INSTITUTO UNIVERSITARIO DE INVESTIGACIÓN EN NANOCIENCIA DE ARAGÓN

icma INSTITUTO DE CIENCIA DE MATERIALES DE ARAGÓN

"BIOMEDICAL APPLICATIONS OF MAGNETIC NANOPARTICLES:

I: DRUG DELIVERY"

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II: ELECTROMAGNETIC RADIATION"

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ZARAGOZA (SPAIN)

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OUTLINE OF THE TALK

Biomedical applications of magnetic nanoparticles I: Drug delivery

-Introduction

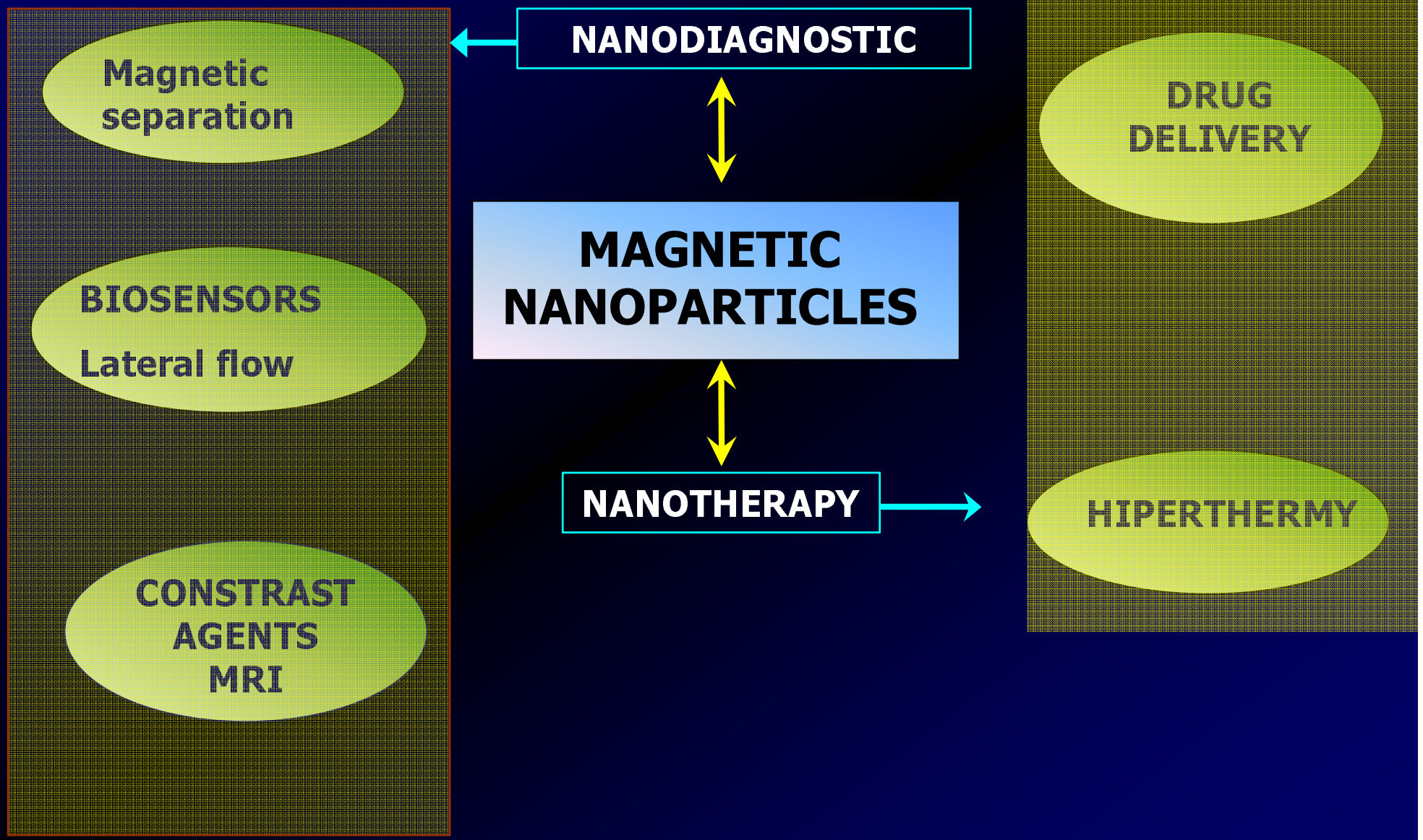
-Encapsulated nanoparticles:preparation and charaterization: Morphology, Structure and Magnetism

-Bioferrofluids for local drug delivery:

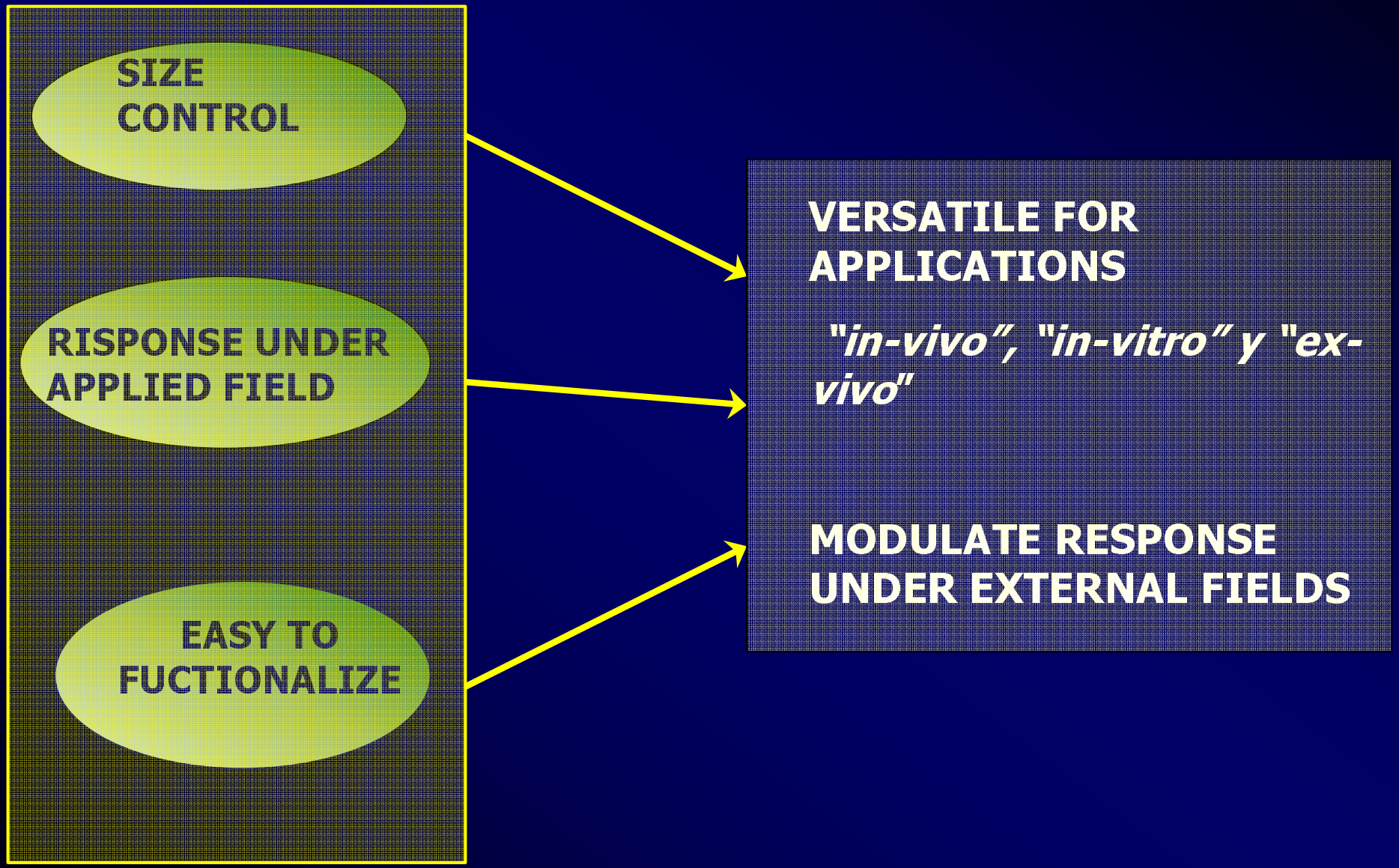
i) In vitro experiment: blood biocompatibility and drug carrier

ii) Invivo experiments: magnet implant and magnetic localization

Medical applications of magnetic nanoparticles

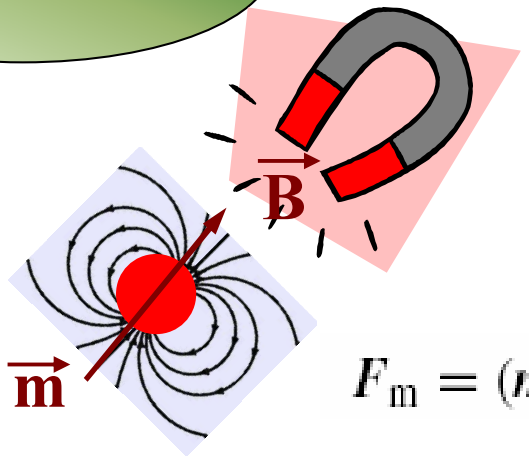


WHY MAGNETIC NANOPARTICLES ?



Magnetic nanoparticles response

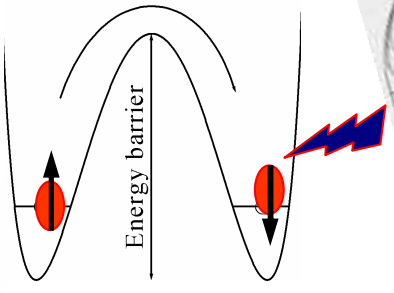
STATIC



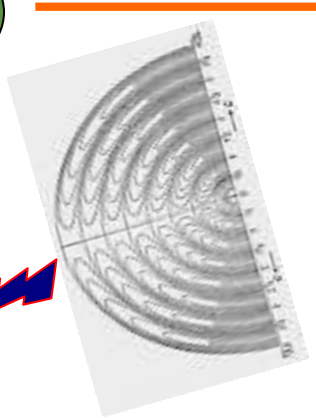
$$F_{\mathrm{m}} = (m \cdot \nabla)B.$$

- -Drug delivery (Therapy)
- -Detection and quantification of biomolecules (Cell sorting and inmunoassays)
- -Contrast enhance in MRI

DYNAMIC



$$\tau = \tau_0 \exp\left(\frac{\Delta E}{k_{\mathrm{B}} T}\right)$$

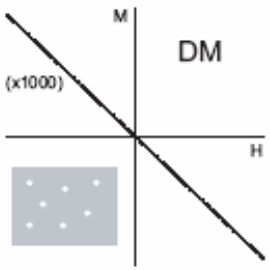


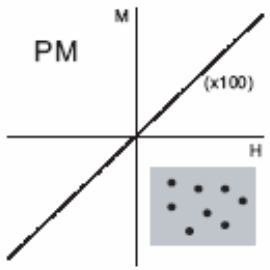
- -Resonant adsorption of electromagnetic rdiant energy (Hiperthermy, drug release)
- -Magnetic resonance

Magnetic behaviour of the matter

Diamagnetism

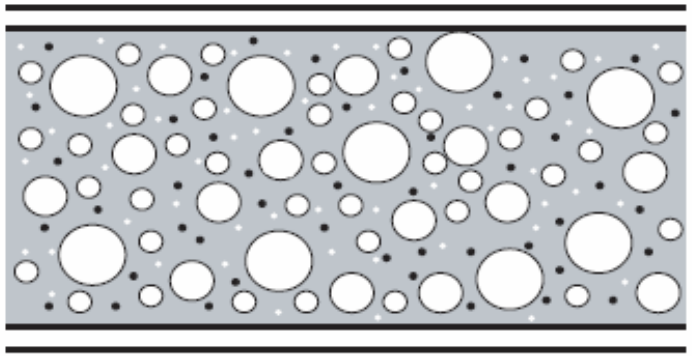
All matter





Paramagnetism

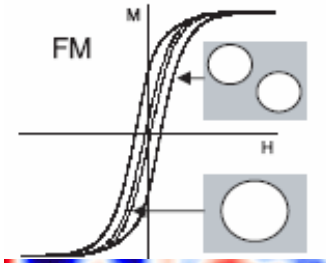
Isolated atoms

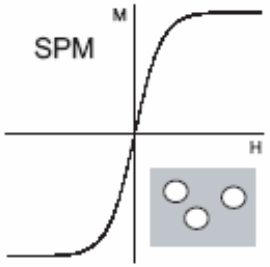


Q.A. Pankhurst et al. J. Phys. D.:Appl. Phys. 36 (2003) R167

Ferromagnetism

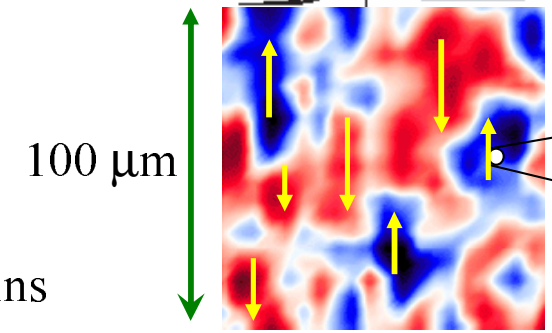
Mainly metals



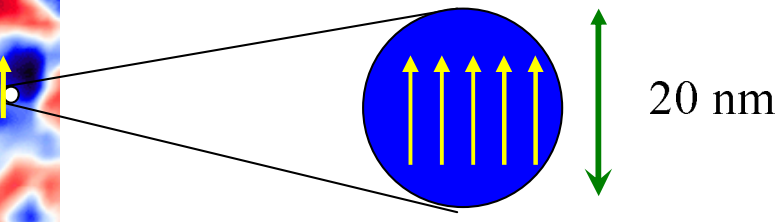


Superparamagnetism

Nanoparticles

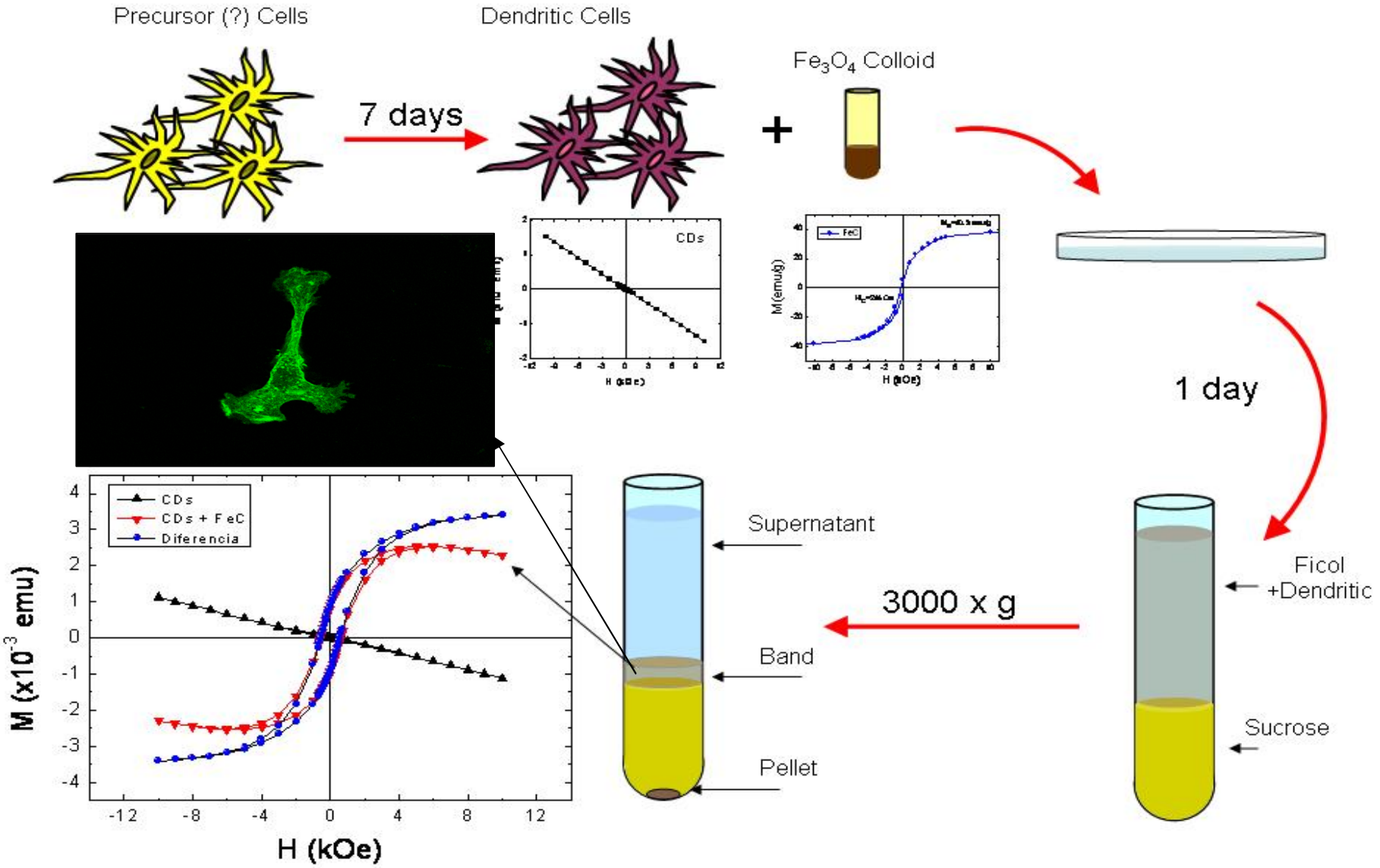


Magnetic domains



Single-domain particles

Dendritic cells as MRI contrast agent



G. Goya et al. INA (2006)

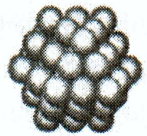
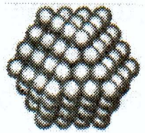
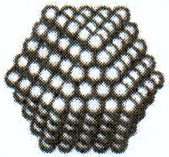
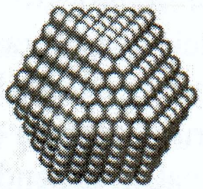
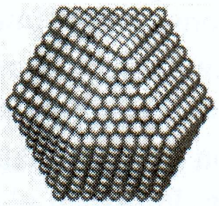
How small?

