WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 8, 341-366. Review Article ISSN 2277–7105

# A REVIEW ON MAGNETIC MICRO/NANOPARTICLES

**Gera. Latha*, P. Dinesh Kumar , Kodela. Gopi, Palla. Srikanth, Y. Kusumalatha, Gudeti. Veera Babu**

Gorantla, Guntur, A.P., India.

Article Received on
01 June 2017,

Revised on 22 June 2017,
Accepted on 12 July 2017

DOI:10.20959/wjpr20178-8889

***Corresponding Author**
**Gera. Latha**
Gorantla, Guntur, A.P., India.

## ABSTRACT

This review focuses on the recent advances in the preparation and application of magnetic micro/nanoparticles specially it covers (a) methods of preparation (such as by co precipitation, pyrolysis, hydrothermal, solvent thermal, sol-gel, micro-emulsion, sonochemical methods, and (b) application such as hyperthermic therapy, magnetic gene therapy, magnetic hyperthermia, diagnosis of cancer cells, magnetic separation, magnetorelaxometry, test of prothrombin time for leukemia, solid phase extraction, atherosclerosis imaging, nanomagnetic molecular sensing.

**KEYWORDS:** This review focuses on the recent advances in the preparation.

## INTRODUCTION

Magnetic micro/nanoparticles are generally ferromagnetic elemental, alloy, oxide, or composite structures of Fe-, Co-, and Ni. Based on the different properties, materials are divided into three types: paramagnetic, antimagnetic and ferromagnetic materials. Paramagnetic materials, which magnetization intensity is directly proportional to exterior field and their susceptibility are positive value. Antimagnetic materials, which magnetization intensity also is proportional to exterior magnetic field, but its susceptibility is negative value. The saturation phenomenon is the magnetization intensity of ferromagnetic materials increases with the strengthening of exterior magnetic field observably at the beginning, but when the exterior magnetic field intensity increases to a value, their magnetization intensity will not continue to increase. To provide magnetism in magnetic micro particles various inorganic materials included they are $Fe_3O_4$, $\gamma$-$Fe_2O_3$, $CoFe_2O_4$, $MnFe_2O_4$, $CoPt_3$ and FePt among which $Fe_3O_4$ nanoparticles are most commonly selected because of their easy availability and superparamagnetism. Ideal micro

particles can be easily trapped by an external magnet but appear individually dispersed when the magnetic field is removed.

Magnetic micro particles have good affinity.[1,2] and easy surface modification. by adsorption or covalent bonding micro particles can combine with the different functional groups and bioactive substances, such as enzymes, cells, antibodies, DNA and so on. It is easy for them to separate from medium under external magnetic field due to their good magnetic conductivity, so they are widely used in the area of cell separation.[3-6] biological detection.[7-12] enzyme immobilisation.[13,14] solid- phase extraction and targeted drug delivery.[15-19] magnetic micro particles have good chemical stability, biocompatibility and biodegradability, which makes them safe in the clinical applications, so they also are used for MRI.[20,22] and targeting drug delivery. Micro particles have broad application prospect for serious diseases, especially the premenstrual syndrome and effective treatment for cancer.

**Advantages of Magnetic Microspheres[23]**

1. Increased duration of action.
2. First pass effect can be avoided.
3. Protein and peptide drug delivery can be improved
4. They enable controlled release of drug. Ex: narcotic, antagonist, steroid hormones.
5. Increase the therapeutic effect and decrease the side effects.
6. 6. Toxicity is reduced.
7. Ability to bind and release high concentration of drugs.
8. Patient compliance is good.
9. Method of preparations is easy.
10. Can be injected into the body using hypodermic needle.
11. Microsphere morphology allows a controllable variability in degradation and drug release.[24]
12. Dosing frequency is reduced.[24]
13. Incorporation of magnetically responsive materials into microspheres makes them susceptible to applied magnetic field, so that they are concentrated to the target site by application of magnetic field externally to that site. Due to this, rapid clearance of these microspheres by RES is prevented.[25]
14. Difference occurs maximally in capillary network so efficient delivery of drug to diseased tissue is achieved.[25]

15. Microspheres can transit into extra vascular space creating an extra vascular depot of drug for sustained release of drug within the targeted areas.[25]

**Disadvantages of Magnetic Microspheres**[23]

1. Removal once injected is difficult.
2. Non-uniformity to drug content may result while preparation**.**
3. Unknown toxicity of beads.
4. It is an expensive technical approach and requires specialized manufacture and quality control system.
5. It needs specialized magnet for targeting, for monitoring, and trained personnel to perform procedures.
6. By the use of magnetic microspheres in the delivery system, the drug cannot be targeted to deep seated organs in the body.

**Methods for the preparation of magnetic micro/nano particles**

**Pyrolysis method**

In pyrolysis method magnetic micro/nanoparticles are prepared by the thermal decomposition of precursors such as metal compounds ( $Fe(acac)_3$, $Fe(CO)_5$, $Co_2(CO)_8$ and $Fe(Cup)_3$ (acac and Cup are acetyl acetone and cup ferrate respectively) at high temperature and high pressure. Then they are further oxidized to magnetic metal oxide nanoparticles. The method has advantages of high nanoparticle crystallinity, tunable size and narrow diameter distribution.[26] Under high temperature, Murray, Sun et al.[27] prepared ferromagnetic nanoparticles, whose size ranged from few nanometres to tens of nanometres, by refluxing ferric acetylacetonate, long chain alcohol, oleic acid, lauryl amine and so on, with diphenyl oxide as medium.

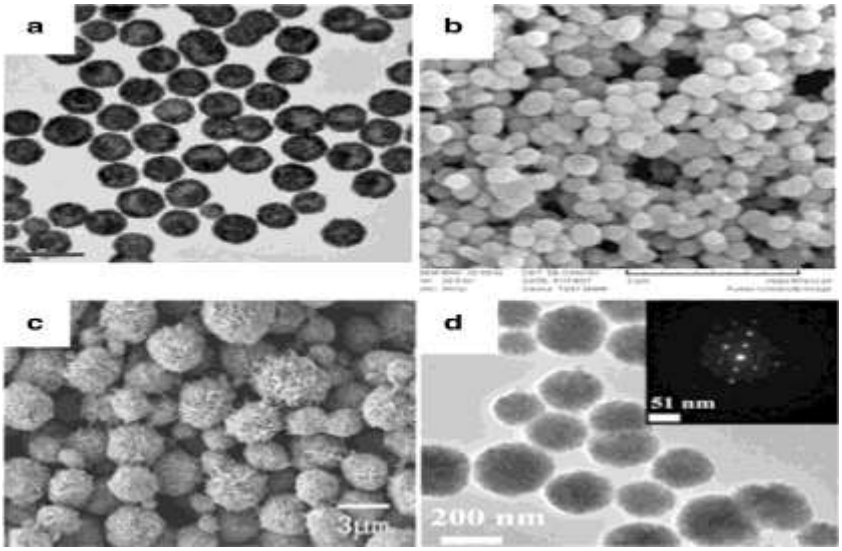


**Fig. 1: Small magnetic nanoparticles prepared by. (a) in situ solvothermal synthesis, (b) sol-gel method, (c) thermaldecomposition and (d) microemulsion progress.**

Reprinted with permissions (a,c) from.[26,29] copyright 2003, 2001 American Chemical Society; (b) from[28] copyright 2008 Elsevier; (d) from[30] copyright 2005 John Wiley and Sons

