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Review on Magnetic Microsphere

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Abstract: Recently a number of novel drug delivery systems have emerged to minimize drug degradation and loss, to prevent harmful side-effects, to increase drug bioavailability and to achieve controlled and targeted drug delivery. Magnetic microsphere is one of the newer approach in pharmaceutical field due to their biocompatibility, easy of surface modification and magnetic properties. The magnetic properties of these particles add a new dimension where they can be manipulated upon application of an external magnetic field. This property opens up new applications where drugs that are attached to a magnetic particle to deliver the drug at a rate directed by the needs of the body during the period of treatment and target the activity entity to the site of action in the body using a magnetic field. Magnetic microsphere is prepared by various techniques and has various applications in diagnosis and treatment of various diseases. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Its use is limited by toxicity and side effects. This review gives an overview of the benefits, drawbacks, limitations, preparation, characterization and biomedical applications of magnetic microsphere.

Key words: Magnetic Microsphere · Magnetic targeting · Magnetic Properties · Biomedical Application

INTRODUCTON

Microsphere is a term used for small spherical particles, with diameters in the micrometer range (typically 1 µm to 1000 µm (1 mm)). Microspheres are sometimes referred to as microparticles. These are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 µm [1,2]. Microsphere are classed into different types such as bio-adhesive microsphere, magneticss microsphere, floating microsphere, radioactive microsphere, mucoadhesive microsphere etc., Among this magnetic microsphere plays very important role. Because magnetically targeted drug delivery system will be a promising way, which involves binding a drug to a small biocompatible magnetically active component, entrapped in the biodegradable polymeric matrix and formulating into a pharmacologically active stable formulation, which is injected into the blood stream and using a high-gradient magnetic field to pull them out of suspension in the target region. Magnetic microspheres will be formulated with an intension to produce a depot near the target organ, by placing a suitable magnet near it. From the depot, drug will be released slowly and carried to the target organ through blood. By localizing the drug carrier near the target organ, unwanted distribution of drug to non-target organ can be avoided. This approach will localize the drug only at target site and minimize the drug-induced toxicity (Figs. 1,2). For example Vimal *et al.* prepared Diclofenac sodium-containing ethyl cellulose micro particles were prepared by the Emulsion-solvent evaporation method with a view for use in the application of magnetic carrier technology [3].

Moreover magnetic polymer microspheres are usually composed of magnetic cores to ensure a strong magnetic response and polymeric shells to provide favorable functional groups and protect from particle aggregation. These microspheres exhibit many unique features such as small and uniform size, different shapes and morphologies and various functional groups on the surface and hence have received much attention in recent years for wide potential applications such as enzyme immobilization, cell and protein separations and drug delivery processes. Among these applications, it is becoming increasingly apparent that the key issues are surface modification and

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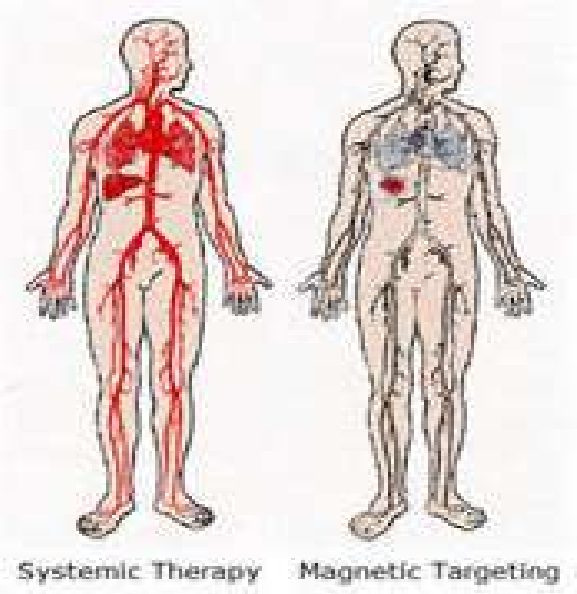

Fig. 1: Representation of systemic drug delivery and Magnetic targeting [15].

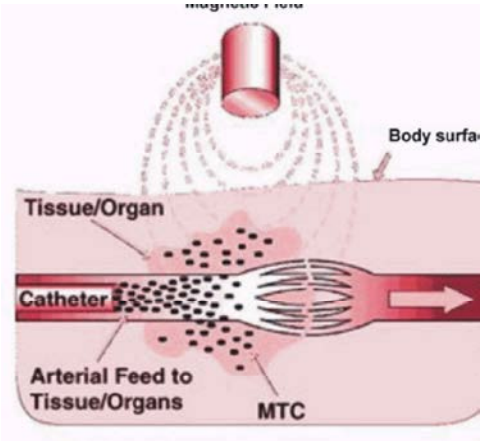

Fig. 2: Magnetic drug targeting [15].

morphology control. Therefore, synthesis of surface-functionalized magnetic microspheres with controllable morphology is particularly important both for fundamental studies and for applications [4].

History of Magnetic Targeting: Gilchrist published a seminar paper in 1956 on the selective inductive heating of lymph nodes after injection of 20-100 nm sized magnetite particles into the lymph nodes near surgically removed cancer [5]. Turner and Rand described the radiofrequency heating method with embolization therapy [6]. Meyers described how magnetic carriers were able to accumulate small iron particles intravenously injected into the leg veins of dogs, using a large, externally applied horse shoe Magnet. They imagined that it might be useful for lymph node targeting and as a contrast agent [7]. Hilal Engineered catheters with magnetic ends and described how they could be used to deposit and selectively embolize arterio-venous malformations with small magnets. The use of magnetic particles for the embolization therapy of liver cancer followed and has found renewed interest [8]. Widder more defined spherical magnetic microspheres were made for the first time at the end of the 1970s. Their magnetic albumin microspheres worked well in animal experiments for tumor therapy and as magnet resonance contrast agents, but were not explored in clinical trials [9, 10].