30 nm → 5 % atoms at the surface

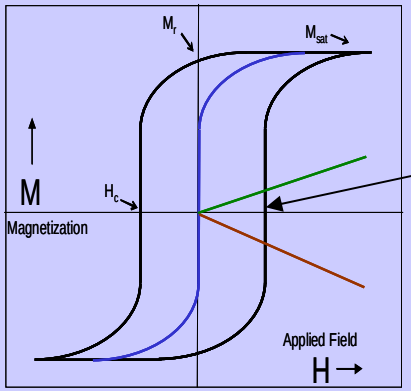
10 nm → 20 % atoms at the surface

3 nm → 50 % atoms at the surface

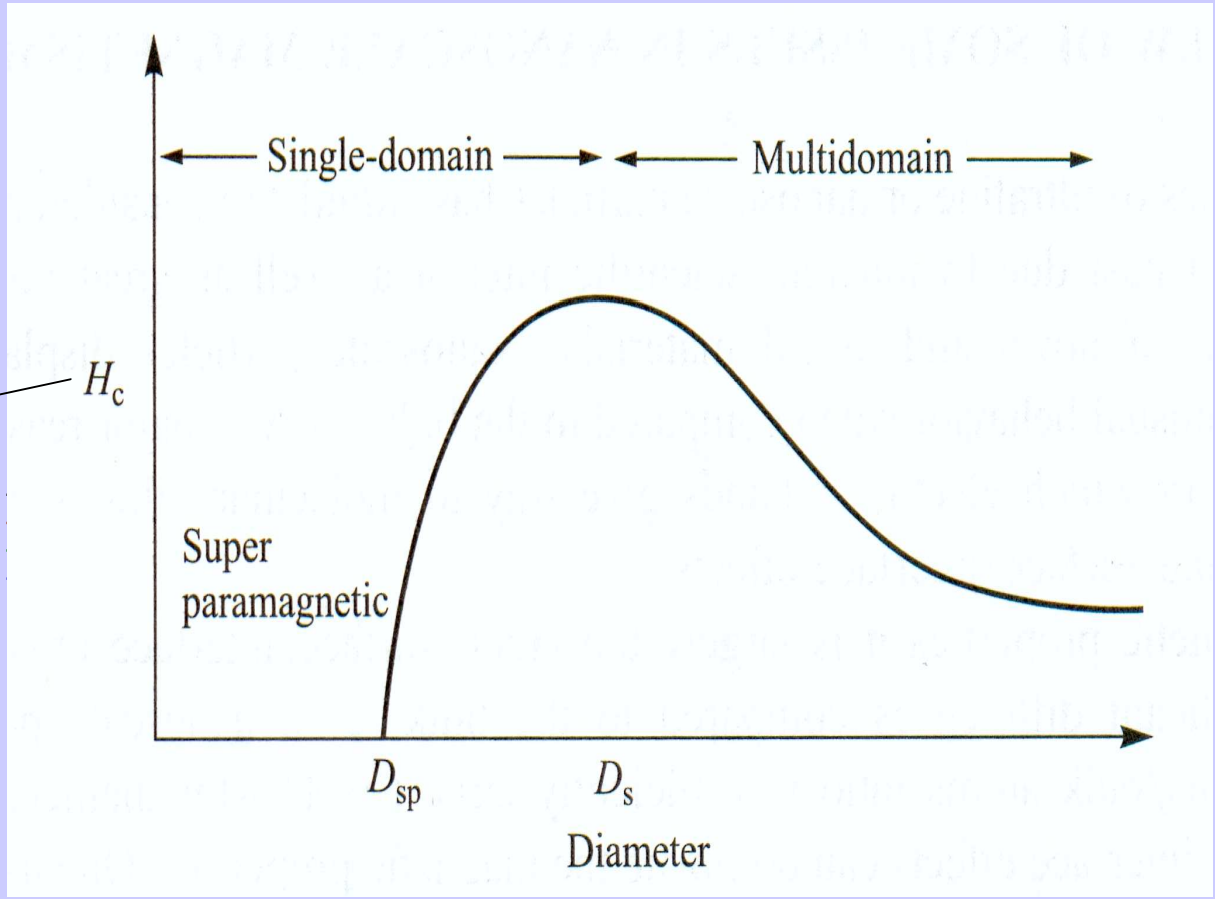
TABLE 2.1 The relation between the total number of atoms in full shell clusters and the percentage of surface atoms

Full-shell Clusters		Total Number of Atoms	Surface Atoms (%)
1 Shell		13	92
2 Shells		55	76
3 Shells		147	63
4 Shells		309	52
5 Shells		561	45
7 Shells		1415	35

Crítical size for single-domain particle



Hysteresis Loop



-Under size reduction the coercive field increases and the the particle becomes single-domain

-When $E_K=KV$ as $V \rightarrow 0$ then $E_K \rightarrow 0$ **superparamagnetic limit** → **$KV = k_BT$**

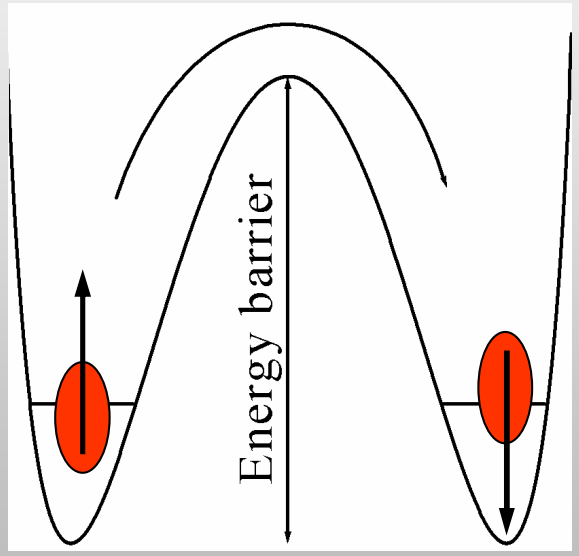
-At this situation the particle magnetic moment will fluctuate independently of the particle

Time effects: relaxation

Due to the stocastic nature of the thermal energy the superparamagnetism is a time dependent effect

$$\tau = \tau_0 \exp(-KV/k_BT)$$

τ time for magnetization reversal (depend on the anisotropy)



Si $\tau < \tau_{measure}$ superparamagnetism

τ_0 tipically 10^{-9} s

$$T_B = \frac{K_{eff}V}{k_B \ln\left(\tau_M / \tau_0\right)}$$

Critical volume to detect superparamagnetism:

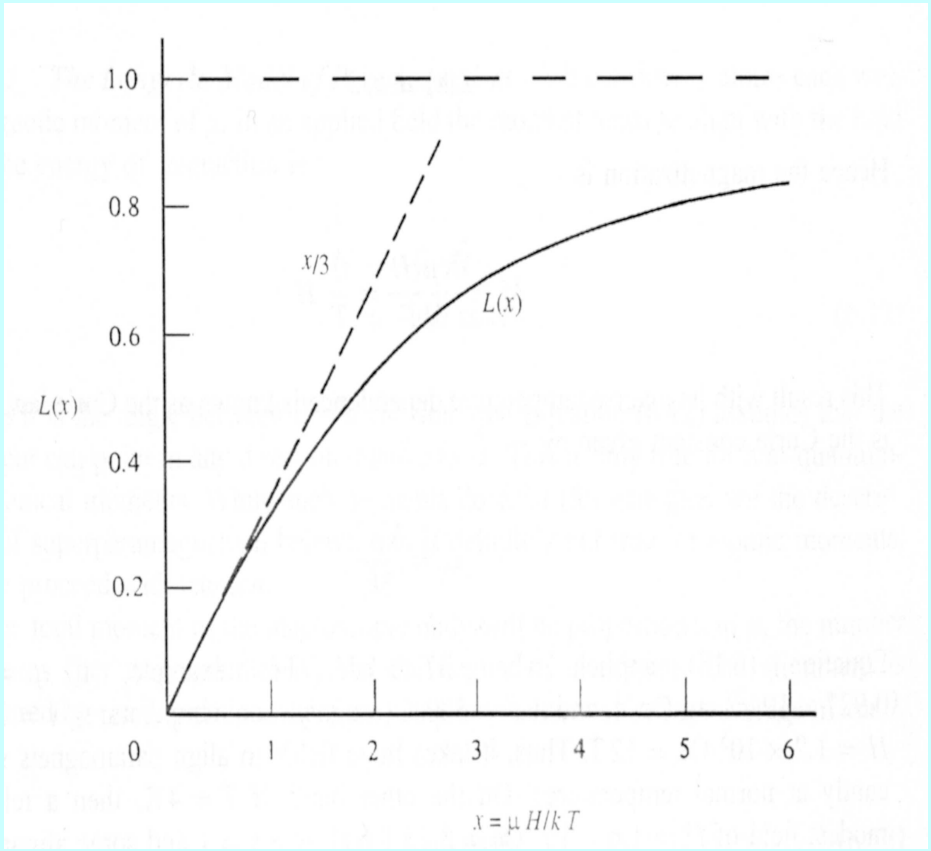
$V_{sp}=25(k_BT/K)$ $\tau_{measure}=100$ s $T_B=KV_{sp}/25k_B$

$V_{sp}=4.5(k_BT/K)$ $\tau_{measure}=10^{-7}$s $T_B=KV_{sp}/4.5k_B$

T_B Mösbauer = 5.5 T_B magnetometry (FC y ZFC)

