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A Review on Targeted Drug Delivery: Magnetic Drug Delivery System

Siddharth K. Gajjar*[1], G. U. Sailor[1], Dr. A. K. Seth[1], Purva B,Patel[2]

1 Dept. Of Pharmacy, Sumandeep vidyapith university, Baroda
2 shree sarvajanik pharmacy college, Mahesana

ABSTRACT:

Presently, several targeted treatment systems including magnetic field, electric field, ultrasound, temperature, UV light and mechanical force are being used in many disease treatments (e.g. cancer, nerve damage, heart and artery, anti-diabetic, eye and other medical treatments). Among them, the magnetic targeted drug delivery system is one of the most attractive and promising strategy for delivering the drug to the specified site. The targeted systems improve therapeutic index of drug molecules by minimizing the toxic side effects on healthy cells and tissues. The use of magnetic carriers for drug delivery of chemotherapeutic agents has evolved since 1970s, when few research has developed albumin microspheres encasing the chemotherapeutic agent adriamycin, and using magnetite as the magnetically susceptible component.

Key words: Magnetic drug delivery, Magnetic delivery, Electric field, Magnetic field delivery, Magnetic targeted drug delivery, Chemotherapeutic agent.

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For Correspondence:

Siddharth K. Gajjar,

Dept. Of Pharmacy, Sumandeep vidyapith university,

Baroda -391760

Gujarat, India.

Phone: +91-9714955889

Email: sid291187@yahoo.co.in

Introduction:

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 morphology control. Therefore, synthesis of surface-functionalized magnetic microspheres with controllable morphology is particularly important both for fundamental studies and for applications [1].

In the past few years, several methods have been developed for synthesis of these materials, including solvent evaporation, dispersion or suspension polymerization, microemulsion polymerization, and Ugelstad's two-step swelling method. Mostly, magnetite (Fe3O4) has been used as the magnetic core and polymers such as polystyrene, poly (glycidyl methacrylate) and polyvinyl alcohol have been used as the shells. Recently, micro- or miniemulsion polymerization has been widely used due to its easiness to obtain surface-functionalized microspheres with uniform particle dispersion. According to the published literature, we find that little attention has been paid to controlling the morphology and size of the surface-functionalized magnetic microspheres. Here, we first report a simple one-step method to prepare magnetic polymer microspheres that have both controllable morphologies and -NH2 groups located on their surface. The properties of these resulting magnetic microspheres are also analyzed and a possible forming mechanism is presented[2],[3],[4].

Magnetic micro and nanoparticles display magnetic properties different from their bulk material counterparts. These unique properties originate from the size of the particles, which are below critical diameter for magnetic domain wall formation. In the absence of external magnetic field, thermal energy can be sufficient to cause magnetic moments in these single-domain particles to equilibrate and overcome any preferential orientation. However, when an external magnetic field is applied, the magnetic moment of particles aligns rapidly in the direction of applied field and the materials display a net magnetization. The magnetization of the magnetite nanoparticles disappears when the external magnetic field is removed. These properties indicate supermagnetic behavior, which suggests that the nanoparticles may be ideal components of vehicles for magnetic field-directed delivery of therapeutic agents. Magnetic particles can be dispersed in carrier fluids through specific interactions between the particle surfaces and selected low molecular weight or polymeric surfactants. Such fluid dispersions are called as ferrofluids [5].

Theory of Magnetic Targeting of Drug [6]

It is assumed that magnetic nanocomposite particles that display high saturation magnetization have potential application for magnetically controlled drug targeting. These particles are relatively magnetic with discrete randomly oriented magnetic moments. When the magnetic particles are placed in the external magnetic field, the moments of the particles rapidly rotate into the direction of the field and improve the magnetic flux density. To control the motion of such particles within a circulating system, a magnetic force due to an externally applied and a hemodynamic drag force due to the fluid flow combine to create a total vectoral force on the particles. In order to effectively overcome the influence of a fluid flow and achieve the desired external magnetic field-controlled guidance, the magnetic force because of the external field must be larger than the drag force or hydrodynamic force. According to this explanation, the magnetic force on the magnetic particles is governed by:

$$\vec{F} = \vec{\nabla}(\vec{m} \bullet \vec{B}_0) \quad \text{(Newtons)}$$

Where F is the magnetic force, m is the total magnetic moment of the material in the microsphere, (is the gradient that is assumed to be derived from characteristics of the field alone, and the magnetic flux density- also known as the Bfield. Each of these quantities thus influence to some degree to which an external magnetic field may be used to internally guide particles in the body. The del operator(is defined for magnetic field distribution at xyz directions:

$$\nabla \equiv \frac{\partial}{\partial x} a_x + \frac{\partial}{\partial y} a_y + \frac{\partial}{\partial z} a_z.$$