Magnetic Properties: Magnetic particles for bio separation consist of one or more magnetic cores with a coating matrix of polymers, silica or hydroxyl apatite with terminal functionalized groups. The magnetic core generally consists either of magnetite (Fe_3O_4) or magnetite (gamma Fe_3O_4) with super paramagnetic or ferromagnetic properties. Some magnetic cores can also be made with magnetic ferrites, such as cobalt ferrite or manganese ferrite. Super paramagnetism is when the dipole moment of a single-domain particle fluctuates rapidly in the core due to the thermal excitation so that there is no magnetic moment for macroscopic time scales. Thus, these particles are nonmagnetic when an external magnetic field is applied but do develop a mean magnetic moment in an external magnetic field (Figs. 3,4) [11]. Ferromagnetism means that the particles have a permanent mean magnetic moment. Here, the larger effective magnetic anisotropy suppresses the thermally activated motion of the core moments. Ferromagnetic particles are generally recommended for the separation of DNA/RNA (SiMAG/MP-DNA) (Figs. 5, 6) [12]. Magnetite also called as ferric ferrous oxide, tri iron tetra oxide and black iron oxide. Magnetic iron oxide chemical formula $FeOfe_20_3$ having a molecular weight of 231.55 with a chemical composition of Fe=72.36%, O=27.64%. The Ferro magnetic material when incorporated into microspheres makes them magnetically responsive, so that they can be concentrated to the desired site by applying some external magnetic field. To prepare magnetite, nitrogen gas flushed through 500 mL round bottom flask fitted with condenser. Charged the flask with 8.9 g (0.1 mol) of goethite, 9.94 g (0.05 mol) of $FeCl_2.4H_2O$ along with 250 mL deionized water. Added 50 mL of 2 M Sodium hydroxide. Reaction mixture was heated to reflux for 12 h. Its pH fell from 14 (orange) in to 8-9 (black precipitates). Particles washed and air dried [13,14].

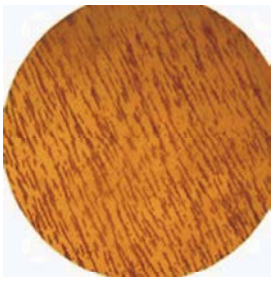

Fig. 3: Super paramagnetic particles under the influence of an external magnetic field

Fig. 4: Super paramagnetic particles in absence of an external magnetic field, monodisperse particle distribution

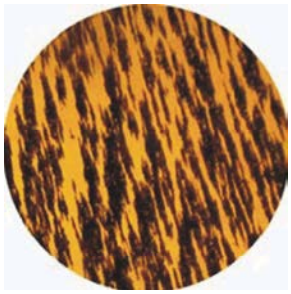

Fig. 5: Ferromagnetic particles under the influence of an external magnetic field

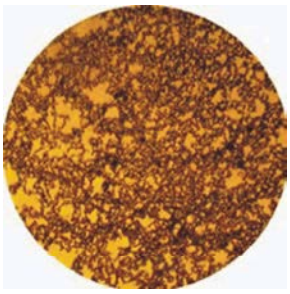

Fig. 6: Ferromagnetic particles in absence of an external magnetic field

Materials Used in Magnetic Microspheres [15]:

- Synthetic polymers
- Biodegradable: Glycolides, Epoxy polymers,
- Non-biodegradable: polyanhydrides, Lactides, Polymethylmetharylate, Acrolein, Glycidyl methacrylate
- Natural polymers
- Proteins: Albumin, Gelatin, Collagen
- Carbohydrates: Agarose, Starch, Chitosan
- Chemically modified carbohydrates: Polydextran, Polystarch

Advantages of Magnetic Microspheres [1, 15, 16]: Magnetic microcarriers are site specific and by localization of these microcarriers in the target area, the problem of their rapid clearance by RES is also surmounted. Linear blood velocity in capillaries is 300 times less i.e.0.05cm/sec as compared to arteries, so much smaller magnetic field, 6-8 Koe, is sufficient to retain them in the capillary network of the target area. Other benefits includes avoidance of acute toxicity directed against endothelium and normal parenchyma cell, controlled release within target tissue for intervals of 30 minutes to 30 hrs. as desired, adaptable to any part of body. This drug delivery system reduces circulating concentration of free drug by a factor of 100 or more. Magnetic carrier technology appears to be a significant alternative for the bimolecular malformation (i.e. composition, inactivation or deformation). Microspheres can transit into extra vascular space creating an extra vascular depot of drug for sustained release of drug within the targeted areas. Controlled and predictable rate of drug release with smaller doses of drug can be achieved. In case of tumor targeting, microsphere can internalize by tumor cells due to its much increased phagocytic activity as compared to normal cells. So the problem of drug resistance due to inability of drugs to be transported across the cell membrane can be surmounted.

Disadvantages of Magnetic Microspheres [1, 15, 16]: Apart from these advantages, this novel approach suffers from certain drawbacks i.e. drug cannot be targeted to deep seated organs in the body, so this approach is confined to the targeting of drugs in superficial tissue only like skin, superficial tumor or to joints only. In addition, unknown toxicity of magnetic beads, possible unwanted localization of the product in the liver and the regions of RES, the dangerous effect of self-flocculation of the magnetic particle and thrombosis at the site of catheterization. Moreover, magnetic targeting is an expensive and requires specialized technical approach, manufacturer and quality controlled system. The magnet

must have relatively constant gradients in order to avoid local overdosing with toxic drugs. It needs specialized magnet for targeting, advanced technique for monitoring and trained personnel to perform the procedure. A large fraction (40-60%) of the magnetite, which is entrapped in carriers, is deposited permanently in target tissues. Due to this limitation magnetic drug targeting is likely to be approved only for very severe diseases that are refractory to other approaches[2].

Factors Affecting Magnetic Targeting of Drug [17]

- Factors related to ferrofluids:
- Size of the particles in ferrofluid.
- Surface characteristics of particles.
- Concentration of the ferrofluid.
- Volume of the ferrofluid.
- Reversibility and strength of drug/ferrofluid binding (desorption characteristics).
- Access to the organism (infusion route).
- Duration or rate of injection/infusion.
- Geometry, strength and duration of the magnetic field application.

- Physiological parameters related to patient (or animal):

- Size, weight and body surface of patient (or animal).
- Total blood volume.
- Cardiac output and systemic vascular resistance.
- Circulation time.
- Tumor volume and location.
- Vascular content of tumor.
- Blood flow in tumor.

Limitations of Magnetic Drug Targeting [18, 19]:

- Magnetic targeting is an expensive, technical approach and requires specialized manufacture and quality control system.
- It needs specialized magnet for targeting, advanced techniques for monitoring and trained personnel to perform procedures.
- Magnets must have relatively constant gradients, in order to avoid focal over dosing with toxic drug.
- A large fraction of magnetite, which is entrapped in carriers, is deposited permanently in targeted tissue.