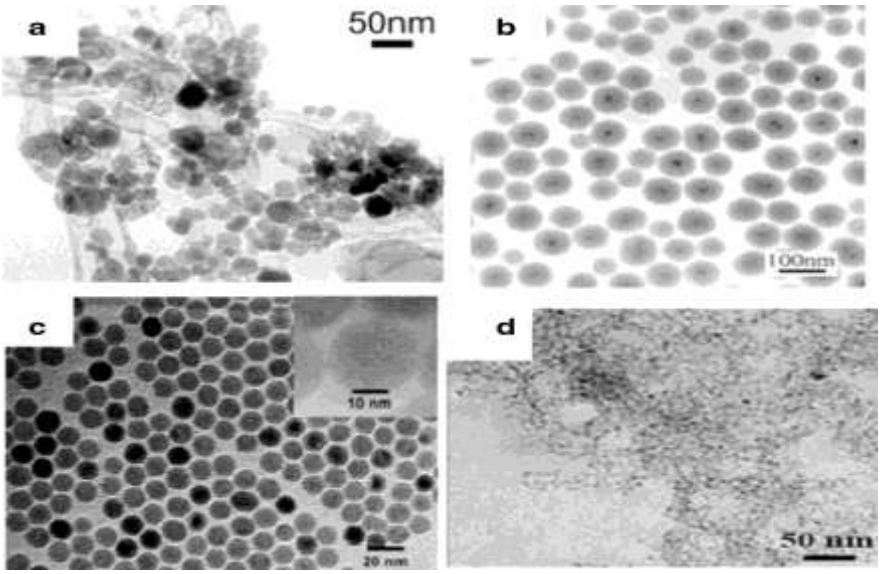


**Fig. 2: Large magnetic nanoparticles prepared by (a) one-pot hydrothermal process,(b) sol-gel method, (c) solvothermal method and subsequent calcining process and (d) self-assembly of nanocrystal in mixed solvents of ethylene glycol and water.**

Reprinted with permissions (a,c) from[26,29] copy right 2003, 2001 American Chemical Society; (b) from[28] copyright 2008 Elsevier; (d) from [3o] copy right 2005 John Wiley and Sons.

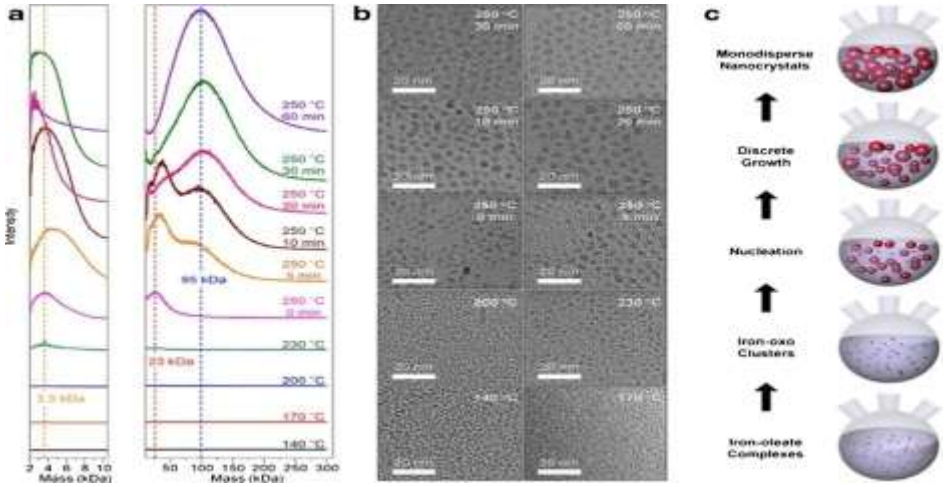


**Fig. 3a: Mass spectra of sample aliquots drawn from the reaction solution during heating. For clarity, the spectra in the mass ranges of <10.5 kDa (left) and >11kDa (right) have been plotted using different scales. b. TEM images of the NCs in the sample aliquots. C. Schematics describing the formation mechanism proposed on the basis of the ex situ MS measurements.**

Reprinted with the permissions (a,b,c,d) from[31,32,33,34] copy right 2015,2009,2013 Elsevier.

**Co precipitation method**

Co precipitation is a widely used simple and efficient method for the preparation of iron oxide (Fe3O4, Fe 2O3, etc.) and ferrite (Zn-Mn ferrite, NiZn ferrite, Co-Zn ferrite). In this method, to precipitate metal ions alkaline solution (ammonia, sodium, hydroxide solution) is added into metallic salt solution as precipitant. This method has the advantages of shorter process, simple reaction conditions and higher product purity. During washing, filtering and drying agglomeration phenomenon of product will happen easily. The co precipitation method is most widely used to prepare ferroferric oxide magnetic micro particles: Fe2þþ2Fe3þþ8OH−→Fe3O4 þ4H2O Fe2+ with Fe3+ were mixed up according to certain proportion, and then excess ammonia water or sodium hydroxide solution was added as precipitant, as a result Fe3O4 magnetic micro particles were made. Kim et al.[35] added sodium oleate as surfactant to disperse precipitates in the process of preparingFe3O4 magnetic micro particles by co precipitation method, thus getting monodisperse Fe3O4 magnetic micro particles. Thapa et al.[36] prepared Fe 3O4 magnetic micro particles by co precipitation method, and they found out that when the particle size was 10 nm, the magnetic micro particles had best magnetism. When preparing ferromagnetic micro particles, precursor powder was first prepared by co precipitation method, and then the final product was fabricated after high temperature solid state reaction.

**Hydro-thermal method**

The hydrothermal method is done under high temperature and pressure condition of autoclave, with water as reaction medium, those substances, which are usually sparingly soluble and insoluble, are dissolved and react, then recrystallize, thus getting ideal product. High temperature is used to improve magnetism, and high pressure is good to improve product purity. Hydrothermal method can control the size and shape of particles effectively, and particles seldom agglomerate. Besides, the particles have a good dispensability.[39] and uniform size distribution.[37] The hydrothermal method can make substances react in static or dynamic closed environment, and the latter can speed up synthetic rate greatly due to the magnetic stirrer in autoclave. Chen et al.[40] prepared Fe 3O4 magnetic micro particles with different diameters in hydrothermal reaction vessel. By two-step hydrothermal reactions Yao.[38] et al. prepared magnetic carbonaceous (MC) microparticles,

Fe3O4 micro particles assembled at low temperature by specific nanoparticles.[39] which are obtained via hydrothermal method and the products still have good dispersibility. Combined with other synthetic processes, core–shell structured magnetic composite micro particles with ordered hexagonal mesopores, bright luminescence, and high magnetization saturation value are prepared.[15] This multifunctional system shows positive sustained properties by the surface modification, which can be potentially used as targeted drug delivery system. More recently, Liu et al.[31] prepared Water-dispersible monodisperse hollow Fe3O4 micro particles via a one-pot hydrothermal process which exhibited superparamagnetic properties with high saturation magnetization value of about 76.7 emu·g−1 at room temperature, and whose bet surface area was 50.04 m 2· g−1. The microwave hydrothermal synthesis method combines traditional hydrothermal method with microwave field. With microwave field as heat source, the reaction makes place in special reactor, through which microwave field can pass.

