drugs to the body include oral (gastric, enteric, and colonic), injections, implants, transdermal, mucosal (ophthalmic, nasal, vaginal, anal, buccal, and sub-lingual) (Hoffman et al., 2007). The conventional administration routes and the controlled drug delivery are schematically illustrated in Fig. 5. Conventionally, the administration routes of drugs includes periodic dosages (A) while in controlled release, polymer systems deliver drugs in the optimum dosage for long periods (B), thus increasing the efficacy of the drug, maximizing patient compliance and enhancing the ability to use highly toxic, poorly soluble or relatively unstable drugs. Even if the drug concentration in plasma were to remain reasonably constant, small short-term fluctuation would always be seen due to factors such as physical activity, emotional stimulation (stress), eating and sleeping, etc.

The main advantages of the use of controlled drug delivery systems include: (1) sustained constant concentration of therapeutically active compounds in the blood with minimum fluctuations; (2) predictable and reproducible release rates over a long period of time; (3) protection of bioactive compounds having a very short half-life; (4) elimination of side-effects, waste of drug and frequent dosing; (5) optimized therapy and better patient compliance; and (6) solution of the drug stability problem.

Controlled drug delivery as well as many other applications using polymers requires complete control over the architecture of the polymer microstructure, especially when synthesizing materials such as microspheres, macrogels and nanogels (Liu et al., 2007). Hydrogels are three-dimensional high-molecular weight networks composed of a polymer backbone, water and a crosslinking agent, which swell in aqueous medium imbibing water into the network structure. The administration routes of hydrogel-based formulations include transdermal, oral, nasal or parenteral. Drug delivery systems can be classified according to the mechanism controlling the drug release: (1) diffusion-controlled systems, including membrane systems (reservoir) and monolithic systems (matrix); (2) chemically controlled systems, including biodegradable systems and Pendent chain systems; (3) solvent-activated systems, including osmotically controlled systems and swelling-controlled systems and finally (4) modulated-release systems. Most drug delivery devices act by a combination mechanisms (Bajpai et al., 2008).

Nanoscale polymeric gels or nanogels can be used as drug delivery vehicles to develop highly selective and effective therapeutic and diagnostic modalities. These nanomaterials can travel through the blood stream without sedimentation or blockage of the microvasculature and can circulate in the body and penetrate tissues such as tumours. In addition, they can be taken up by the cells through natural means such as endocytosis. Nanoparticles have already been used to deliver drugs to target sites for cancer therapeutics (Gref et al., 1994) or deliver imaging agents for cancer diagnostics (Lemarchand et al., 2004). These vehicles can be engineered to recognize biophysical characteristics that are unique to the target cells and therefore minimize drug loss and toxicity associated with delivery to non-desired tissues (Chung et al., 2007).

Stimuli-responsive polymeric hydrogels do not dissolve in water at physiological temperature and pH but exhibit a phase transition in response to change in external conditions such as pH, ionic strength, temperature and electric currents are known as "stimuli-responsive" or "smart" gels. Recently, these materials have attracted increasing attention in many fields of academic as well as industrial research due to their wide applications (Okubo and Nakagawa, 1994). Their three-dimensional hydrophilic networks can retain a large amount of water that not only contributes to their good blood compatibility but also maintains a certain degree of structural integrity and elasticity (Li et al., 2006).

As it was mentioned before, in thermally-sensitive polymers, the balance between segment–segment interactions and segment–solvent intermolecular interactions can be shifted by temperature changes. The polymer–solvent interactions decrease upon increasing temperature above the LCST due to the dominating effect of hydrophobic interactions at an elevated temperature. Concerning thermoresponsive micro/nanogels, water diffuses into the hydrogel at temperature below the LCST and, consequently, the hydrogel swells. Diffusion involves migration of water into pre-existing or dynamically formed spaces among hydrogel chains. Swelling of hydrogel involves a larger-scale segmental motion, resulting, ultimately, in an increase of the separation distance among hydrogel chains (Berens and Hopfenberg, 1989). Above the LCST, there are strong intermolecular and/or polymer–polymer interactions, such as hydrogen bonds and hydrophobic interactions, which remain in a decrease in the distance among hydrogel chains and, consequently, in the collapsed state of the gels (Yoshida et al., 1994). In the same way, this decrease of the gels volume leads to a decrease in charge density on the surface of the gel particles. As result, the zeta potential of the thermoresponsive gels will change as a function of the temperature. However, the value of the zeta potential depends on the ions presented on the surface of the thermally-sensitive gels.

Rahimi et al. (2008) developed temperature sensitive nanoparticles based on poly(*N*-isopropylacrylamide-co-acrylamide-coallylamine) (NIPAam-AAm-AH) via free radical polymerization. The use of AAm and AH as comonomers aimed the increase of the LCST, compared with the NIPAam homopolymer and to provide the functionalization with amine groups. Transmission electron microscopy (TEM) and laser scattering technology revealed the sizes of the nanoparticles, which was inversely proportional to the surfactant concentrations. The chemical composition of the nanoparticles was determined with Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) and confirmed the presence of functional groups of each monomer. The nanoparticles did not show significant cytotoxicity activity against human fibroblast cells. Finally, doxorubicin (DOX) was used in order to investigate the drug release profiles of the NIPAam-AAm-AH nanoparticles at different temperatures and the results indicated that higher amounts of DOX was released at 41 °C compared to that of 37 °C and 4 °C.

While nanogels based on temperature-responsive polymers are generally designed to be altered by external stimuli, pH-responsive polymers can respond to variations in the intracellular or tissue environment. The pH-dependent swelling behaviour of a nanogel can be useful not only for drug release, but also for its loading. The permeability of pH-responsive nanogels increases when they swell, allowing either the incorporation of molecules/nanoparticles or the release of previously trapped substances (Deka et al., 2010). Thus, several pH-responsive polymers have been widely used as a controlled drug delivery system. Dandekar et al. (2009) incorporated curcumin, a natural anti-cancer agent, for the treatment of colon cancer in pH-sensitive nanoparticles by emulsion-evaporation technique, aiming to enhance the bioavailability and, consequently, the required dose of this active agent. Eudragit® S100 was used in order to aid targeting due to the fact that this polymer dissolves at colonic pH, resulting in selective colonic release of the entrapped drug. The effect of various process parameters on the particle size distribution and encapsulation efficiency was previously evaluated. The nanoparticles were characterized in terms of particle size, drug content, DSC studies, and particle morphology. The results revealed that spherical particles were obtained with encapsulation efficiency of the curcumin of 72%. MTT assay in HT-29 cell line demonstrated higher inhibition of the cancerous cells by the nanoparticles, as compared to curcumin alone. The author attributed this result to the effect of particles size on the uptake, resulting in reduction of overall dose requirement.

However, nowadays the most hydrogels for biomedical applications are those sensitive to temperature and/or pH. Polymer–polymer and polymer–solvent interactions show an abrupt readjustment in small ranges of pH or temperature (Bajpai et al., 2008). Stimuli-responsive sensitive polymer gels offer potential economic alternatives to conventional separation processes for industrial applications (Kucuk and Kuyulu, 2005). Controlled permeability variations of responsive gels have also been used to achieve a variety of size- or charge-selective separations. In order to study the effect of the addition of acid molecules on the LCST of thermally-sensitive polymers, acrylic acid is the most commonly co-monomer employed. However, this ionisable monomer restricts the thermosensitivity of the copolymer to a limited range of pH range because at high pH the carboxylic groups are ionized

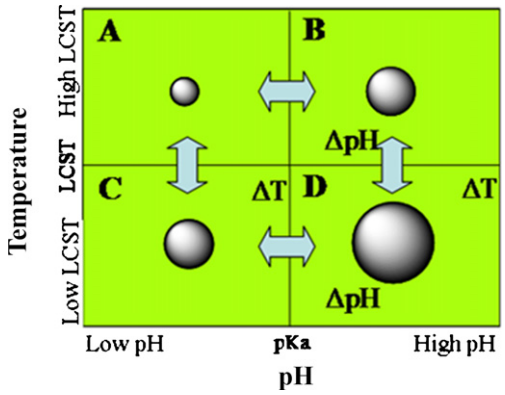

Fig. 6. Phase variations of the equilibrium swelling states of the temperature and pH-sensitive nanogels (adapted from Asoh et al., 2006).

and the polymer becomes rather hydrophilic, thus decreasing its thermosensitivity. This fact can be attributed to the combined ability of the residual ionic monomer to break the chain sequences of the thermoresponsive polymer into short uncooperative segments. Asoh et al. (2006) defined four main transition stages for the swelling of systems comprising thermo- and pH-sensitive polymers such as hydrogels (Fig. 6).

We can see in Fig. 6 that the first stage (A) (temperature above the LCST and pH below the *pK*a of the acid monomer) is a complete-shrinking state; the thermoresponsive polymer networks are dehydrated above the LCST, whereas the segments of acid monomer are non-ionized. In contrast, there is a stage (D) (temperature below the LCST and pH above the *pK*a of the monomer acid) where the thermoresponsive polymer networks are hydrated and in a full-swelling state because the segments of the acid monomer are sufficiently ionized to hydrate above the *pK*a. In addition, there are two intermediate states: in the first one (B), the thermoresponsive polymer is dehydrated above the LCST but the pH-sensitive chains are hydrophilic due to the ionized state of carboxyl groups while in the second one (C), the thermoresponsive polymer is hydrated below the LCST but the pH-sensitive chains are hydrophobic due to the non-ionized state of the carboxyl groups.

3. Combination of magnetic and stimuli-responsive properties

Nanobiomagnetism is the intersection of nanomagnetism and medicine that focuses on biological systems and/or processes (Leslie-Pelechy et al., 2006). Some hydrogels and nanoparticles have been developed to combine field and stimuli-responsive mechanisms within a single polymer system (Wong et al., 2008). Magnetic colloids, also known as a ferrofluid, are stable dispersions of nanometer-sized magnetic particles in a suitable carrier solvent (Häfeli et al., 1997). The liquid carrier can be polar or nonpolar. Since the last decades, when these materials were initially synthesized, their technological applications did not stop to increase (Scherer and Neto, 2005).

There are several techniques to obtain magnetic materials such as magnetite as a microcrystaline powder. Among theses techniques, physical methods are known to form nanoparticles without any control in sizes. Thus, researches have been performed in the sense to obtain good control of the magnetic properties, size distribution and chemical composition of the nanoparticles (Elaissari, 2003).