METHODS OF PREPARATION OF MAGNETIC MICROSPHERES [15]"

Selection of Drugs: In the selection of a drug for formulation of magnetic microspheres, following points are taken into consideration:

- The drug is so dangerous or labile that we cannot allow it to circulate freely in the blood stream.
- The agent is so expensive, that we cannot afford to waste 99.9% of it.
- Requires a selective, regional effect to meet localized therapeutic objective. Requires an alternative formulation essential to continue treatment in patient whose systemic therapy must be temporarily discontinued due to life threatening toxicity directed at selective organs [20].

Methods

Continuous Solvent Evaporation Method: In this method the drug and polymer (Carrier) are dissolved in appropriate volatile organic solvent and then magnetite (if magnetic microspheres) is added to this solution along with stirring in order to form a homogeneous suspension. This suspension is added to an immiscible auxiliary solution along with vigorous stirring. Now the volatile organic solvent is evaporated slowly at 22-30 °C to form microspheres. Microspheres are centrifuged then freeze dried and stored at 4 °C [21-23].

Phase Separation Emulsion Polymerization Method: Homogenous aqueous suspension is prepared by adding albumin water-soluble drug and agent with magnetite in appropriate quantity of water (if magnetic microspheres). This aqueous suspension is then emulsified in the presence of suitable emulsifying agent to form spheres in emulsion. This aqueous proteineous sphere thus formed in the emulsion are stabilized either by heating at 100-150°C or by adding hydrophobic cross linking agents like formaldehyde, glutraldehyde or 2-3 butadiene, microspheres thus produced are centrifuged out and washed either in ether or some other appropriate organic solvent to remove excess of oil. Microspheres are freeze dried and stored at 4 °C [21-23].

Multiple Emulsion Method: Water dispersible magnetite with a PEG/PAA coating was added to the BSA-containing inner water phase. 0.2 mL of a 1 mg/mL BSA solution added to a 4 mL mixture of DCM and EA at a ratio

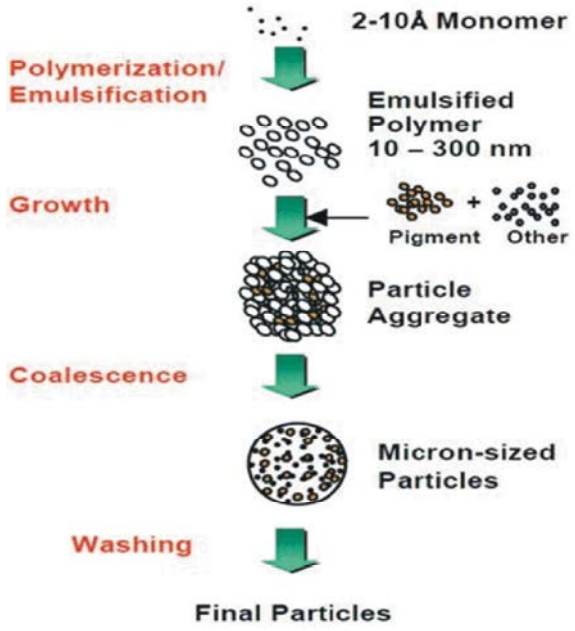

Fig. 7: Preparation of microspheres by multiple emulsion method.

of 3 to 1 containing 200 mg of PLGA (first w/o emulsion was prepared using a homogenizer (Polytron PT10-35; Kinematica, Luzern, Switzerland) in an ice bath at 26 000 r/min for 2.5 min). Fifteen mL of a 1% PVA solution poured directly into the primary emulsion and re-emulsified using the same homogenizer under the same conditions for another 2.5 min. W/o/w emulsion immediately poured into a beaker containing 85 mL of 1% PVA solution and stirred in a hood under an overhead Propeller for 2 h, allowing the solvent to evaporate. Solidified microspheres harvested by centrifugation at 2500 r/min for 10 min and washed with distilled water three times (Fig. 7) [24,25].

Cross Linking Method: Reagents Used: Acetate buffer–used as solvent for the chitosan polymer; Glutraldehyde-used as the cross-linker; Sodium hydroxide solution-used as medium. Synthesis of magnetic fluid: A 35% (w/v) ferrous sulfate solution, 54% (w/v) ferric chloride solution and 36% (w/v) sodium hydroxide solution were prepared using distilled water. Then the ferric salt and ferrous salt were mixed, stirred and heated. When the temperature reached 55 °C, the alkaline solution was added. The mixture was stirred for 30 min and then 5 g of polyethylene glycol-10000 (PEG- 10000) was added. The temperature was raised to 80 °C and maintained for 30 min. The mixture was then neutralized while cooling and the magnetic fluid was prepared. 1% (w/w) chitosan was dissolved in acetate buffer at pH 4.5. The dissolved chitosan was added drop wise on the magnetic fluid. Formed chitosan magnetic microspheres were washed with deionized water and soaked in 1, 3 and 5 mol% glutraldehyde solution for 2 h and then washed with deionized water [26].

Alkaline Co-Precipitation Method: Treat poly (acrylic acid–divinylbenzene) microspheres with dilute aqueous NaOH solution (0.5 M) for hours at suitable temperature to transform the carboxylic acid groups to sodium carboxylates and then washed thoroughly with water to remove the excess NaOH till neutral pH. Purged the microsphere suspension with nitrogen for 30 min. To this suspension add an aqueous solution of $FeCl_3$ and $FeCl_2$ that had been purged with nitrogen. Stirred the mixture overnight under nitrogen atmosphere for ion exchange. The resulting microspheres were washed repeatedly with water under nitrogen atmosphere to remove excess iron salts. Added drop wise an aqueous NaOH solution (3M) to a suspension of the microspheres taken up with iron ions under nitrogen atmosphere to adjust the pH value to be >12. The mixture was then heated to 60 °C and kept for another 2 h. The resulting magnetic microspheres were suspended in an aqueous HCl solution (0.1 M) to transform the –COONa to –COOH and then washed thoroughly with water to neutral pH, dried under vacuum at 50 °C overnight, giving magnetic microspheres[27].

Inverse Phase Suspension Polymerization Method: A 250 mL three-neck flask fitted with a mechanical stirrer used for performing the reaction. Continuous phase includes: 100 mL of castor oil and 10 mL of span 80. Determined amount of itaconic acid (IA), Styrene (St), divinylbenzene (DVB) and N, N-Methylene-bisacrylamide (BIS) dissolved completely in DMSO and the organic phase was added drop wisely into the flask, with 70°C heating using an oil bath. Ammonium persulfate (INITIATOR) added drop wise using a syringe. The reaction proceeded for 8 h with continuous stirring. The resulting microspheres were separated by centrifugation. Further washed with diethyl ether and then by deionized water (Fig. 8) [28].

