Magnetic Carriers: A Promising Device for Targeting Drugs Into the Human Body

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Abstract: Suboptimal disposition behavior of drugs requires innovative delivery approaches. Magnetic drug targeting seems to be a promising one. Magnetic particles develop magnetic polarization and magnetophoretic mobility, and because of such unique properties, these carriers may be eligible candidates for delivering drugs to specific locations within the body. Their special properties also allow other uses, such as those in magnetic separation, hyperthermia, and magnetic resonance imaging. This review focuses on a brief discussion of magnetic drug targeting, the properties and fate of magnetic carriers, the methods used to produce and characterize them, and their other uses in biotechnology.

Key Words: Magnetic particles, magnetic drug targeting, biotechnology.

1. INTRODUCTION

Adsorption of drugs into the blood is followed by a non-specific distribution to tissues. Therefore, the inability to target a specific area of the body is a matter of concern. In order to reach an acceptable therapeutic level at the desired site, large amounts of a drug must be administered, but only a small fraction of the dose will actually reach the intended organ or disease. The residual dose may be inactivated or express undesirable effects on organs and tissues that are not involved in the pathological process [1, 2].

Suboptimal disposition behavior of drugs requires innovative delivery approaches [3]. Drug targeting is pointed out as one of the most promising means of overcoming the drawbacks presented above. This consists of associating an active ingredient to an appropriate carrier. Thus, the properties of the system will depend on those of the carrier. The carrier, in turn, will be chosen according to the desired aim [4]. By this technique, the affinity of the drug to the target may be successfully increased, and the active ingredient may be remarkably protected from a potentially hostile environment, such as hydrolytic enzymes and acid pH [5].

Taking into account their nature, drug carriers fall mainly into two groups: biological and synthetic. Biological carriers are represented by red blood cells, bacteria, viruses and prions. However, they present several drawbacks like heterogeneity and high costs related to their processing and storage. Synthetic carriers, such as liposomes, micro and nanoparticles, seem to be more eligible [6-8].

Drug carriers can be directed to the target either in a passive or in an active way. Passive drug targeting refers to the spontaneous drug accumulation into sites with leaky vasculature (tumors, infarcts, inflammation). On the other hand, active drug targeting requires guidance of drug carriers to specific sites by means of pH- and temperature-sensitive drug carriers or antibody-cojugated ones, for instance [2, 4, 9].

Magnetic systems have been proposed as physicochemical carriers to actively direct drugs to target sites. When a magnetic field is applied, such systems develop magnetic polarization and magnetophoretic mobility [10, 11]. Therefore, by means of a selective application of a magnetic field on a desired area, active ingredients bound to these particles can be successfully carried to their site of action with high accuracy, minimum or no surgical intervention, and maximum concentration [11]. Regional therapy efficacy may be then improved by increasing local drug concentration while systemic drug biodistribution and toxic side effects may be limited [10, 12].

In this approach, the drug is first bound or loaded into the magnetic systems. While this suspension is injected through a catheter into a regional artery feeding the targeted site, an external magnetic field (generated by a source outside the organism) is superimposed onto this area [13]. Because of their high magnetic susceptibility, magnetic systems are responsive to the physical force of the magnetic field and extravasate into the targeted area without any detectable physiologic damage to the arterial wall. Once located, the systems do not redistribute but do start releasing the drug, creating a high local concentration of the drug in the tumor tissue, while minimizing the amount of the drug throughout the rest of the patient's body [13, 14].

2. MAGNETIC CARRIERS- PROPERTIES AND *IN VIVO* FATE

For therapeutic purposes, magnetic carriers must be water-based, biocompatible, biodegradable, and nonimmunogenic [13, 15]. Iron oxide particles present low toxicity and are well tolerated in the human body. Inside the cells, such systems are expected to be degraded relatively fast [16].

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Degradation into iron and oxygen is presumed to occur in intracellular lysosomes of macrophages under the influence of a variety of hydrolytic enzymes, low pH, and proteins participating in iron metabolism. Iron oxides have been shown to degrade *in vivo* by iron mobilization and utilization according to natural iron pathways [17]. Magnetite is one of the iron oxides approved by FDA for *in vivo* use [18]. Magnetic fluids have demonstrated good cardiovascular tolerance. Its infusion has shown not to change blood pressure, heart rate, or respiratory rate [19].