In order to avoid agglomeration, the magnetic particles must be coated with a shell of an appropriate material. According to the coating, the ferrofluids can be classified into two main groups: surfacted, if the coating is a surfactant molecule, and ionic (cationic or anionic), if it is an electric shell. In recent years, the design and development of magnetic nanoparticles have been the focus of intense fundamental and applied research, with special emphasis on their unusual properties and promising new possibilities in the biomedical area (Lacava et al., 2004). Due to its biocompatibility this material can find applications in biomedicine, such as: (a) cellular therapy in cell labeling, separation and purification (Wilhelm and Gazeau, 2008); (b) protein immobilization (Liu et al., 2004); (c) contrasting enhancement in magnetic resonance imaging (MRI) (Hong et al., 2008); (d) localized therapeutic hyperthermia (Hiergeist et al., 1999); (e) biosensors (Astalan et al., 2004), etc.

3.1. Magnetic nanoparticles for in vitro applications

For many years, polymer-based particles have been used in the biomedical field such as *in vitro* biomedical diagnostic (i.e. immunoassay tests, cells separation and analysis, nucleic acids concentration) or as solid-phase supports for immobilization of biomolecules (Hatakeyama et al., 1998). For these *in vitro* diagnosis applications, the phase separation is a crucial step and it is usually achieved by centrifugation, precipitation or filtration. In this sense, magnetic particles can offer important advantages over classical polymer-based particles due to the rapid and easy separation upon applying an external magnetic field (Elaissari, 2009). The pioneer work in this field has been done by Ugelstad et al. (1993) who reported the preparation of magnetic microspheres with narrow size distribution and also described their utilization as a support for biomolecules. The dispersions of magnetic nanoparticles are attractive in separation applications as they offer high surface area and can be functionalized to selectively discriminate between different molecular or cellular species. Kondo et al. (1994) prepared poly(styrene/*N*-isopropylacrylamide/methacrylic acid) latex particles containing magnetite by a two-step emulsifier-free emulsion polymerization. The author investigated the effect of the magnetite to monomer weight ration on the colloidal properties of the particles and they verified that the hydrodinamic diameter decreased with increasing the weight ratio. The effect of thermoflocculation of the magnetic latex particles on their separation properties was studied and it was observed that the separation time of the magnetic particles with higher magnetite content was shorter. In addiction, they studied the immobilization of bovine serum albumin (BSA) on the magnetic latex particles and the purification of anti-BSA antibodies it was possible to verify that these antibodies were successfully purified from the antiserum by the BSA-immobilized magnetic latex particles.

In vitro applications of the MNPs aim the detection (magnetorelaxometry assays) or the separation (magnetic cell separation) (Safarik and Safarikova, 1999) of biological species like proteins (Burns et al., 1965; Kouassi and Irudayaraj, 2008), oligonucleotides (Horák et al., 2001; Krizová et al., 2006) or cells. Magnetic cell separation can be done using batch or flow processes, depending on the specific application. In batch processing, the magnetic beads and the material to be analyzed are mixed. It is necessary to know the reaction kinetics to determine the time necessary for a sufficient amount of binding. A magnet is used to separate the magnetically targeted cells from the non-targeted cells, as shown in Fig. 7. The most commonly used materials for cell separations are micron-sized polymer beads into which a magnetic material has been embedded.

Another potential application of magnetic particles is the real-time detection and monitoring of bacterial, viral and other pathogenic contamination. Integrated structures utilizing nanolithography can perform sorting and quantitative analysis in a single device. The main advantages of these materials include the low interference, low background signal, no requirement of pretreatment, and the fact that they can be small enough to be portable.

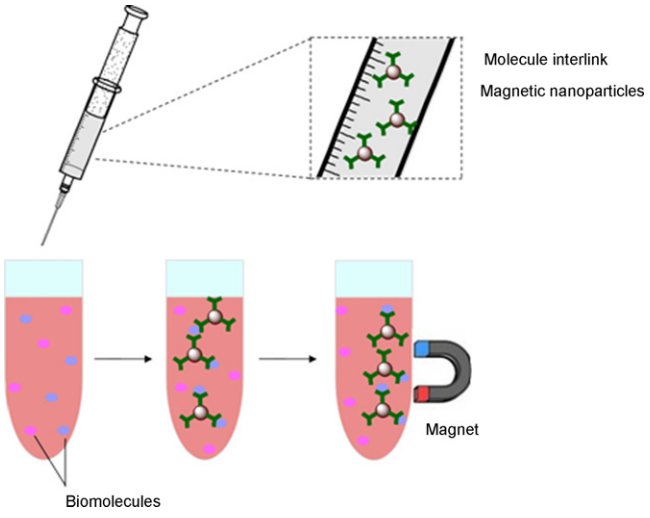

Fig. 7. A schematic illustration of the magnetic cell separation process.

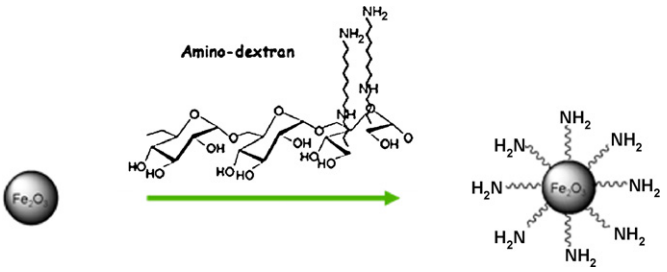

Fig. 8. Schematic illustration of the amino-dextran coated magnetic nanoparticles.

Moreover, magnetic nanoparticles have the potential to detect more than one molecule at the same time.

In the case of the diagnosis of infectious diseases, in order to obtain an early diagnosis, the detection of very low quantities of pathogen within a large sample volume is required. Thus, the diagnosis should be specific and sensitive. Nucleic acid concentration is an efficient way to gain sensitivity. Today, total nucleic acid extraction from crude biological samples can easily be performed using various methods (Boom et al., 1990; Elaissari et al., 1999). The literature describes the development of some fast and automated nucleic acid extraction processes using silica magnetic beads (Pinto et al., 2007). However, some biological samples as sputum can contain very high amounts of non-specific RNA/DNA which can strongly affect the final analysis. In this context, an efficient capture (i.e. specific and at low target concentration) is required and the extraction of the target nucleic acid can be performed with colloidal particles, in general on magnetic carriers.

Blood purification using magnetic carriers has been also used to treat autoimmune and inflammatory diseases. T4 and T8 cells in HIV-infected patients have been isolated using magnetic separation, thus allowing to study of the effect of different drugs on specific types of cells. Isolation of rare cell populations such as endothelial cells in blood down to 10 cells/mL has been accomplished (Ugelstad et al., 1998).

3.2. Magnetic nanoparticles for in vivo applications

The large surface-to-volume ratio of MNPs provides abundant chemically active sites for biomolecule conjugation, allowing delicate design and engineering of these MNPs for intended functions such as long-circulating in the blood stream, target specificity to lesion tissue, optical detectability, and therapeutic delivery (Fang and Zhang, 2009). Other biomedical applications of magnetic nanoparticles include drug delivery and magnetic resonance imaging. Fe^{3+} ions are the most wanted in formation of intracellular and macromolecular biologically active or magnetic resonance contrast materials because of their non-toxicity and their existence in nature in many tissues. For these *in vivo* applications, the magnetic nanoparticles needs to exhibit high magnetic saturation. Biocompatible and non-toxic magnetic nanoparticles, well-dispersed in the solvent carrier, have a lot of potential for *in vivo* applications in which superparamagnetic particles are of interest because they do not retain any residual magnetism after removal of a magnetic field (Nagy et al., 2008).

Coating of colloidal particles with a layer of a different material in the nanometer scale is an interesting route in order to modify the surface properties. Due to their liquid properties and sensitivity to an applied magnetic field, such materials can deliver an anticancer drug to a targeted region of the body, such as a tumour (Zablotskaya et al., 2009). In this sense, we have been interested in the synthesis of aqueous iron oxide colloids bearing biologically active organic ligands. For the *in vivo* applications dextran coating agent has been used mostly because of its biocompatibility (Pradhan et al., 2007). Dextran is a component of plasma substitute and has been used in drug transportation for many years (Yu et al., 2008). The association of a biocompatible natural polymer such as dextran with functional compounds for adsorption of some molecules is an interesting task as shown by Mouaziz et al. (2009). In this work, the authors reported not only the preparation of amino-dextran containing submicrometer magnetic particles but they also studied the colloidal properties of the final dispersion as well they evaluated the use of these particles in nucleic acids extraction. The schematic illustration of the interaction between the iron oxide particles and amino-dextran molecules is shown in details in Fig. 8. Yu et al. (2008) studied the biocompatibility of dextran-coated magnetic fluids as well as the fluid-body interaction *in vivo*. The acute toxicity and irritation of the magnetic fluid injected into mice subcutaneous tissues were examined. The authors achieved a lethal dosage only when the magnetic fluid concentration was equivalent to 4409.61 ± 514.93 mg/kg of the animal. When injected a 30 mg/0.3 mL dextran-aqueous fluid, activities of glutamicoxalacetictransaminase (AST) and glutamicpyruvictransaminase (ALT) and cell number of mice blood did not change statistically. The infiltration of the magnetic fluid in subcutaneous tissues was observed and these phenomena almost disappeared 72 h later. With these results, they could conclude that the dextran-magnetic fluid was tolerable, safe, and biocompatible. It was also evaluated the biocompatibility of the magnetic particles using enzymatic nucleic acid amplification reaction. The capture of plasmid DNA was pointed to be effective. Moreover, the amplification reactions showed non-inhibition of enzymes activities in the investigated conditions.

Another promising biologically active ligand is oleic acid. Zablotskaya et al. (2009) studied the interaction of iron oxide with oleic acid. The physico-chemical properties, such as magnetization, magnetite concentration and particle diameter were investigated. Magnetic nanoparticles with 11 nm in diameter were obtained. The authors also performed *in vitro* tests and they observed that the iron oxide-to-oleic acid molar ratio, varied from 2.6:1 to 4.7:1, had some effect against human fibrosarcoma and mouse hepatoma cells. The authors performed tests *in vitro* using crystal violet coloration in order to evaluate the effect of iron oxide concentration on the nitric oxide (NO) production and they verified that the magnetic fluid with higher content of iron oxide possessed the higher NO induction ability. NO is known for its effect on a diverse array of physiologic and pathologic processes (Bredt and Synder, 1994). Moreover, this gas radical, produced by many cells in the human

body, not only controls important functions in tumour progression, but may have a major influence on the outcome of cancer therapies, particularly those that are mediated by increased generation of reactive oxygen species (oxidative stress) (Hirst and Flitney, 1997). A number of studies have described the complex relationships between iron and NO (Gow and Stamler, 1998; Kagan et al., 2001). It is known that the NO production by macrophages depends on the inducible isoform of NO synthase (iNOS) (Nathan and Xie, 1994), while iron is required for dimerization and activation of iNOS (Stuehr, 1999). However, it still remains unclear whether iron increases or decreases NO production *in vivo*. Liver is the main organ for iron storage (Papanastasiou et al., 2000) and the Kupffer cells, which are macrophages located in the liver lining the walls of the sinusoids that form part of the reticuloendothelial system (RES), show the ability of expressing iNOS in the presence of excess iron (Hida et al., 2003). Thus, as suggested by Hida et al. (2003), the increase in the iron concentration appears to stimulate the NO production by the Kupffer cells cytokines in rats. Finally, the antitumour action of the iron oxide nanoparticles was demonstrated *in vivo* by Zablotskaya et al. (2009) using sarcoma mouse model.