Fe y Co at 300K V_{sp}=16 y 7.6 nm

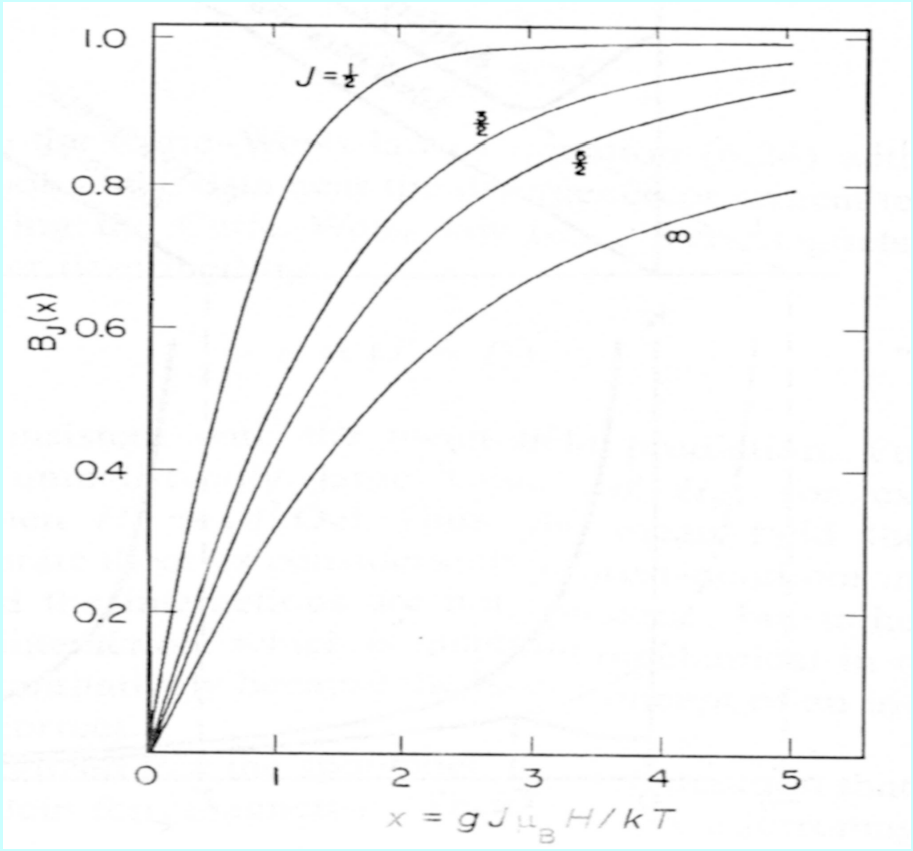
Classic paramagnet



If K → 0

The supermoment follows the Langevin law

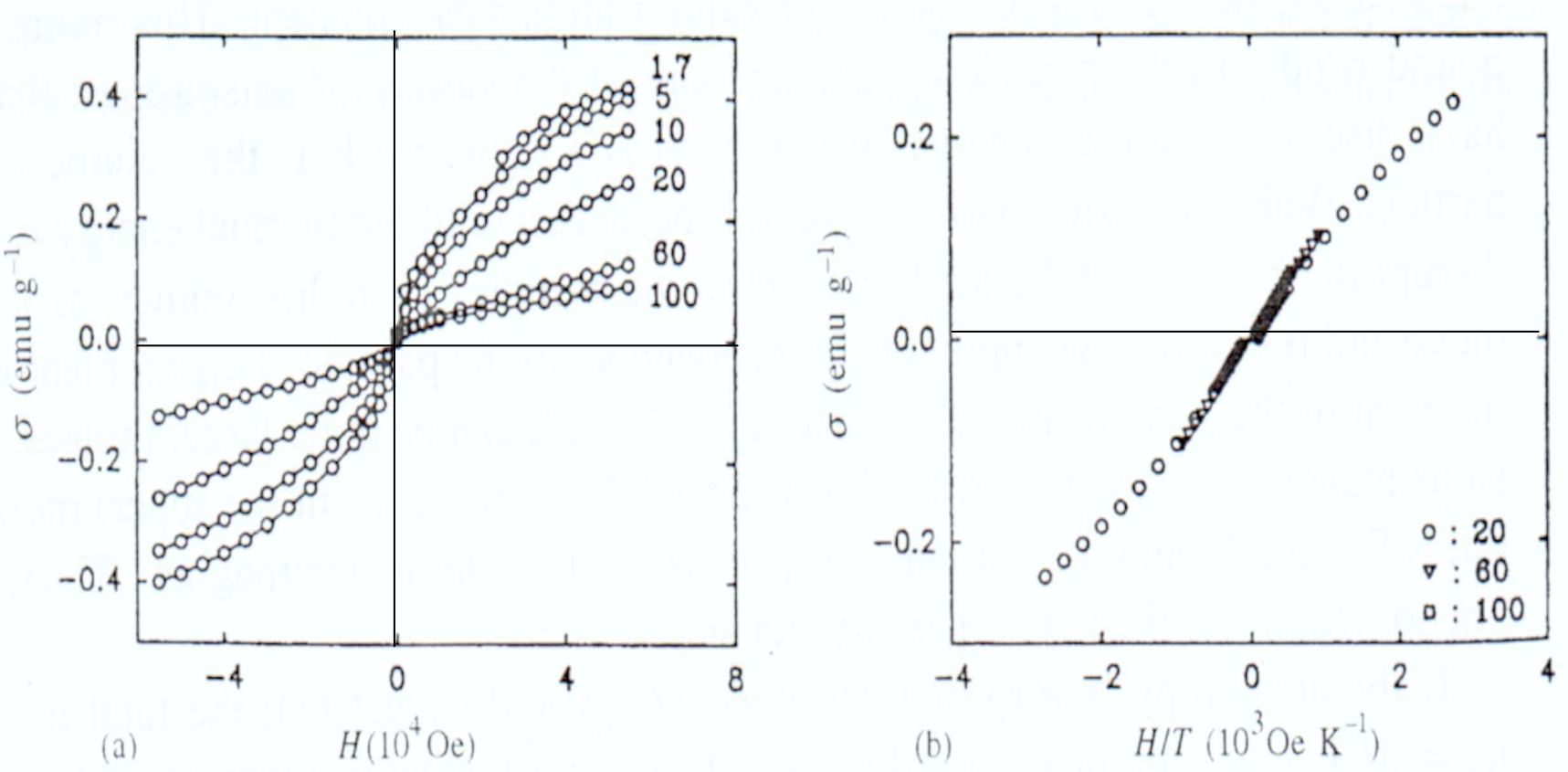
Quantum paramagnet



If K>>>

The supermoment follows the Brillouin J=1/2 law

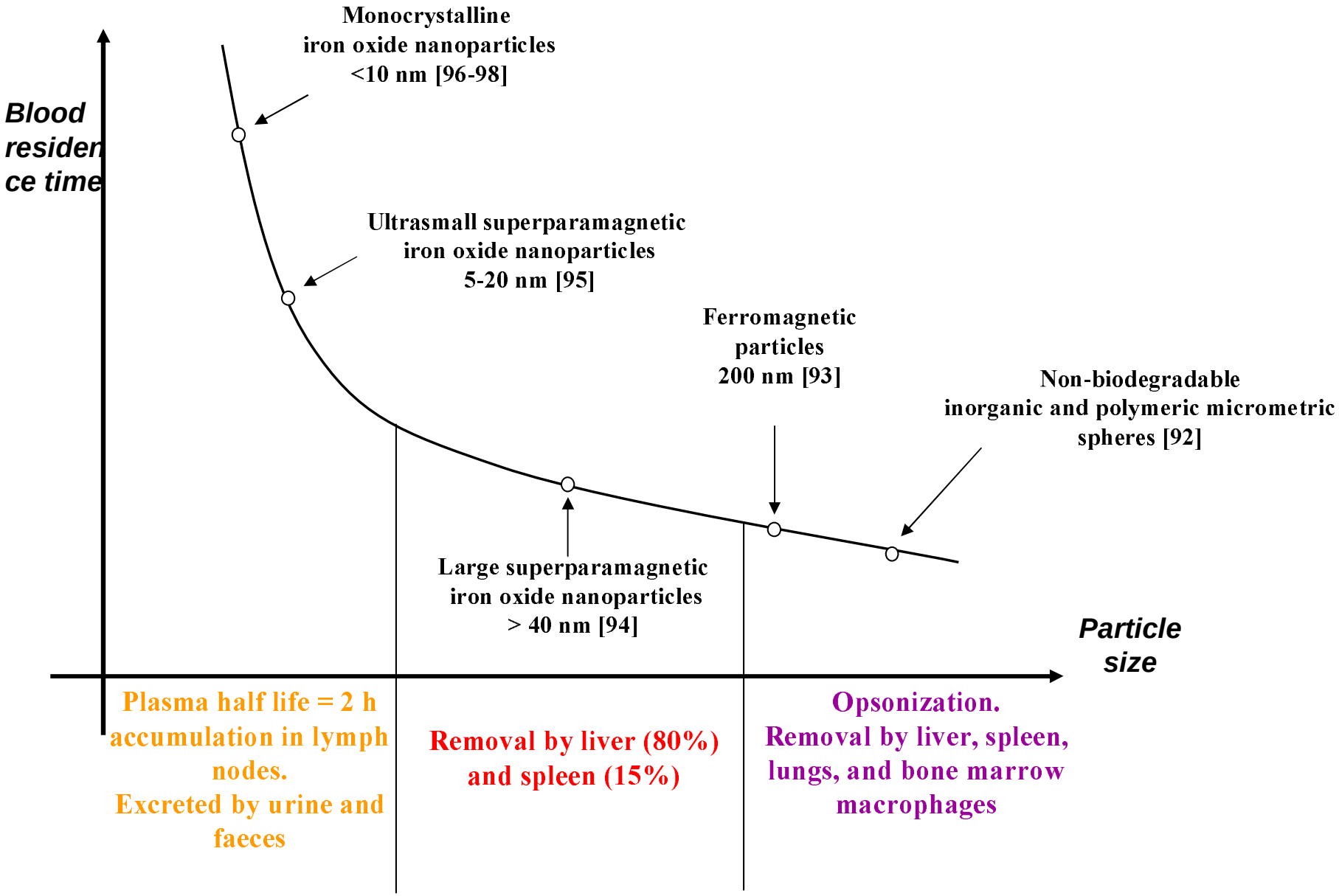
Real superparamagnetic system



-No hystheresis

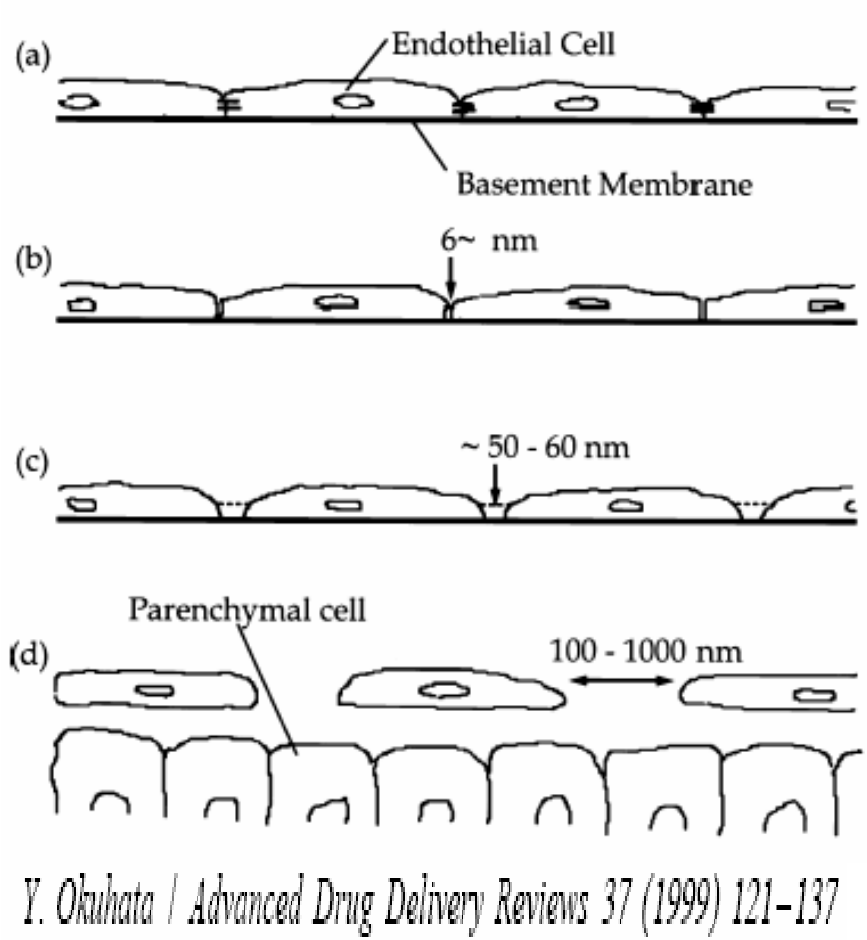
-The isotherm presents a universal H/T behaviour

Qualitative diagram showing the evolution of blood residence time with particle size



Different classes of blood capillaries

For agents to distribute in a tissue they must go through the vascular capillary wall; The permeability of this wall is essential



Y. Okuhata / Advanced Drug Delivery Reviews 37 (1999) 121–137

Lining cells are connected with a tight junction (BBB "Blood-Brain-Barrier)(Central nervous system)

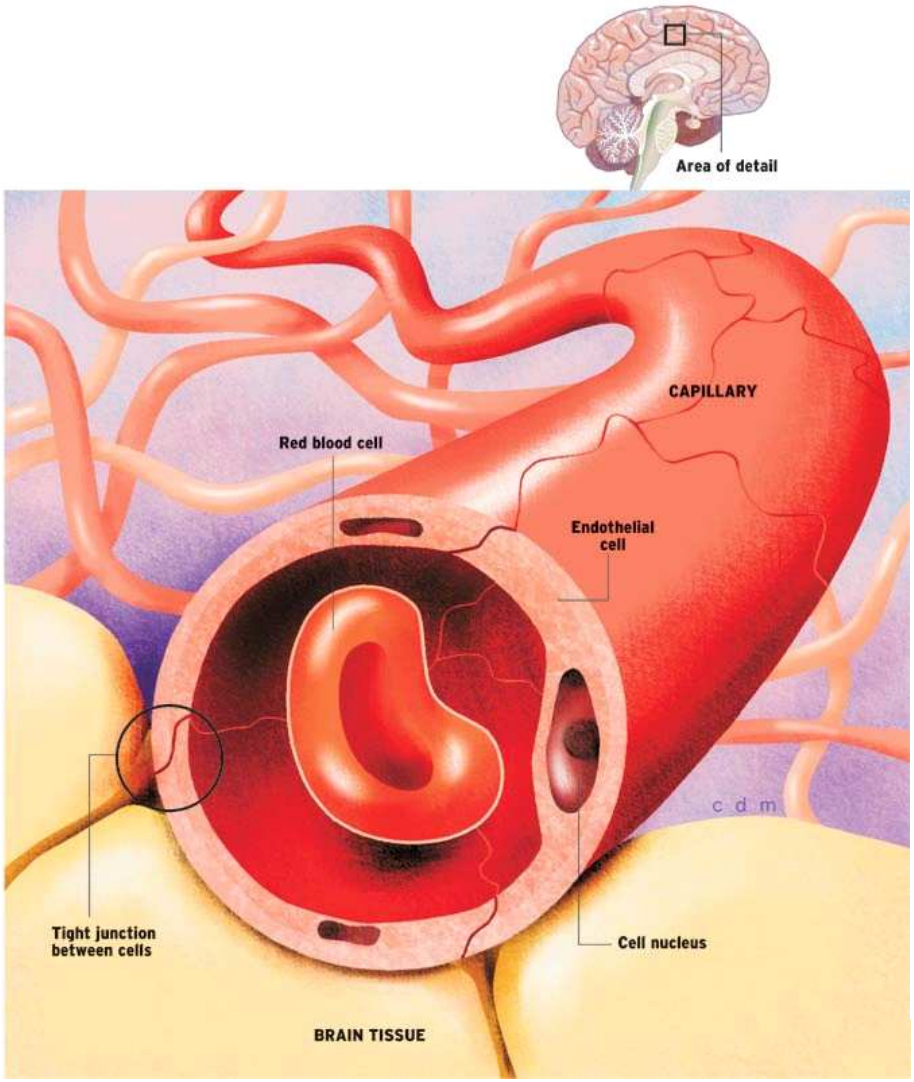
Continuous capillaries (muscles,skin, lung…

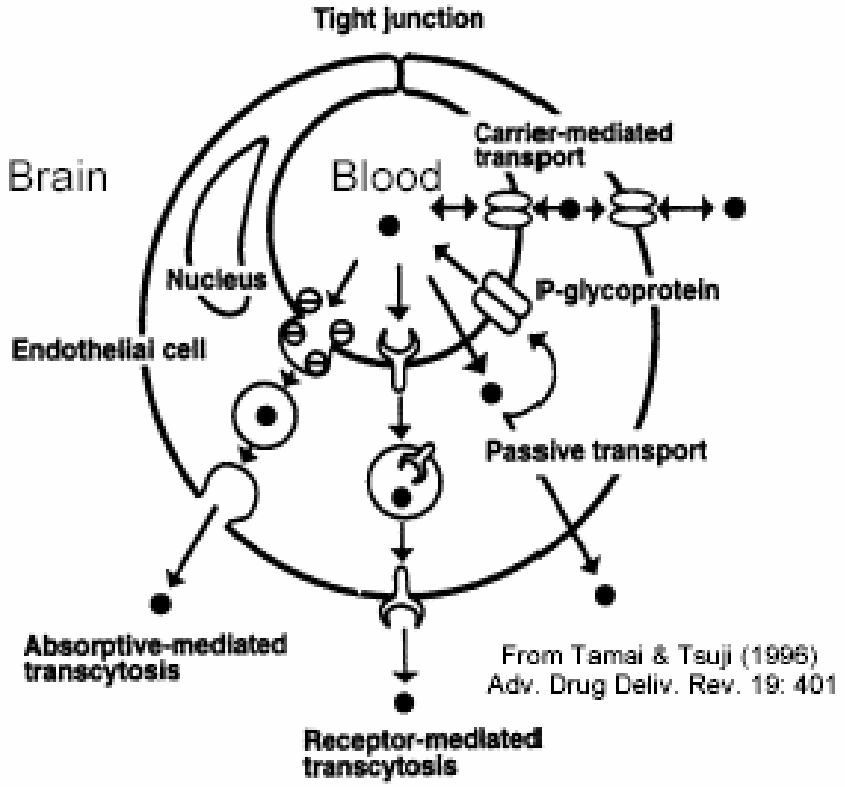
Fenestrated capillaries (kidney, instentine and glands..)

Sinusoid capillaries (liver, spleen and bone marrow…)

Nanoparticles between 60-80 nm are removed from the blood stream in 8-10 minutes

Brain Blood Barrier (BBB)





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-Bioferrofluids for local drug delivery:

i) In vitro experiment: blood biocompatibility and drug carrier

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FOR MEDICAL APPLICATIONS WE NEED:

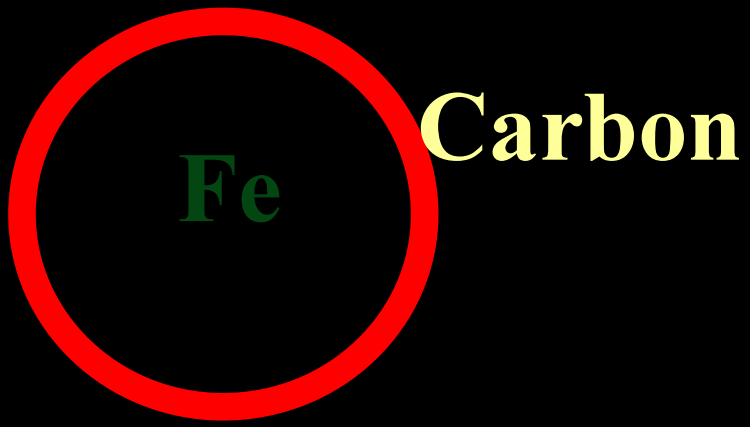
-Small particles

-Strong magnetic response

-Biocompatibles

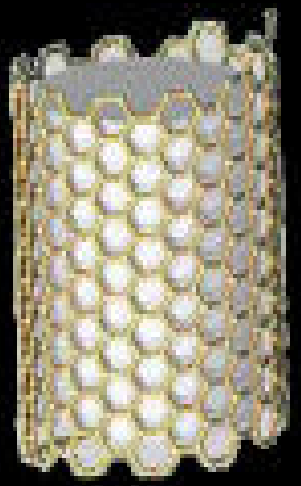
-Capability to adsorb the drug in short time

-Release the drug during long time



Particles coating: Carbon and Silica nanocages

The discovery of graphitic nanostructures as fullerenes and nanotubes offers the possibility to fill nanoscale cavities with transition metals

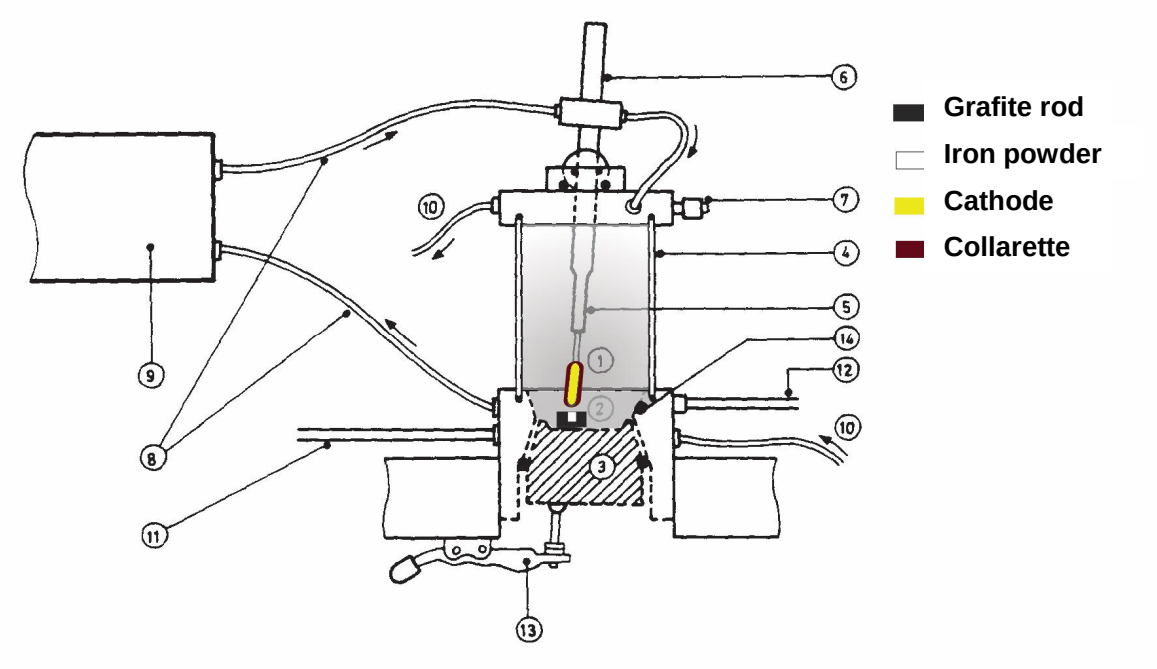


The confinenement of this small amount of material promises:

-Novel physical properties

-Protection of the encapsulated metals from oxidation by resistant carbon cages

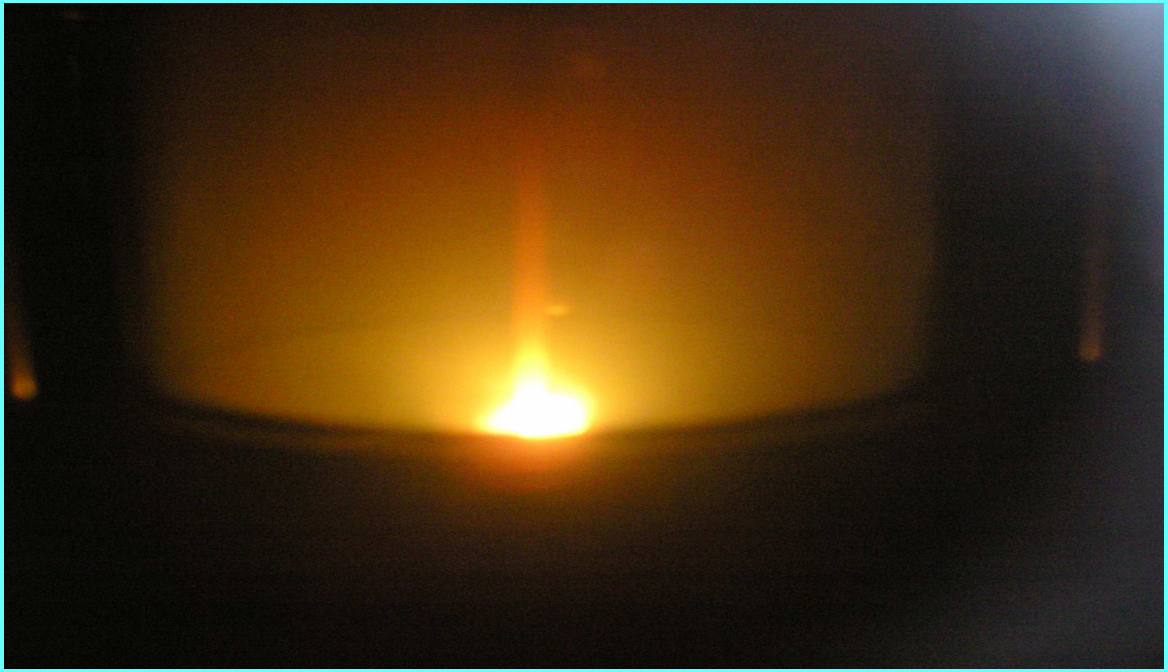
Plasma Furnace

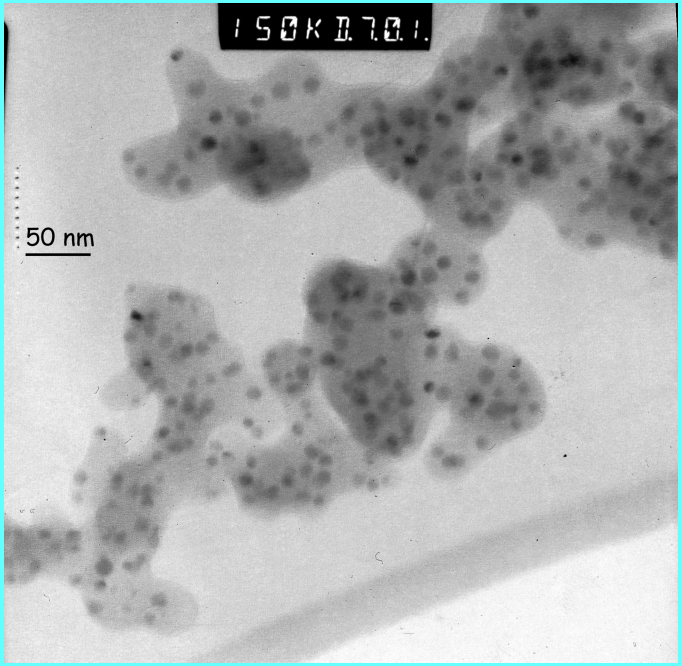


(Kratschmer W. et al., (1990) Nature 347, 354)