It is noted that the gradient of a scalar function at any point is the maximum spatial change of the magnetic field. The Bfield tends to align the net magnetic moment of a particle in a fixed direction while the gradient leads to a force that moves the particles. The second factor characterizes the magnetic properties of the particles. The magnetic moment of a material m, is proportional to the applied magnetic field H, and the intrinsic magnetic susceptibility of the material, χm

$m = \chi_m H$

The magnetic volume susceptibility for various materials ranges from aluminium at 2.07 x 10-5 to magnetite 1.0 x 106 5.7 x 106 and as high as 106 for various ferromagnetic rare-earth materials. The force that counteracts the magnetic force on the particle in the fluid stream is due to the liquid flow (blood flow). Stokes law governs the hemodynamic forces on a particle in the liquid. The equation is given by:

$F = 6 \Pi \eta \upsilon r$

Where, F is the drag force, is the viscosity of fluid, is the relative velocity of a spherical particle and r is the radius of the particle.

Also, there are other variables for drug delivery including tissue porosity, particle distribution, allowable cell damage caused by incompatible sphere size and variable blood flow and viscosity. A highly porous tissue allows small particles to be easily manipulated out of the blood stream and into tissue. However, a relatively tight tissue structure would require more magnetic field induced force to pull the nanoparticles out of the bloodstream, and such interfacial transport could also cause damage to the tissue. As a result, the magnetic nanocomposite particle size and external forces needed for effective particle manipulation are highly dependent on the area in which drug delivery is performed.

Factors Affecting Magnetic Targeting of Drug[7]

1. **Factors related to ferrofluids:**
   1. Size of the particles in ferrofluid.
   2. Surface characteristics of particles.
   3. Concentration of the ferrofluid.
   4. Volume of the ferrofluid.
   5. Reversibility and strength of drug/ferrofluid binding (desorption characteristics).
   6. Access to the organism (infusion route).
   7. Duration or rate of injection/infusion.
   8. Geometry, strength and duration of the magnetic field application.
2. **Physiological parameters related to patient (or animal):**
   1. Size, weight and body surface of patient (or animal).
   2. Total blood volume.
   3. Cardiac output and systemic vascular resistance.
   4. Circulation time.
   5. Tumor volume and location.
   6. Vascular content of tumor.
   7. Blood flow in tumor.

Methods of Preparations for Magnetic Particles [8]

The methods of preparation of magnetic particles resemble to the general methods to form microspheres, nanoparticles and liposomes. The methods described here are some modifications of general methods which have been tried successfully by researchers. Some of the methods are as follows:

1.Ramazan Asmatulu et al. method [1]

There are two steps discussed by Ramazan Asmatulu et al.

A. Synthesis of Magnetite nanoparticles

Magnetic nanoparticles were prepared by the chemical co-precipitation of Fe2+ and Fe3+ salts in the presence of a strong base. An exemplary procedure is given using 2 g of FeCl3 and 0.736 g FeCl2 dissolved in 2ml of deoxygenated water. A 25ml3-necked round bottom flask was purged with dry nitrogen to ensure an inert atmosphere. The prepared salt solutions were injected in the flask followed by the addition of deoxygenated ammonium hydroxide (50/5aqueous) until the pH of the solution was 9.5. The solution turns black immediately upon addition of the base, signifying that magnetite (Fe3O4) was formed. After allowing the reaction to continue under a nitrogen purge for 3min, a stabilizer solution (oleic acid and dichloromethane) was added by a syringe. The reaction was stirred for an additional 0.5-2 hrs whereupon it was neutralized with 1M HCl to a pH 7 and allowed to stir for an additional 3min. The produced material was decanted into a beaker where it separated into aqueous and organic layers. The magnetite nanoparticles were located in the organic phase as they were stabilized by oleic acid. After removing the aqueous layer, the organic layer was rinsed with fresh deionized water to remove any remaining salt.