**Solvent-thermal method**

The process of solvent-thermal method is similar to hydro-thermal method, except the solvent of the former is non-aqueous solution. By the solvo thermal method and subsequent calcining process Liang.[33] et al. firstly prepared a kind of magnetite micro particles with such high surface area of 82.7 m2·g−1. Recently, Lu et al.[34] synthesized sodium poly acrylate modified Fe3O4 magnetic microspheres (SPMFMs) with quasi-superparamagnetic behaviour and high saturation magnetization by a solvothermal method. The loading capacity of the SPMFMs for bovine haemoglobin was 95mg·$g^{-1}$ and 33% of the loaded bovine haemoglobin was released

in a period of 75 h. The SPMFMs after the adsorption of the organic pollutants can be recycled and reused effectively with a slightly reduced adsorption capacity. Jiang et al. fabricated magnetically separable BiOBr/CoFe2O4 micro particles assembled from nanoparticles by a facile solvothermal method at 160 °C for 12 h.[41] Zhu et al. reported an immobilization of Candida rugosa lipase (CRL) onto PAMAM- dendrimer grafted magnetic nanoparticles synthesized by a modified solvothermal reduction method. And this immobilized lipase exhibited excellent reusability.[42]

**Sol-gel method**

For the preparation of sol with metal organic compound solution or metal inorganic compound the sol-gel method is frequently performed, and then the sol is dehydrated under certain conditions s(heating) to get gel, at last nano-scale product is prepared with the gel after drying and roasting. This method has the advantages of mild reaction conditions, high product purity, accurate stoichiometry, simple process and a short reaction period, and usually used to prepare the core-shell SiO2 magnetic composites.[43,44,45] Liu[28] etal .prepared a new kind of magnetic luminescent nano composite (MLNC) particles by a combination of sol-gel process and electrostatic self-assembly techniques. Xu et al.[48] prepared Fe3O4 magnetic micro particles with different size after annealing treatment under the vacuum condition of 200 to400 degrees Celsius. Shao.[45] etal. first made a study on the separation of protein by submicron-size micro particles grafted with flexible polymer chains. Liu et al. successfully prepared Silica encapsulated core–shell structured carbonyl iron (CI) magnetic particles (CI@SiO2) via a facile sol–gel method based on the silane grafted CI particles, which shows enhanced property of heat-induced oxidation resistance with decreased particle density.[46] Xu etal. Successfully fabricated novel core–shell structured magnetic Fe3O4/silica nano composite with gridlock-copolymer grafted on their surface (Fe3O4@SiO2@MDN) by sol–gel method and a seeded aqueous-phase radical copolymerization approach.[47] It has the excellent characteristics of the strong magnetic responsivity, outstanding hydrophilicity and abundant π electron system.

**Micro-emulsion method**

For the preparation of magnetic micro particles the micro-emulsion method has been developed into an effective method. The micro-emulsions are transparent, isotropic and low viscosity thermodynamic stability system, which is made from oil (hydrocarbon), water (electrolyte aqueous solution) and surfactant (sometimes with alcohols as co surfactant.).

They are classified into water in oil type (W/O)and oil in water type (O/W). The droplet size of them is nanometre and the droplet separates from each other. The reaction space is limited to this micro-reactor – droplet. As an example, surfactant succinic acid-1-ethyl hexyl sodium sulfonate (AOT) dissolving in n hexane can become water in oil type reversed-phase micro emulsion system.[50] This method can make particles avoid agglomerating effectively, so it is easy to prepare magnetic micro particles with narrow particle size distribution, regular shape and good dispersion property.

Zhou et al.[49] prepared $Fe_3O_4$ magnetic micro particles with particle size of smaller than 10nm,which have high correctivity, in the O/W micro emulsion system with cyclohexyl amine as oil phase,$Fe_2SO_4$ and $Fe(NO_3)_3$ as aqueous solution. Chen et al.[51] prepared chitosan /montmorrillonite $Fe_3O_4$(CTS/MMT-$Fe_3O_4$) microparticles, a magnetically separable adsorbent, by micro emulsion process.

**7. Sonochemical method**

The ultrasonic vaporization bubble produced by ultrasonic wave.[52] can make local high temperature and high pressure environment come into being and has micro-jet with strong impulsive force, which promotes oxidation reaction, reduction reaction, decomposition reaction, hydrolysis reaction and so on for preparing micro/nanoparticles. Compared with conventional stirring technology, ultrasonic cavitational effect produces shear action on agglomeration, which is beneficial to form small particles, and it makes the uniform mixing of medium easier to be realized, avoiding inhomogeneous local concentration, improving reaction rate.

Vijayakumar et al.[53] prepared super paramagnetic $Fe_3O_4$ magnetic micro particles which is having particle size of 10 nm from acetic acid ferric salt solution at Atmosphere of 0.15M Pa in the high-intensity ultrasonic wave environment. In the presence of hydrophobic $Fe_3O_4$ nanoparticles and tetracycline Gedanken etal.[55] prepared Fe-Fe $_3$C magnetic micro particles with stability in air and controllable particle size by this method . Most recently, Wu et al.[54] prepared magnetic targeted antibiotic microspheres (MTAMs) with ultrafine size, high biocompatibility, biodegradability, controlled-release, and antibiotic effect by a sonochemical method.

**Applications of magnetic micro/nanoparticles**

**Diagnostic Application**

**Diagnosis of cancer cells**

Cancer is a difficult disease to treat and identify. There are many ways to treat cancer such as surgery, chemotherapy, radiation and many others. These methods are effective if the cancer tumour is caught soon enough. However, these methods are not effective enough because they are not only target the affected cells, but also affect healthy cells. For detecting cancer cells 'Nanotechnology' has found many new ways and how far the disease has spread throughout the body. A couple of these new cancer detecting nanoparticles are gold nanoparticles and magnetic iron oxide nanoparticles encased in a biocompatible material. Magnetic iron oxide nanoparticles encased in a biocompatible material can make detecting cancer cells easier, even if the cancer cells are small and clearer so there is less mistakes in the detecting process. The particles stick to the tumour cells turning them into little magnets which are then attracted to the tip of a biopsy needles.[56] MRI's can be used to distinguish malignant lymph nodes (which can help in telling how far cancer has spread) instead of using biopsies**.**

**Magnetic separation**

This includes techniques and devices for using SPIONs for use in targeting and magnetic extraction of cellular compounds and their chemical, optical, and physical analysis including proteomics. To separate cell from blood samples, bone marrow, tissue grinds or culture media this is a very convenient way. Magnetic separation involves no interference of iron or charged solvents in the sample under the static magnetic field. Advantage of magnetic separation is less damage to the separated cells. One way of separation of targeted cells is by normal phase separation. From the mixed solution the cells are separated directly. Another way is by negative phase separation. The unrelated cells from the mixed solutions are separated using an external magnetic field resulting in purified and concentrated targeted cells. For cell separation, a suspension of magnetic nanoparticles with specific antibody is added directly to a biological fluid sample. After 10-20 minutes of incubation the solution is placed in a magnetic separator, where the desired magnetically labelled cells are retained on the magnet while the supernatant is removed. Magnetic separation of cells finds application in clinical diagnosis. These magnetic methods have reached high efficiency levels for cell separation, as compared with electric and centrifugal, and also standard methods based on fluid-fluid interface separation. Magnetic separation has been successfully tested for precise

separation of specific cells in blood.[57] gram positive pathogens.[58] and protein purification.[59]

**Magnetorelaxometry**

For the evaluation of immunoassay Magnetorelaxometry was introduced.[60] It measures the magnetic viscosity, i.e., the relaxation of the magnetic moment of a system of magnetic nanoparticles after removal of a magnetic field. There are two different relaxation mechanisms.

1. Neel relaxation
2. Brownian relaxation

Neel relaxation is called the internal magnetisation vector of a nanoparticle relaxes in the direction of the easy axis inside the core.

Brownian relaxation is the particles accomplish rotational diffusion in a carrier liquid. Neel and Brownian relaxation can be distinguished by their different relaxation times.[61] Furthermore, Brownian relaxation can take place only in liquids, whereas Neel relaxation does not depend on the dispersion of the nanoparticles. Magnetorelaxometry depends on the core size, hydrodynamic size and anisot ropy allows this technique to distinguish between free and bound conjugates by their different magnetic behaviour, therefore can be used as an analytical tool for the evaluation of immunoassays.