For many controlled drug delivery applications, zero order release of therapeutics over prolonged period of time is the goal. In this context, the use of high frequency alternating magnetic fields (AMF) to actuate magnetic particles is also rapidly emerging as an important research area in externally controlled drug delivery systems (Satarkar and Hilt, 2008). The first use of external magnetic fields to achieve pulsatile release from polymer composites was demonstrated by Kost et al. (1987). These authors observed an externally controlled insulin release from magnetic composite of ethylene vinyl acetate by application of low frequency oscillating magnetic field. More recently, Paoli et al. (2006) demonstrated enhancement in dextran release by application of low frequency oscillating magnetic field to magnetic nanocomposites of collagen. It is known that the low frequency oscillating magnetic field application relies on interactions between magnetic particles and resultant mechanical deformation of the gel to squeeze out the drug (Liu et al., 2008). It was recently observed that pulsatile release from magnetic nanocomposites of gelatin hydrogels was obtained by application of high frequency (50–100 kHz) magnetic field (Hu et al., 2006), but the applications of AMF in drug delivery systems still remain unexplored.

Nowadays, cancer therapy is an important and active field of biomedical research given its relevance in the design of new anti-tumour devices. In this context, polymers-coated magnetic nanoparticles have been intensively studied and can find innumerous applications in cancer therapy in order to increase the specificity of anticancer drugs in cancer cells (Scherer and Neto, 2005). In one of these applications, the idea is that polymeric magnetic nanoparticles bounded drug are firstly injected in a cancer tumour and they are kept there during some time (approximately 15 min) by a suitably focused magnetic field, with very intense action. In this case, the amount of drug necessary is much less than what would be necessary if it were dispersed in the whole body. When the magnetic field is turned off one percentage of the drug will disperse in the body but, since the total amount is very small, there will be practically no side effects.

3.3. Stimuli-responsive gels encapsulated magnetic nanoparticles

There is a considerable interest in preparation of particles, which can be manipulated in different systems by external stimuli such as thermal, electric or magnetic field. In this context, some hydrogels and nanoparticles have been developed to combine field- and stimuli responsive mechanisms within a single polymer system. Incorporation of magnetic iron oxides in polymeric particles can be an interesting route for preparation of hybrid particles, which can provide this interesting feature.

The development of stimuli-responsive gels is often complicated by the fact that structural changes (such as volume changes), are kinetically restricted by relatively slow swelling and deswelling. Therefore, optimized design of materials for different purposes is a challenge. In order to accelerate the response rate and to achieve agitation without contact, new mechanisms, involving magnetic and electric fields could be applied and appears to be very promising. Moreover, for any technical application it is of great importance to have a quick and reliable control system.

Electric and magnetic fields are the most practical stimuli with respect to signal control. To provide an enhanced influence of external fields on the gel properties, it is necessary to combine solid-like and fluid-like behaviour. Therefore, new colloidal solutions termed "complex fluids" based on ferrofluids and polymer gels have been investigated. Since polymer gels contain a substantial amount of liquid as a swelling agent, it is possible to design field-sensitive gels by using a polymer network swollen in a complex fluid. The colloidal particles incorporated within the gel, which are characterized by strong adsorptive interactions between solid particles and polymer chains allow fast response to an external field. These field-sensitive gels can be used to construct new types of sensors, microengines, biomimetic energy-transducing devices and controlled delivery systems.

In general, those systems are characterized by particle size and particle size distribution, scanning electron microscopy (SEM) or transmission electron microscopy (TEM), X-ray diffractometry, density of the charges on the surface of the nanoparticles, thermogravimetric analyses, fourier transform infrared (FTIR), magnetic properties, dynamic light scattering (DLS), among other techniques.

In the search for a more generally applicable drug targeted delivery method for cancer therapy, magnetically controlled targeted chemotherapy has been proposed (Gupta and Hung, 1994). The main objectives of this method are to reduce the amount of systemic distribution of the cytotoxic drug, consequently, eliminating the associated side effects, and to reduce the dosage required by more efficient, localized targeting of the drug (Chunfu et al., 2004).

When the particles are administered intravenously, external, high-gradient magnetic fields are used to concentrate the complex at a specific target site within the body. The process of drug localization is based on the competition between forces exerted on the particles by the blood compartment and magnetic forces generated from the magnet, i.e., the applied field. When the magnetic forces exceed the linear blood flow rates in arteries (10 cm/s) or capillaries (0.05 cm/s), the magnetic particles are retained at the target site and may be internalized by the endothelial cells of the targeted tissue. The drug can be released from the drug/carrier system either via enzymatic activity or via changes in physiological conditions such as pH, osmolality, temperature. Once the drug is released it can be taken up by the tumour cells (Alexiou et al., 2000).

pH and temperature-sensitive polymer sequences with their dual function are an example of systems that can respond to a combination of external stimuli in different forms. Talking about the pH-sensitive behaviour, acrylic acid is one of the most studied monomer in literature. For poly(acrylic acid), in acidic pH, the units of the carboxylic monomer are in a non-ionized state. In this state, the nanoparticles are stable keeping the drug into the particles. On the other hand, in basic pH, the carboxyl groups are in an ionized state. Repulsion forces make the swelling of the particles, promoting the release of the drug and magnetic nanoparticles. Concerning the effect of temperature on thermally-sensitive magnetic systems, at temperature values below the LCST the particles are stable keeping the drug. Above the LCST, the presence of strong attraction forces between the polymeric chains leads to the for-

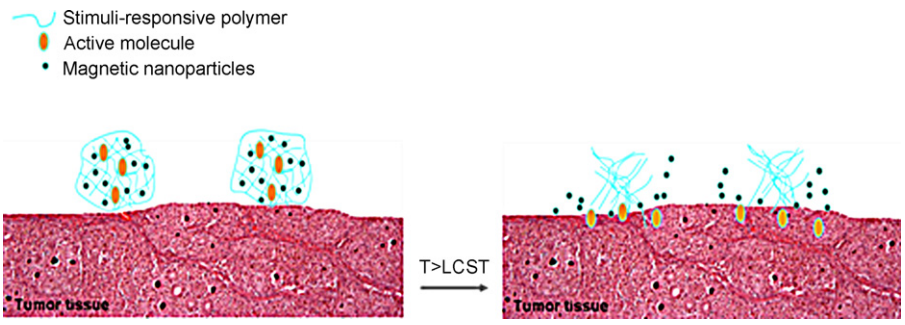

Fig. 9. Schematic illustration of the thermally-sensitive polymeric magnetic nanoparticles behaviour in temperatures below and above the LCST.

mation of aggregates expelling the active agent inside the polymer particles (Fig. 9).

The first application of thermally-sensitive magnetic particles for *in vivo* applications was described by Chen and Su (2001), who synthesized core-shell-type latex particles composed of styrene, *N*-isopropylacrylamide (NIPAam) and *N*-acryloxysuccinimide (NAS) by surfactant-free emulsion polymerisation. The latex particles revealed thermo-flocculation behaviour due to the presence of thermally-sensitive segments composed by NIPAam. The authors investigated the immobilization of the enzyme α-chymotrypsin through covalent bonding with the reactive ester groups from NAS. Magnetite particles were prepared and incorporated to the thermally-sensitive latex particles during the polymerisation procedure in order to enhance the sedimentation rate upon the action of a magnetic field, which was reported to be 6 times faster. The average hydrodynamic diameters were found to be 250 nm and enzyme activity investigation revealed a tendency to increase of this parameter with temperature. On the other hand, the latex aggregation at higher temperature tends to decrease enzyme activity. Finally, the immobilization system showed a gain of 54.3% in enzymatic activity, compared with previous studies.

Magnetic carriers were first used to target cytotoxic drugs (doxorubicin) to sarcoma tumours implanted in rat tails (Widder et al., 1983) as well as to target cytotoxic drugs to brain tumours (Pulfer et al., 1999). Gaharwar et al. (2009) prepared surface-modified nanoparticles with a core-shell structure. The core consisted of magnetic nanoparticles and the shell was composed of biocompatible and biodegradable thermoresponsive hydroxypropyl cellulose. A coupling agent was used in order to bind covalently the core to the shell. The surface modification of the magnetic nanoparticles was confirmed by X-ray diffractometry and the superparamagnetic behaviour was observed by magnetization measurements. The thermoresponsive behaviour of the particles was observed with a lower critical solution temperature of 41 °C, which is the same temperature at which hydroxypropyl cellulose undergoes a coil-to-globule transition.

Chunfu et al. (2004) prepared a human serum albumin (HSA)-coated magnetic nanoparticle as a radioisotope carrier labeled with ^{188}Re and explored the optimal labeling conditions with ^{188}Re, which is a precondition for further studying the targeting behaviour of the particles *in vivo*. First of all, Fe_3O_4 nanoparticles were prepared by adopting a partial reduction method. After, the HSA-coated magnetic nanoparticles were prepared using a microemulsion approach. Finally, the magnetic particles were labeled with ^{188}Re. They concluded that the microemulsion method can be used for the preparation of HSA-coated magnetic particles with diameters of about 200 nm. Moreover, the particles can be labeled with ^{188}Re for the purpose of regional target therapy. The optimum labeling conditions include: $SnCl_2$-$2H_2O$ (8 mg/mL), citric acid (20 mg/mL), vitamin C (8 mg/mL), reaction volume of 500 mL and reaction time 3 h, with a labeling efficiency of about 90%. The labeled particles are stable up to 72 h in BSA, suitable for study *in vivo* delivery and can be used for magnetically targeted radiotherapy.

On the other hand, Schmidt (2005) has developed a new strategy for thermoresponsive stabilization of magnetic particles by the use of a well-defined polymeric shell. Thermoresponsive ferrofluids are first synthesized by combining a magnetic core with a polycaprolactone shell showing LCST in DMSO carrier medium. Under the influence of a magnetic field, the core warms up owing to magnetic induction and causes a thermal transition in the shell.