B. By using this method four different types of microspheres were prepared. Theproportion ofvarious components is as follows (Table-1,2)

The magnetite was stabilized by oleic acid and then it was dissolved in given amount of above mentioned organic solvent. The given amount of polymer matrix (phenoxy resin) was added in the above solution to make the organic phase. The poly (vinyl alcohol) was dissolved in 10ml of nanopure water to make aqueous phase. The organic phase was injected into aqueous phase with 18-gauge needle over a 2 min time period while the solution was being homogenized at 13,70rpm. The solution was homogenized for 5 min. whereupon it was poured into a round bottom flask containing 10ml of 4% isopropanol/water solution stirred for 6 hrs. The resulting solution was centrifuged to isolate the microspheres, which were dried under vacuum.

2. Xian-Ming Liu et al. method [5]

The maghemite sub-microspheres were synthesized by hydrothermal treatment using FeCl3.6H2O as the iron source materials. In a typical experimental procedure, FeCl3.6H2O (0.5 g) and PVP (0.5 g, MW 58000) were dissolved in ethylene glycol (35 ml) and formamide (5 ml, 99%) to form a clear yellow solution. After vigorously stirring for 1min, the mixture was then transferred into a teflon-lined stainless steel autoclave with a capacity of 5ml for hydrothermal treatment at 160C for 8 hrs. After the autoclave had cooled down to room temperature naturally, the precipitates were separated by centrifugation, washed several times with absolute alcohol and deionized water, and subsequently dried under vacuum at 60C. And a brown product was obtained. The resulting microspheres were 200-40nm in diameter.

3. U. K. Dutta et al. method [6]

About 1 g of polymer (Eudragit RS 100) was dissolved in 1ml of methylene chloride in a suitable container containing 1 g of cimetidine and 0.5 ml of ferrofluid. The mixture is dispersed in purified water containing sodium lauryl sulphate (0.2% w/v solution) as an emulsifying agent at a temperature of 30C for 5 min. at a higher stirrer speed. Then the temperature is raised upto 42C gradually for 2 hrs. After filtration and washing with purified water and drying in open air, the spherical discrete microspheres are obtained.

The above procedure was repeated using 1 ml of ferrofluid instead of 0.5 ml of ferrofluid.

The diameter of majority of particles formed by using the above two methods were 2, 3 or 5 m.

4. Ekapop Viroonchatapan et al. method [7]

Ekapop Viroonchatapan and co-workers prepared thermosensitive liposomes by this method. The method is as follows-

Dipalmitoylphosphatidylcholine (DPPC) 294 mg was dissolved in 12ml of an isopropyl ether and chloroform mixture (1:1, v/v). Then it was emulsified with 2ml of dextran magnetite (DM) suspension (167 mM as magnetite) containing 192 mM 5-Fluorouracil (5-FU) by 5 min sonication at 40C in a bath sonicator (150/30W) fixed at 35 kHz. The organic solvent was evaporated from the w/o emulsion in a rotary evaporator at 42C and low pressure (260-40mmHg). Thermosensitive liposomes were separated from non-encapsulated DM and 5-FU by centrifugation (96g) at 4C for 2min and thermosensitive liposomes were resuspended in 2mM (N-tris [hydroxymethyl] methyl-2-amino-ethanesulfonic acid) buffer solution containing 0.8% sodium chloride (NaCl) (pH 7.0). This step was repeated three times. The concentration of DPPC was adjusted to 8.21 mM. The average diameter of TMs was measured using a laser particle size analyzing system.

5. Kathryn M. Spiers et al. method [8]

Magnetite particles were synthesized by oxidative hydrolysis of a ferrous sulphate solution in an alkaline medium. A solution of 6.46 g potassium nitrate (KNO_3) and 44.9 g

potassium hydroxide (KOH) in 240 ml oxygen-free double distilled water was added drop wise over 5 min to a solution of 80 g ferrous sulphate ($FeSO_4 \cdot 7H_2O$) in 560 ml water, heated to 90ºC and flushed with N_2. The solution was stirred continuously using a magnetic stirrer. This reaction typically yields approximately 23 g of magnetite. To limit the growth of the magnetite particles, 14.4 ml oleic acid was added 15 min after the addition of a KNO_3/KOH solution. The reaction vessel was immediately removed from the heat, and allowed to cool, reaching 40ºC in an hour under continuous stirring. Subsequently, the solution was acidified to pH 3 by the addition of dilute nitric acid. Addition of hexane to this solution caused magnetite to separate into the organic phase, indicating functionalisation of the magnetite by the oleic acid. The magnetite powder was collected and dried **(Figure-1)**.