Sonochemical Method: The microspheres composed of iron oxide-filled and coated globular bovine serum albumin (BSA). The magnetic microspheres were prepared from BSA and iron penta carbonyl, or from BSA and iron

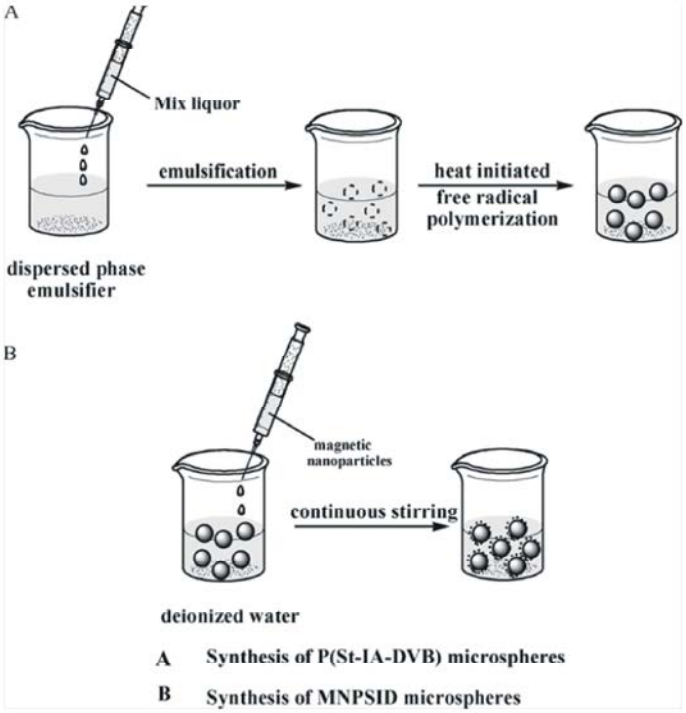

Fig. 8: Synthesis of P(St-IA-DVB) microspheres and MNPSID

acetate. Protein microspheres have a wide range of biomedical application, i.e. use as echo contrast agents for sonography. The microsphere were formed by either heat denaturation at various temperatures, or by cross linking with carbonyl compounds in the ether phase. Cross linking was done as: the microspheres are formed by chemically cross-linking cysteine residues of the protein with HO_2 radical formed around a non-aqueous droplet. The chemical cross-linking is responsible for the formation of the microspheres and is a result of the chemical ejects of the ultrasound radiation on an aqueous medium. Two sonochemical methods for the fabrication of iron oxide nanoparticles were (i) Water as the solvent and (ii) Decalin as solvent. Decane and iron pentacarbonyl Fe $(CO)_5$ (7.43U1034 M) were layered over a 5% w/v protein solution. The bottom of the high-intensity ultrasonic horn was positioned at the aqueous organic interface. The mixture was irradiated for 3 min, employing a power of W150 W/ 32cm with the initial temperature of 23°C in the reaction cell. The pH was adjusted to 7.0 by adding HCl. This procedure was performed again with an aqueous solution of iron acetate, Fe $(CH_3CO_2)2$ 95% (Sigma) (7.66U1033 M). After the synthesis, the products were separated from the unreacted protein and from the residues of iron acetate or iron pentacarbonyl by centrifugation (1000 r/min for 5 min). The magnetic microspheres were washed a few times with sufficient volumes of water to remove the residues of the precursors [29-32].

Swelling and Penetration Method: For swelling of polymer micro particles, 0.25 g of PS (Micron-size polystyrene) particles was mixed with 35 mL of a NMP/water solution in a specific v/v NMP (N-methyl-2-pyrrolidone)-to-water ratio. In later preparations of magnetic microspheres, SDS (Sodium dodecyl sulfate) was added to the NMP/water solution. Whenever SDS was used, 0.025 g of SDS were added to each NMP/water solution. The NMP/water mixture with PS spheres was left soaking for 24 h at room temperature while stirring. 2.5 mL of the superparamagnetic nanoparticle dispersion (24 mg/mL or other specified concentration) was added to the mixture of PS sphere and NMP/water solution at 30°C while shaking (at 140 r/min) for 1-5 days to allow the magnetic nanoparticles to penetrate into the interior of the PS particles. Afterwards, the polymer particles were separated from the solution by centrifugation. Finally, particles were sequentially washed with methanol, deionized water and vacuum dried at room temperature for 1-2 days to yield the magnetic polymer microspheres [33].

Low-temperature Hydrothermal Method: 0.1g Fe_3O_4 was dispersed in the aqueous glucose solution without additives, the hydrothermal reaction catalyzed only by Fe_3O_4 was kept at 180°C for 5 h [34].

CHARACTERIZATION OF MAGNETIC PARTICLES [35, 16]

Particle Size and Shape: Magnetic particles synthesized by above methods are of variable sizes. Their properties are quite different from other type of micro and nanoparticles. The most widely used procedures to visualize microparticles are conventional light microscopy (LM) and scanning electron microscopy (SEM). Both techniques can be used to determine the shape and outer structure of the microparticles. Particle size and its distribution are determined by light microscopy, scanning electron microscopy, transmission electron microscopy, etc.Confocal laser scanning microscopy (CLSM) is applied as a nondestructive visualization technique for microparticles. CLSM allows visualization and characterization of structures not only on the surface, but also inside the particles, provided the material is sufficiently transparent and can be fluorescently labeled. By collecting several coplanar cross sections, a three-dimensional reconstruction of the inspected object is possible.

Chemical Analysis: The surface chemistry of the microspheres can be determined using the electron spectroscopy for chemical analysis (ESCA). ESCA provides a means for the determination of the atomic composition of the surface. Fourier Transform Infrared Spectroscopy (FTIR) is used to determine the degradation of the polymeric matrix carrier system. The surface of the microspheres is investigated measuring total attenuated reflectance (ATR). The surface carboxylic acid residue is measured by using radioactive glycine. The radioactive glycine conjugate is prepared by reaction of 14C-glycine ethyl ester hydrochloride with the microspheres. The radioactivity of conjugate is measured using scintillation counter. Surface associated amino acid residue is determined by the radioactive 14C- acetic acid conjugate. The carboxylic acid residue is measured through the liquid scintillation counter and hence the amino acid residue can be determined indirectly.