Nowadays, cancer is a major social and health issue. Cancer causes seven million deaths every year, corresponding to 12.5% of deaths worldwide (WHO website). Different cancer therapy strategies include combined or separated protocols of surgery, chemotherapy and radiotherapy. The radiotherapy can be realized by different forms such as: (1) external beam radiation, (2) arterial embolization, (3) metabolic radiotherapy, (4) immunoradiothepary and (5) brachytherapy (Chakarova et al., 2005; Alevizaki et al., 2006; Rivera et al., 2006; Andratschke et al., 2007; Hacker and Alken, 2007). Unfortunately, the external radiotherapy destroys nearby healthy tissue along with the cancer cells resulting in various side effects or major complications (Buono et al., 2007). Therefore, recent studies have been performed in the sense to treat the tumour area by delivering the radioactivity locally. In order to reduce the mortality rate of conventional radiotherapy and to enhance treatment effects on malignant tumour, it is highly desirable to accurately restrict the radiation to the localized tumour area (Sun et al., 2000; Hamoudeh et al., 2008). In this sense, much research has been devoted to the synthesis of magnetic Nan particles.

Saravanan et al. (2004) studied the use of magnetic gelatine micro spheres to promote the controlled release of sodium diclofenac. They observed that the microspheres contained 8.9% (w/w) of sodium diclofenac and 28.7% (w/w) of magnetite. An emulsification/cross-linking method was applied using glutaraldehyde as croslinker. The formulated microspheres were characterized by particle size distribution, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray diffraction. The *in vivo* distribution and target ability of these gelatin magnetic microspheres after intravenous administration were studied in rabbits. They verified that the formulated microspheres were below 5 μm in size as well the DSC and X-ray diffractometry revealed the absence of drug-polymer interaction. Sodium diclofenac was slowly released for more than 18 days. The application of sonification as external stimuli to enhance drug release increased the release rate. The formulated microspheres were injected intravenously after keeping a suitable magnet near the target area. However, about 5.5% of injected dose localized near the target organ.

Wong et al. (2008) described the synthesis, characterization and surface modification of magnetic nanoparticles and a

poly(*N*-isopropylarylamide) (PNIPAam) microgel, followed by the assembly and characterization of magnetic nanoparticles on the microgel. To facilitate this deposition, the surface of the microgel was firstly modified via layer-by-layer assembly of polyelectrolytes. Inductive heat study revealed that the heat generated by the magnetic nanoparticles was sufficient to cause the collapse of the microgel above its volume phase transition temperature. Successful confinement of positively and negatively charged magnetic nanoparticles between polyelectrolytes layers was achieved. Dynamic light scattering measurements showed that each layer was successfully deposited and the thermo-sensitivity was preserved in the coated microgel, as there was no detachment of the magnetic nanoparticles during the phase transition of the microgel.

4. Preparation and properties of stimuli-responsive magnetic based-materials

4.1. Synthesis of iron oxide magnetic nanoparticles

Co-precipitation, as illustrated below, is an easy and largely used method to prepare iron oxide based nanoparticles, either magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) from aqueous ferric and ferrous salt solutions by the addition of a concentrated base solution with or without heating. The final product properties depend on parameters such as the ratio between Fe^{2+} and Fe^{3+}, pH and ionic strength of the medium, among others.

$$Fe^{2+} + Fe^{3+} \rightarrow Fe(OH)_2 + Fe_2O_3{\cdot}H_2O \rightarrow Fe_3O_4{\cdot}H_2O$$

$$Fe(OH)_2 = FeOH^+ + OH^-(+HFeO^{2-})$$

$$FeOH^+ + O_2 \rightarrow [Fe_2(OH)_3]^{3+}$$

$$[Fe_2(OH)_3]^{3+} + FeOH^+ \rightarrow Fe_3O_4$$

In this way, colloidal suspensions of magnetic particles, also known as ferrofluids, have very interesting properties due to their fluidity and ability to respond to an applied magnetic field. However, they have shown to be not stable and tend to form aggregates, which result in higher particles diameter. The solution for this problem can be found by an adequate coating of the magnetic particles with inorganic or organic substances. In this sense, the challenge is the development of materials that can be used for *in vivo* applications.

Magnetic nanoparticles for MRI applications, for example, can be prepared by adding an alkaline solution (e.g. NH_4OH) to ferrous- and ferric chlorides in the presence of dextran (poly-D-glucose) or a stabilizing polysaccharide, which is then grafted on the surface of the core. Usually, ferrous and ferric oxyhydroxides ("green" and "brown" rusts, respectively) are initially formed, and then transformed into a maghemite (γ-Fe_2O_3) or magnetite (Fe_3O_4) core as the pH and temperature is further increased. The core can vary substantially in composition and magnetic properties, since several different reaction pathways are possible (Elaissari, 2009). The formation of different types of iron oxide depends on the initial Fe^{2+}/Fe^{3+} ratio, the type and molecular weight of the polysaccharide used, and, finally, on pH and temperature. The control of synthesis parameters is necessary in order to avoid the preparation of extremely polydisperse particles and the requirement of several purification steps. The post-synthesis steps can include centrifugation, ultra- and diafiltration, column chromatography, magnetophoresis and sonification.

Another method used to obtain magnetic nanoparticles is biomineralization. By using biologic principles to specially confine the synthesis within specific subunit compartments, novel materials with a narrowly defined crystal size can be produced.

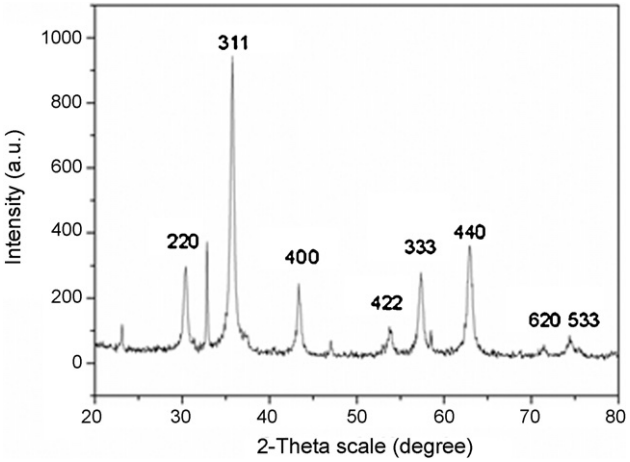

Fig. 10. X-ray diffraction pattern of Fe_3O_4 magnetic particles.

In particular, magnetoferritin is of interest for use as an MRI contrast agent. Its biomineralization is made using the protein ferritin. Then, ferrous iron is added at high pH and temperature under strictly anaerobic conditions, resulting in the formation of a strongly superparamagnetic core. Physicochemical characterization of the spherical particles has shown that the iron oxides with a diameter of about 7.3 nm are essentially composed of maghemite as the mineral phase. However, some *in vivo* studies showed a rapid biexponential clearance of the magnetoferritin from blood, with a short initial half-life less than 2 min (Ugelstad et al., 1993).

The magnetization measurements of the magnetic particles are essentially obtained using vibrating sample magnetometer (VSM). The output is a hysteresis curve, which shows the relationship between the induced magnetic flux density and the magnetizing force and gives important information about the magnetic saturation, the remanence, the coercivity and the level of residual magnetism left in the material.

Fig. 10 illustrates a typical X-ray diffractogram (XRD) of a Fe_3O_4 nanocrystallite sample. The diffraction peaks are clearly and sharp, which indicates that the particles are in a good crystal condition. The peaks could be indexed to (2 2 0), (4 0 0), (4 2 2), (5 1 1), (4 4 0), and (5 3 3) planes of a cubic unit cell, which corresponds to that of an iron oxide structure. From X-ray diffraction investigation, it is possible to confirm the good crystallization of Fe_2O_3 particles.

The superparamagnetic property of magnetic microgel particles is critical for their application in biomedical and bioengineering fields, which prevents magnetic microgels from aggregation and enables them to redisperse rapidly when the magnetic field is removed (Häfeli et al., 1997). Fig. 11 illustrates the separation and redispersion process of the oleic acid coated magnetic nanoparticles. In the absence of an external magnetic field, the dispersion of the MNPs was saddle brown and homogeneous (Fig. 11A). When the external magnetic field was applied, the MNPs were enriched, leading to transparency of the dispersion (Fig. 11B).

4.2. Constructing polymer-based magnetic materials

There are different routes to prepare polymer-based magnetic nanoparticles. Among these forms the most used are the polymerization in dispersed media and the magnetic latex particles from preformed polymers (Elaissari, 2009). The first one includes the classical heterogeneous polymerization processes such as emulsion, suspension, dispersion, miniemulsion, inverse emulsion or inverse microemulsion as well some multi-step synthesis procedures. These processes include the polymerization

reaction in the presence of magnetic particles. The obtained magnetic latex nanoparticles can present the following morphologies: well defined magnetic core with polymer shell, polymer particles with heterogeneous iron oxide nanoparticles distribution in the polymer matrix and polymer seed bearing magnetic nanoparticles in the shell. However, in some techniques, such as miniemulsion polymerization, polymer particles with heterogeneous iron oxide nanoparticles distribution in the polymer matrix are preferentially obtained. The miniemulsion polymerization process in the presence of the magnetic nanoparticles as well as the possible morphologies or structures of the obtained nanocomposites is schematically illustrated in Fig. 12.

On the other hand, magnetic latex particles can be also prepared from preformed polymers. This method involves, in general, the preparation of emulsions in which the polymer, which can be hydrophilic or hydrophobic, is dissolved in aqueous or organic solvents according to its properties. Iron oxide fine nanoparticles are dispersed in the same phase that the polymer and this phase is then emulsified in an external continuous phase that can contain a polymer as stabilizer, such as poly(vinyl alcohol) (PVA), or a surfactant, such as Pluronic®, in order to form an oil in water emulsion. Fig. 13 represents a schematic illustration of an example of a method used to obtain this system. The process consists of the utilization of a preformed polymer and a co-precipitation method.

Hamoudeh and Fessi (2006) have shown the incorporation of magnetite in poly(ε-caprolactone)-based microparticles by an emulsion-solvent evaporation method. One of their most promising utilization of this material is the magnetic resonance imaging (MRI). In this method, the polymer is usually dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate. The magnetic particles is dispersed into the preformed polymer solution and this mixture is then emulsified into and aqueous solution containing a surfactant/emulsifying agent in order to make an oil in water (O/W) emulsion. After the formation of a stable emulsion, the organic solvent is evaporated by increasing the temperature/under pressure or by continuous stirring (Soppimath et al., 2001). However, this method is often performed using microencapsulation technology and is not recommended for nanoencapsulation. In general, nanocapsules do not resist direct evaporation of the solvent, possibly due to the mechanical stress caused by the gas bubbles formed inside the aqueous suspension (Mora-Huertas et al., 2010).