6. Huiling Bao et al.method [9]

A) Preparation of hydroxylated P (BMA-b-GMA) by A novel living radical polymerization (ATRP)

Polymerization was carried out using schlenk techniques under argon atmosphere. To a dry, 1ml, round-bottom schlenk flask, with a magnetic stir bar, ligand (4 equivalent for Phenanthroline), Cu (4 equivalent) and CuCl2 (2 equivalent) were added. The flask was closed with a stopcock. Then the contents of the flask were placed under vacuum and the flask was backfilled with argon to remove oxygen. The degassed monomer n-butyl methacrylate (BMA) and solvent were then added by syringe technique. After the mixture was allowed to stir at room temperature until it was homogeneous, the initiator was added and the flask was immersed in an oil bath kept at the desired temperature with magnetic stirring. After a certain time, the flask was removed from the oil bath and the mixture was diluted with tetrahydroflourane (THF). The solution was passed over a column with neutral alumina to remove the catalyst. Then the rest of the solution was concentrated by rotary evaporation, and the product was dried at 6C under vacuum. The block copolymerization was carried out by the successive addition technique. The second monomer glycidyl methacrylate (GMA) was directly added until the first monomer had reached the desired conversion.

P (BMA-b-GMA) (0.7821 g) was dissolved with 1ml THF, and 3 ml H2O containing 1drops of HClconc was added dropwise with magnetic stirring. After 3min, hydroxylatedP (BMA-b-GMA) was produced. The copolymer was finally isolated by rotary evaporation.

B) Preparation of magnetic nanoparticles

The hydroxylated copolymer was dissolved in 1ml THF, and 10ml Fe2+ solution was added. The reaction was continued for 4 h with magnetic stirring at 70C. Thereafter, the reaction mixture was cooled to room temperature and the latex was separated from the medium by centrifugation. Then the obtained latex was filtered with methanol, and the precipitation was washed with water and methanol three times in turn. After vacuum dried at 60C, the final product was obtained.

8. A. Pich et al. method [10]

A) Synthesis of iron oxide particles

Solutions of FeCl2 and FeCl3 were prepared in separate flasks and added to stirred dispersion under nitrogen blanket (molar ratio FeCl3:FeCl2 was kept constant at 2:1). Water solution of NH4OH was added drop-wise to start iron oxide formation process. Immediately after base addition solution became dark-brown indicating that iron oxide has been formed in the system. After 30-min formed composite particles were removed from reaction vessel and cleaned by precipitation to remove all by-products. Magnetic nanoparticles were washed with 0.01-M HCl solution. Magnetic dispersion with HCl solution was centrifuged (Universal 16 A) at a speed of 3000-U/min for 25-min to precipitate the particles. This procedure was repeated for four times. After that precipitated magnetic particles were cleaned with distilled water. Then calculated amount of sodium oleate was added to the required amount of magnetic dispersion (15-mg Na-oleate in 50-ml of 1.1-mg/ml magnetic dispersion, keeping the ratio constant at 1:1). Then the dispersion was heated to 80C for 5-min and finally sonified for another 5-min.

B) Synthesis of PS/AAEM/Iron oxide particles

Double-wall glass reactor equipped with stirrer and reflux condenser was purged with nitrogen. Appropriate amounts of iron oxide dispersion (from 0.92 to 9.2-g) were diluted with water to keep the total water amount 20-g and then Styrene (ST) (1.33-g) and acetoacetoxyethyl methacrylate (97%) (AAEM) (0.07-g, 5% to ST) were added into reactor and stirred at room temperature. After 10-min temperature was increased to 70C and solution of water soluble azo initiator (0.07-g in 2-g water) was added to start the polymerization process.

9. Ji Zhang et al. method [11]

A) Preparation of magnetic fluid

Magnetic fluid was synthesized as followsa 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-1000(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.

B) Synthesis of magnetic composite microspheres

In a typical procedure, a 1% suspension (w/v) of artemisia seed gum was prepared using distilled water containing a certain amount of magnetic fluid (2-ml). Then a 1.5% chitosan

solution, prepared using 2% acetic acid glacial solution was added. The mixture was added drop wise to the dispersion medium, which was composed of mineral oil, petroleum ether (15:45, v/v) and emulsifier (Tween-80, 0.7-ml). During this process, the dispersion medium was stirred at 500-1000-rpm at room temperature. Twenty minutes later, 1-ml glutaraldehyde was added to the dispersion medium and stirring was maintained for about 1-h. At the end of this period, the composite magnetic microspheres were collected using a magnet and washed consecutively with petroleum ether and acetone. The microspheres were dried in an oven at 40C for 2 days.