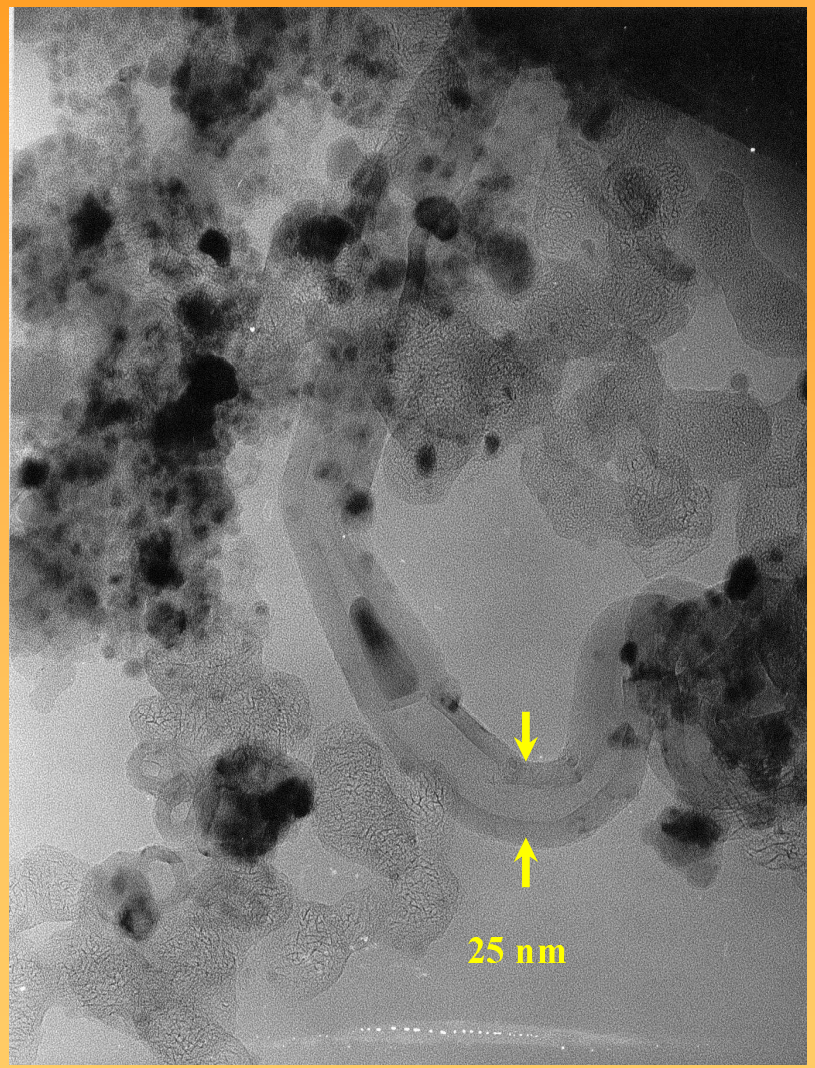
-Helium plasma vaporizes the iron and carbon electrode

-Small drop of Fe condensed in nanoparticles coated by carbon cages

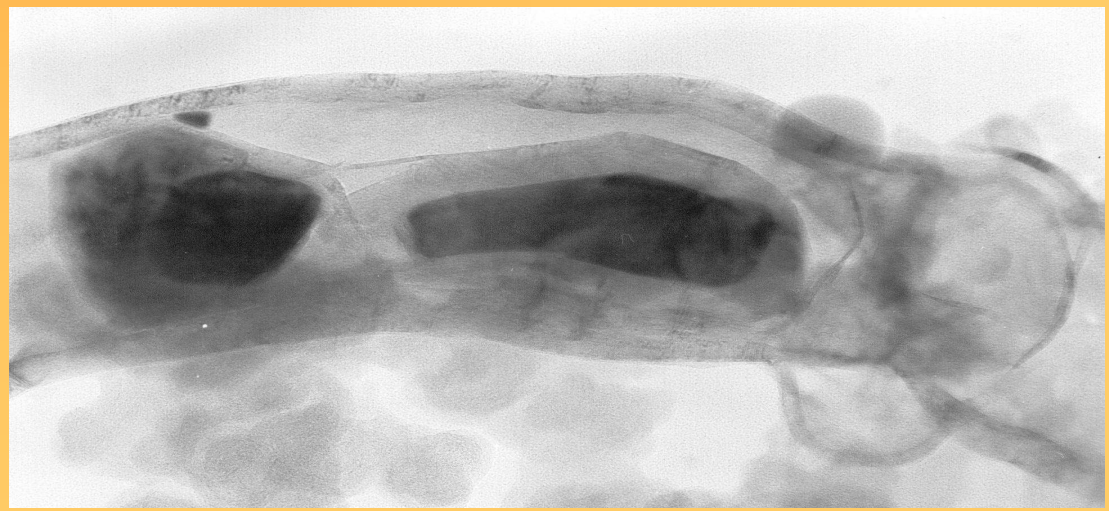




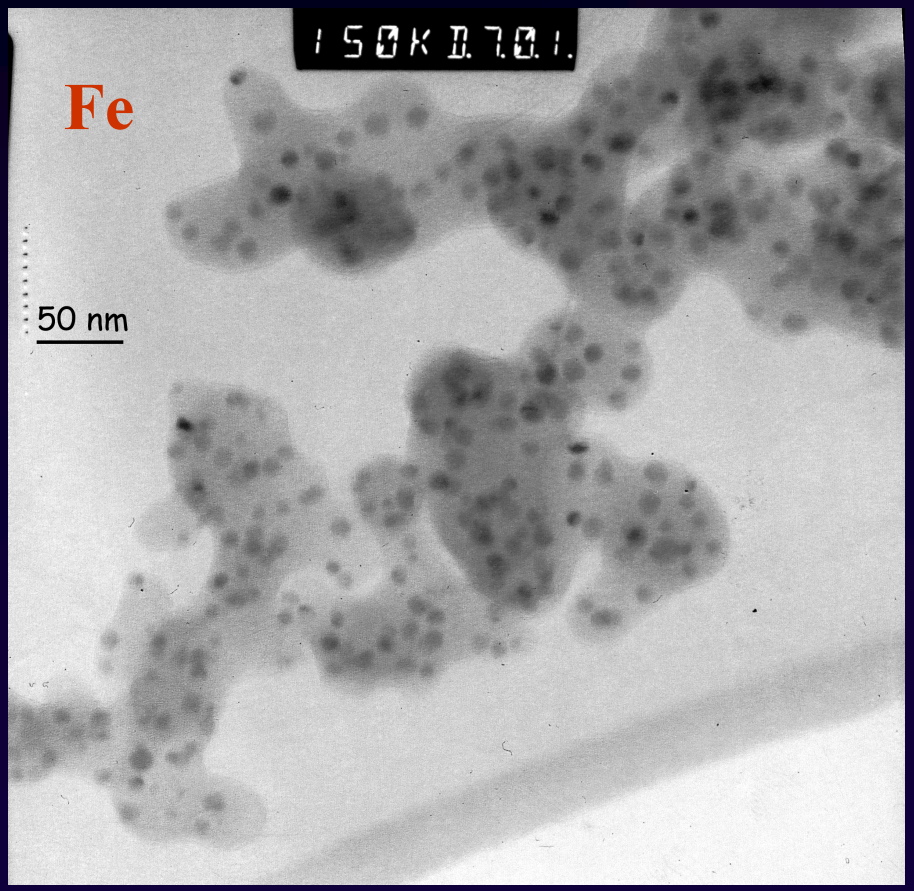
Transition metals encapsulated nanoparticles

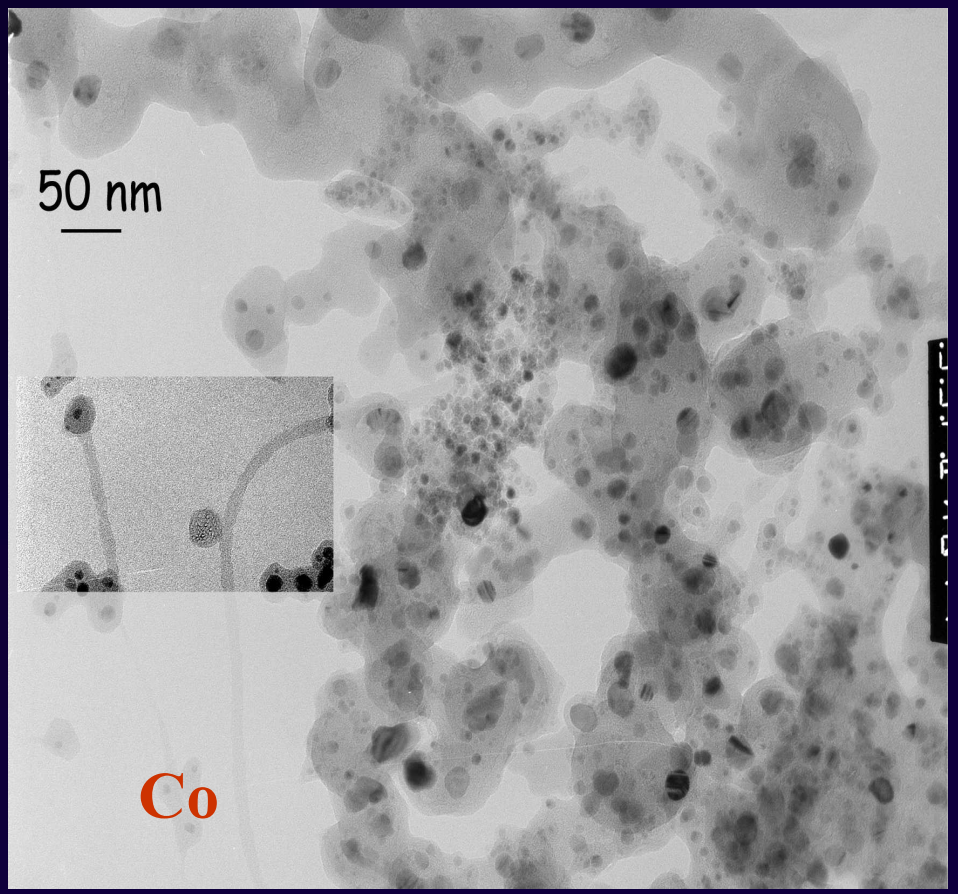


-Graphitic multiwall nanotubes
-Catalytic particles forming large single wall nanotubes
-Small particles sourronded by polygonal layers: Onions
-Metallic inclusions in nanotubes
-Nanoparticles encapsulated in graphitic layers and glassy carbon

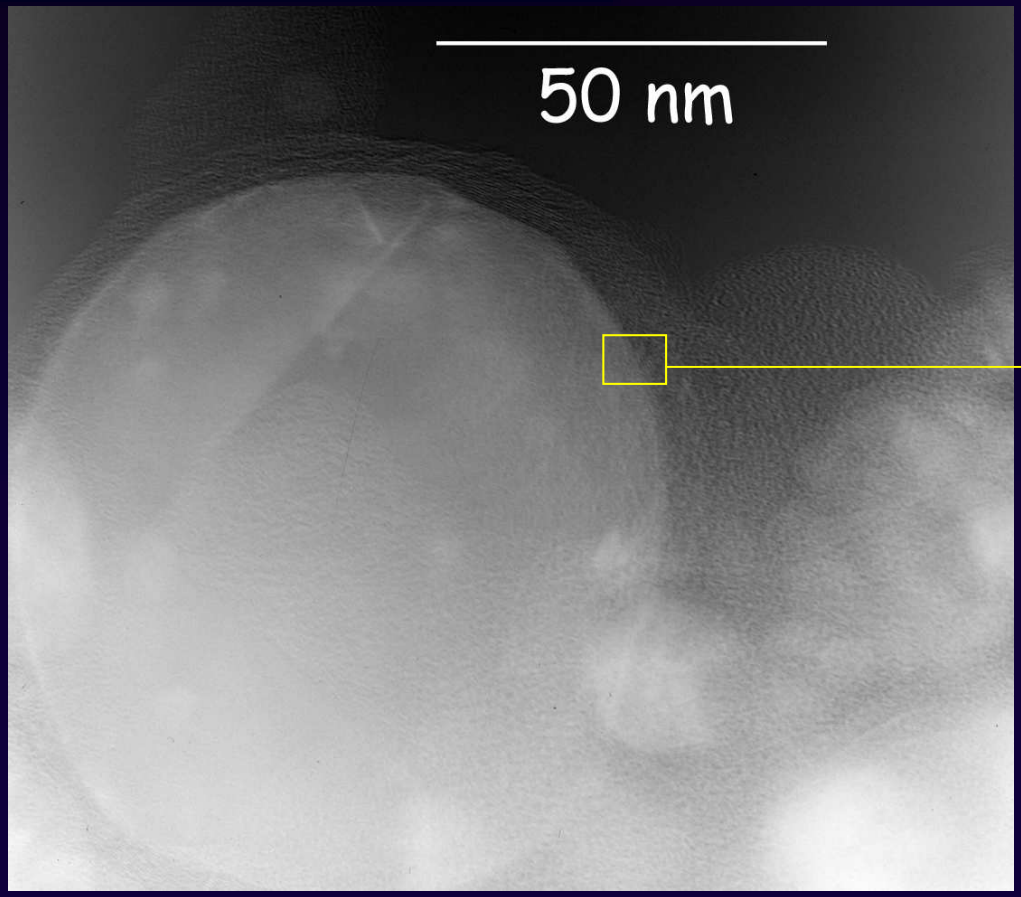


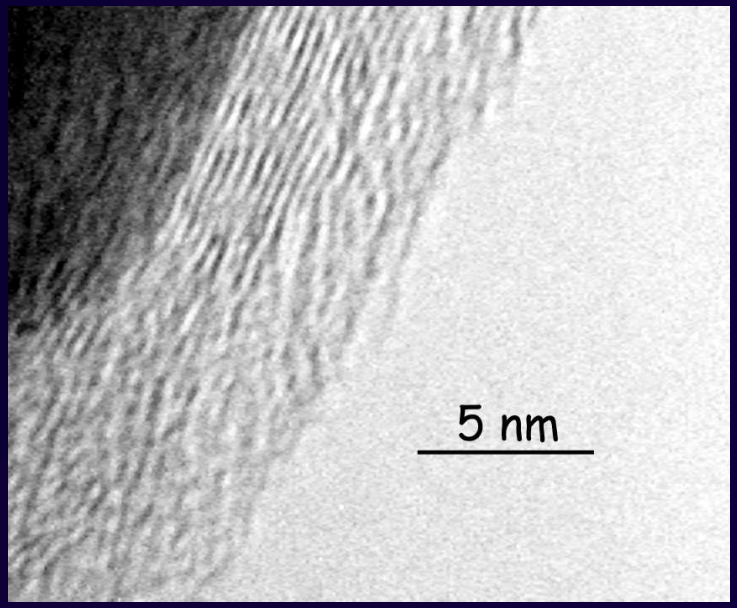
TEM images of Fe & Co encapsulated nanoparticles





Fe coated by graphitic layers



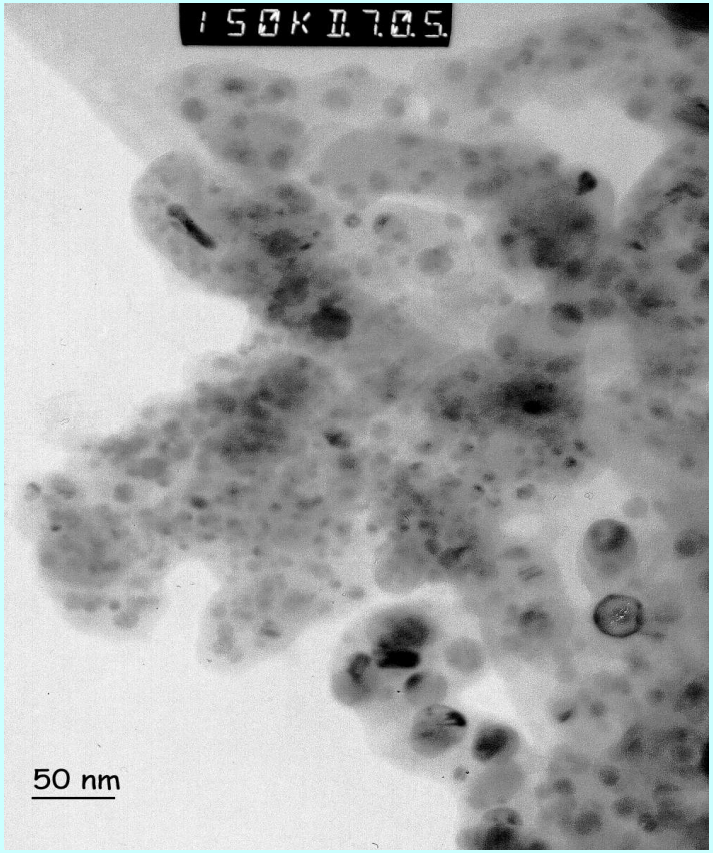


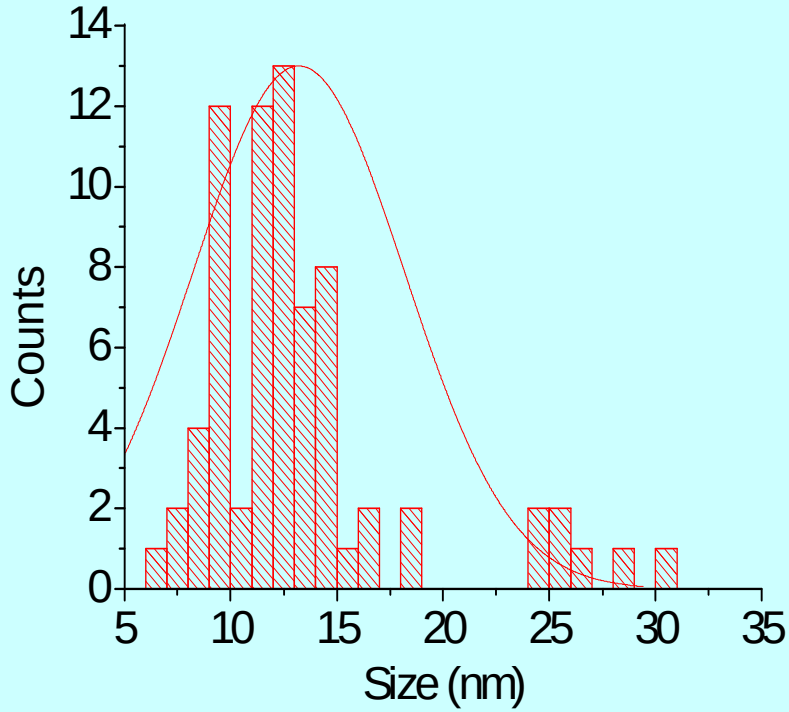
Sample treatment an average size

-Samples are sonicated in a dilution of surfactant (SDS and distilled water (5g/l))