In this context, another method, including preformed polymers, used to prepare magnetic polymeric nanoparticles is the solvent diffusion process. This method consists of using a partially water-miscible solvent such as ethyl acetate as an organic solvent. This solvent can be emulsified in an aqueous solution of a stabilizing agent, followed by diluting the internal phase with an excess of water to induce the precipitation of the polymer.

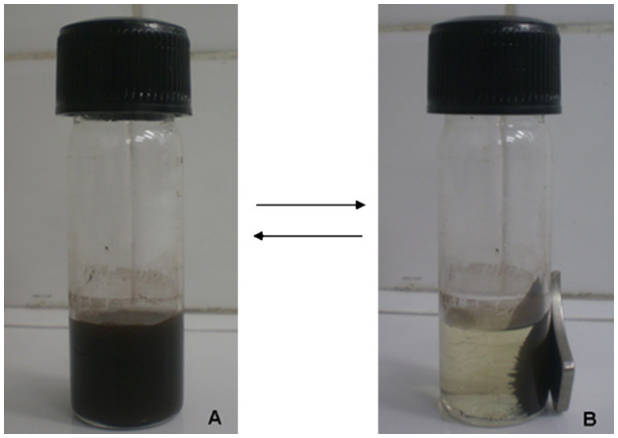

Fig. 11. Photographs of the separation (A to B) and dispersion (B to A) of the oleic acid coated magnetic nanoparticles: (A) without external magnetic field, (B) with external magnetic field.

In general, the established procedures for the preparation of spherical polymer beads require reaction times between 3 and 4 h. However, Müller-Schulte and Schimitz-Rode (2006) developed a novel inverse suspension polymerization technique that allows bead preparation within minutes. For this purpose, it is necessary to find appropriate conditions for preparation of stable droplets, evaluating the oil phase, and particularly its viscosity and surfactant composition. This approach can result not only in a fast synthesis of the beads but also in a very simple encapsulation of drug model substances and the necessary magnetic colloids. This technology provides a broad platform for a simple, simultaneous encapsulation of diverse species such as magnetic colloids and bioactive substances. In addition, it offers a basis for a combinatorial application of two basic therapeutic procedures and one diagnostic procedure. Thus, the systems can be used as a contrast agent in tumour tissue imaging and as a drug delivery agent. In magnetically targeted therapy, a cytotoxic drug is attached to a biocompatible carrier including magnetic nanoparticles.

For all applications, the control of size, shape and composition of nanoparticles depends on the type and concentration of the stabilizers, the Fe^{2+} and Fe^{3+} ratio, pH and ionic strength of the media, among other parameters (Sjogren et al., 1994). The major problem of such nanoparticles (for *in vitro* diagnostic) is related to low magnetic separation under applied magnetic field, due to the Brownian motion compared to magnetic force (Mouaziz et al., 2009).Then, various roots have been investigated in order to elaborate large functionalized magnetic particles. The stability of the magnetic suspensions may be accomplished with micron-sized or nanometer sized particles (Radbruch et al., 1997). Smaller nanoparticles produce suspensions that are stable against sedimentation due to gravity or an applied magnetic field, while larger particles can be used to take advantage of sedimentation as part of the separation process (Kriz et al., 1998). The particles size is also important when using magnetic nanoparticles for detection of biological molecules. This technique is based on changes in the Brownian relaxation due to binding between the magnetic nanoparticles and the biological molecules (Chung et al., 2004). The relaxation frequency of a nanoparticle changes when it binds to another molecule due to the increase of its hydrodynamic size. The shift is proportional to the hydrodynamic size of the nanoparticle, allowing discrimination between target molecules with different sizes (although molecules with different functionalities, but similar sizes cannot be independently detected) (Leslie-Pelechy et al., 2006). The main advantage of this technique is that the signal can be observed before and after binding, which allows for reliability checks.

Both *in vitro* and *in vivo* biomedical applications usually require that the nanoparticles have size smaller than 100 nm with overall narrow particle size distribution, so that the particles have uniform physical and chemical properties. The optimum particle size for *in vivo* applications depends on a number of factors, especially for multi-modal applications such as combined magnetic drug targeting and hyperthermia. For *in vivo* applications, smaller particle size helps in evasion of the reticulo-endothelial system (RES), the body's immune system responsible for detection and clearance of foreign particles from circulation (Purushotham et al., 2009). The nanoparticles accumulate due to their specific size and their extravasation within the tumour, where the microvasculature is hyperpermeable and leaky, a process also aided by the tumour-limited lymphatic drainage (Gupta and Gupta, 2005). In combination, these factors lead to the selective accumulation of nanoparticles of sizes generally between 80 and 200 nm in tumour tissue, a phenomenon known as enhanced permeation and retention (EPR) (Maeda et al.,

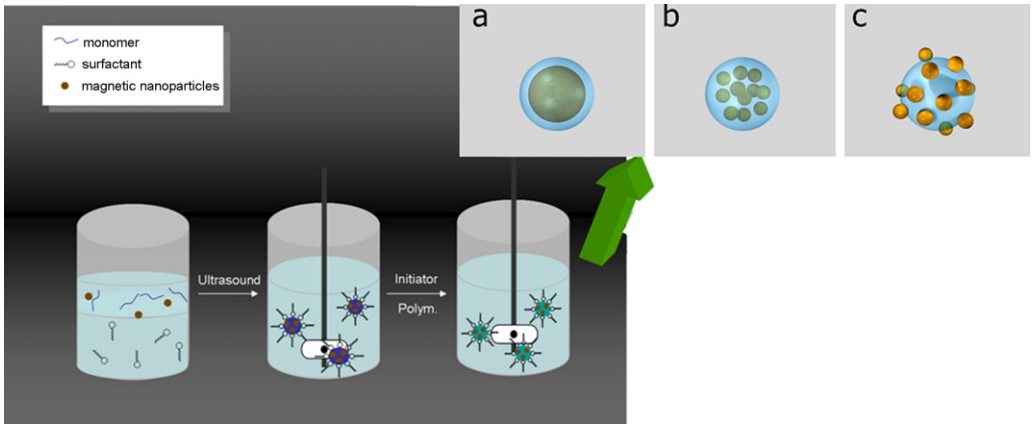

Fig. 12. Schematic representation of the miniemulsion polymerization process in the presence of magnetic nanoparticles and the possible morphologies or structures of the obtained nanocomposites (a) core(magnetic)-shell, (b) Distribution inside the polymer matrix and (c) Distribution on the particles surface.

2000). Particles smaller than 10 nm are well suited for tumour penetration, but are rapidly cleared by renal filtration and extravasation. Particles in the size range of 50–150 nm are typically cleared by the Kupffer cells of the liver and those that are larger than 200 nm are likely to be filtered by the venous sinuses of the spleen (Neuberger et al., 2005). However, nanoparticles with larger surface area/volume ratios tend to agglomerate and adsorb plasma proteins. When the nanoparticles agglomerate, or are covered with adsorbed plasma proteins, they are quickly cleared by macrophages in the reticulo-endothelial system before they can reach target cells (Torchilin and Trubetskoy, 1995). One possible approach, therefore, to increasing the circulation time of these nanoparticles in the blood stream is to coat the particles with hydrophilic polymers in order to disperse them and minimize or eliminate the protein adsorption (Florence et al., 1997).

Concerning the *in vitro* and *in vivo* applications, the superparamagnetic behaviour is also of the magnetic nanoparticles is also of interest because they do not retain any magnetism after removal of magnetic field. This parameter is also related with the size of the particles. For biomedical applications, particles in the 10–50 nm size range, with appropriate coatings, provide a good balance of circulation times, ability to be manipulated by an external magnetic field and heat generation for hyperthermia. Particles size smaller than 10 nm usually results in lower magnetization of saturation (*MS*) (Kim and Shima, 2007; Lu et al., 2007) resulting in a decreased response to externally applied magnetic fields. This increases the difficulty of targeting tumours deep inside the body. Generation of large static or alternating magnetic fields with sufficient penetration depth is then a technical challenge, once it is important to ensure that the magnetic nanoparticles have the highest *MS* values possible. Moreover, a successfully magnetic hyperthermia is also strongly dependent on *MS* (Jordan et al., 2001): higher *MS* values result in higher heating power (for magnetic hyperthermia, the theoretical ideal magnetic particle core size for heat generation is in the 10–20 nm range). In summary, particles size is an important parameter to be controlled in order to maximize the power of magnetization at any field with superparamagnetism and to obtain high saturation magnetization values (Chatterjee et al., 2003). The particles in the appropriate range are rapidly removed through extravasations and renal clearance (Pratsinis and Vermury, 1996).

Many different polymers have been studied as coating agents of magnetic particles. However most of these materials can be only used for *in vitro* biomedical applications, limiting the use of these systems. Nanoparticulate systems for *in vivo* applications need to improve the pharmacokinetics of drugs including their rate of adsorption, in situ distribution, metabolism effect, toxicity and their specific targeting. The *in vivo* applications of these materials include the use of the nanoparticles as imaging tools for *in vivo* nanodiagnosis, liquid perfluorcarbons for ultrasonic imaging and paramagnetic or superparamagnetic contrast agent for magnetic resonance imaging (MRI). This last one application is the most studied in the literature (Häfeli, 2004). Moreover, these nanoparticles can be also used in medicine and biotechnology such as in hyperthermia, DNA separation, and drug targeting and enzyme purification. In this context, Poly(*N*-vinylcaprolactam) (PNVCL) is a promising polymer to be used as coating agent of magnetic particles due to its biocompatibility and low cytotocixity, besides its thermoresponsive properties (Vihola et al., 2005). Pich et al. (2004) described the preparation of hybrid temperature-sensitive microgels, which include magnetite nanoparticles in their structure. The polymeric microgels were firstly prepared by surfactant-free emulsion copolymerization of acetoacetoxyethyl methacrylate (AAEM) and *N*-vinylcaprolactam (NVCL) in water using 2,2′-azobis-(2-methylpropyonamidine) dihydrochloride (AMPA) as initiator. Microgels with a low critical solution temperature (LCST) in water solutions were obtained. Then, magnetite was deposited directly into microgels, leading to the formation of composite particles, which combine both temperature-sensitive and magnetic properties. The influence of magnetite load on microgel size, morphology,

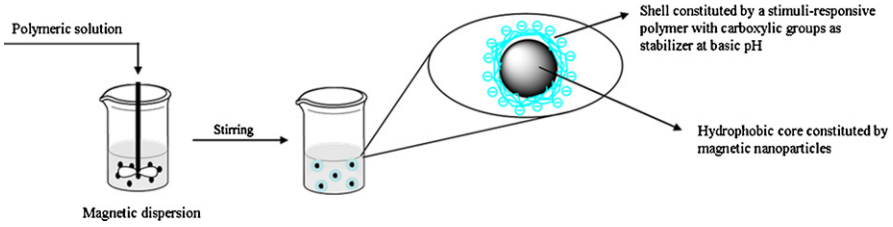

Fig. 13. Schematic illustration of an example to obtain stimuli-responsive polymer with magnetic properties.

swelling–deswelling behaviour, and stability was investigated. The authors observed that the stability of hybrid microgels was not influenced by the magnetite content and that the obtained particles were sterically stabilized in aqueous medium by the hydrophilic biocompatible NVCL-rich shell. They could conclude that the obtained results open the door for many applications in different fields of material science and technology.