10. A. Dhawan et al. method [12]

An aqueous internal phase (3ml) of an emulsion (w/o type) containing 40mg bovine serum albumin, 1mg actinomycin D and 75 mg magnetite was emulsified with 25ml of pure olive oil under constant stirring at 120rpm while the temperature was maintained at 4C. This emulsion was gradually (10-15 drops/min) to preheated (120C) pure olive oil under constant stirring at 750 rpm for 15 min and mixture was allowed to cool down to room temperature . The heat stabilized albumin microspheres were separated by centrifugation at 3000 rpm for 5 min. The separated microspheres were washed with 60 ml of anhydrous ether thrice and separated by centrifugation. Finally the microspheres were suspended in 10 ml of ether and stored at -4C in air tight container. The average diameter of microspheres was 4.5 m.

11. A. Ibrahim et al. method [13]

Magnetically responsive polyalkylcyanoacrylate nanoparticles were prepared by anionic polymerization of the monomer in the presence of ultrafine magnetite particles of between 0.01 to 0.05 m. After 1g of glucose and 1g of citric acid had been dissolved in 100 ml of distilled water, 0.7 g of magnetite particles were dispersed by ultrasonic treatment for 15 min. The suspension was passed through a fritted glass filter (pore size 9-15 m) to avoid magnetite agglomerates. Dactinomycin (2 ml) and isobutylcyanoacrylate monomer (1.5 ml) were added and stirred ultrasonically (40W). After 3 hrs, nanoparticles were formed and filtered through a fritted glass filter (suspension A). To separate magnetized nanoparticles, the suspension was allowed to flow through a magnetic field at a rate of 1 ml per 3 min, using a pumping circulation tub system. Four permanent magnets were attached to the external surface of the circulation tubes. After removal of the magnets, the nanoparticles attached to the internal surface are washed with 10ml of an aqueous solution containing sodium chloride (0.7 %) and calcium chloride (0.2 %). This magnetically responsive particle suspension was finely resuspended by ultrasonic treatment for 15 min, at 40W and filtered through fritted glass.

Characterization of Magnetic Particles [14]

1. 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.

2. 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.

3. 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

4. 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 -800and

+800Gauss 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.

5. 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.

6. 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.

7. 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.

8. 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.

9. 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 formulaWe/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

1. Study by Andreas Jordon et al. [15]

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 -3.

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. 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-4, Table-5 and Figure-2.

2. Study by YousefHaik et al [16]

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 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.

There was increase in the viscosity for the blood under the influence of magnetic field **(Figure-3)**. 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 η *.

3. Study by Ekapop Viroochatapan et al [7]

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 mM dipalmitoylphosphatidyl 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 Na2HPO4, 13.3 mM NaH2PO4, 75.2 mM NaCl; 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 (42C). After being exposed to the field for the given period of time (5, 10, 20, 30, 6and 12min after reaching 42C), 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.

4. Study by A.Dhawan et al [12]

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 145C and plain drug was also studied in the same manner.

***In-vivo* Studies**

1. Study by A. Ibrahim et al [13]

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.

Limitations of Magnetic Drug Targeting[17],[18]

- 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.

Applications [19]

1. The proteinase of *Balillus sublitis* is widely used in industrial fields, such as in leather tanning, drug-producing, and food making, but its stability is poor and it easily loses its activity. The proteinase is bound by support material containing aldehyde groups and magnetic fluid and the stability is improved. Also the free poteinase was immobilized on the magnetic polymer microspheres carriers containing hydroxyl group, activated by *p*- benzoquinone.

2. Doxorubicin was loaded on magnetic targeted carriers and was used for clinical studies in swine was proved successful in treating artificially induced tumor.

3. Epirubicin was successfully targeted to tumor in rat, mice and human. The epirubicin loaded magnetic carrier was able to cause successful remission of tumor in human volunteers.

4. Cimetidine was successfully loaded on magnetic microspheres and release profile of the microspheres was determined.

5. Metronidazole is used as radiosensitizing agent in cancer radiotherapy. It was loaded on magnetic microspheres and targeted to tumor, thus sensitizing the tumor cells to radiations.
6. Submicron magnetic polyglutaraldehyde nanoparticles were synthesized and loaded by poly-l-lysine methotrexate.