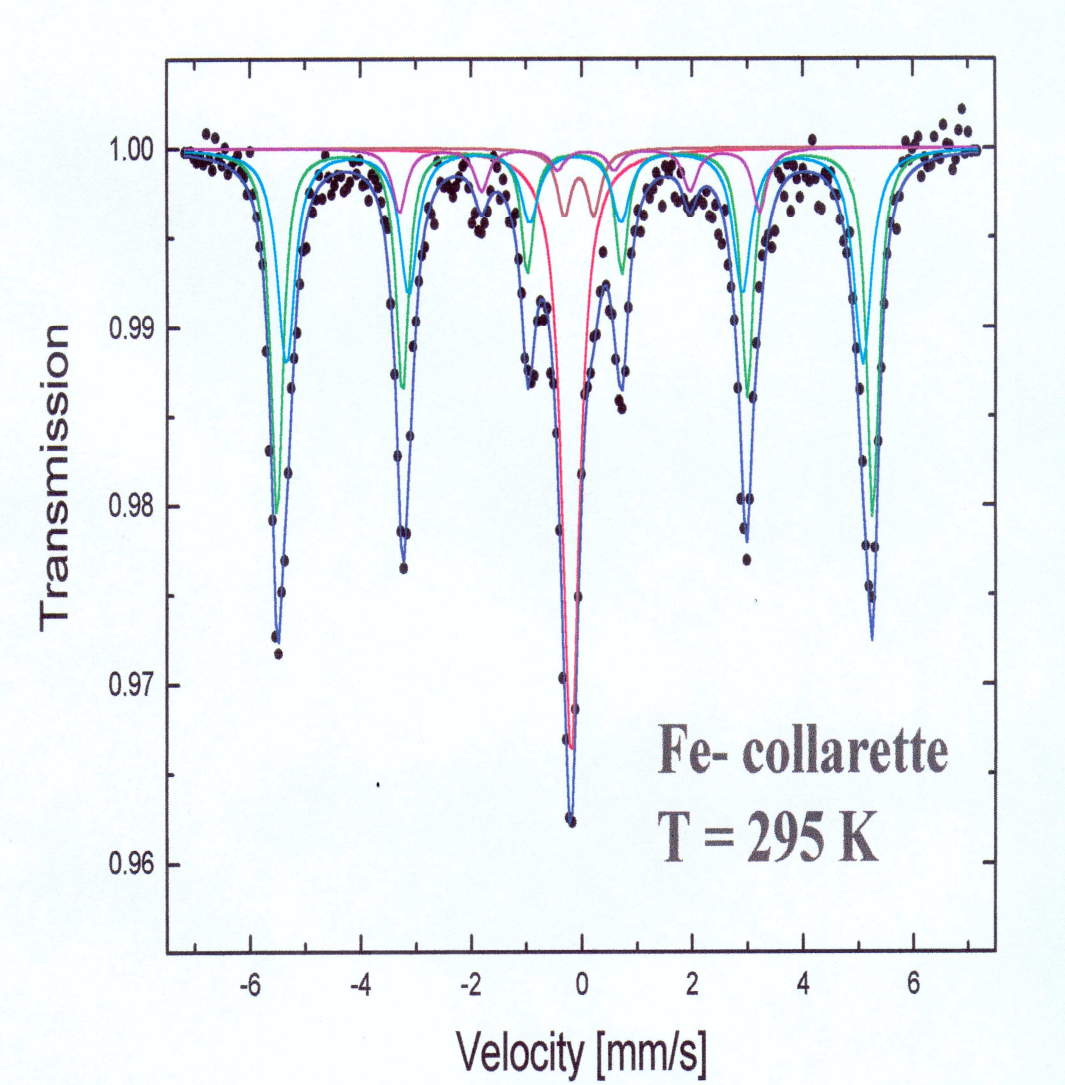
-Magnetic separation is acheived in a field gradient of 3 kOe/cm

-Chemical etching with aqua regia is made to remove the uncovered metallic particles





Mössbauer spectroscopy



No indication of SP relaxation at room temperature

Estimated particle size 13-9 nm (interparticle interaction)

α–Fe

Two sextets (34T and 31T)

Fe_3C

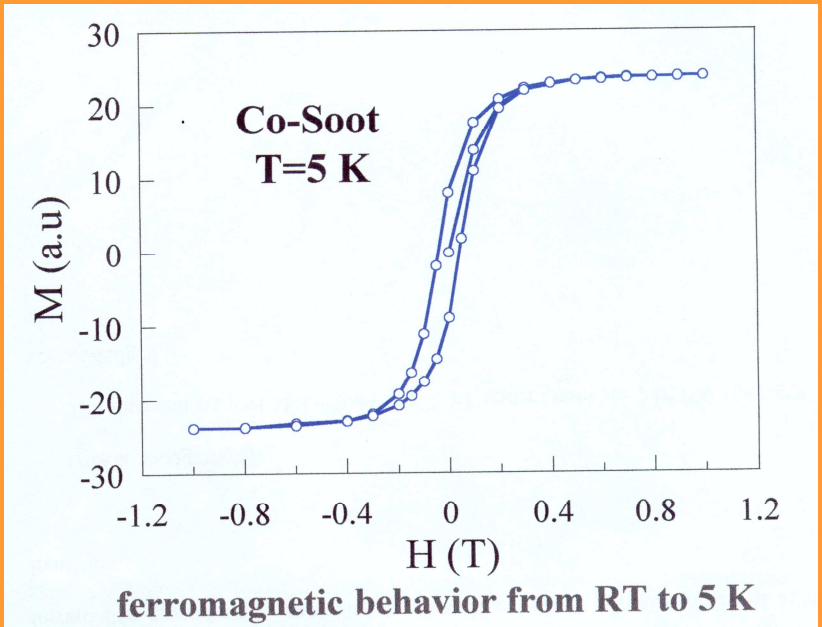
A sextet (25.1 T)

γ-Fe

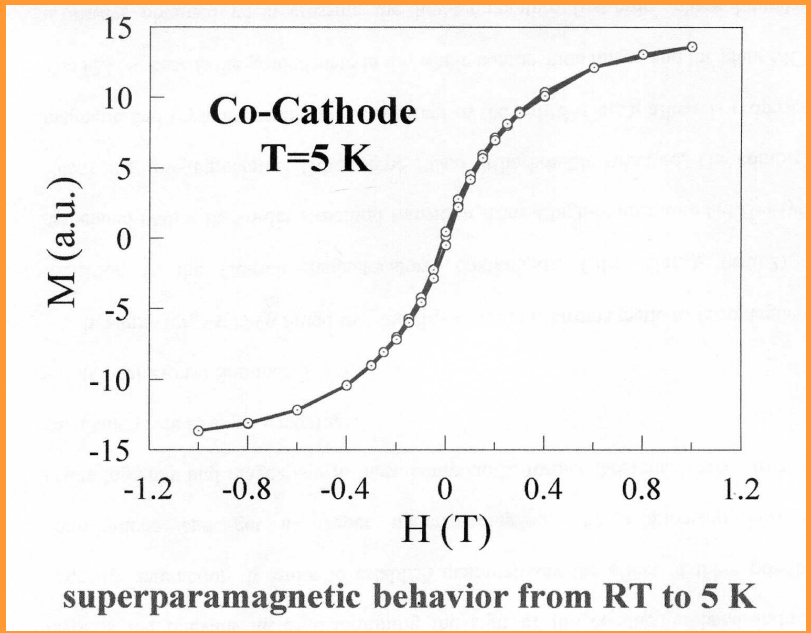
Singlet and doublet

H.R. Rechenberg et al. J. Magn. Magn. Mat 226-230 (2001) 1930

Magnetization measurements



ferromagnetic behavior from RT to 5 K



superparamagnetic behavior from RT to 5 K

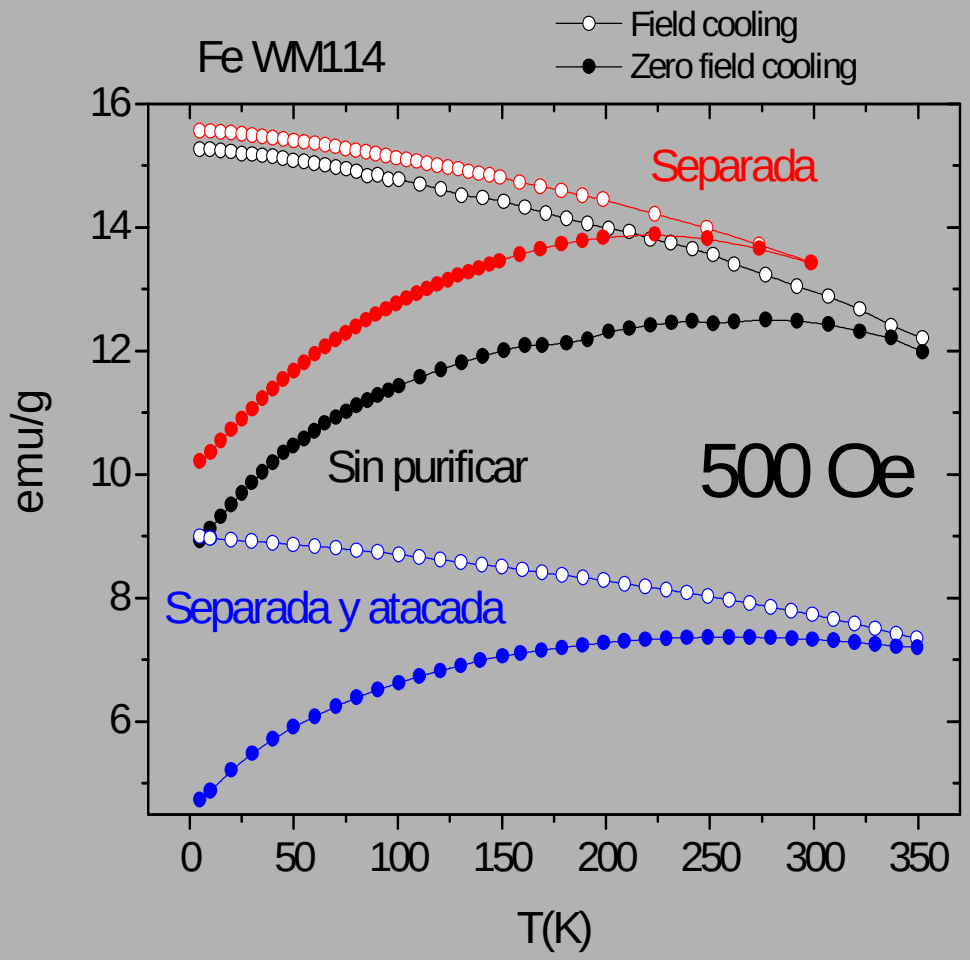
Blocked particles

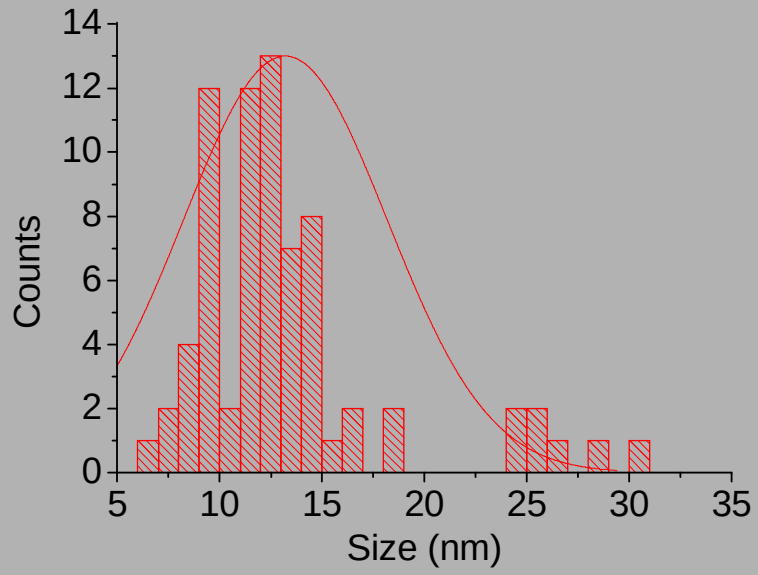
Large/correlated particles

Superparamagnetic particles

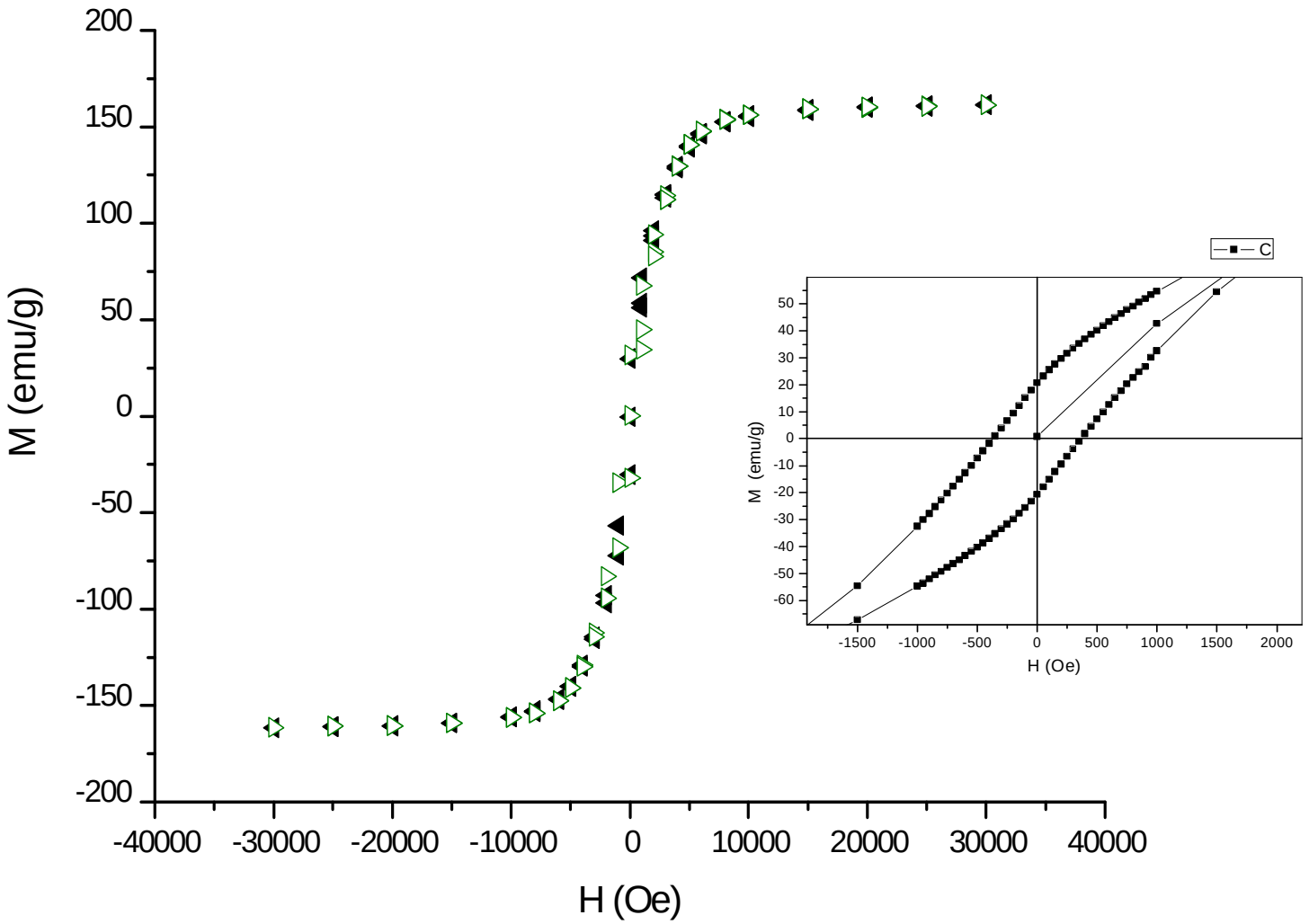
Small particles

Blocking temperature is determinaed from FC and ZFC

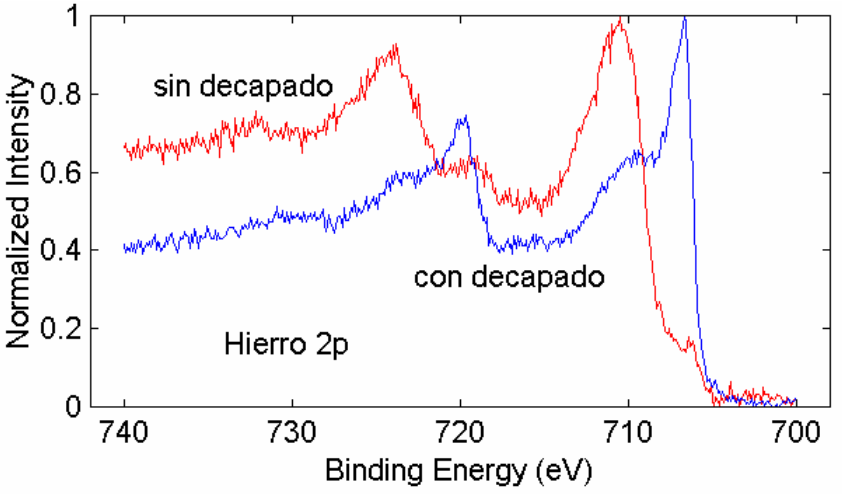


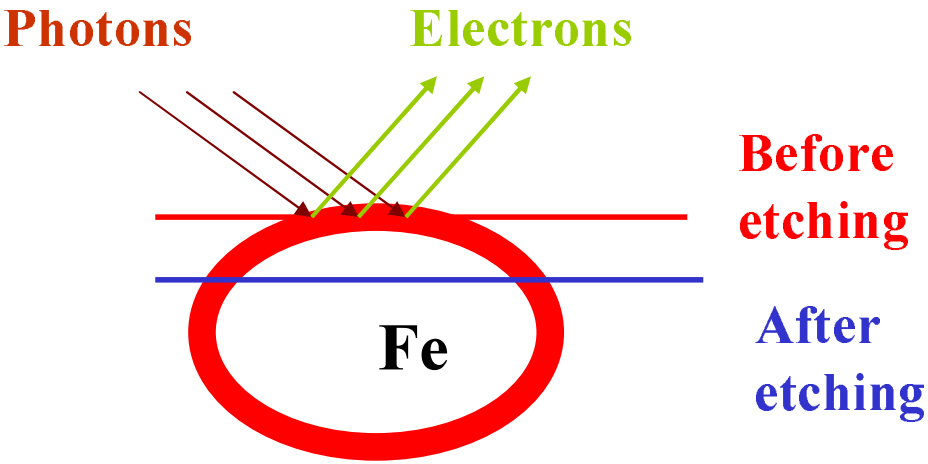


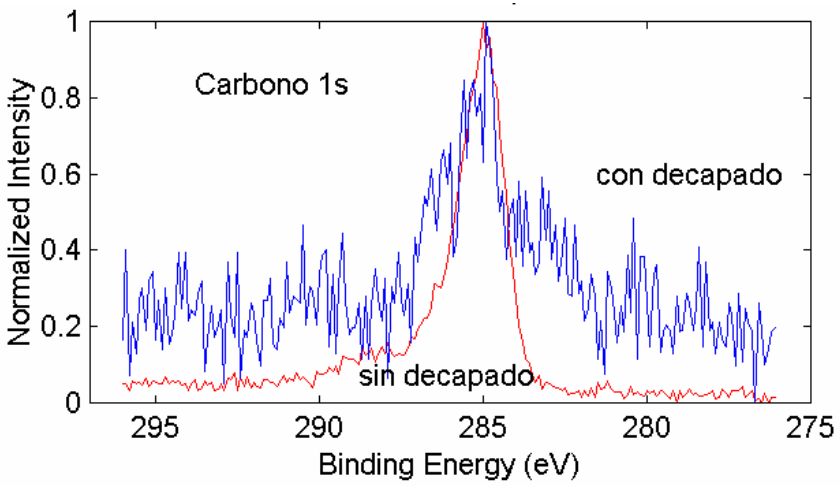
Silica encapsulated Fe nanoparticles

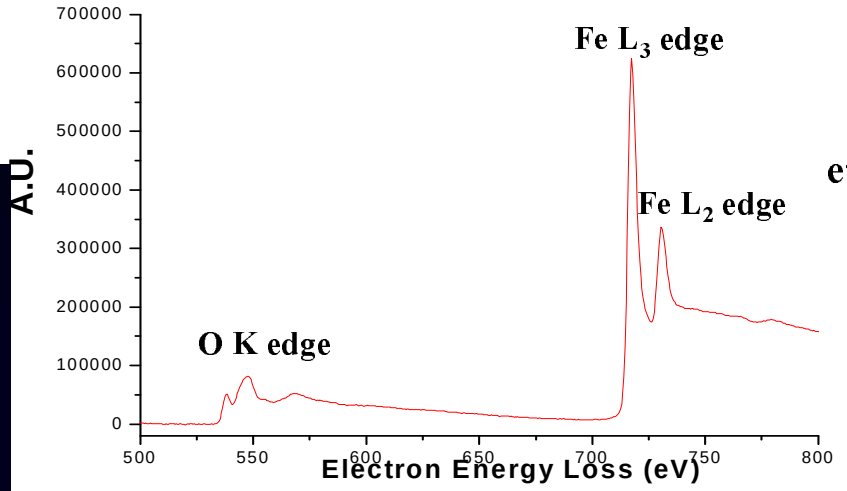


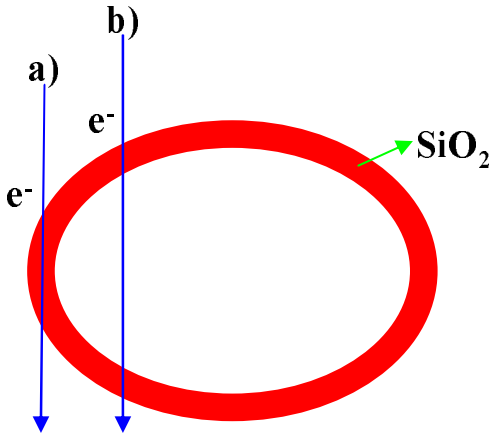
Fe encapsulated in Silica X-Ray Photoelectron Spectroscopy