Magnetic drug targeting imposes several requirements on the magnetic carriers. The following parameters of the ferrofluid/drug compound and the magnetic field are critical: (a) particle size, (b) surface characteristics of the particle, (c) concentration of the fluid, (d) volume of the fluid, (e) reversibility and strength of the drug/ferrofluid binding (desorption characteristics), (f) access to the organism (infusion route), (g) duration/rate of the injection/infusion, (h) geometry and strength of the magnetic field, (i) duration of magnetic field application, (j) particle stability, and (k) magnetic properties. Physiological parameters of the patient organism are also important. They comprise: a) size, weight, body surface, (b) blood volume, (c) cardiac output and systemic vascular resistance, d) circulation time, (e) tumor volume and location, (f) vascular content of target area, and (g) blood flow on it [13, 20, 21]. Relevant remarks on some parameters deserve special attention.

Markedly, size is a crucial factor. Large microspheres can physically irritate the surrounding tissue or even embolize small blood vessels and capillaries [22]. Besides, stable suspensions of dense particles larger than 2 μm are hardly prepared, and it is difficult to inject suspensions of such particles through a catheter. On the other hand, very small particles (less than 0.1 μm in diameter) have a small magnetic moment. In such a case, magnetic forces may not be high enough to counteract the linear blood-flow rates in the tissue. As a consequence, the magnetic field may fail in successfully concentrating particles at the target organ, with also the possibility of a significant fraction of them accumulating in the liver [15, 19]. However, the use of magnetic microparticles (0.5 to 5 μm) was found to overcome such difficulty even in organs that lie deeply in the body cavity (8-12 cm from the body surface) [10, 23].

Surface charge is known to play an important role in blood half-lives of particles. It is generally agreed that strong positive and negative charged particles present a decreased circulation time. In such a case, particles undergo phagocytosis, resulting in distribution mainly in the liver or spleen. The clearance from circulation is mediated by interaction with cells, especially those of the reticulo-endothelial system (RES) [24]. Functional groups on cell surfaces alter the circulation time [17]. A usual approach consists of grafting magnetic systems with PEG (polyethylene glycol). By such a technique, sterically stabilized carriers are produced due to the induced sterical hindrance, which avoids protein binding and macrophage recognition [25]. The modification of magnetite nanoparticles with both PEG [26] and folic acid is also feasible. PEG immobilization on the surfaces of magnetic nanoparticles protects them from phagocytosis and promotes particle dispersion, improving their cell internalization [27]. By means of folic acid immobilization, cancer cells are preferentially targeted since the folate receptor is frequently over-expressed on their surface [28].

Concerning the reversibility of the drug/ferrofluid binding, carriers must have high drug-binding capacity and the rate of drug desorption in an organism needs to be slow. Therefore, high drug concentration may be maintained in the target area for a prolonged period of time [13].

Since the particles must be effectively controlled by the applied magnetic field, their magnetic properties, their dispersity, and degree of agglomeration are important. It has been observed that an increase in stability of the particles leads to a decrease in toxicity [20]. Low coercive force will prevent aggregation of the particles prior to superimposition of the field [13]. As a result, superparamagnetic particles seem to be ideal. Superparamagnetism takes place when single-domain particles are above a critical size. In such a case, energy fluctuations are able to overcome the anisotropy forces and spontaneously reverse the magnetization of a particle from one easy direction to the other. In the superparamagnetic behavior there is no hysteresis. This means that the demagnetization curve, during the removal of the applied field, follows the initial magnetization curve. Therefore, the remanence magnetization (M_R), which is the magnetization remaining at zero applied field, is zero. Besides, the coercive force (H_c), which is the magnetic field applied in the negative direction required to return the magnetization to zero, is zero [29]. M_R and H_c are identified in a hysteresis curve, as seen in Fig. (**1**). Besides superparamagnetism, high magnetic susceptibility and high saturation magnetization allow the particles to be effectively controlled by a relatively weak field [13].