**Solid-phase extraction**

Solid phase extraction (SPE) is a way to isolate and pre concentrate desired components from a sample matrix. SPE offers an excellent alternative to the conventional sample concentration methods, such as liquid –liquid extraction.[62] The separation and pre concentration of the substance from large volumes of solution can highly time consuming when using standard column SPE, and is in this field where the use of magnetic or magnetisable adsorbents called magnetic solid phase extraction (MSPE) gains importance. In this procedure, the magnetic adsorbent is added to a solution or suspension containing the target. This is adsorbed onto the magnetic adsorbent and then the adsorbent with the adsorbed target is recovered from the suspension using an appropriate magnetic separator. for separation and selection the advantage of using magnetic nanoparticles instead magnetic microparticles is that we can prepare suspension that are stable against sedimentation in absence of applied magnetic field.

**Atherosclerosis imaging**

Macrophages such as monocytes are in highly active state of phagocytosis, promote atherosclerosis by secreting mediators and secretion of cytokines and chemokines. Atherosclerosis lesions show accumulation of macrophages. Research on the use of SPIONs for the detection of atherosclerosis plaques by MRI showed as a potential marker of inflammation for plaque imaging. In this study, MAC-1 expressing Chinese Hamster Ovary (CHO) cells, expressing MAC-1 either in native, low affinity state (wild type, WT) or a high affinity state (GFFKR-deleted cells, DEL) were used to simulate the type of activated macrophages found in atherosclerotic plaques. CHO-cells not expressing MAC-1 (CD11b/CD18) as a mediator for the super paramagnetic iron oxide nanoparticles to endocytose into monocytes/ macrophages and showed that MAC-1 is also a central mediator of inflammation. Dextran –coated particles, which are mostly used as a contrast agents, are commonly attached to cells but not taken up by them, while SPIONs coated with amino functionalized polyvinyl alcohols interact with different cells.[63] and therefore underline a receptor based uptake of SPIONs by cells.

**Nanomagnetic molecular sensing**

Highly sensitive detection and accurate analysis of biomarker molecules in human samples are essential for the early detection, treatment, and management of diseases.[64] Biosensors are widely used in medicine to monitor or detect biological molecules for applications ranging from diabetes to cancer. In a bioisomer, a ligand and a receptor bind together in a reaction that is collected as a signal to a transductor using different methods, including optical, magnetic, electrochemical, radioactive, piezoelectric, mechanical, mass spectrometric, and so forth. Like any sensor, a biosensor should be cheap, compact, selective, portable, reusable, and have a fast readout. The introduction of nano particles in the molecular diagnosis field has represented an advantage in many cases to well established detection techniques based on fluorophores, such as Polymerase Chain Reaction (PCR) and Enzyme – Linked Immuno Sorbent Assay (ELISA). Nanopaticles offer their physical properties to the biosensor. In some cases, nanoparticles are used simply as carriers of antibodies to recognize them by association in biosensors. The absence of magnetic material in biological samples allows a controlled application of magnetic paramagnetic particles are therefore powerful because they can be easily manipulated and reliably detected inside complex biological fluids.

**Test of prothrombin time for leukemia**

The time of prothrombine (TP), and the values the derive from it, the International Normalilized Ratio (INR) are used for determining the tendency of the blood to coagulate in the presence of possible biological disorders like hepatic failure or K vitamin deficiency. A sensor, based on a magnetoelastic material, can be used to determine the TP and the INR in patients under anticoagulation treatment, without the need for specialized staff or installations. The method is based on the variation in the magnetic permeability of a magneto elastic microwire induced by change on the blood viscosity when it coagulates. When the blood coagulates, the viscosity force applied to the immersed wire dissipates, as heat, a portion of the magnetic energy supplied by the magnetic field. Therefore the apparent magnetic permeability of the microwire decreases due to magnetoelastic coupling. The sensor consists of two identical microwires, with an iron base, that are placed into two capillaries with tenths of mm. inner diameter and around 5cm in length. These capillaries are surrounded by one coil each, which are fed by two power amplifiers driven by a signal generator. The capillaries are filled with a fluid of reference, respectively. The difference between both signals increases when the blood coagulation process begins and its absolute value tends to a maximum when the blood coagulation process begins and its absolute value tends to a maximum when the blood is fully clotted. The experimental setup compares this permeability with that of a reference wire immersed in an inalterable fluid.[65] The absolute value of the measured signal tends to a maximum when the blood coagulates. The time to raise this maximum enables the TP and the INR to be calculated.

**Therapeutic Applications**

**Hyperthermic therapy**

Magnetic induced therapy also called as Magnetic Fluid Hyperthermia (MEH) was first reported by Gilchrist in 1957. The heat induced cell death with magnetic nanoparticles (MNPs) generates numerous cellular changes, leading to morphological changes, cell detachment, and death. Cellular alterations include changes in the membrane, nuclear and cytoskeletal structures, cellular metabolism, macromolecular synthesis etc. Its use is based on the fact that tumour cells are more sensitive to temperature in the range of 42-45°C which yields necrosis, coagulation, or carbonisation than normal tissue cells. This temperature range has become critical for cancer treatment due to damaging the cancerous cells without altering the healthy cells by selective heating (up to 45°C) and controlling heating rate and time. This process not only enhances the effectiveness of other cancer treatments, but it also kills tumor

cells that are resistant to other forms of cancer. Increased tumor tissue perfusion facilitates the absorption of chemotherapeutic drugs through cell membrane without being more toxic.[66,67] As a result, the action of combination of hyperthermia with radiotherapy becomes more efficient. Consequently, hyperthermia allows reducing of tumours resistant to various chemotherapeutic drugs such as doxorubicin, cisplatin, bleomycin, nitrosoureas, and cyclophosphamide. It has been demonstrated that hyperthermia also has an anti-angiogenic action and immunotherapeutic role, due to thermal shock proteins, which are produced by stressed tumour cells.[68,69] Thus, the magnetic materials with cutie temperature ~45°C, having sufficient compatibility are the best candidates for effective cancer hyperthermia treatment to avoid overheating. Because of unique capability of turning on and off the magnetic properties depending on temperature, the tumours will be continuously heated at a self-controlled temperature equal to the curie temperature of the magnetic nanoparticles. This approach will allow to heat the tumour cell and vasculature selectively and to prevent overheating with subsequent damage to neighbouring healthy tissues. Tumour cells have shown a greater sensitivity to heat treatments compared to healthy cells.[70] This has led to the use of thermo ablation and hyperthermic therapies in the clinic, often in combination with other treatments.

**Magnetic hyperthermia**

There are four different mechanisms by which magnetic materials can generate heat in an alternating field:[71]

a. Generation of eddy currents in magnetic particles with size >1 μ.
b. Hysteresis losses in magnetic particles >1 μ and multidomain magnetic particles.
c. Relaxation losses in ‘superparamagnetic’ single-domain magnetic particles.
d. Frictional losses in viscous suspensions.