5. Applications

With the development of physical and chemical methods of preparation of colloidal iron oxides, the first biomedical application of ferrofluids also gradually appeared in early 1960s. In 1963 Meyers et al. (1963) developed an experimental approach for controlled delivery of magnetic particles *in vivo* as lymphatic and vascular contrast and isotopic agent in dogs. Four years later, a magnetic probe for stereotactic thrombosis of intracranial aneurysm was proposed (Alksne et al., 1967). Then in 1975 Turner et al. (1975) reported the idea of portable liquid helium superconducting magnetic for external targeting of iron microspheres (in silicon oil) for thrombosis of selected arteries, a device which was later developed for treatment of hypernephromas. Thus, a number of notable events in the gradual evolution of biomedical applications of ferrofluids were succeeded for the utilization of this material for *in vitro* and *in vivo* biomedical applications.

Nowadays, magnetic nanoparticles can be used in a wide variety of biomedical applications. The most promising applications of the MNPs are shown in Fig. 14. Among the *in vivo* applications, it seems convenient to highlight the magnetic resonance imaging (MRI) and the cancer detection.

In the context of *in vivo* biomedical applications, an ideal biocompatible stimuli-responsive and magnetic system is able to possibility the monitoring, the kinetics and biodistribution of the medication in the organism non-invasively using MRI followed by the treatment which can be made by different ways such as the local drug delivery using the sensitive properties of the polymer and thus, preserving most of the healthy tissues and cells or hyperthermia. Fig. 15 illustrates the action of an ideal magnetic system for *in vivo* biomedical applications.

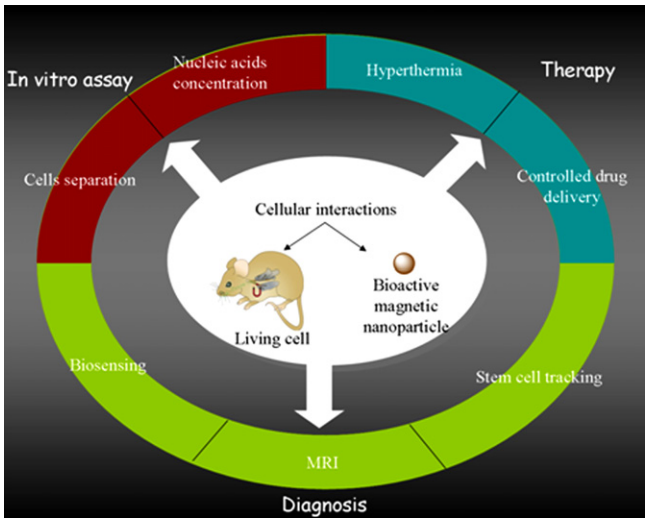

Fig. 14. Biomedical applications of magnetic nanoparticles (adapted from Nanomedicine © 2009 Future Medicine Ltd).

As cancer grinding away the lives of a large section of the society, the importance of nanomedicine is gaining new heights. Novel nanomedical anticancer therapies with much less pain and side effects are becoming the ultimate anticancer solutions. The small size of nanoparticles associated with their properties can

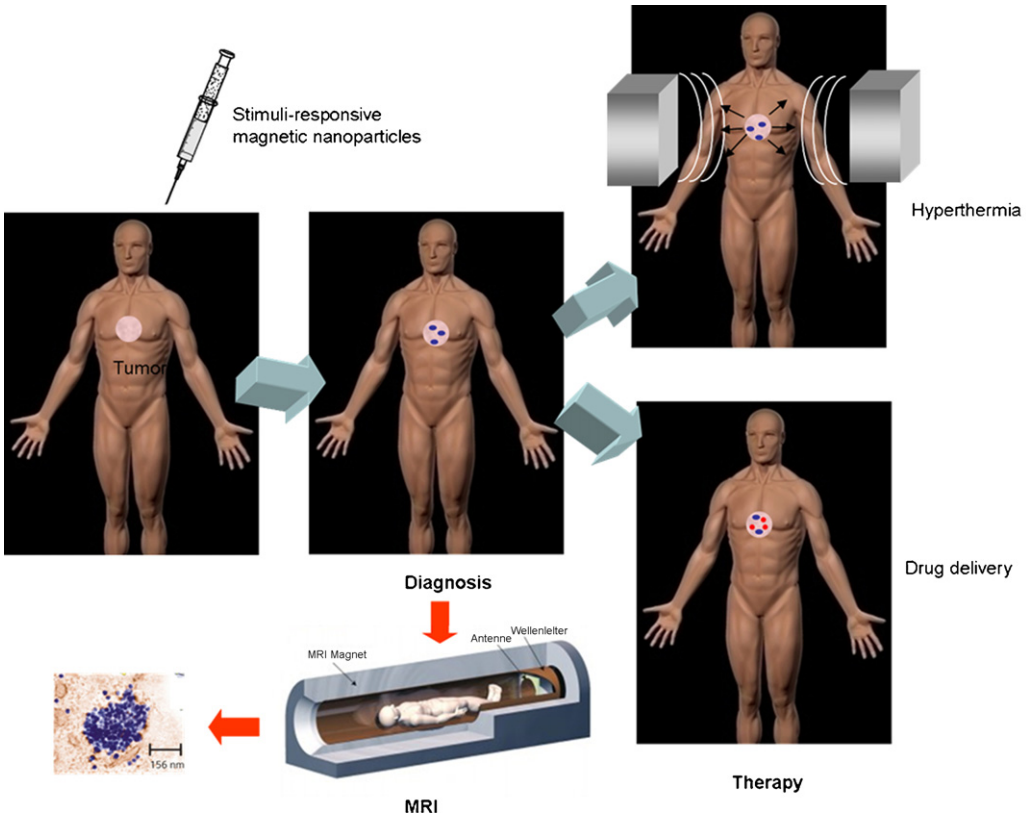

Fig. 15. Schematic representation of the drug-loaded magnetic nanoparticles localization by MRI followed by the treatment of the tumour either by hyperthermia or by the drug release.

be very useful in oncology, particularly in imaging. In this context, iron oxide nanoparticles, when used in combination with MRI, can produce exceptionably sharp images of tumour sites. Another particularity of magnetic nanoparticles is that they can be encapsulated by a functional or a stimuli-responsive polymer and this system can also contain a specific drug for cancer therapy that leads to the possibility to combine diagnosis and therapeutic applications in the same system.

5.1. Magnetic resonance imaging (MRI)

Developed in 1973 by Paul Lauterbur (1973), magnetic resonance imaging (MRI) has become widely used in hospital, around the world, since it received the FDA (Food and Drug Administration) approval for clinical in 1985 (Häfeli et al., 1997). During the last decades, many researches have been performed and great progress has been achieved in the field of pharmaceutical technology towards the synthesis of sophisticated nanoparticulate systems for pharmaceutical applications as novel agents for drug delivery (Burns et al., 1985). These systems in general, include materials nanoscale designed, biocompatible and able to degrade into eliminable fragments after drug release. Since one of the main objectives of these materials is to promote controlled and local drug delivery, the incorporation of a paramagnetic or superparamagnetic contrast agent for magnetic resonance imaging has been studied. MRI contrast agents are a unique class of pharmaceuticals that enhance the image contrast between normal and diseased tissue and indicate the status of organ function or blood flow after administration by increasing the relaxation rates of water protons in tissue in which the agent accumulates (Hong et al., 2008). Paramagnetic substances have been used as MRI contrast agents due to their net positive magnetic susceptibility. While superparamagnetic and ferromagnetic materials have very large net positive magnetic susceptibilities when magnetized in the presence of an external magnetic field (Nelson and Runge, 1996).

In MRI, image contrast is a result of the different signal intensity produced by each tissue in response to a particular sequence of applied radiofrequency (RF) pulses. This response is dependent on the proton density and magnetic relaxation times (Häfeli et al., 1997). Once the proton density of soft tissues has a significantly narrow range throughout the body, natural contrast is mainly determined by the relaxation times, which depends on the chemical and molecular structure of the tissue (Koenig, 1996). Tissue contrast can be manipulated by adjusting instrumental parameters including the applied pulse sequence. The detection of iron can be improved by choosing an imaging sequence with greater sensitivity for its relaxation effects.

Many studies have been performed in the sense to explain the effect of the paramagnetic or superparamagnetic nanoparticles on the MRI. Basically, magnetic relaxation is described by the time constants T1 (longitudinal) and T2 (transversal). Both relaxations are exponential and the time constants represent the time for 63% of the relaxation to take place. If a magnetic field B_0 is applied, the absorption of the electromagnetic radiation by the nuclei of interest, usually protons, leads to their return or relax to the lowest energy state of alignment with the applied magnetic field. This is an exponential process and is described by the longitudinal time constant T1. The RF pulse also creates a temporary transverse magnetization that process about B_0, inducing a signal in the surrounding coil (which is used to generate the MRI image). The signal dies away quickly because of the dephasing caused by the local field, the lack of homogeneity, among other reasons. Thus, due to these effects T2 is usually shorter than T1. Then, the signal intensity can be calculated from the T1 and T2 constants according to

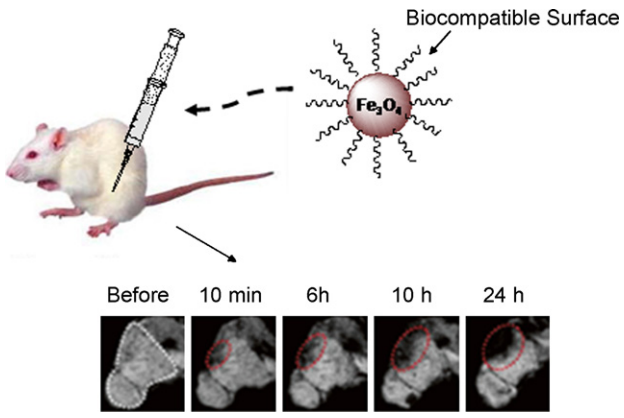

Fig. 16. Schematic representation of the magnetic nanoparticles *in vivo* detection using MRI (adapted from Hu et al. (2006)).