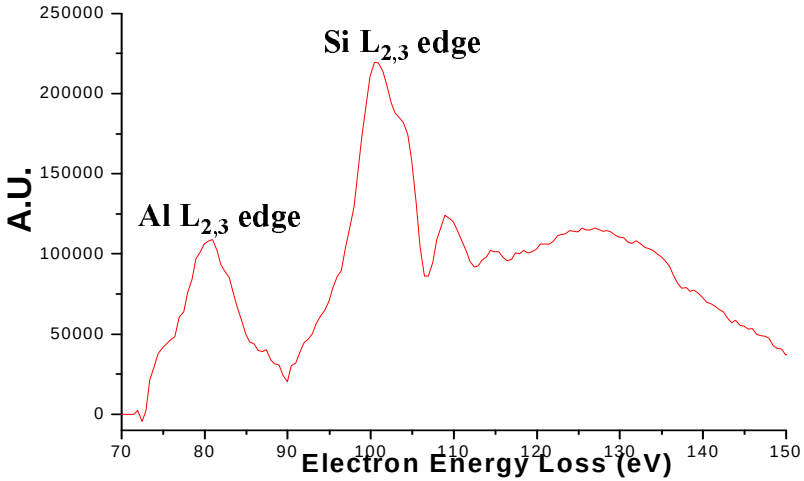


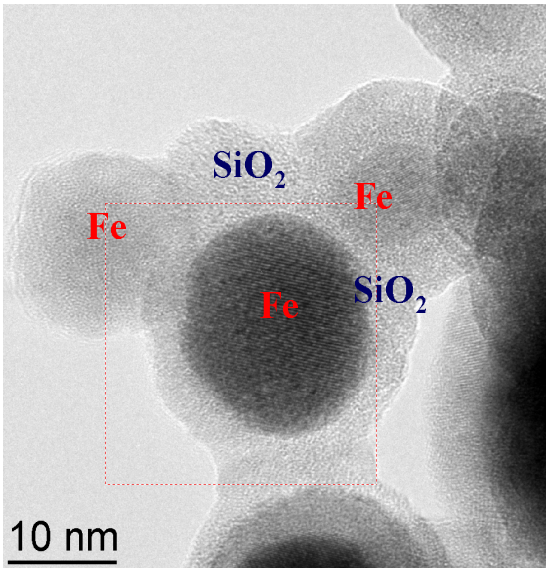





A.U.
700000
600000
500000
400000
300000
200000
100000
0
500
550
600
650
700
750
800
Fe L3 edge
Fe L2 edge
O K edge
Electron Energy Loss (eV)

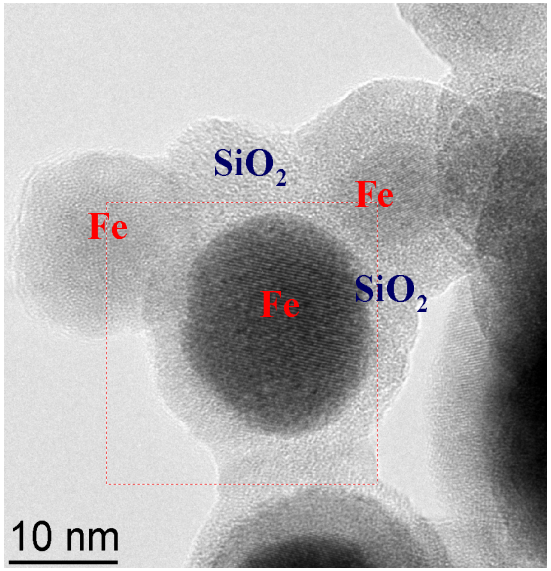

a)
b)
e-
e-
SiO2

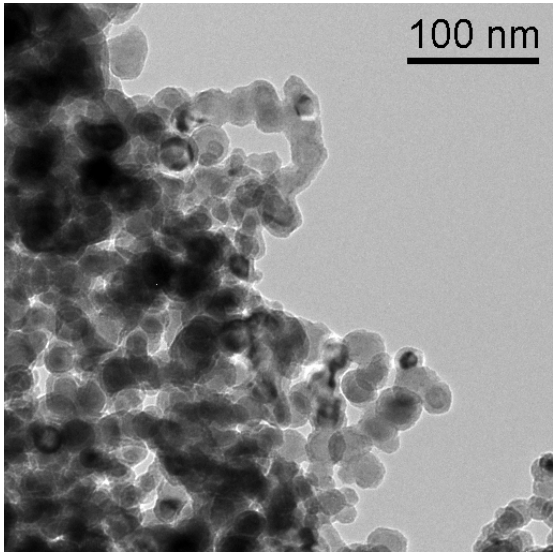

A.U.
250000
200000
150000
100000
50000
0
70
80
90
100
110
120
130
140
150
Si L2,3 edge
Al L2,3 edge
Electron Energy Loss (eV)

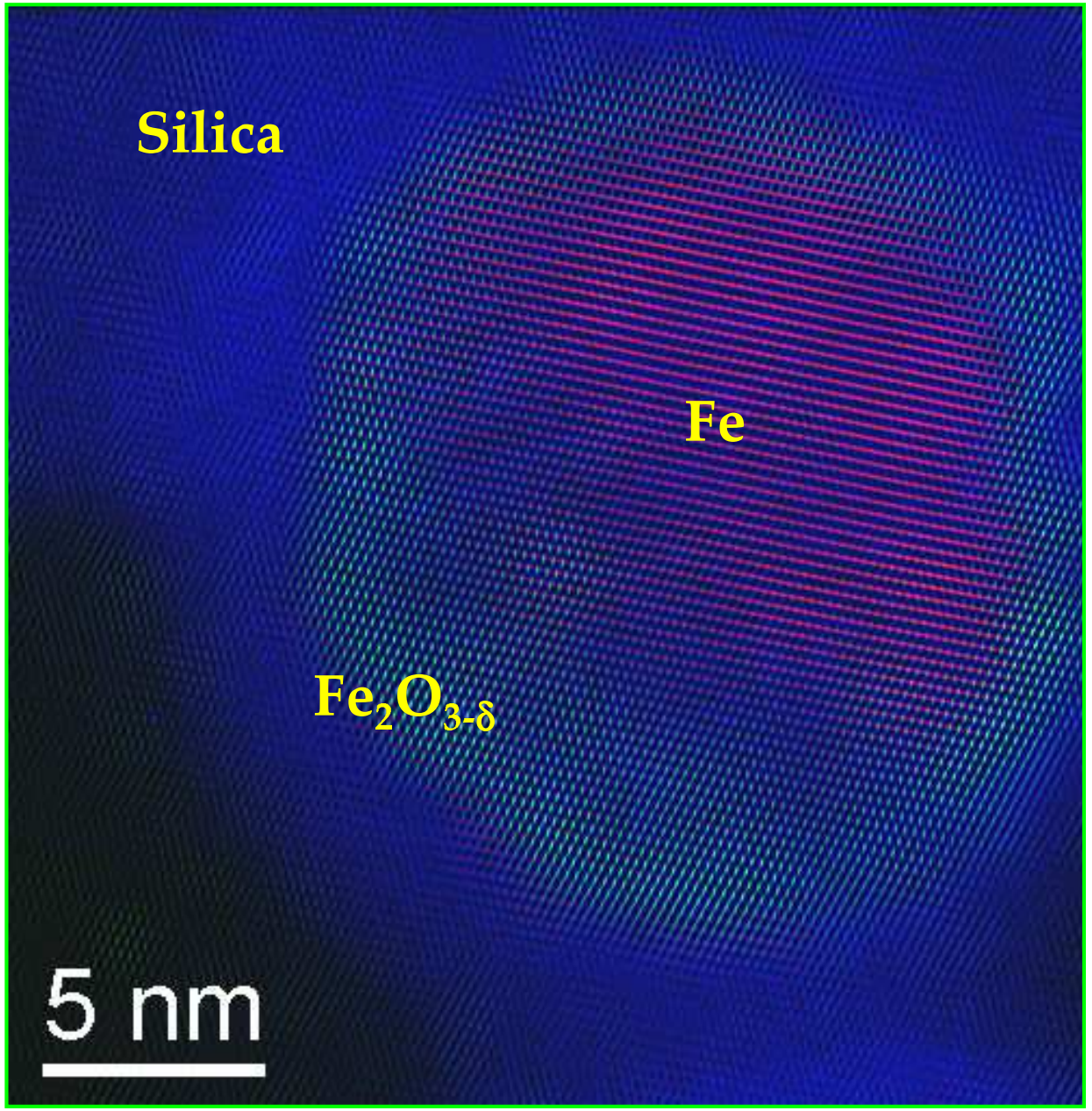

SiO2
Fe
Fe
Fe
SiO2
10 nm

Iron nanoparticles encapsulated in Silica

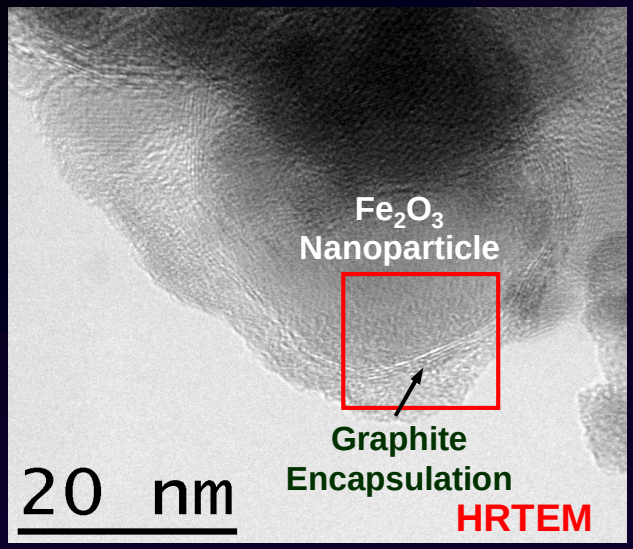
(M. Arruebo et al. Adv. Funct. Mat. 2007)

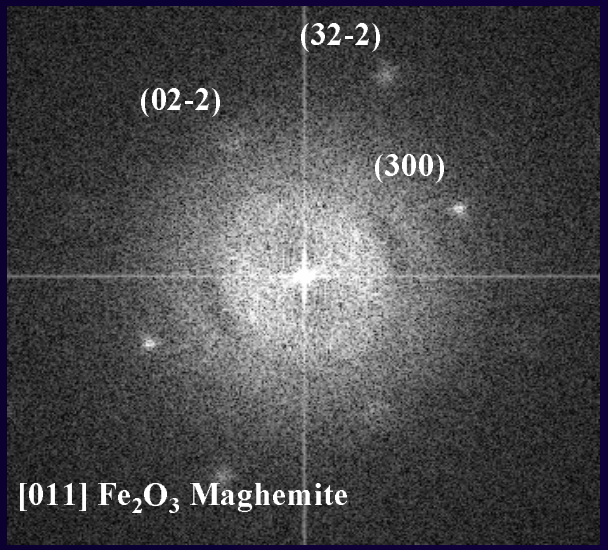


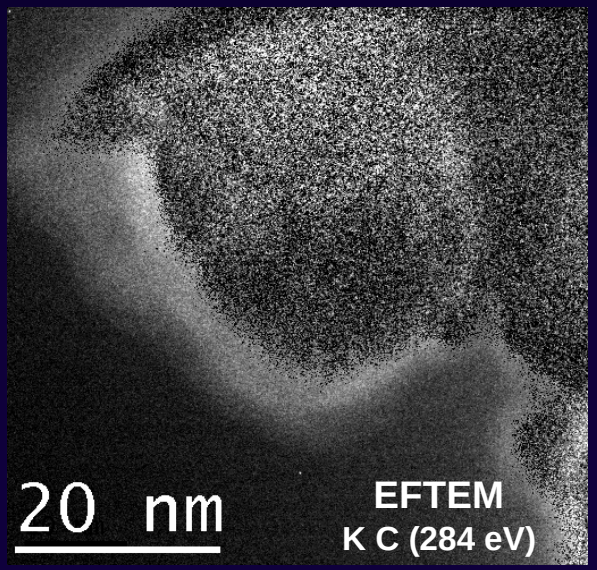


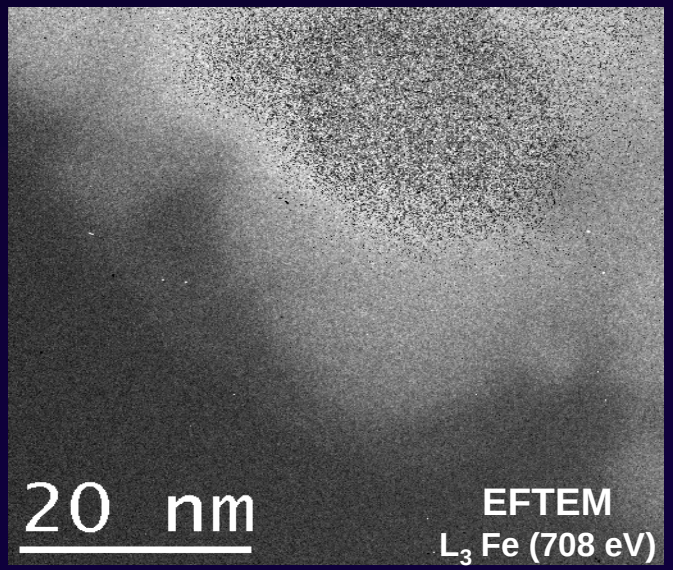


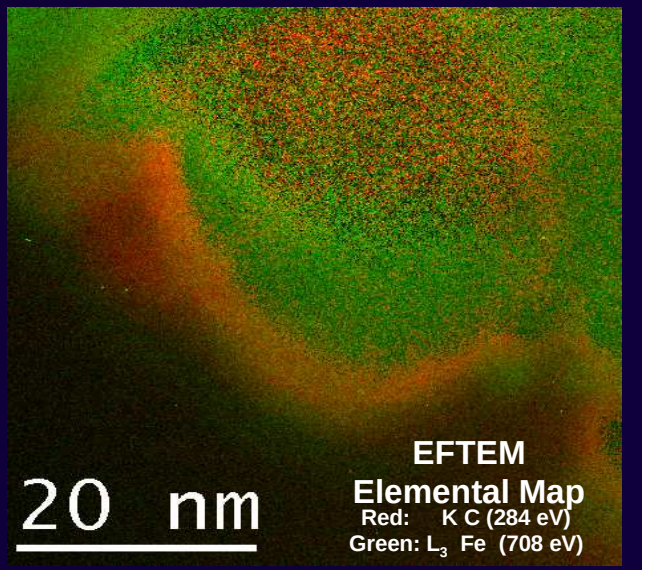
High Resolution Transmision Electron Microscopy of carbon encapsulated iron nanoparticles