Drug Loading: The capture efficiency or the drug loading of the microspheres or the percent entrapment can be determined by allowing washed microspheres to lyse the lysate is then subjected to the determination of active compound by suitable method. The percent encapsulation efficiency is calculated using following equation:

% Entrapment = (actual content/theoretical content) x 100

Magnetic Properties: Magnetic properties of nanocomposite particles were characterized by using vibrating sample magnetometer (VSM). The magnetic moment of each dried magnetic particles measured over a range of applied fields between -800 and +800 Gauss with a sensitivity of 0.1 emu/g. The prepared samples can be characterized by weight or volume in VSM. The dry samples are weighed (0.075 g), while the fluids are injected into the sample holder (~ 0.05 ml). In this system, when a magnetic sample is placed between two coils of an electromagnet creating a uniform magnetic field gradient, the applied field induces the magnetic domains to line up with the field through dipole interactions. As the magnetic field is increased, number of domains will be also enhanced until the particles reach saturation levels. During magnetic field alignment, the particles undergo a sinusoidal motion and produce an electrical signal in a set of stationary pick-up coils. This signal is proportional to magnetic moment, vibration amplitude and vibrational frequency. After the measurements, magnetic saturation values of the materials are calculated for each sample by dividing the saturation magnetization by the weight of samples.

Thermo Gravimetric Analysis: Differential scanning calorimetry and other gravimetric methods are used to determine the extent of interaction of polymers with magnetite and such other magnetic materials. Moreover the stability of ferrous and ferric ions can be assessed by thermogravimetric methods.

Measurement of Swelling Kinetics of Microspheres: Swelling kinetics of the composite magnetic microspheres can be determined by swelling rate at given time. Dried microspheres are immersed in distilled water at each predetermined time at room temperature. Then the sample is removed from distilled water and is frequently weighed after trapped with filter paper. Thus, the wet weight of the microspheres is recorded during the swelling period at regular time intervals. The SR, (Ws + Wd)/Wd, is defined as the ratio of total weight of water in swollen microspheres to the weight of the dried microspheres, where Ws is the weight of adsorbed water and Wd is the weight of the microspheres at dry state.

Stability Measurements: Stability measurements can be performed by using separation analyser (e.g. LUMiFuge). Measurements are made in glass tubes at accelerated velocities from 50to 300rpm. The slope of sedimentation curve can be used to calculate sedimentation velocity and stability data can be found.

ξ - Potential Measurements: ξ - Potential measurements can be made using an instrument like Zetasizer 2000. The zeta potential is measured at different pH values and stability of magnetic particles can be predicted.

Effect of pH on Magnetic Microspheres: Measurement of pH sensitive behavior is similar to the measurement of swelling kinetics of the microspheres. It is determined by the equilibrated swelling rate (ESR) at given pH data. ESR of the microspheres is measured by immersing dry and known weight of microspheres into buffer solution with different pH data for at least 1h at room temperature. Then the microspheres are removed from the buffer solution and frequently weighed after trapped with a filter paper to remove excess of water on the surface. ESR is calculated from the following formula We/Wd, where We is the weight of the solution in equilibrated swollen microspheres at each predetermined buffer solution with different pH data, the symbol of Wd is the same as defined earlier.

***In-vitro* Studies:** Study by Andreas Jordon *et al*. [36]: The ferrofluids used in their study were the dextran magnetite # P6 (supplied by Schering AG) and the aminosilan-coated magnetite preparation # BU48 (supplied by INM). The characteristics of the ferrofluids are as per Table 1.

Cell lines used were the normal human cerebral cortical neuronal cell line HCN-2, the human mammary carcinoma line BT20 and the colonic adenocarcinoma line WiDr. The cells were grown in either # P6 or # BU48 containing medium (0.6 mg ferrite/ml) and the intracellular iron concentration determined after 0, 6, 24, 48, 72, 144, 168, 192 h. To determine particle uptake and distribution throughout the cytoplasm, into phagosomes or lysosomes, transmission electron microscopy of selected cell preparations was done. The attachment of both particle types on the cell surface was determined by scanning electron microscopy. Hyperthermia was performed either in a precisely controlled water bath according to standard procedures or in a special designed AC magnetic field. Magnetic fluid hyperthermia was performed by inserting a small vial containing the test cells (5 x 107 1 x 108 cells per pellet) into a thermostatized bolus which in turn was inserted in a water-cooled copper coil, the AC magnetic field applicator.

Table 1: Characteristics of Ferrofluids

Ferrofluidcharacteristics	#P6	#BU48
Average particle core diameter	3.3nm	13.1nm
Average hydrodynamic particle diameter	50-70nm	17nm
Type of nanoparticle coating	Dextran	Aminosilan
Suspension stability as sterilized fluid	Years	Months
Biocompatibility	High	High
Formation of intracellular particle aggregates	Yes	No
MagneticSusceptibility	117.2emu/g	50-100emu/g
Surface charge	Negative	Highly positive
Specific absorption Rate	120mW/mgFe	146mW/mg
Super paramagnetic	Yes	Yes

Table 2: Uptakeof#P6bycells

Time	Malignant human glioma cells(pgof iron per cell)	Normal humancerebral cortical neuronal cell(pg of iron per cell)
6h	Nil	Nil
144h	110	60
Maximum Uptake	120	60

Table 3: Uptakeof#BU48bycells

Time	Malignant human glioma cells(pg of iron per cell)	Normal humancerebral cortical neuronal cell(pg of iron per cell)
6h	350	Nil
144h	400	20

Before any treatment, the cells were washed ten times with phosphate-buffered saline to remove loosely attached particles from the cell surface. The obtained results were as per Table 2, Table 3 and Fig. 9.