Magnetophoresis is also a key issue. Such property plays a major role in targeting drugs to the desired area. Magnetic particles, submersed in a liquid medium and placed in the static magnetic field, experience magnetic force, friction force, and gravitational force. If the sum of them is larger than zero, magnetic particles will develop magnetophoresis [30]. In fact, such property is governed by various forces including (a) the magnetic force due to all field sources, (b) viscous drag, (c) inertia, (d) gravity,(e) buoyancy, (f) thermal kinetics, (g) particle/fluid interactions (perturbations to the flow field), (h) interparticle effects including magnetic-dipole interactions, electric double-layer interactions, and van der Walls force [31], and (i) magnetic field gradient [11].

A recent review on some of these properties is provided elsewhere [32, 33].

3. DEVELOPMENT OF MAGNETIC CARRIERS

In most applications reported in the literature, iron oxides, like magnetite and maghemite, are the magnetic material of choice [18]. The synthesis, coating, and stabilization of such particles will be discussed below.

The most common synthetic route to produce magnetite (Fe_3O_4) is the coprecipitation of hydrated divalent and trivalent iron salts in an alkaline medium [34]. The precipitated powders are black in color. The chemical reaction of Fe_3O_4 precipitation is expected as follows in Equation 1. A complete precipitation of Fe_3O_4 should be expected between pH

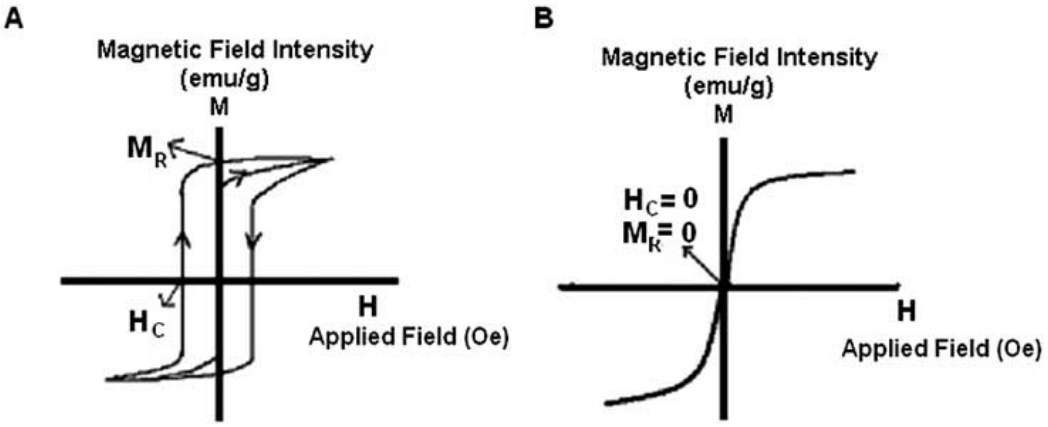

Fig. (1). Hysteresis curve of a ferromagnetic, non-superparamagnetic material (A) and a superparamagnetic one (B).

= 7.5 -14, while maintaining a molar ratio of Fe^2: Fe^3 = 1: 2 under a non-oxidizing environment. Otherwise, Fe_3O_4 might also be oxidized (Equation 2) [35]. The structure, dispersity, and morphology of the particles depend on their synthesis conditions, the order and rate of reagent mixing, intensity of their intermixing, temperature of the process, concentration of the original solutions, pH value of the medium, nature of the precipitator, and the presence of surfactant [36].

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \longrightarrow Fe_3O_4 + 4H_2O \text{ (Equation 1)}$$

$$Fe_3O_4 + 0.25O_2 + 4.5H_2O \longrightarrow 3Fe(OH)_3 \text{ (Equation 2)}$$

Maghemite (γ-Fe_2O_3) particles are usually obtained by oxidizing magnetite nanoparticles. Magnetite particles are oxidized to maghemite at 90°C for 30 min by ferric nitrate. On the other hand, oxidation may also take place under air exposition, and mixtures of magnetite and maghemite may be produced by a coprecipitation technique [37-39].

Iron oxides are also formed by partial oxidation of Fe (II) salts [40], or by the partial reduction of Fe (III) salts. In the latter, ferrous ions are not added. Instead, they are formed from ferric ions by partial reduction with Na_2SO_3 before a precipitation agent is added [41].