Equation (1).

$$SI = N(1 - e^{-TR/T1})e^{-TE/T2} \quad (1)$$

where N is the proton density and TR is the repetition time used in the pulse sequence.

In general, tissue or organ-specific contrast agents consist of two components: a magnetic label capable of altering the signal intensity on MR images and a target-group molecule having a characteristic affinity for a specific type of cell or receptor (Hatakeyama et al., 1998). After successful *in vitro* experiments, Hu et al. (2006) demonstrated the potential *in vivo* applications of a poly(ethylene-glycol) (PEG) (HOOC-PEG-COOH)-coated Fe_3O_4 nanocrystals conjugated with a cancer-targeting antibody, that is, the anti-carcinoembryonic antigen (CEA) monoclonal antibody rch 24 (rch 24 mAb) for detecting human colon carcinoma xenograft tumours implanted in nude mice at their proximal thigh region. Fig. 16 illustrates the use of biocompatible magnetic systems for *in vivo* MRI and shows the stages of signal intensity after the injection of the magnetic material.

We can observe from Fig. 16 that a fraction of the tumour turned dark as early as 10 min after the injection of the rch 24 mAb conjugates. With time, this fraction of the tumour showing hypo intensity and becomes darker and bigger until the end of the experiment, demonstrating a successful binding of Fe_3O_4 nanocrystals to the tumour via rch 24 mAb. Quantitative analysis revealed a decrease of the signal intensity in approximately 10%, 24 h after injection (considering the signal intensity equal to 100% before the injection).

Magnetic nanoparticles have been also used as a contrast agent for the reticulo-endothelial system (RES). Clinical applications include contrast-enhanced imaging of the liver, spleen, and lymph nodes. However, the particles are also explored as a perfusion agent. The use of magnetic nanoparticles as imaging of the RES and perfusion agents presents some advantages. Some of these advantages are: site-specific *in vivo* mapping, cellular tagging and *in vivo* transport, *in vivo* mapping of blood-brain barrier disruption and development of combined "drug systems" (Ugelstad et al., 1993). Site-specific *in vivo* mapping implies the attachment of site-specific molecules, such as sugars and proteins, to the magnetic nanoparticle. Mapping of the asialoglycoprotein receptor on hepatocytes has been made possible by either attaching arabinogalactan or asialofetuin to the iron oxide. When the specific sites are not directly accessible from the bloodstream, and the magnetic particles are required to penetrate through the endothelium, small size and long blood half-life become important factors.

Cellular tagging and *in vivo* transport mean the use of MRI to study dynamic cellular processes. For this purpose, MRI needs to be non-invasive. An example of this study is the use of cellular tagging

of fetal cells using wheat germ agglutin (WGA)-conjugated magnetic particles (Norman et al., 1992) or reconstituted Sendai virus enveloped containing magnetite (Hawrylak et al., 1993) followed by cerebral implantation and serial MRI for up to two months. This system allows the monitoring of *in vivo* cell migration in neural grafting procedures.

In vivo mapping of blood-brain barrier (bbb) disruption describes the easy *in vivo* detection of systems based on magnetic nanoparticles in the bbb disruption, which is naturally associated with some brain lesions. The bbb represents an obstacle for a large number of drugs, including antibiotics, antineoplastic agents and a variety of central nervous system (CNS)-active drugs, especially neuropeptides. One of the possibilities to overcome this barrier is a drug delivery to the brain using nanoparticles. The nanoparticles may be helpful for the treatment of the disseminated and very aggressive brain tumours (Leslie-Pelechy et al., 2006). An example of this study consists of the use of MION-46 to demonstrate lesions following osmotic bbb-disruptions, as described by Neuwelt et al. (2008). These authors found that the MION was taken up by neuronal cells in what is thought to be a dextran-specific process. The resulting MRI maps correlated well with the histopathological results. Thus, it was purposed that MION particles may be applied as both a diagnostic and therapeutic vector, e.g. a combined drug system.

Coupling *in vivo* imaging with drug delivery provides performance feedback as to the biodistribution and pharmacokinetics of the vehicle, supplying valuable insight to the formulation scientist seeking to improve vehicle localization or persistence (Norman et al., 1992). In summary, a combined "drug delivery system" consists of a carrier that contains both a therapeutic and diagnostic (contrast) agent. Following administration or implantation of such system, it could be possible to track medication uptake kinetics and biodistribution non-evasively using MRI. Some gels have been developed to track controlled and drug release and transport in the brain (Reisfeld et al., 1993). For implanted systems, a local sustained release (with low systemic toxicity) of conjugated doxorubicin (Weissleder et al., 1993) and gentamicin (Weissleder et al., 1995) has been evaluated.

5.2. Drug delivery

Controlled drug delivery has the potential to improve drug efficacy, as well as patient convenience and compliance (Kumar et al., 2007). As the overall drug dosage can be reduced by 50–80%, dosage at the target site is increased and systemic uptake is decreased. Local drug delivery also reduces the patient-to-patient pharmacokinetic variability inherent in oral and intravenous applications. Protecting the drugs until they reach their target area increases the usability of drugs that have a short half-life in the body. Moreover, the release of a drug over a prolonged period of time maximizes the effect of drugs such as chemotherapeutics, which are effective only during a specific part of a cell's life cycle.

Nanoparticles were first used for drug delivery around 1970, with the development of carriers for vaccines and anticancer drugs, and are now widely used and target of intense study (Andratschke et al., 2007). Nanoparticles for drug delivery must be able to efficiently incorporate a reasonably high weight fraction (loading) of the drug, must form a stable suspension in an aqueous media, must be biocompatible and biodegradable, and must not be cleared too rapidly from the blood stream. Moreover, they should be able to be made in a range of sizes, but with uniform size distribution, and should be able to be further functionalized. The main attraction of magnetic nanoparticles from the perspective of drug delivery is the ability to use the magnetic properties to either limit the drug to a particular region using magnetic targeting, or to release the drug remotely.

These systems can include the encapsulation of the drug in conjugated to, or adsorbed onto the surface of a nanoparticle. The release may be made via degradation of the carrier particle or may be triggered by heat or pH. For this purpose, many drug delivery systems use poly(lactic acid-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL) and their copolymers, as these are biodegradable, FDA-approved materials and the degradation rate can be controlled by the particle formulation (Panyam and Labhasetwar, 2003). Careful choice of surfactants can allow hydrophobic drugs to be transported throughout the body. Ionically bound pharmaceuticals have the advantage that the active-low molecular weight substances can desorb from the carriers after a defined time span and diffuse from the vascular wall into the tissue. The diffusion through the vascular wall can significantly change the desorption kinetics of the pharmaceutical (Bergemann et al., 1999). As an example, Epirubicin chemoadsorptively bound to a polymer-coated particle can desorb according to physiological environment (pH, osmolarity, and temperature) (Lübbe et al., 1999). The half-life of the drug desorption can be fixed to be approximately the same as the desired time for magnetic field targeting.

Smart materials such as thermally- and pH responsive polymers are largely studied like carriers for controlled drug delivery. Moreover, as previously seen, a combined drug delivery system which consists of a carrier that contains both a therapeutic and diagnostic (contrast) agent is desired.

Magnetic systems are attractive candidates for delivery of high doses of anticancer drugs in intracavitary therapy (Gupta and Hung, 1989). There are many studies in literature performed with the chemotherapeutic anthracycline drugs loaded into magnetic microcapsules. However, this kind of drugs must be released near the tumour, and then bind to (or diffuse into) malignant cells in order to be effective. On the other hand, most of chemotherapeutic drugs encounter physiologic barriers in many tumours (Jain, 1991), and consequently, parts of the tumour do not receive sufficient drug concentration.

Radioactivity is another factor to be considered which exerts action over a defined radionuclide-dependent distance and the resulting radiation penetrates the tissue irrespective of diffusion barriers, membrane and pressure differences. Beta emitters penetrate up to 10 mm in soft tissue, and γ emitters up to several centimeters. In magnetic brachytherapy the radiopharmaceutical should ideally be distributed as homogenously as possible in the tumour, and remain there until radioactivity is completely decayed. However, it is just possible if the magnetic systems can be targeted to tumours by planting a magnet in the tumour or in its vicinity, and holding it there during the decay period.

According to Wootton (1991), magnetic targeting of radiolabeled microcapsules presents various advantages and can be achieved as follows:

1. Inject a diagnostic dose of magnetic microcapsules labeled with a γ emitting tracer (for imaging).
2. Apply an adjustable magnetic field to target area.
3. Produce an image from the tracer radiation with gamma camera.
4. Adjust the magnetic field to target the microcapsules as required.
5. Inject a therapeutic dose of magnetic microcapsules loaded with the required β (or α) emitter.
6. Apply and maintain the adjusted magnetic field for a defined period.
7. Schütt et al. (1997) performed a similar therapy including an additional step as follows (either 7a or 7b):
8. Remove the nontrapped radiolabeled magnetic microcapsules via a blood vessel different from the one used for injection by means of an extracorporeal magnetic separation.
9. Magnetically confine the microcapsules to target area for a defined period of time, followed by removal of the (still) radio-

labeled or drug loaded microcapsules from the body in the same way as in 7a.

In other words, this last step is similar to the dialysis procedure in which undesirable substances are removed from the body during or after treatment. This study is an example of the possibility to minimize the undesirable effects of the treatment by magnetically controlling both tumour targeting and removal of unwanted circulating microcapsules.

Attempted therapeutic use of radiolabeled magnetic microcapsules for cancer therapy dates back to 1969, when Mosso and Rand (1973) conceived the idea of a portable liquid helium superconducting magnet that could be applied externally to embolize selected tumour feeding arteries by *in vivo* formed ferrosilicone microcapsules.

In vivo evaluation of radiolabeled magnetic microcapsules normally includes the study of biodistribution in an appropriate animal model, target tissue and whole body toxicity, treatment parameters (radiation dose, strength and duration of magnetic field), and monitoring interventions. In all cases, biocompatibility and toxicity are parameters hardly discussed.