Study by Haik *et al.* [37]: The experiment was carried to study the fluid dynamic behavior of blood flow, from which the magnetic viscosity of blood was determined. The blood used was drawn from a healthy volunteer. Two different blood volumes of blood of 30ml and 10ml were used. For 30ml volume, the initial flow rate only under gravity was adjusted to approximately 0.6 ml/s and for the 10ml it was adjusted to 0.1 ml/s. for both volumes; the flow was adjusted using valve. The tubing connecting the two bags was 130mm long; the midpoint of the tubing was centered about the magnet center. The time by the blood to empty from the bag above the magnet was first measured with no magnetic field applied.. Subsequently, the time required to empty the blood bag was measure again with the magnetic field applied. The higher field measurements at 1Tesla (T) were made first followed by the lower field measurements at 5 and 3 T. the temperature were measured along the length of the tubing to determine

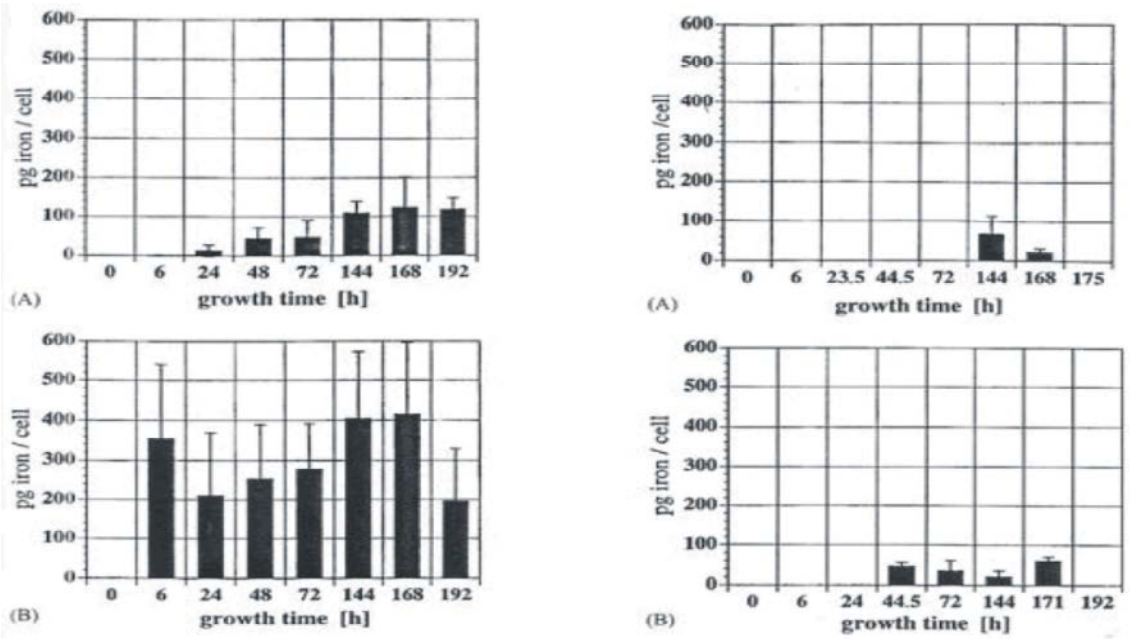

Fig. 9: (a)#P6(A)and#BU48(B)iron uptake of malignant human glioma cells.
(b)#P6(A)and#BU48(B) iron uptake by normal human cerebral cortical neuronal cells

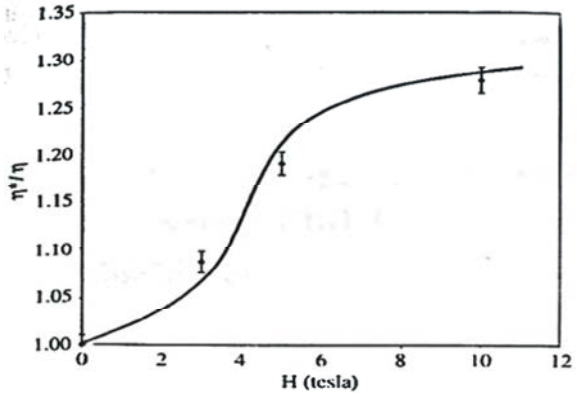

Fig. 10: Viscosityofhuman blood undertheinfluenceof magnetic field

whether thermal changes were occurring during the experiment. The Reynolds number was 390. The effect of magnetic field on the flow time and viscosity of blood was observed as shown in following graphs (Fig.10).

There was increase in the viscosity for the blood under the influence of magnetic field (Fig. 8). This behavior can be related to the existence of the magnetic torque, which will case cell to orient with the magnetic field. When the red cells are suspended in the plasma without magnetic field. When the magnetic field is applied, the viscosity of blood at that time is denoted by ç *.

Study by Viroochatapan *et al.* [17]: This study was carried out to study thermosensitive drug release of thermosensitive magnetoliposomes (TM) caused by an electromagnetic field. 1 ml of TMs with 8.21 mMdipalmitoyl phosphatidyl choline (DPPC) was put into a microtube and centrifuged at 1920xg for 2min. The supernatant was decanted and the precipitate (5l of TMs) was redispersed with 950 l of Sorensen buffer (53.4 mM Na_2HPO_4, 13.3 mMNa_2HPO_4, 75.2 mMNaCl; pH 7.4) or 50% calf serum in Sorensen buffer, followed by centrifugation at 1920xg for 20 min to obtain concentrated TMs with 164 mM DPPC. The double jacketed beaker was placed 5 cm out of the center of the pancake coil to keep the TM suspension near the phase transition temperature of DPPC (42°C). After being exposed to the field for the given period of time (5, 10, 20, 30, 6and 12min after reaching 42°C), TMs were dispersed and centrifuged at 192x g for 2min and the supernatant was collected. The supernatant obtained (50l) was mixed with 3.5 ml of methanol filtered through the high gradient magnetic filtration apparatus. 5-Fluorouracil (5-FU) in the supernatant was determined by spectrophotometer at 266 nm. This amount was compared with the amount of 5-FU released at 0 min exposure to magnetic field.

Study by Dhawan *et al.* [38]: Drug release from the loaded albumin microspheres determined by means of a dynamic dialysis system employing cellulose tubing. 3ml of

phosphate buffer (pH 7.0) was taken in a cellulose tube and 84 mg of albumin microspheres (equivalent to 1 mg of drug, Actinomycin D) were suspended in it. 1ml of the fluid was removed from the beaker every 24 h and replaced by the same amount of fresh fluid. The experiment was carried out for a week and the drug content was determined spectrophotometrically at 445 nm. The drug release from albumin microspheres prepared at 145°C and plain drug was also studied in the same manner.

***In-vivo* Studies:** Study by A. Ibrahim *et al.* [39]: Mice kidneys were chosen for targeting the magnetized drug-carrier behavior. This is because of their accessibility and ease of comparing the drug concentration in a magnet-bearing kidney with the paired kidney. A magnet was placed on the left kidney of each mice; the right kidney was used as a reference. The mice were then intravenously injected with 0.3 ml of nanoparticle suspension (which was radioactive). After 10 min, they were killed. Each kidney was isolated and separately homogenized suspension (100 l) was treated with tissue oxidizer and its radioactivity determined. To test the possibility of avoiding excessive accumulation of the carrier in the liver, the same experiment was made on 8 mice with a magnet on each kidney. 10 min after intravenous administration of magnetized radioactive nanoparticles, average three times higher radioactive concentration was found in the kidney bearing the magnet compared with the control.