Nanoreactors can be employed for the precipitation reaction. They provide a constrained domain, which limits the growth of the particles. This method offers numerous advantages over previous ones when higher homogeneity of size and shape are concerned [42].

Microemulsions are colloidal nano-dispersions of water in oil (or oil in water) stabilized by a surfactant film. The synthesis of magnetic particles by this means is carried out when water droplets interact and exchange their contents [43]. Experimental results have confirmed that the microemulsion method allows good control of the particles by preventing their growth and providing particles small enough to get stable magnetic fluids [43-45]. On the other hand, magnetic particles prepared by coprecipitation may undergo aggregation [44, 46]. Microemulsions, which are thermodynamically stable dispersions, can be considered as truly nanoreactors that can be used to carry out chemical reactions and, in particular, to synthesize nanomaterials. The main idea behind this technique is that by appropriate control of the synthesis parameters, these nanoreactors can produce smaller and more uniform particles than are found in other standard methods. Particle size was found to depend on the molar ratios of water and surfactant [43, 45, 47].

Magnetoferritin may have considerable importance as a biocompatible ferrofluid, with many possible biomedical and industrial applications based on its magnetic properties [48]. The iron storage protein ferritin consists of a spherical polypeptide shell (apoferritin) surrounding a 6-nanometer inorganic core of the hydrated iron oxide ferrihydrite ($5Fe_2O_3.9H_2O$). The ferritin cavity has attracted wide interest as a template for constrained material synthesis and producing nano-particles. The cavity size is invariant because each subunit is produced from the same gene. Therefore, the obtained nano-particles are the same size, which is ideal for many applications [49]. Experimentally, it was observed that the produced particles were discrete 6-nanometer spherical single crystals of the ferrimagnetic iron oxide magnetite (Fe_3O_4) [50]. After accomplishing the synthesis, the protein coat can be removed by heat treatment at 450ºC. Therefore, it acts perfectly as a temporary scaffold, which determines the nature of the final structure without being a part of it [49].

Liposomes are also used as nanoreactors for the precipitation as they provide a constrained domain, which limits the growth of the particles [42]. Alternatively, encapsulation of magnetic particles into liposomes may be performed after synthesis [51]. Magnetoliposomes have been found to be a promising approach that offers some unique advantages when the magnetic nanoparticles are applied in biological systems. Lipid systems present the advantage of their low toxicity due to their composition of physiological lipids compared with polymeric particles. In fact, encapsulation of the magnetic nanoparticles in liposomes increases their biocompatibility under physiological conditions, making them suitable for a large variety of biological applications. Furthermore, it is known that magnetic particles tend to agglomerate, and are chemically unstable with respect to oxidation in air. Encapsulation of the magnetic nanoparticles in liposomes protects them from aggregation and oxidation [42, 52]. Cholesterol is a compound that is commonly used in magnetoliposome formulation [42, 51]. It improves the fluidity of the bilayer membrane and reduces the permeability of water soluble molecules through the membrane. Further-

more, it improves the stability of the bilayer in biological fluids [53]. Phosphatidylcholine is a very promising biocompatible surfactant for magnetoliposomes. It can be totally biodegraded and metabolized. Such a phospholipid is an integral part of biological membranes. Therefore, it is a well-tolerated and non-toxic compound [54]. Magnetoliposomes may also contain phospholipids whose transition temperature is slightly above normal physiological temperature. In this case, magnetic particles may be heated by means of either an alternating magnetic field or laser pulses. The transfer of thermic energy to the liposome bilayer would result in phospholipid melting followed by drug release [55, 56].