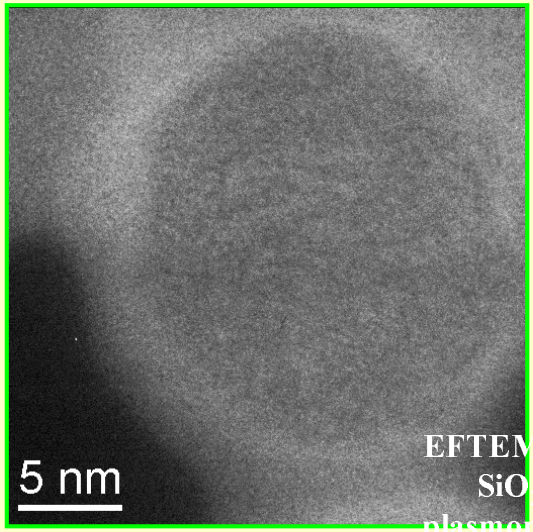








Cryatalline structure at the nanoscale



Fourier transform

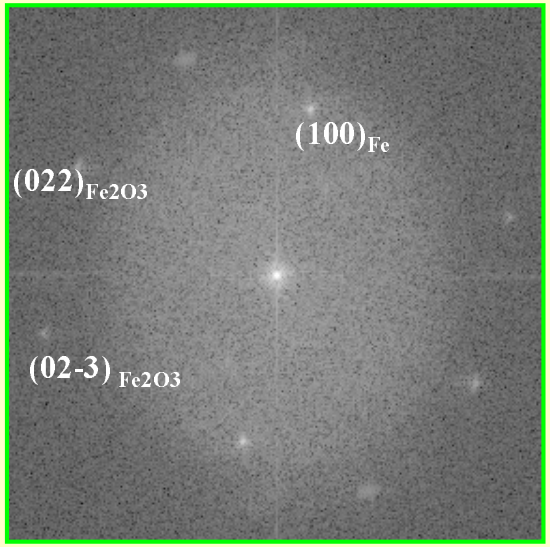
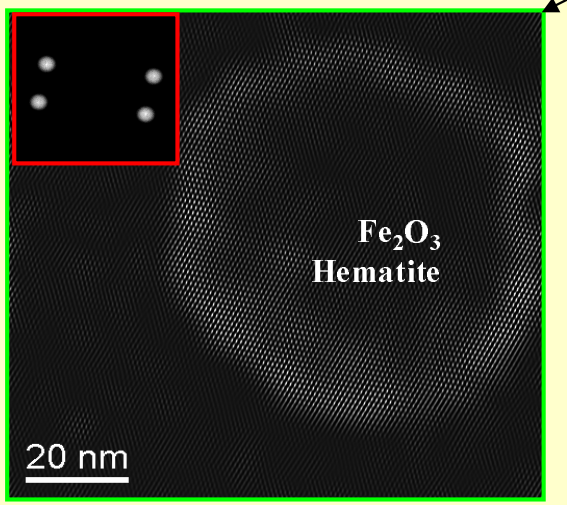
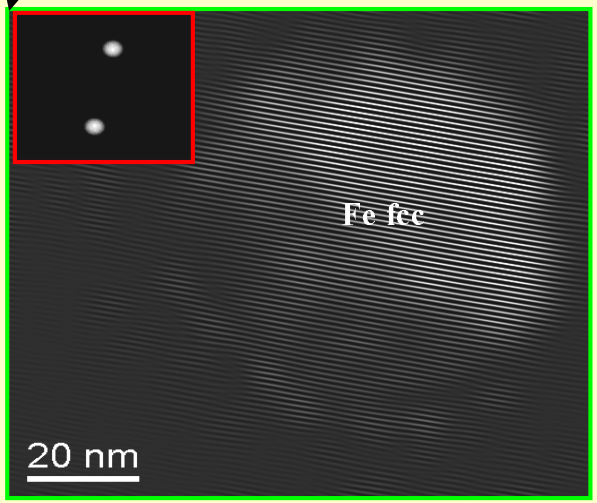


Image (real space)

Image (reciprocal space)

FILTERING





OUTLINE OF THE TALK

Biomedical applications of magnetic nanoparticles I: Drug delivery

-Introduction

-Encapsulated nanoparticles:preparation and charaterization: Morphology, Structure and Magnetism

-Bioferrofluids for local drug delivery:

i) In vitro experiment: blood biocompatibility and drug carrier

ii) Invivo experiments: magnet implant and magnetic localization

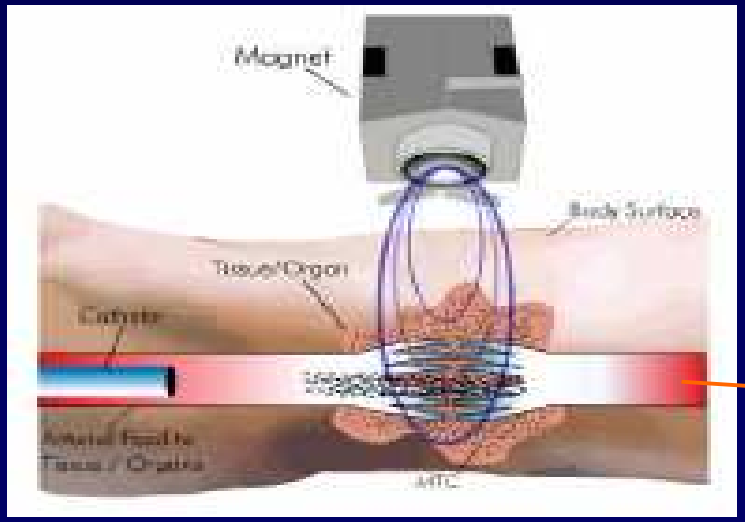
BIOFERROFLUIDS AS THERAPEUTIC CARRIERS

-They should be magnetic to be guided by applied magnetic fields

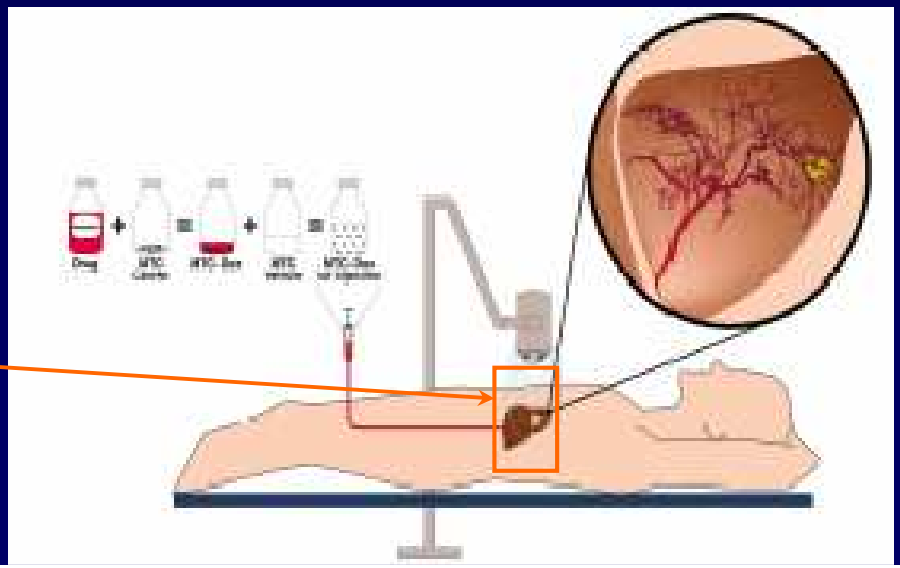
-The magnetic materials are not biocompatibles

-The nanoparticles should be encapsulated

-The sourrounded material should be able to adsorb and desorb the drug



J.Johnson et al., EC&M 3 (2002) 12

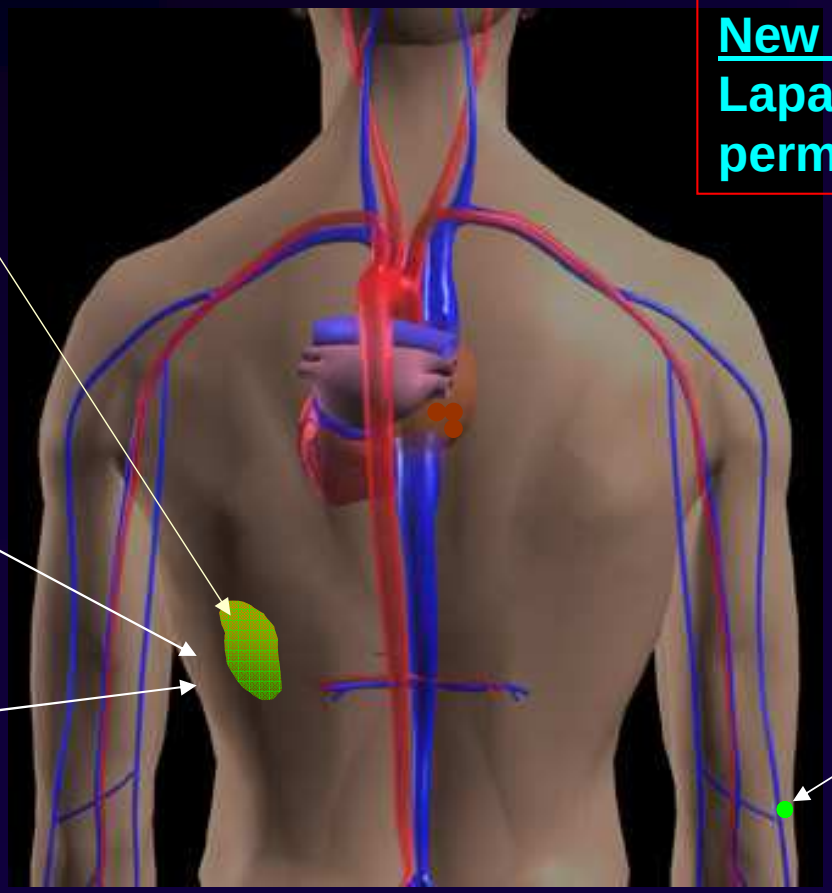


Local drug delivery by using magnetic carriers

Solid tumor

Magnet implantation

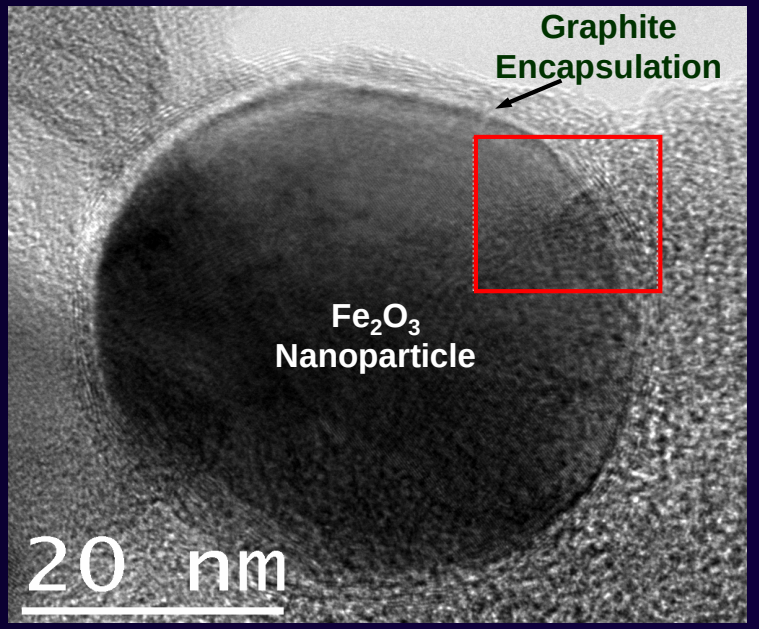
External applied magnetic field

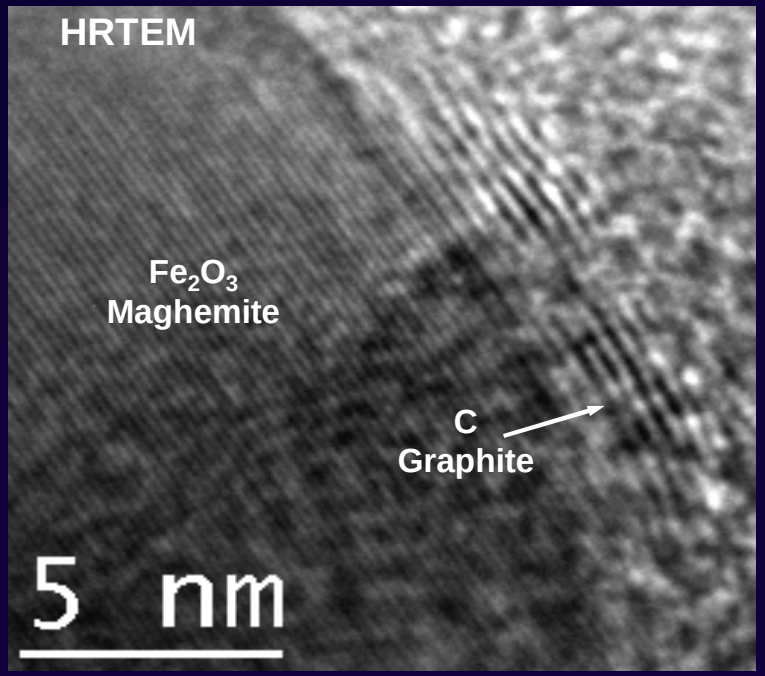


Intravenous administration of magnetic carriers

New development at the INA
Lapararoscopic implant of a permanent magnet

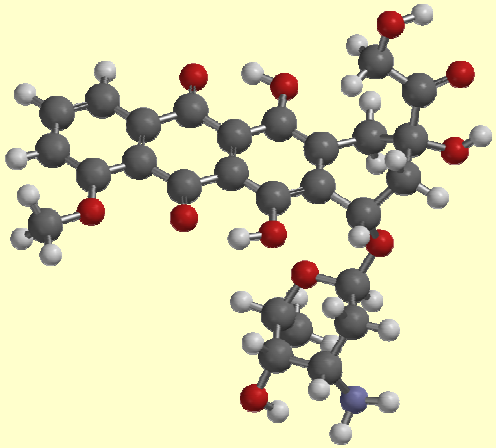
BIOFERROFLUIDS

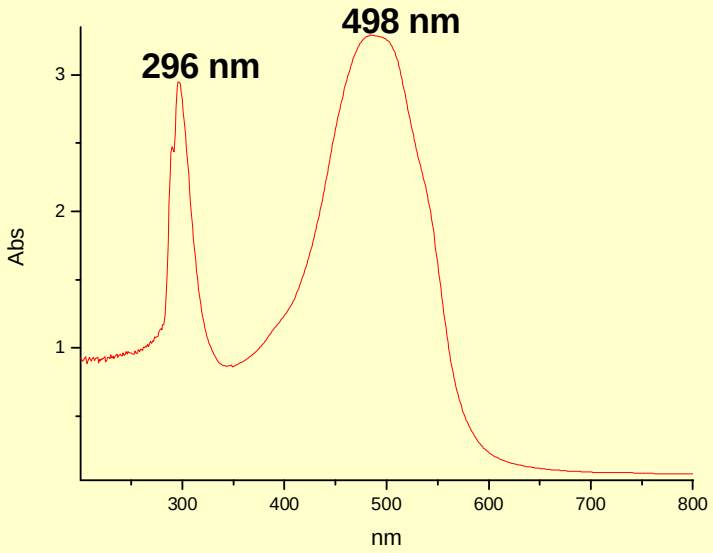


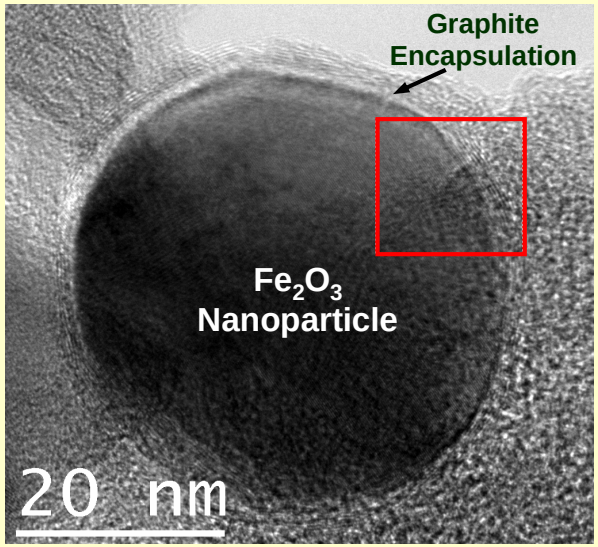


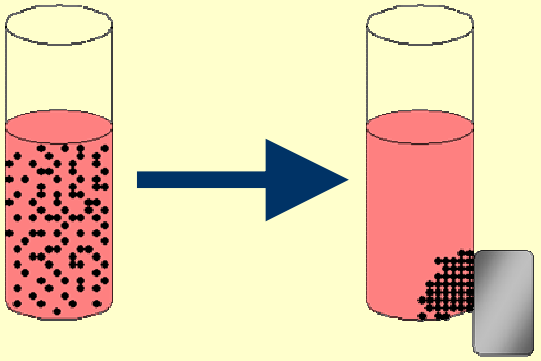
Drug adsorption and desorption of drugs

Doxorubicin (11x 13 Å)