Hyperthermia is classified into two categories; thermo- ablation and mild hyperthermia. In thermo-ablation cell necrosis is occurred when temperature rise exceeds 46°C.[72] Mild hyperthermia is where the temperature increases is between 41-46°C.[73] this temperature rise is high enough to cause partial cell kill and to damage and sensitize cancer cells to chemotherapy and radiotherapy.[74] Studies have shown to cause increase blood flow into radio-resistant, hypoxic, low pH areas, causing oxygenation of the tissues, in turn increasing the cells radio-sensitivity.[75] Furthermore, thermal treatment can enhance the toxicity of chemotherapy. By exposing magnetic nanoparticles to an Altering Magnetic Field (AMF),

energy is created in the form of heat, this thermal energy dissipates into the surroundings tissues and if the temperature is high enough can destroy or weaken cancerous cells. Using MNPs to generate heat can specific tissue heating, as the application of the alternating magnetic field can be localised to the area of the body where the MNPs have collected. Through varying the frequency, current parameter of the AMF and the size and composition of the MNPs, the temperature generated from the MNPs can be controlled. MNPs are thought to generate heat through Neel relaxation and Brownian motion. In the case of Neel relaxation, the AMF causes the magnetic moments within the MNPs to rotate, generating internal friction. When the field is off the moments return to equilibrium, this is where energy is released in the form of heat.[76,77] Brownian motion requires the rotation of the MNPs as a whole therefore; the heat is generated through frictional movements in its surroundings. However, MNPs can become trapped within biological tissues, this in-turn blocks free rotation of the particles, preventing the generation of frictional heat.[78]

**Magnetic Gene Therapy**

Gene therapy is technique that uses genes to treat or prevent diseases. The most common form of gene therapy involves inserting a normal gene to replace an abnormal gene. Other approaches including swapping an abnormal gene for a normal one, repairing an abnormal gene or altering the degree to which a gene is turned on or off.

There are two types of gene therapy:

1. Somatic gene therapy and
2. Germ line gene therapy

In somatic gene therapy, the therapeutic genes are transferred into the somatic cells, or body of a patient. Any modifications will not be inherited by the off springs of patients or later generation.

In germ line gene therapy, germ cells are sperm or eggs modified by the introduction of functional genes. This are inherited and passed on to later generation. There are three primary gene delivery systems that employ viral vectors (retroviruses and adenovir uses), nucleic acid electroporation, and snucleic acid transfection.[79] Gene delivery by viral vectors can be highly efficient (80-90%) but may insert viral vector nucleic acid sequences into the host genome, causing inappropriate expression of deleterious genes. Electroporation is also a highly efficient technique for introducing foreign genes into a host (50-70%); however, half of the recipient cells die due to the electrical stimulation. Transfection reagents do not

efficiently deliver nucleic acids into cells (20-30%); however, cell viability is largely preserved and the method is safe enough for clinical use. Therefore, this method holds relatively more promise for medical applications, provided that its efficiency can be improved. MNPs are already in use by basic researchers to increase transfection efficiencies of cultured cells.[80]

**Characterization Properties of Microspheres**[81-91]

1] Particle size analysis

2] Scanning electron microscopy (SEM) study

3] Flow properties

4] Thermal analysis

5] Determination of percentage yield

6] Drug content

7] Determination of drug loading

8] Incorporation efficiency of microspheres

9] Determination of solubility

10] Dissolution studies of microspheres

**1] Particle size analysis**

Microscopic method is used for detecting particle size of recrystallized sample, pure samples, spays dried microspheres A microscopically image analysis techsnique for determination of particle size was applied. The morphology and particle sizes were determined in a Digital microscope equipped with a 1/3”CCD camera imaging accessory. The microspheres were dispersed on a microscope slide. A microscopically field was scanned by video camera. The images of the scanned field are analyzed by the software.

**2] Scanning electron microscopy (SEM) study**

Using scanning election microscopy the morphology of microspheres was examined. A small amount of powder was spread on an aluminium stub, which was placed latter gold sputtering in san SEM chamber. Photographs are taken at an acceleration voltages of 20 KV electron beam. Obtained photograph to identify and confirm spherical nature and Surface topography of the crystals.

**3] Flow properties**

Flow properties of the microspheres were evaluated by determining the angle of repose and the compressibility index.

**Angle of repose of microsphere**

fixed funnel method is used for measuring angle of repose of microspheres and commercial crystals. Static angle of repose was measured according to the fixed funnel and free standing cone method of Banker and Anderson.

A funnel with the end of the stem cut perpendicular to the axis of symmetry is secured with its tip at a given height (1 cm), H, above graph paper placed on a flat horizontal surface. The microspheres were carefully poured through the funnel until the apex of the conical pile so formed just reached the tip of the funnel. Thus, the R being the radius of the base of the microspheres conical pile:

$\tan \theta = H/ R$

or

$\theta = \tan^{-1} (H/ R)$ Where

$\theta$ = angle of repose

**Compressibility index (I)**

Carr's index was determined from powder volumes at the initial stage and after 1250 tapings to constant volume. Compressibility index (I) values of the microspheres are determined by measuring the initial volume (V0) and the final volume (V) after subjecting to 100 stapping in a graduated measuring cylinder using the equation.

$$I = [1- (V/V0)] \times 100$$

A Pycnometer is used for measuring apparent particle densities of microsphere

4**] Thermal analysis**

During the crystallization process Differential scanning calorimeter (DSC) study was carried out to detect possible polymorphic transition. DSC measurements were performed on differential scanning calorimeter (DSC DuPont 9900) with a thermal analyzer. Differential scanning calorimeter (DSC) was performed on ketoprofen and ketoprofen loaded microspheres. DSC measurement were done on a Mettle Toledo DSC 822c C/ min over a

temperature range of 30 to 30000 C under an inert atmosphere flushed with nitrogen at a rate of 20 ml/min.

**5] Determination of percentage yield**

The percentage yield of microspheres was determined by the following formula,

$$\%yield = \frac{\text{Total Weight of Microspheres}}{\text{Total Weight of Raw Material}} \times 100$$

The percentage yield of each formulation was determined according to the total recoverable final weight of microsphere and the total original weight of Indomethacin.

**6] Drug content**

Microspheres in a particular quantity were dissolving in a solvent and at specified λ max of drug the drugcontent of microspheres is estimated. 10 ml of water is added to microspheres (50 mg) and triturate. Allowed to stand for 10 min with occasional swirling and methanol was added to produce 100 ml. After suitable dilution, samples are measured at particular λ max value of drug. Plot a standard graph. And drug content was determined from standard plot.

**7] Determination of drug loading**

UV-Visible spectrophotometer is used to determine the drug loading. The microspheres were stirred with 100 ml particular solution as dissolution media (pH 7.40 phosphate buffer) for 2hr. The drug concentration will be determined at particular λ max value of drug after suitable dilution. The readings were taken in triplicate.

$$\text{Drug loading (\%)} = \frac{\text{M actual}}{\text{Weighed quantity of powder of micros}} \times 100$$

**8] Incorporation efficiency of microspheres**

$$\text{Incorporation efficiency (\%)} = \frac{\text{M actual}}{\text{M theoretical}} \times 100$$

Where,

M actual is the actual drug content in weighed quantity of powder of microspheres &

M theoretical is the theoretical amount of drug in microspheres calculated from the quantity added in the fabrication process.

**9] Determination of solubility**

The solubility of particular drug microspheres in specific solution as microspheres or microcapsule to be soluble in that particular environment (water and pH 7.4 phosphate buffers) is determined by taking excess quantity of microspheres in 50 ml to screw-capped glass vials filled with water. The vials were shaken for two hours on mechanical shaker. The solution is filtered through Whatman filter paper No.1 and drug concentration is determined at particular λ max value of drug.

**10]. Dissolution studies of microspheres**

USP dissolution apparatus XXIV Type II is used for the dissolution of microspheres. 900 ml 7.4 Phosphate buffer is used as dissolution medium. The amount of dissolved drug was determined using UV spectrophotometric method at specified λ max of particular drug. The readings were taken in triplicate.

**Summery and conclusion**

This paper has provided an overview of the synthetic approaches and highlighted applications of micro/nanoparticles.