Conclusion

In this era of novel drug delivery systems, magnetic targeted drug delivery systems offer great flexibility and ease of site specific drug delivery. Their main advantage is the targeting of drug using an external magnet, which can be accomplished very easily. Despite of such an advantage, the main drawback of this system i.e. accumulation of magnetic material at the targeted site and difficulty of removal poses a challenge for the scientists in the development of magnetically targeted drug delivery system. Thus system seems to be promising if the drawback can be overcome.

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Tables:

Table 1: Preparation of nanocomposite microspheres.

Samples	Amount of stabilized magnetite (g)	Amount of polymer matrix (g)	Amount and type of organic solvent	Amount of poly(vinyl alcohol) (g)
1	0.85	0.5	10 ml CH_2Cl_2	0.5
2	0.85	0.25	10 ml CH_2Cl_2	0.5
3	1.9	0.5	10 ml C_3H_6O	1
4	0.45	0.5	12 ml CH_2Cl_2	1

Table 2: Properties of nanocomposite particles prepared by above methods

Method	Sample	Saturation magnetization (emu/g)	Particle size (nm)
A	Synthesized Fe_3O_4	54-60	8-10
	1	27.13	500-3000
B	2	21.08	500-4000
	3	15.31	< 7000
	4	0.94	500-5000

Table 3: Characteristics of ferrofluids-

Ferrofluid characteristics	**# P6**	**# BU48**
Average particle core diameter	3.3 nm	13.1 nm
Average hydrodynamic particle diameter	50-70 nm	17 nm
Type of nanoparticle coating	Dextran	Aminosilan
Suspension stability as sterilized fluid	Years	Months
Biocompatibility	High	High
Formation of intracellular particle aggregates	Yes	No
Magnetic Susceptibility	117.2 emu/g	50-100 emu/g
Surface charge	Negative	Highly positive
Specific absorption rate	120 mW/mg Fe	146 mW/mg Fe
Superparamagnetic	Yes	Yes

Table 4: Uptake of # P6 by cells

Time	**Malignant human glioma cells (pg of iron per cell)**	**Normal human cerebral cortical neuronal cell (pg of iron per cell)**
6 h	Nil	Nil
144 h	110	60
Maximum uptake	120	60

Table 5: Uptake of # BU48 by cells

Time	**Malignant human glioma cells (pg of iron per cell)**	**Normal human cerebral cortical neuronal cell (pg of iron per cell)**
6 h	350	Nil
144 h	400	20
Maximum uptake	420	60

Figures:

Figure 1: TEM image of magnetite particles.

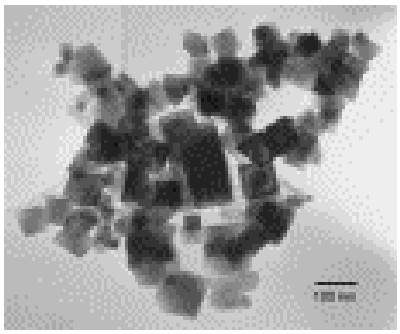

Figure 2 - (a) # P6 (A) and # BU48 (B) iron uptake of malignant human glioma cells. (b) # P6 (A) and # BU48 (B) iron uptake by normal human cerebral cortical neuronal cells.

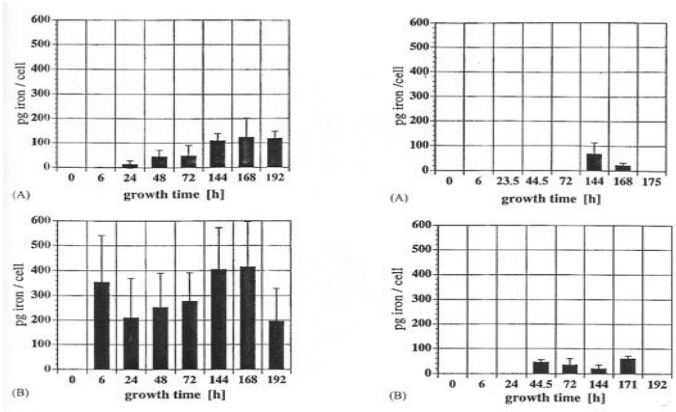

Figure 3: Viscosity of human blood under the influence of magnetic field