APPLICATIONS [40]

Tumor Targeting via Magnetic Microspheres: Magnetism can play very important role in cancer treatment. The first clinical cancer therapy trials using magnetic microspheres were performed by Lubbe *et al.,* in Germany for the treatment of advanced solid tumor while current preclinical research is investigating use of magnetic particles loaded with different chemotherapeutic drugs such as mitoxantrone, paclitaxel. Permanent magnetic field for one hour way found to induces lethal effects on several rodent and human cancers [41]. Anticancer drugs reversibly bound to magnetic fluids and could be concentrated in locally advanced tumors by magnetic field that or arranged at tumor surfaceoutside of the subject. Various novel biodegradable magnetic microspheres are synthesized andtheir targeting to brain tumor is evaluated *in vitro* and in animal models. New cationic magnetic aminodextran microspheres have been synthesized. Its potentiality for drug targeting to tumor was studied. These particles were retained in brain tissue for longer period of time [42].

Locoregional Cancer Treatment with Magnetic Drug Targeting: The specific delivery of chemotherapeutic agents to their desired targets with a minimum of systemic side effects is an important, ongoing challenge of chemotherapy. One approach, is the i.v. injection of magnetic particles [ferrofluids (FFs)] bound to anticancer agents that are then concentrated in the desired area (e.g., the tumor) by an external magnetic field. Whereas an external magnetic field was focused on the tumor. Application of FF-MTX is successful in treating experimental squamous cell carcinoma. This “magnetic drug targeting “offers a unique opportunity to treat malignant tumors locoregionally without systemic toxicity. Furthermore, it may be possible to use these magnetic particles as a “carrier system” for a variety of anticancer agents, e.g., radionuclide’s, cancer-specific antibodies and genes [43].

Magnetically Induced Hyperthermia for Treatment of Cancer: Heat treatment of organs or tissues, such that the temperature is increased to 42–46°C and the viability of cancerous cells reduces, is known as hyperthermia. It is based on the fact that tumor cells are more sensitive to temperature than normal cells. In hyperthermia it is essential to establish a heat delivery system, such that the tumor cells are heated up or inactivated while the surrounding tissues (normal) are unaffected [44].

Magnetic Delivery of Chemotherapeutic Drugs to Liver Tumors: The first clinical cancer therapy trial using magnetic microspheres (MMS) was performed by Lubbe *et al.* in Germany for the treatment of advanced solid cancer in 14 patients. Their MMS were small, about 100 nm in diameter and filled with 4-epidoxorubicin. The phase I study clearly showed the low toxicity of the method and the accumulation of the MMS in the target area. However, MRI measurements indicated that more than 50% of the MMS had ended up in the liver. This was likely due to the particles’ small size and low magnetic susceptibility which limited the ability to hold them at the target organ. The start-up company FeRx in San Diego developed irregularly shaped carbon coated iron particles of 0.5–5 m in diameter

with very high magnetic susceptibility and used them in a clinical phase I trial for the treatment of in operable liver cancer. They have treated 32 patients to date, are able to super selectively (i.e. well directed) infuse up to 60 mg of doxorubicin in 600 mg MMS with no treatment related toxicity [45].

Magnetic Targeting of Radioactivity: Magnetic targeting can also be used to deliver the therapeutic radioisotopes. Advantage of these method over external beam therapy is that the dose can be increased, resulting in improved tumor cell eradication, without harm to adjacent normal tissues. Magnetic targeted microspheres, which are more magnetically responsive iron carbon particles, have been radiolabel led in last couple of years with isotopes such as 188Re 90Y, 111In, 125I are currently undergoing animal trials [46].

Human Cholangiocarcinoma Xenografts: Cholangiocarcinoma, a malignant disease, poses a severe hazard to human health. It constitutes2.32% of biliary tract disease and the incidence ratio of male to female is 1.46-1. The incidence of cholangiocarcinoma has shown a tendency to rise in recent years. Treatment includes mainly operation and combined chemotherapy and radiation. But cholangiocarcinoma can be located deep, be anatomically concealed and difficult to diagnose early. As a result, the outcome of operation can be unsatisfactory and the survival rate is very low. Single or combined application of chemotherapeutic drugs is usually less than 30% successful in the clinic. The targeting drug with magnetic microspheres is used to treat human cholangiocarcinoma xenografts. It scan inhibit the growth of human cholangiocarcinoma xenografts in nude mice [47].

Magnetic Control of Pharmacokinetic Parameter and Improvement of Drug Release: Magnetite or iron beads into a drug filled polymer matrix and then showed that they could activate or increase the release of drug from the polymer by moving a magnet over it or by applying an oscillating magnetic field. The microenvironment within the polymer seemed to have shaken the matrix or produced 'micro cracks' and thus made the influx of liquid, dissolution and efflux of drug possible thereby achieving magnetically controlled drug release. Macromolecules such as peptides have been known to release only at a relatively low rate from a polymer controlled drug delivery system, this low rate of release can be improved by incorporating an electromagnetism triggering vibration mechanism into the polymeric delivery devices with a hemispheric design; a zero-order drug release profile is achieved [41].

Magnetic Systems for Magnetic Cell Separation: One important application of magnetic cell separation is the purging of malignant cells from autologous stem cell products, depletion of T cells and selection of specific lymphocyte subsets with potential anti-leukemic activity. In this way, a cancer patient's stem cells can be extracted, purified and then injected again after he has gone through a harsh cancer. The therapeutic applications of immune magnetic cell selection are based on antibodies that bind to cancer cell antigens such as CD10, CD19 or CD20. Two machines for magnetic cell separation have recently received FDA approval, Cellpro's "Ceprate SC stem cell collection system" and Baxter's "Isolex 300I." A 3rd system is approved in Europe, Miltenyi's "ClinicMACS" system [41].

Combination Therapy: There also exists the combination therapy which would induce hyperthermia treatment followed by chemotherapy or gene therapy. A combination of chemotherapy or radiation therapy with hyperthermia is found much more effective than hyperthermia itself. The approach involves use of magnetic microspheres containing a drug to cause hyperthermia using the standard procedure, followed by the release of encapsulated drug that will act on the injured cells. It is anticipated that the combined treatment might be very efficient in treating solid tumor. Ongoing investigations in magnetic hyperthermia are focused on the development of magnetic particles that are able to self-regulate the temperature they reach. The ideal temperature for hypothermia is 43°C-45°C and particles with a curie temperature in this range have been described by kuznetsov *et al*. [48].