**REFERENCES**

1. 1.Sun Y, Wang B, Wang H, Jiang J Controllable preparation of magnetic polymer microspheres with different morphologies by miniemulsion polymerization. J Colloid Interface Sci., 2007; 308(2): 332–336.
2. 2.Gao Z, Zhang Q, Cao Y, Pan P, Bai F, Bai G Preparation of novel magnetic cellulose microspheres via cellulose binding domain-streptavidin linkage and use for mRNA isolation from eukaryotic cells and tissues. J Chromatogr A., 2009; 1216(45): 7670–7676.
3. 3.Chen W, Shen H, Li X, Jia N, Xu J Synthesis of immunomagnetic nanoparticles and their application in the sepa- ration and purification of CD34(+) hematopoietic stem cells. Appl Surf Sci., 2006; 253(4): 1762–1769.
4. Fegan C, Poynton CH, Whittaker JA The gut mucosal barrier in bone marrow transplantation. Bone Marrow Transplant, 1990; 5(6): 373–377.
5. Chung T-H, Chang J-Y, Lee W-C Application of magnetic poly (styrene-glycidyl methacrylate) microspheres for immunomagnetic separation of bone marrow cells. J Magn Magn Mater, 2009; 321(10): 1635–1638.

6. Hallier-Soulier S, Guillot E An immunomagnetic separa- tion polymerase chain reaction assay for rapid and ultra-sensitive detection of Cryptosporidium parvum in drinking water. FEMS Microbiol Lett, 1999; 176(2): 285–289.
7. Perez JM, Simeone FJ, Saeki Y, Josephson L, Weissleder R Viral-induced self-assembly of magnetic nanoparticles allows the detection of viral particles in biological media. J Am Chem Soc, 2003; 125(34): 10192–10193.
8. Chen A-Z, Lin X-F, Wang S-B, Li L, Liu Y-G, Ye L, Wang G-Y Biological evaluation of Fe3O4-poly(L-lactide)-poly(eth- ylene glycol)-poly(L-lactide) magnetic microspheres prepared in supercritical CO2. Toxicol Lett, 2012; 212(1): 75–82.
9. 9.Chen X, Ding N, Zang H, Yeung H, Zhao R-S, Cheng C, Liu J, Chan TWD Fe3O4@MOF core-shell magnetic micro- spheres for magnetic solid-phase extraction of polychlorinated biphenyls from environmental water samples. J Chromatogr A, 2013; 1304: 241–245.
10. Candido RRF, Favero V, Duke M, Karl S, Gutierrez L, Woodward RC, Graeff-Teixeira C, Jones MK, St Pierre TG The affin- ity of magnetic microspheres for Schistosoma eggs. Int J Parasitol, 2015; 45(1): 43–50.
11. de la Escosura-Muniz A, Plichta Z, Horak D, Merkoci A Alzheimer's disease biomarkers detection in human samples by efficient capturing through porous magnetic microspheres and la- belling with electrocatalytic gold nanoparticles. Biosens Bioelectron, 2015; 67: 162–169.
12. Demirel D, Ozdural AR, Mutlu M Preparation and characterization of magnetic duolite-polystyrene composite particles for enzyme immobilization. J Food Eng, 2004; 62(3): 203–208.
13. Lei H, Wang W, Chen LL, Li XC, Yi B, Deng L The preparation and catalytically active characterization of papain immobilized on magnetic composite microspheres. Enzym Microb Technol, 2004; 35(1): 15–21.
14. Liang HF, Yang TF, Huang CT, Chen MC, Sung HW Preparation of nanoparticles composed of poly (gamma-glutamic acid)-poly(lactide) block copolymers and evaluation of their up- take by HepG2 cells. J Control Release, 2005; 105(3): 213–225.
15. Yang P, Quan Z, Hou Z, Li C, Kang X, Cheng Z, Lin J A magnetic, luminescent and mesoporous core-shell structured com- posite material as drug carrier. Biomaterials, 2009; 30(27): 4786–4795.
16. Hafeli U, Pauer G, Failing S, Tapolsky G Radiolabeling of magnetic particles with rhenm-188 for cancer therapy J Magn Mater, 2001; 225(1-2): 73-78.

17. Lubbe AS, Bergemann C, Riess H, Schriever F, Reichardt P, Possinger K, Matthias M, Dorken B, Herrmann F, Gurtler R, Hohenberger P, Haas N, Sohr R, Sander B, Lemke AJ, Ohlendorf D, Huhnt W, Huhn D Clinical experiences with magnetic drag targeting: a phase I study with 4'-epidoxorubicin in 14 patients with advanced solid tumors. Cancer Res, 1996; 56(20): 4686–4693.
18. Ren J, Hong H, Ren T, Teng X Preparation and character- ization of magnetic PLA-PEG composite nanoparticles for drug targeting. React Funct Polym, 2006; 66(9): 944–951.
19. Fahlvik AK, Holtz E, Klaveness J Relaxation efficacy of paramagnetic and superparamagnetic microspheres in liver and spleen. Magn Reson Imaging, 1990; 8(4): 363–369.
20. Gellissen J, Axmann C, Prescher A, Bohndorf K, Lodemann KP Extra- and intracellular accumulation of ultrasmall superparamagnetic iron oxides (USPIO) in experimentally induced abscesses of the peripheral soft tissues and their effects on magnetic resonance imaging. Magn Reson Imaging, 1999; 17(4): 557–567.
21. Muhler A, Zhang X, Wang H, Lawaczeck R, Weinmann HJ Investigation of mechanisms influencing the accumulation of ultrasmall superparamagnetic iron oxide particles in lymph nodes. Investig Radiol, 1995; 30(2): 98–103.
22. Moghimi SM, Hunter AC, Murray JC Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol Rev, 2001; 53(2): 283–318.
23. Vimal M, Amareshwar P, Hemamalini K, Sreenivas K et al. Preparation and evaluation of Diclofenac sodium loaded Ethyl cellulose composite magnetic microspheres. Int J Pharm Analysis, 2009; 1(2): 40-45.
24. Kunchu K, Raje VA, Ganesh NS Albumin Microspheres: AUnique system as drug deliverycarriers for non-steroidal antiinflammatory drugs (NSAIDs), 2010; 5(2): 5:12.9.
25. Vimal M, Amareshwar P, Hemamalini K, Sreenivas K. Preparation and evaluation of Diclofenac sodium loaded Ethyl cellulose composite magnetic microspheres. Int J Pharm Analysis, 2009; 1(2): 40-45.
26. Hyeon T, Lee SS, Park J, Chung Y, Bin Na H Synthesis of highly crystalline and monodisperse maghemite nanocrystallites with- out a size-selection process. J Am Chem Soc, 2001; 123(51): 12798–12801.
27. Sun SH, Murray CB Synthesis of monodisperse cobalt nanocrystals and their assembly into magnetic superlattices (invit- ed). J Appl Phys, 1999; 85(8): 4325–4330.
28. Liu B, Xie W, Wang D, Huang W, Yu M, Yao A Preparation and characterization of magnetic luminescent nano- composite particles. Mater Lett, 2008; 62(17–18): 3014–3017.