5.3. Chemotherapy

One of the numerous applications of MNP in medicine is the cancer treatment (Alexiou et al., 2006). Cancer is the second cause of death in the developed world, killing over 500,000 people in the United States in 2000 (Zhang et al., 2004). Chemotherapy is a major therapeutic approach for the treatment of localized and metastasized cancers. The cancer detection is one of the major goals in modern diagnostics because early detection and accurate staging of the primary tumour is a prerequisite for or successful cancer therapy. Effective cancer treatment is based on accurate tumour staging. The selective increase in tumour tissue uptake of anticancer agents would be of great interest in cancer chemotherapy, because anticancer drugs are not specific to cancer cells (Hu et al., 2006). Routes of administration, biodistribution, and elimination of available chemotherapeutic agents can be modified by drug delivery systems in order to optimize the cancer therapy. Conventional cancer therapy and diagnostics involve the application of catheters, surgery, biopsy, chemotherapy, and radiation. Most current anticancer agents do not greatly differentiate between cancerous and normal cells. This leads to systemic toxicity and adverse effects. Consequently, the systemic application of these drugs often causes severe side effects in other tissues (e.g., bone marrow suppression, cardiomyopathy, and neurotoxicity), which greatly limits the maximal allowable dose of the drug. In addition, rapid elimination and widespread distribution into non-targeted organs and tissues requires the administration of a drug in large quantities, which is uneconomical and is often complicated because of non-specific toxicity.

New therapeutic systems for neurodegenerative illness/pain are under research. Because they are chronic illnesses, it is desirable to develop new materials in order to prolong the delivery time and to reduce the drawbacks of the typical application systems such as chemotherapy (Häfeli et al., 1997). The most accurate method for evaluating tumour spread is still lymph node resection and histopathological examination of each individual lymph node. However, this technique is too invasive for routine use. Thus, lymph node is clinically performed by non-invasive imaging modalities like ultrasound (US), computer tomography (CT) or resonance tomography (MRT).

In the search for an efficient method for cancer therapy, biocompatible-based magnetic systems have been proposed (Gupta and Hung, 1989). Ideally, such materials bear on their surface or in their bulk an anticancer drug that can be driven to the target organ and released there (Chen et al., 2009). The main disadvantage with drug targeting to other sites in the human body is the rapid uptake of intravenously injected particulate drug carriers by the mononuclear phagocyte system (Hu et al., 2006).

The first evidence for the utility of magnetic nanoparticles in cancer treatment was demonstrated in the early 1980s, when Widder et al. (1979, 1983) showed significant remission of Yoshida sarcoma without drug toxicity in rats using magnetically targeted albumin microspheres with doxorubicin. Some other drugs for cancer therapy, such as Mitoxantrone, mitomycin C, etoposide, paclitaxel, oxaliplatin and epirubicin have been bound to iron oxide or Fe-C fluids for magnetically targeted cancer treatment (Widder et al., 1979, 1983; Bergemann et al., 1999; Goodwin et al., 1999; Lübbe et al., 1999; Johnson et al., 2002).

It is also important to remember that the multidrug resistance (MDR) is the primary cause for almost 90% of cancer treatment failures (Kohler et al., 2005). Cancer cells are becoming resistant to the cytotoxic effects of a wide range of structurally and mechanistically unrelated anticancer drugs. It is known that various molecular mechanisms can contribute to reduce this effect. Some agents confers cancer cells the strongest resistance to the widest variety of compounds. In this field, Chen et al. (2008) developed tetraheptylammonium-capped Fe_3O_4 in order to facilitate the accumulation of daunorubicin (DNR) inside leukemia K562/A02 cells and to enhance the response of DNR in leukemia K562/A02 cells *in vitro*. It was demonstrated that DNR-loaded Fe_3O_4 have shown higher activity than those of DNR alone. The cytotoxicity test *in vitro* revealed that the MNPs exhibit excellent biocompatibility. More recently, Chen et al. (2009) demonstrated that DNR-loaded Fe_3O_4 particles are able to reverse leukemic K562-n/VCR cells *in vivo* by inducing apoptosis. However, the system failed in the enhancement of the cytotoxicity response in sensitive leukemic K562-n cells *in vivo*.

Nanotechnology could offer a less invasive alternative, enhancing the life expectancy and quality of life of the individual with cancer. The diameter of human cells spans from 10 to 20 μm. The size of cell organelles ranges from a few nanometers to a few hundred nanometers. Nanoscale devices can interact with biomolecules on the cell surface and within the cells in a non-invasive manner, leaving the behaviour and biochemical properties of those molecules intact (Kim et al., 2003a,b). In this sense, macromolecular compounds composed by a polymer backbone loaded paramagnetic or superparamagnetic iron oxide nanoparticles can be used such as contrast agents. The biodistribution of these systems to different organs and tumours depends on the composition of the lipid bilayer, substances bound to it, such as antibodies, and the preparation method of the material, which influences the particle surface and size. In this way, there is a diversity of possible variations of materials to synthesize and consequently, a previous study is required to examine their accumulation behaviour in the tumour and to choose that best adapted to the application.

5.4. Hyperthermia

The use of hyperthermia (heat) in the treatment of malignant tumours is as old as medicine itself. Magnetic induction hyperthermia, one of the therapies for cancer treatment, means the exposition of cancer tissues to an alternating magnetic field (Häfeli et al., 1997). The idea of hyperthermia has been recognized as a useful therapeutic modality for treating malignant tumours, leading to the possibility to kill cancer, not with drugs, but with targeted nanoscale heaters that would essentially cook malignant cells to death (Gazeau et al., 2008). Hyperthermia has thus been suggested as a non-invasive and non-toxic method of inducing gene expression locally using a thermo inducible promoter (Walther et al., 2002).

In this context, intracellular hyperthermia methods have been suggested and developed using MNPs, whereby the particles are concentrated at the tumour site and they are remotely heated using an applied magnetic field in order to achieve the required hyperthermic temperatures (42–45 °C) (Masashige, 2002; Fortin et al., 2008). It is known that when MNPs are injected into an organ with a tumour, they tend to accumulate in the tumour due to the unorganized vasculature, thus effectively heating the tumour as opposed to the surrounding healthy tissue (Berry, 2009). Moreover, if they are subjected to a variable magnetic field, some heat is generated due to magnetic hysteresis loss. The amount of heat generated depends on the nature of magnetic material and of magnetic field parameters. Direct injection into solid tumours, followed by exposure to an alternating magnetic field, has been shown to be capable of inducing tumour regression (Ito et al., 2004). Cancer cells are destroyed at temperatures higher than 43 °C, whereas the normal cells can survive at these temperatures (Häfeli et al., 1997).

There are many works in the literature describing the use of magnetic particles for hyperthermia in order to manifest a therapeutic effect on several types of tumours by performing experiments with animals (Luderer et al., 1993) or using cancerous cell cultures (Chan et al., 1993). Recent studies have highlighted the potential of MNPs in human cancer models, such as breast cancer, whereby 10–30 nm iron oxide particles were heated effectively (Jin and Kang, 2007). Recently, Johannsen et al. (2007) published the first clinical experiences with MNP-mediated thermotherapy on prostate carcinoma. Wust et al. (2006) studied the effect of the association of hyperthermia and MNPs on the tolerance of intestinal tumours.

6. Concluding remarks and perspectives

Stimuli-sensitive polymers have been known for over 40 years. However, they still remain a subject of vigorous investigation, both in academic and in industry fields. Thus, they can be expected to result in valuable applications in the near future. There are numerous reasons for this continuing interest. However, it is difficult to foresee in detail the potential applications of systems that rely on stimuli-sensitive polymers. The development of devices based upon these materials calls for a fine-tuning of their properties. They should respond to the external stimuli in a way that precisely fits the application proposed. In order to achieve this approach, a better understanding of the relations between the polymer properties (dependence of behaviour at the transition point upon external conditions) and the structure of the macromolecules is necessary. Certainly their application in biology, medicine and other related sciences is already obvious and will be further developed. Diagnosis tools, intelligent carriers of drugs and prodrugs, and devices for delivery of active species triggered by external stimuli are just a few examples. However, the biomedical area is not likely to remain the only field of potential application. Nanosensors, nanoactuators (e.g. self-controlled nanovalves) and stimuli sensitive electrochemical and electro-optical devices will remain objects of studies that will lead to numerous potential applications.

The concept of drug delivery using magnetic nanoparticles greatly benefit from the fact that nanotechnology has developed to a stage that it makes possible not only to produce magnetic nanoparticles in a very narrow size distribution range with superparamagnetic properties but also to engineer particle surfaces to provide site-specific delivery of drugs. Due to its strong magnetic properties magnetite was used first in biology and then in medicine for the magnetic separation of biological products and cells as well as magnetic guidance of particle systems for site-specific drug delivery. The size, charge, and surface chemistry of magnetic particles could strongly influence their biodistribution. Another important point is that the magnetic properties depend strongly on the size of the magnetic particles. In the last decade, the activities in the clinical applications of magnetic carriers and magnetic particles have been very high, due to the demand for better diagnostic procedures as well as better treatment modalities. Precise delivery of anti-inflammatory drugs to the exact area of inflammation is a desirable task, since it can reduces drug dosages, eliminates side effects on the other healthy tissues and increases rapidity the drug action. Application of external magnetic field to the area of inflammation may provide an important enhancement of such treatments.

Magnetic nanoparticles are rapidly becoming an important and indispensable tool for the non-invasive study of biologic process with MRI. The development of very small stable preparations, the understanding of the underlying relaxation mechanisms, and the clinical use are all factors that increase the use of magnetic carriers in MRI. Therefore, successful development in this area will aid the growth of the biomedical industry as well as improving the quality of life of the population. The main demands on magnetic radioactive systems for this purpose are biocompatibility and biodegradability, a high magnetic susceptibility for an effective magnetic enrichment in the target area and a high circulation time in the blood to allow for the enrichment process and the extracorporeal removal of the materials. The applied particles should have a small size distribution, uniform surface properties and to present a good colloidal stability.

There are many publications about the utilization of the magnetic polymer based nanoparticles, but most of this literature can only be used for *in vitro* applications, not *in vivo*. For this last purpose, the polymeric matrix needs to be composed of a biocompatible polymer as coating agent for para- and superparamagnetic particles. Using this concept, associated with the knowledge of the magnetic properties of iron oxide nanoparticles, it is possible to develop new materials able to make possible monitoring the kinetics and biodistribution of the medication in the organism non-invasively using MRI as to promote a local drug delivery using the sensitive properties of the polymer and, thus, preserving most of the healthy tissues and cells.

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