Concerning the production of polymer-based magnetic carriers, three different methods may be used. The emulsification/polymerization method has been successfully used to produce magnetic microcapsules. In this process, particles are synthesized in the internal aqueous phase of an inverse emulsion/microemulsion. Afterwards, polymerization by a cross-linking agent takes place. In such microcapsules, the drug and the magnetic particles are in the inner compartment [57-59]. Alternatively, polymer-covered magnetic particles can be produced by *in situ* precipitation of magnetic materials in the presence of a polymer that acts as a stabilizer. Magnetic polymer nanoparticles have been produced in the presence of water-soluble dextran [60], poly(vinyl alcohol) [61], sodium poly(oxyalkylene di phosphonates) [62], and amylose starch [63], just to name a few. In all cases, magnetic particles are surrounded by a hydrophilic polymer shell. Such systems are functionalized by the introduction of chemical groups so that they are able to bind active molecules [24]. For instance, dextran-coated magnetic particles, which are highly hydrophilic, uniform, and nontoxic magnetic carriers, may be activated by the periodate oxidation method. Thus, magnetic polyaldehyde dextran is formed and may be conjugated to different molecules [60]. Particles coated by starch polymers may be functionalized with phosphate groups. Ionic binding of cationic drugs takes place due to the charge of the phosphate group. A remarkable feature of ionically binding pharmaceutical drugs to the surface of particulate drug delivery systems is that the active substances can desorb from the carriers after a defined time span. Afterwards, they can diffuse from the vascular wall into the tissue. In contrast, strong binding makes the desorption of these ligands a difficult task [10, 64, 65]. Another method for producing magnetic polymer particles consists of separately synthesizing magnetic particles and polymer particles and then mixing them together to enable either physical or chemical adsorption of the polymer onto the magnetic material to be achieved [66].

Some of the methods cited above and other ones were recently reviewed [26, 67, 68].

4. CHARACTERIZATION

Many techniques have been used to characterize magnetic carriers. A detailed view of them is beyond the scope of this paper, whose focus is only on the most commonly used ones.

Scanning Electron Microscopy [69] and Transmission Electron Microscopy [67] are very important techniques. SEM offers better information on particle shape, especially for coarser materials. TEM provides images of individual particles, and also gives, besides particle shape, information on the internal structure (strain, grain boundaries, dislocations) [70].

Unlike the electron microscopic imaging techniques, X-ray diffraction gives a measure of the coherent scattering volume, i.e., the size of single crystalline domains of the particles. The mean X-ray size is smaller than the values deduced from imaging techniques because particles obviously are not completely single crystalline, but contain grain boundaries [70]. Therefore, X-ray diffraction is an additional technique used to establish the structure of the ultrafine particles. Despite the broad peaks, the x-ray diffraction data unequivocally show the crystal structure and lattice parameter of the granules, in spite of their small sizes [71].

Concerning the magnetic properties, saturation magnetization (Ms), remanent magnetization, and coercivity are the main technical parameters to characterize the magnetism in a particle sample [72]. Such data may be provided by vibrating sample magnetometry. This technique is based on a flux change in a coil when a sample is vibrated near it. Since it is very versatile and sensitive, this technique may be applied to both weak and strong magnetic substances [29].

For hyperthermia application, the main parameter characterizing the magnetic sample is the specific absorption rate (SAR). The SAR values of the samples are determined from the time-dependent calorimetric measurements [72].

5. OTHER USES IN BIOTECHNOLOGY

Hyperthermia is a promising approach to cancer therapy. It is a minimal invasive method for regional selective heat treatment, which is based on heating the target tissue to temperatures between 42 to 46ºC. By this technique, the viability of cancer cells is reduced because they are more sensitive to temperatures above 41ºC than are the normal cells. The magnetic materials generate heat in an alternating magnetic field, which enables the induction of hyperthermia. The subdomain superparamagnetic particles produce substantially more heat per unit mass than the 1000 times larger multidomain ferrite particles of similar composition. The mechanism of heating is based on the Brown effect (rotation of the particle as a whole according to external magnetic field) and the Neél effect (reorientation of the magnetic moment across an effective anisotropy barrier within each particle) [70, 73, 74]. Such parameters are strongly size dependent. For small particles, the Neélian process will dominate while large particles will relax following Brownian relaxation. For magnetite, the critical size for the transition from Neél to Brown relaxation is about 13 nm [72, 75]. The frequency of 300 kHz ($w = 2x\ 10^6 s^{-1}$ together with a field amplitude of 6.5 kA/m) is at the higher end of what may be applied to a tumor patient [70].