29. Jiang LQ, Gao L Carbon nanotubes-magnetite nanocompos- ites from solvothermal processes: formation, characterization, and en- hanced electrical properties. Chem Mater, 2003; 15(14): 2848–2853.
30. Lee Y, Lee J, Bae CJ, Park JG, Noh HJ, Park JH, Hyeon T Large-scale synthesis of uniform and crystalline magnetite nano- particles using reverse micelles as nanoreactors under reflux con- ditions. Adv Funct Mater, 2005; 15(3): 503–509.
31. Liu Y, Li C, Zhang H, Fan X, Liu Y, Zhang Q One-pot hydrothermal synthesis of highly monodisperse water-dispersible hollow magnetic microspheres and construction of photonic crystals. Chem Eng J, 2015; 259: 779–786.
32. Shao D, Xu K, Song X, Hu J, Yang W, Wang C Effective adsorption and separation of lysozyme with PAA-modified Fe3O4@silica core/shell microspheres. J Colloid Interface Sci, 2009; 336(2): 526–532.
33. Liang X, Xi B, Xiong S, Zhu Y, Xue F, Qian Y Porous soft magnetic material: the maghemite microsphere with hierarchical nanoarchitecture and its application in water purification. Mater Res Bull, 2009; 44(12): 2233–2239.
34. Lu B-Q, Zhu Y-J, Zhao X-Y, Cheng G-F, Ruan Y-J Sodium polyacrylate modified Fe3O4 magnetic microspheres formed by self-assembly of nanocrystals and their applications. Mater Res Bull, 2013; 48(2): 895–900.
35. Kim DK, Zhang Y, Voit W, Rao KV, Muhammed M S ynthesis and characterizatio n o f s urfac tant-coated superparamagnetic monodispersed iron oxide nanoparticles. J Magn Magn Mater, 2001; 225(1–2): 30–36.
36. Thapa D, Palkar VR, Kurup MB, Malik SK Properties of magnetite nanoparticles synthesized through a novel chemical route. Mater Lett, 2004; 58(21): 2692–2694.
37. Mishra D, Anand S, Panda RK, Das RP Studies on char- acterization, microstructures and magnetic properties of nano-size barium he xa-ferrite p r ep ared through a hydrothermal precipitation-calcination route. Mater Chem Phys, 2004; 86(1): 132–136.
38. Yao G, Qi D, Deng C, Zhang X Functionalized magnetic carbonaceous microspheres for trypsin immobilization and the application to fast proteolysis. J Chromatogr A, 2008; 1215(1–2): 82–91.
39. Lv Y, Wang H, Wang X, Bai J Synthesis, characterization and growing mechanism of monodisperse Fe3O4 microspheres. J Cryst Growth, 2009; 311(13): 3445–3450.
40. Chen D, Xu R Hydrothermal synthesis and characterization of nanocrystalline Fe3O4 powders. Mater Res Bull, 1998; 33(7): 1015–1021.

41. Jiang R, Zhu HY, Li JB, Fu FQ, Yao J, Jiang ST, Zeng GM Fabrication of novel magnetically separable BiOBr/CoFe2O4 mi- crospheres and its application in the efficient removal of dye from aqueous phase by an environment-friendly and economical ap- proach. Appl Surf Sci, 2016; 364: 604–612.
42. Zhu W, Zhang Y, Hou C, Pan D, He J, Zhu H Covalent immobilization of lipases on monodisperse magnetic microspheres modified with PAMAM-dendrimer. J Nanopart Res, 2016; 18(2): 32.
43. Lou MY, Wang DP, Huang WH, Chen D, Liu B Effect of silane-coupling agents on synthesis and character of core-shell SiO2 magnetic microspheres. J Magn Magn Mater, 2006; 305(1): 83–90.
44. Xu H, Tong N, Cui L, Lu Y, Gu H Preparation of hydro- philic magnetic nanospheres with high saturation magnetization. J Magn Magn Mater, 2007; 311(1): 125–130,
45. Shao D, Xu K, Song X, Hu J, Yang W, Wang C Effective adsorption and separation of lysozyme with PAA-modified Fe3O4@silica core/shell microspheres. J Colloid Interface Sci, 2009; 336(2): 526–532.
46. Liu YD, Choi HJ, Choi S-B Controllable fabrication of silica encapsulated soft magnetic microspheres with enhanced oxidation-resistance and their rheology under magnetic field. Colloids Surf A Physicochem Eng Asp, 2012; 403: 133–138.
47. Xu M, Liu M, Sun M, Chen K, Cao X, Hu Y Magnetic solid-phase extraction of phthalate esters (PAEs) in apparel textile by core shell structured Fe3O4@silica@triblock-copolymer mag- netic microspheres. Talanta, 2016; 150: 125–134.
48. Xu J, Yang H, Fu W, Du K, Sui Y, Chen J, Zeng Y, Li M, Zou G Preparation and magnetic properties of magnetite nanopar- ticles by sol-gel method. J Magn Magn Mater, 2007; 309(2): 307–311,
49. Zhou ZH, Wang J, Liu X, Chan HSO Synthesis of Fe3O4 nanoparticles from emulsions. J Mater Chem, 2001; 11(6): 1704–1709.
50. Gupta AK, Gupta M Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials, 2005; 26(18): 3995–4021.
51. Chen D, Li W, Wu Y, Zhu Q, Lu Z, Du G Preparation and characterization of chitosan/montmorillonite magnetic micro- spheres and its application for the removal of Cr (VI). Chem Eng J, 2013; 221: 8–15.
52. Gedanken A Using sonochemistry for the fabrication of nanomaterials. Ultrason Sonochem, 2004; 11(2): 47–55.

53. Vijayakumar R, Koltypin Y, Felner I, Gedanken A Sonochemical synthesis and characterization of pure nanometer- sized Fe3O4 particles. Mat Sci Eng A-Struct, 2000; 286(1): 101–105.
54. Wu S, Jiang W, Zhang X, Sun H, Zhang W, Dai J, Liu L, Chen X, Li F A sonochemical route for the encapsulation of drug in magnetic microspheres. J Magn Magn Mater, 2012; 324(2): 124–127.
55. Sivakumar M, Gedanken A, Zhong W, Du YW, Bhattacharya D, Yeshurun Y, Felner I Nanophase formation of strontium hexaferrite fine powder by the sonochemical method using Fe(CO)(5). J Magn Magn Mater, 2004; 268(1–2): 95–104.
56. Charles SW, Popplewell J Properties and applications of magnetic liquid. Hand Book of Magnetic Materials, 1986; 2: 153.
57. Toner M, Irimia D Blood-on-a-chip. Annu Rev Biomed Eng, 2005; 7: 77-103. (http://www.ncbi.nlm.nih.gov/pubmed/16004567).
58. Lin YS, Tsai PJ, Weng MF, Chen YC Affinity capture using vancomycin-bound magnetic nanoparticles for the MALDI-MS analysis of bacteria. Anal Chem, 2005; 77: 1753-1760. (http://www.ncbi.nlm.nih.gov/pubmed/15762582).
59. Franzreb M, Siemann-Herzberg M, Hobley TJ, Thomas OR Protein purification using magnetic adsorbent particles. Appl Microbiol Biotechnol, 2006; 70: 505-516. (http://www.ncbi.nlm.nih.gov/pubmed/16496138).
60. Weitschies W, Kotitz R, Bunte T and Trahms L Determination of relaxing or remanent nanoparticle magnetization provides a novel binding- specific technique for the evaluation of immunoassays. Pharm Pharmacol Lett, 1997; 75.
61. Kotitz R, Weitschies W, Trahms L, Brewer W, Semmler W Determination of the binding reaction between avidin and biotin by relaxation measurements of magnetic nanoparticles. J Magn Magn Mater, 1999; 194: 62-68. (http://www.sciencedirect.com/science/article/pii/S0304885398005800).
62. Safarikova M, Safarik I Magnetic solid-phase extraction. J Magn Magn Mater, 1999; 1: 94-108.
63. Steitz B, Hofmann H, Kamau SW, Hassa PO, Hottiger MO, et al. Characterization of PEI-coated superparamagnetic iron Oxide nanoparticles for transfection: Size distribution, colloidal properties and DNA interaction. J Magn Magn Mater, 2007; 311: 300-305. (http://infoscience.epfl.ch/record/109740).