In addition, Magnetic microspheres have wide range of applications. Various applications have been listed in Table 4. Various preparations of Marketed products of magnetic microspheres are available which are characterized by their INCI names, size, oil abs, refractive index and density shown in Tables 5 and 6 shows comparison of magnetic and non- magnetic targeting microspheres.

Table 4: Applications of Magnetic Microspheres [15]

S.No	Applications	Drugs/Carriers presently in current investigation
1.	Tumor targeting	Mitoxantrone, Paclitaxel
2.	Radioembolisationofliverandspleentumors	186re/188re-glass Microspheres
3.	Magneticcontrolofpharmacokinetic parameters	Insulin
4.	Magneticbioseparation	Dynabeads,usedinisolationofmRNA, genomicDNA andproteins
5.	Changingthetiming and/orextentofdrugabsorption instomachorintestines	Diclofenac sodium
6.	Radiosynovectomyofarthritis joints	35s-colloid, microspheres, 169er.citrate
7.	Hyperthermia fortreatmentof cancer	Cisplastin,Paclitaxel
8.	Detectionofmetastasesinnon-enlargedlymphnodes	Supramagneticironoxide
9.	Labeledsandwichimmunoassay	Fluorogeniccompound,AttoPhos (visual colorgeneratingcompound)
10.	DNAdetection	Dynabeads
11.	Bacteria detection	Streptavidin coatedmagneticbeads
12.	Cellseparationinmicrofluidics channel	Dynabeadsforseparation of, proteins, nucleic acids, antigensand antibodies)orcells (e.g.,bloodcells, stemscells orbacteria)
13.	Cell surfacemarkers forthedetectionandlocalization ofantigensandlectinreceptors	Separationofredbloodcells(RBC) andlymphoidcells
14.	Isolationandfunctionality ofcancercells (eukaryotic cells)	Dynabeadsforbreastcancer cells
15.	Immunoprecipitation(isolationofvariousproteins)	Dynabeadsforliposaccharidebindingproteins
16.	Isolationofcell compartmentsofeukaryotic cells	DynabeadsforGolgibodies,endosomalvesicles
17.	Removal ofanti-spermantibodiesandsperm cells	Goatanti-human immunoglobulin
18.	T8depletioninallogenictransplantation	dynabeadsfor bonemarrow
19.	Magneticseparationofpoly(A)mRNA	Homogenizationbymixture ofguanidiumthiocyanateandphenol- chloroform
20.	Magnetocaptureproteininteraction assays(Figure 11) havebeenusedforversatile magnetocaptureassays using6xHis-tagged proteins	Ni-NTA(nitriloaceticacid)taggedmagnetic agarosebeads
21.	Drugtargeting	Elementalironparticlesandactivatedcarbon.Magnetictargeted carriers(1-2μminsize) canadsorbanddesorbspharmaceutical agentssuchasdoxorubicin(DOX)
22.	Contraceptivedrugdelivery	Drugdeliveryisdesignedresponsivetothe changesinsteroid secretionduringmenstrualcycle

Table 5: Marketed Products of Magnetic Microspheres [15, 49]

Trade name	INCI name	Size (μm)	Oil abs (g/g)	Refractive index	Density (g/in3)
EA-209	Ethylene/acrylic acid copolymer	10.0	0.60	1.51	2.6
Flo-beads SE-3107A(soft beads A)	Ethylene/Methacrylate copolymer	11.0	0.62	1.49	3.12
Flo-beads SE-3207 B(Soft beads B)	Ethylene/Methacrylate copolymer	11.6	0.62	1.49	3.9
BPD-800	HDl/trimethylolhexyllactyl cross polymer (AND silica)	6.5	0.63	1.52	6.4
BPD-500	HDl/trimethylolhexyllactyl cross polymer(AND silica)	12.0	0.65	1.52	9.5
BPD-500T	HDl/PPG/Polycaprolactone cross polymer (AND silica)	13.5	0.58	1.52	8.2
BPA-500	Polymethyl Methacrylate	10.0	0.55	1.49	5.2
BPA-500X	Methyl Methacrylate cross polymer	7.0	0.58	1.49	6.7
MSP-822	Polymethyl Methacrylate	7.0	0.55	1.49	6.2
MSP-825	Methyl Methacrylate cross polymer	8.0	0.57	1.49	6.7
MSP-930	Methyl Methacrylate cross polymer	11.0	2.00	1.49	5.0
SUNPMMA-H	Methyl Methacrylate cross polymer	11.7	0.65	1.49	NA
TR-1	NYLON-6	13.0	1.12	1.53	4.0
TR-2	NYLON-6	20.0	1.41	1.53	3.5
POMP-605	NYLON-6	6.0	1.70	1.53	3.3
POMP-610	NYLON-6	11.0	1.80	1.53	2.8
SP-10	NYLON-12	10.0	0.60	1.53	6.2
SP-10L	NYLON-12	10.0	0.62	1.53	5.2
SP-500	NYLON-12	5.0	0.60	1.53	4.7
CL-2080	Polyethylene	12.0	0.60	1.51	4.0
TOSPEARL® 1110A	Polymethylsilsesquioxane	11.0	0.50	1.41	4.5
TOSPEARL® 120A	Polymethylsilsesquioxane	1.2	0.57	1.41	6.5
TOSPEARL® 145A	Polymethylsilsesquioxane	4.5	0.55	1.41	8.2
TOSPEARL® 2000B	Polymethylsilsesquioxane	5.0	0.54	1.41	8.5
TOSPEARL® 3000A	Polymethylsilsesquioxane	5.0	0.54	1.41	7.0

Table 6: Comparison of Magnetic and Magnetic Microspheres [50]

PROPERTY	MAGNETIC	NON MAGNETIC
SITE SPECIFICITY	well tolerated by the body, magnetic fields are believed to be harmless to biological systems and adaptable to any part of the body	Poor site specificity and are rapidly cleared off by RES
TEM features	Jagged edges seen by TEM which means that the magnetic nano-particles are Embedded well by the microspheres' matrix material PLGA.	No jagged edges
Adaptability	Adaptable to any part of the Body	Adaptable to less parts
Drug requirement	Locally congregating high concentrations of the drug at the diseased site thereby minimizing drug requirement and side effects.	More drug requirement than Magnetic microspheres

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