Magnetic particles can also be used to purify or detect cells, cell organelles, and biologically active compounds such as nucleic acids, proteins, and xenobiotics [76]. Magnetic separation implies several advantages in comparison with other techniques used for the same purpose. Direct isolation from crude samples such as blood, bone marrow, or tissue homogenates can be pointed out. Moreover, compared with conventional methods of cell separation, magnetic sepa-

ration is relatively simple and fast, and it is regarded as a sample enrichment step for further chromatographic and electro-migratory analysis. Ligands can be attached to their surface to provide particles with target specificity and selectivity. The separation process for the purification of target cells usually consists of the following fundamental steps: Initially, the suspension containing the cells of interest is mixed with magnetic particles. During incubation, such particles are bound to desired cells or molecules, and thus stable magnetic complexes are formed. Due to their capacity of being attracted by a magnetic field, complexes can be removed from the sample by using an appropriate magnetic separator, and the supernatant can be discarded or used for another application. The magnetic complex is washed several times to remove unwanted contaminants [77]. Isolated cells remain pheno-typically unaltered, and as a result, very pure cell populations with excellent viability can be isolated [65, 76].

Magnetic marker monitoring is a new technique to evaluate the *in vivo* performance of magnetically labeled oral dosage forms. After ingestion, their magnetic dipole field is recorded, and by means of fitting procedures, the location of the marked dosage form is estimated from the recorded data. The disintegration behavior is also assessed by this technique. The induction generated by the magnetic dipole moment of the oral dosage form during disintegration is used for the investigation of its mechanism and quantitative determination of the process [69, 78].

Magnetic particles have also found use in radionuclide therapy. Success in this approach depends on the critical relationship between the amount of radioactive isotopes in the target tissue and in normal tissue [24]. The advantage of this method over external beam therapy is that the dose can be increased, resulting in improved tumor cell eradication, without harm to nearby normal tissue [15]. For this purpose, radionuclides are irreversibly bound to magnetic carriers so that the tumor can be magnetically targeted with the radiolabeled magnetic systems [14]. The advantage of radiation over cytotoxic drugs is that radiolabeled particles can deposit a dose and produce biological damage over a defined radioisotope distance [24].

It has also been proposed to block the blood flow to the tumor using magnetorheological (MR) fluids and an applied magnetic field. The microscopic structures of these fluids change in the presence of a magnetic field, which leads to an embolus formation [79]. This is a safe method with very little toxicity for cancer therapy, using the rheological (mechanical) properties of MR fluids to inhibit the blood supply to the tumor [80]. Embolization with or without anticancer agents may result in tumor regression or necrosis. However, this method is not fully effective in eradicating tumor cells. In fact, tumors quickly regenerate lateral neovasculature and nullify the embolization effect [9]. A collateral microcirculation develops over days to feed the tumor and offset any beneficial effect of obstructing major feeding arteries and arterioles [21].

Additionally, magnetic particles are useful for magnetic resonance imaging (MRI). The primary requirement for an effective MRI contrast agent is a magnetic interaction with hydrogen nuclei. Magnetic particles themselves generate a magnetic field and influence the local area around them. This feature is exploited in MRI. This technique offers the advantage of high spatial resolution of contrast differences between tissues. It is used for localization and diagnosis of brain and cardiac infarcts, liver lesions, or tumors, where the microparticles tend to accumulate at higher levels due to the differences in tissue composition and/or endocytotic uptake processes This technique offers the advantage of high spatial resolution of contrast differences between tissues [81, 82].

The interested reader can refer to [26, 32, 83] for further information on the uses mentioned above and additional ones such as magnetofection and tissue engineering.

CONCLUSIONS

Targeting drugs into the human body imposes several requirements on the magnetic carriers. Even so, they seem to be eligible because of their improved properties. The synthesis of magnetic particles may be carried out by different techniques. The same applies to surface coating, which has been widely used. In fact, it plays an important role in biocompatibility, stability and biokinetics of nanoparticles in the body, as discussed previously. Besides magnetic drug targeting, magnetic particles are used in magnetic separation, magnetic hyperthermia, and magnetic resonance imaging due to their special properties.

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ABBREVIATIONS

RES	=	Reticulo-endothelial System
PEG	=	Polyethylene glycol.
M_R	=	Remanence Magnetization
H_c	=	Coercive Force
SEM	=	Scanning Electron Microscopy
TEM	=	Transmission Electron Microscopy
Ms	=	Saturation magnetization
SAR	=	Specific Absorption Rate
MR	=	Magnetorheological
MRI	=	Magnetic Resonance Imaging

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