64. Liu X, Dai Q, Austin L, Coutts J, Knowles G, et al. A one-step homogeneous immunoassay for cancer biomarker detection using gold nanoparticle probes coupled with dynamic light scattering. J Am Chem Soc, 2008; 130: 2780-2782. (http://www.ncbi.nlm.nih.gov/pubmed/18257576).
65. Dobrovol'skii NA, Kostritso PR, Labinskaia TA, Makarov VV, Parfenov AS, et al. Blood coagulation Analyzer. Med Tekh, 1999; 40-42. (http://www.ncbi.nlm.nih.gov/pubmed/10198897).
66. Kampinga HH Cell biological effects of hyperthermia alone or combined with radiation or drugs: a short introduction to newcomers in the field. Int J. Hyperthermia, 2006; 22: 191-196. (http://www.ncbi.nlm.nih.gov/pubmed/16754338).
67. Dayanc BE, Beachy SH, Ostberg JR, Repasky EA Dissecting the role of hyperthermia in natural killer cell mediated anti-tumor responses. Int J Hyperthermia, 2008; 24: 41-56. (http://www.ncbi.nlm.nih.gov/pubmed/18214768).
68. van der Heijden AG, Kiemeney LA, Gofrit ON, Nativ O, Sidi A, et al. Preliminary European results of local microwave hyperthermia and chemotherapy treatment in intermediate or high risk superficial transitional cell carcinoma of the bladder. Eur Urol, 2004; 46: 65-71. (http://www.ncbi.nlm.nih.gov/pubmed/15183549).
69. Nakano H, Kurihara K, Okamoto M, Toné S, Shinohara K Heat-induced apoptosis and p53 in cultured mammalian cells. Int J Radiat Biol., 1997; 71: 519-529. (http://www.ncbi.nlm.nih.gov/pubmed/9191897).
70. Ito A, Saito H, Mitobe K, Minamiya Y, Takahashi N, et al. Inhibition of heat shock protein 90 sensitizes melanoma cells to thermosensitive ferromagnetic particle- mediated hyperthermia with low Curie temperature. Cancer Sci, 2009; 100: 558-564. (http://www.ncbi.nlm.nih.gov/pubmed/19154416).
71. Van der Zee J Heating the patient: a promising approach? Ann Oncol, 2002; 13: 1173-1184. (http://www.ncbi.nlm.nih.gov/pubmed/12181239).
72. Nedelcu G() Magnetic nanoparticles impact on tumoural cells in the treatment by magnetic fluid hyperthermia. Digest J Nanomat Biost, 2008; 3: 103-107.

(http://www.researchgate.net/publication/237418737_magnetic_nanoparticles_ipact_on_tumoral_cells_in_the_treatment_by_magnetic_fluid_hypertherm.

73. Jordan A, Scholz R, Wust P, Schirra H, Schiestel T, et al. Endocytosis of dextran and silan-coated magnetite nanoparticles and the effect of intracellular hyperthermia on human mammary carcinoma cells in vitro. J Magn Magn Mater, 1999; 194: 185-196.
74. Lao LL, Ramanujan RV Magnetic and hydrogel composite materials for hyperthermia applications. J Mater Sci Mater Med, 2004; 15: 1061-1064. (http://www.ncbi.nlm.nih.gov/pubmed/15516865).
75. Okayama T, Kokura S, Ishikawa T, Adachi S, Hattori T, et al. Antitumor effect of pretreatment for colon cancer cells with hyperthermia plus geranylgeranylacetone in experimental metastasis models and a subcutaneous tumor model of colon cancer in mice. Int J Hyperthermia, 2009; 25: 141-149. (http://www.ncbi.nlm.nih.gov/pubmed/19337914).
76. Griffin RJ, Corry PM Commentary on classic paper in hyperthermic oncology 'Tumour oxygenation is increased by hyperthermia at mild temperatures' by CW Song et al., 1996. Int J Hyperthermia, 2009; 25: 96-98. (http://www.ncbi.nlm.nih.gov/pubmed/19337909).
77. Maier-Hauff K, Rothe R, Scholz R, Gneveckow U, Wust P, et al. Intracranial thermotherapy using magnetic nanoparticles combined with external beam radiotherapy: results of a feasibility study on patients with glioblastoma multiforme. J Neurooncol, 2007; 81: 53-60. (http://www.ncbi.nlm.nih.gov/pubmed/16773216).
78. Pankhurst QA, Connolly J, Jones SK, Dobson J Application of magnetic nanoparticles in biomedicine. J Phys D Appl Phys, 2003; 36: R167-R181. (http://iopscience.iop.org/0022-3727/36/13/201/).
79. Si HY, Li DP, Wang TM, Zhang HL, Ren FY, et al. Improving the anti-tumor effect of genistein with a biocompatible superparamagnetic drug delivery system. J Nanosci Nanotechnol, 2010; 10: 2325-2331. (http://www.ncbi.nlm.nih.gov/pubmed/20355429).
80. Strachnan T, Read AP Human Molecular Genetics 3rd Edition, Garland Publishing, 2004; 616.
81. Kreuter J, Nefzger M, Liehl E, CzokR, Andb Voges R. Investigations on the toxicity of nanoparticles. J Pharm sci, 1983; 72: 1146.

82. Bolster D R, S J Crozier, S R Kimball, L S Jefferson. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. J Biol Chem, 2002; 277(27): 23977-23980.
83. Boonstra A, C Asselin-Paturel M, Gilliet C, Crain G. Trinchieri Y-J, Liu A. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: Dependency on antigen dose and differential toll-like receptor ligation. J Exp Med, 2003; 197(1): 101-109.
84. Collins, A.E and Deasy, Bioadhesive lozenge for the improved delivery of cetypyridinium chloride. J pharm sci, 1990; 79(2): 116-120.
85. Save T. and Venkatachalam P. Bioadhesive tablets of Nifedipine: Standardization of a novel buccoadhesive erodible carrier. Drug Dev Ind pharm, 1994; 20(19): 3005-3014.
86. Dev rajan, Gupta S, Gandhi A.S, Niphadkar P V and Shah. Trans mucosal drug delivery systems of Salbutamol Sulfate. 26th edition. Int Symp Control Rel Bioact Mater 1999; 650. 23. Lopez C R, Portero A, Vila-Jato, J C and Alonso, M J. Design and Evaluation of Chitosan/Ethyl cellulose mucoadhesive bilayered devices for buccal drug delivery. J control Rel, 1998; 55: 143-152.
87. Parodi, B Russo, E Caviglioli, G Cafaggi, and Binardi G. Development & characterization of a buccoadhesive dosage form of Oxydodone hydrochloride. Drug Dev Ind Pharm, 1996; 22(5): 445-450.
88. Chien, Y W, Combo, D C and Live, J C. Mucosal delivery of progestational Steroids from a Controlled release device: in vitro/in vivo relationship. Drug Dev Ind pharm, 1991; 17(17): 2269-2290.
89. Cassidy J P, Landcert N M, and Quardos E. controlled buccal delivery of buprenorphine. J control Rel, 1993; 25: 21-29.
90. Nokhodchi A, Javadzadeh Y, Siahi-Shadbad M R, Barzegar-Jalali M. The effect of type and concentration of vehicles on the dissolution rate of a poorly soluble drug (indomethacin) from liquisolid compacts. J. Pharm Sci, 2005; 8(1): 18-25.
91. Funden berg H H, Stites D P, Caldwel J L and Wells J V. In: Basic and clinical immunology. 2nd edition. Lange Medical, Los Altosca, 1978.