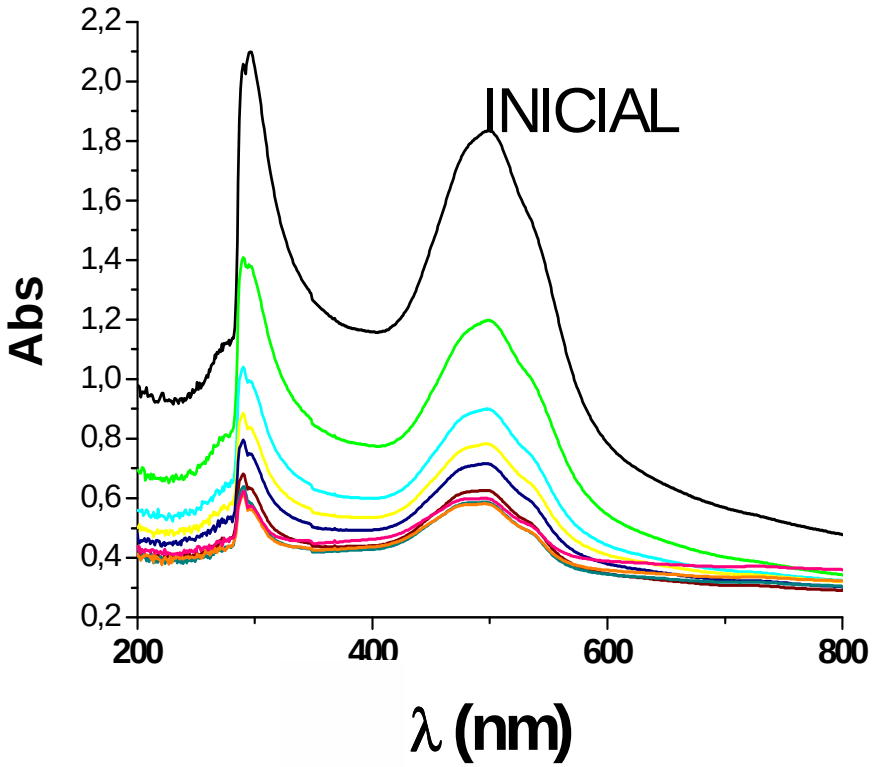


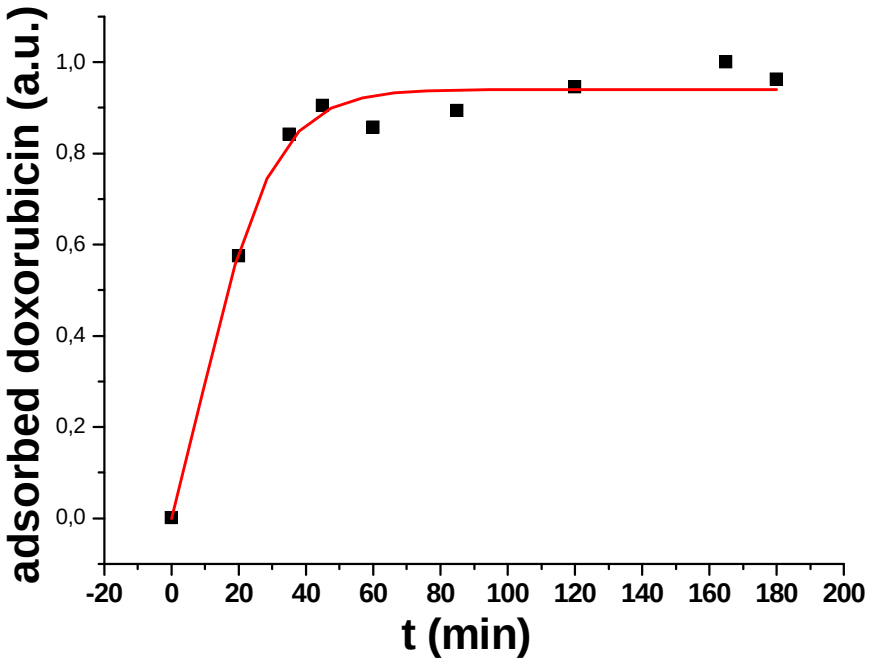


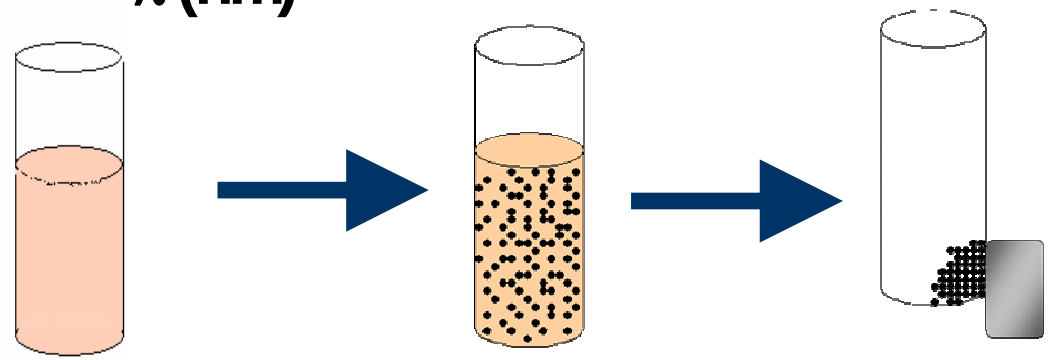




Absorbance spectra of Doxorubicin. Kinetics of adsorption

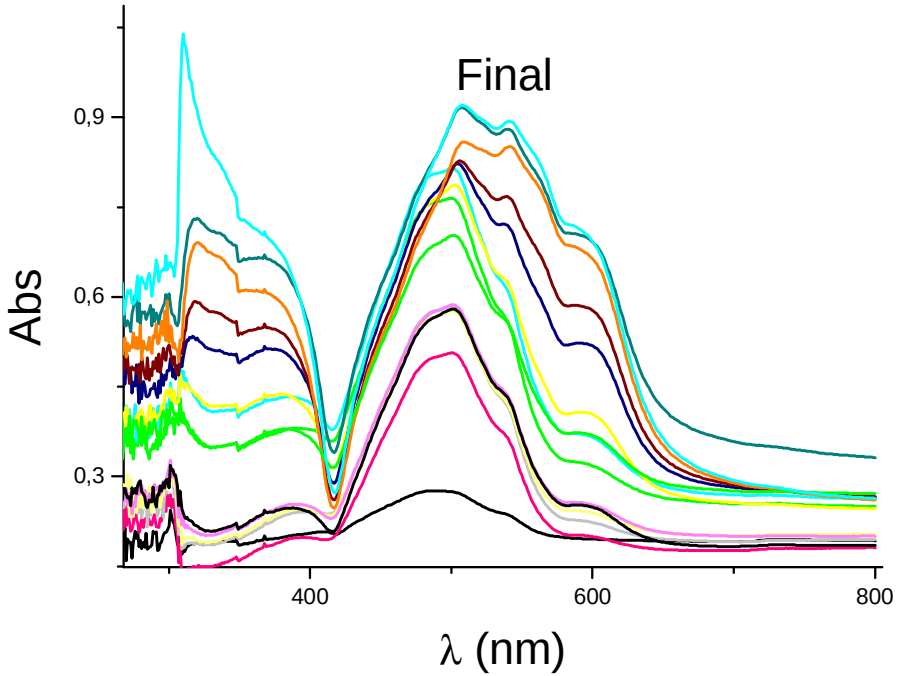


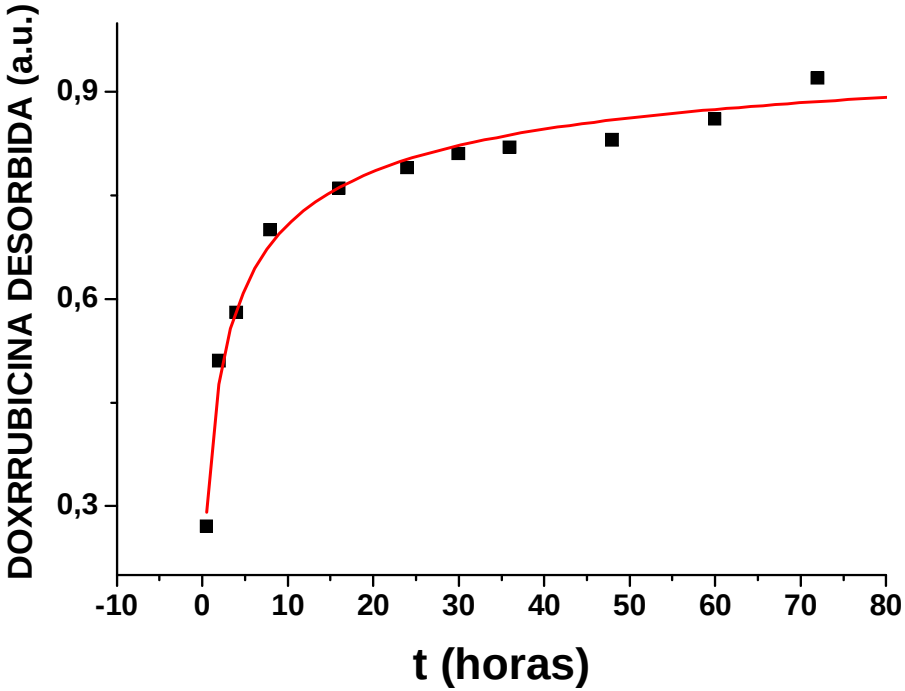


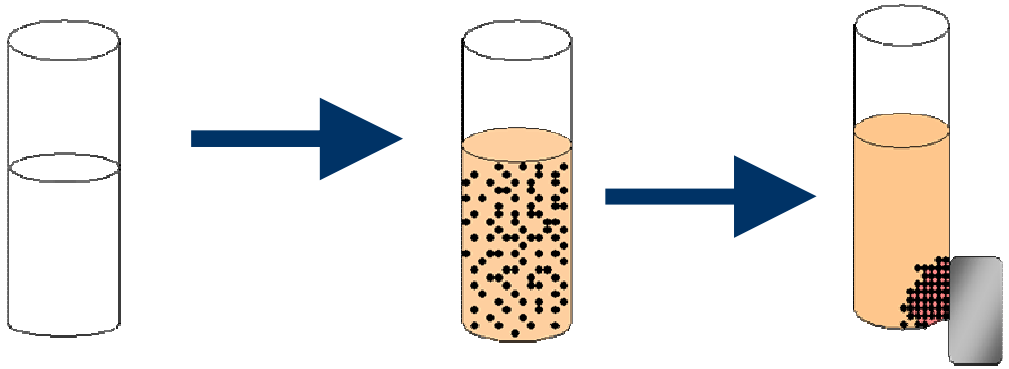


The adsorbent particles were sedimented with a 3 KOe permanent magnet, and the optical density of the supernatant measured with a UV spectrophotometer (method proposed by Kuznetsov, A. et al, (1999) J. Mag. Mag. Mat. 194, 22).

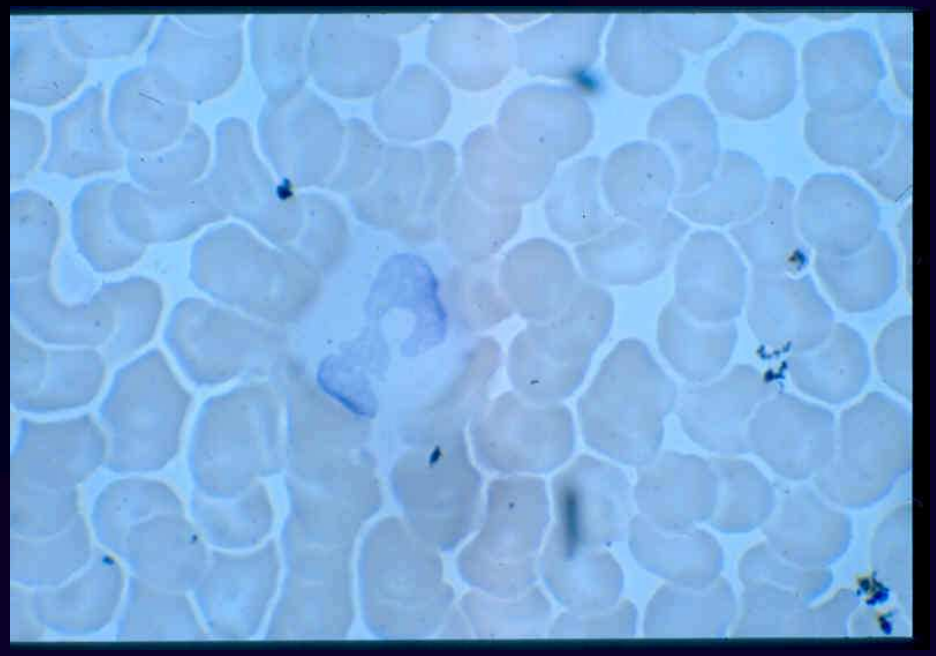
Absorbance spectra of Doxorubicin after desorption





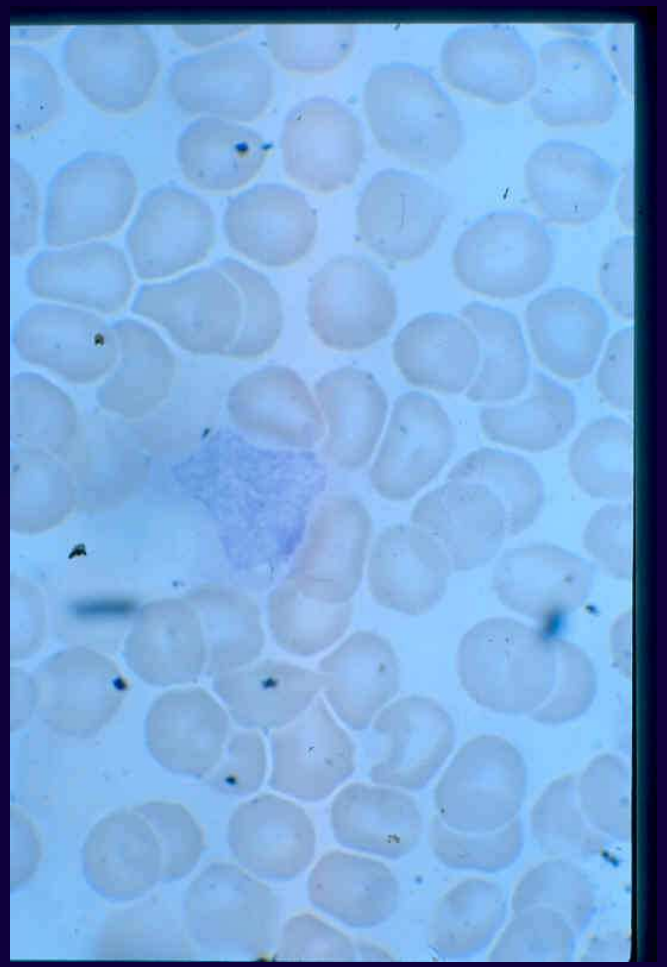


The adsorbent particles were sedimented with a 3 KOe permanent magnet, and the optical density of the supernatant measured with a UV spectrophotometer



Nanoparticles in the blood stream

Test of biocompatibility



The particles can be seen in blood smeared as black point in May-Gruendwald-Giemsa and Perls stain. The green-blue die characteristic of iron in Perls stain is absent, which is an indication of the good encapsulation of the iron by graphitic layers. All the particles are extra-cellular, both in rabbits (circulating particles “in-vivo”) and human (mixture of the ferrofluid and blood “in-vitro”). We did not find magnetic particles neither in circulating monocytes nor granulocytes. This means that iron is well coated and that the particles have not been withdrawn from circulation for phagocytes.

HEMATOLOGIC TESTS

"In vivo" in rabbits

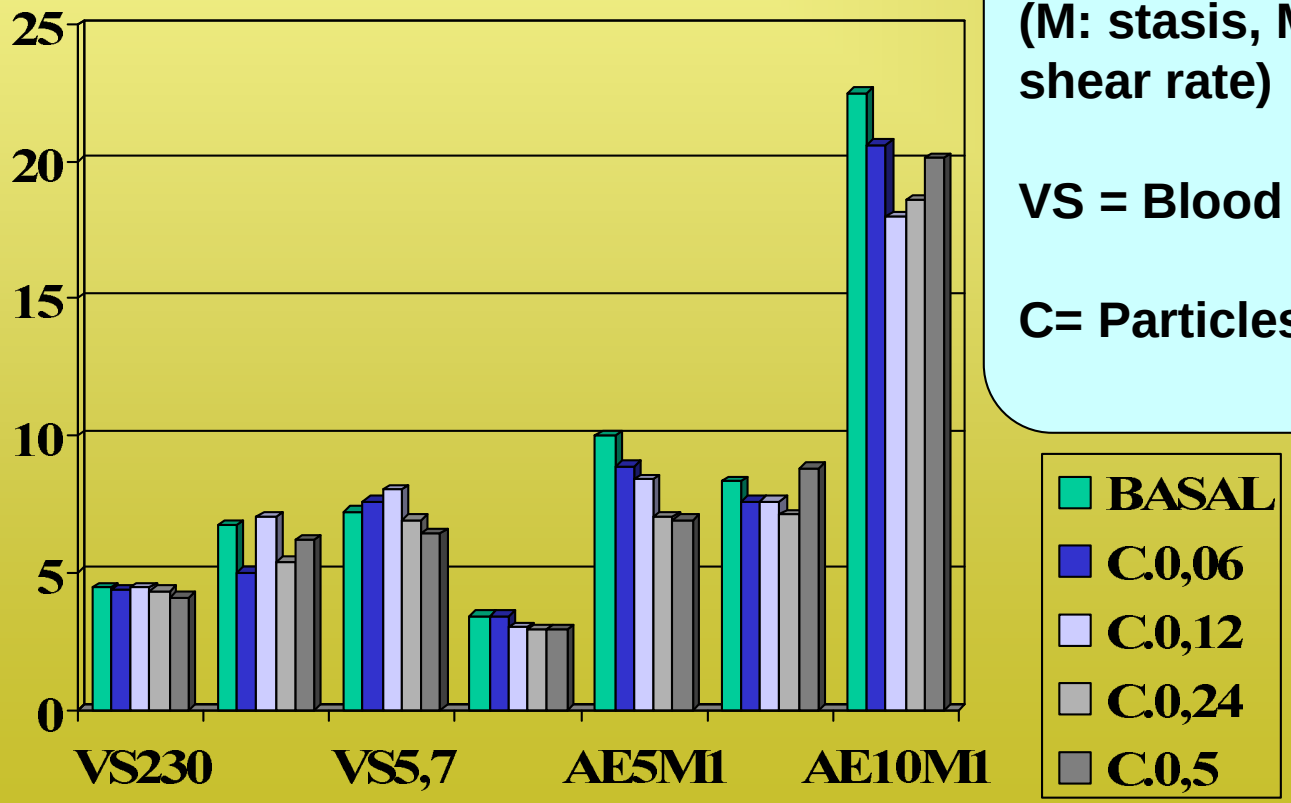
- **Blood samples were taken before, and 10 minutes after the venous injection of the particles.**
- **1 ml of Nanoparticles at a concentration of 12.5 mg/ml of Gelafundine injected in rabbits do not modify blood and plasma viscosity.**
- **Erythrocyte aggregation remains within normal limits, with very small variations without clinical significance.**

"In vitro" in human blood

- **We have tested human blood at different concentration of particles (5 ml of blood and 0, 0.06, 0.12, 0.24 and 0.5 ml of ferrofluid). We obtained normal values in all tests, differing less than 10% in relation to our own series of normal controls.**

RESULTS IN HUMAN BLOOD

	VS230	VS 23	VS5,7	AE5M	AE5M1	AE10M	AE10M1
BASAL	4,44	6,7	7,2	3,4	10	8,3	22,5
C.0,06	4,36	5	7,6	3,4	8,9	7,6	20,6
C.0,12	4,45	7	8	3	8,4	7,6	18
C.0,24	4,33	5,4	6,9	2,9	7	7,1	18,6
C.0,5	4,1	6,2	6,4	2,9	6,9	8,8	20,1



AE = Erythrocyte aggregation (M: stasis, M1 minutes under low shear rate)

VS = Blood viscosity Shear rate (s^{-1})

C= Particles concentration (mg/ml)

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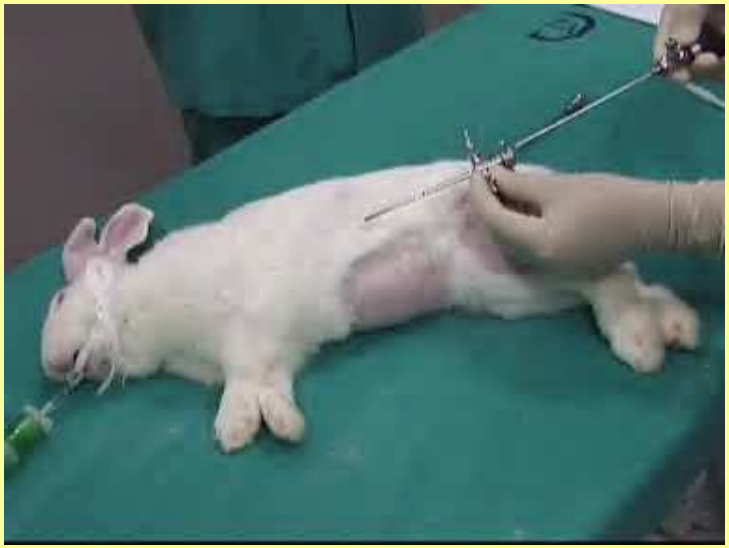
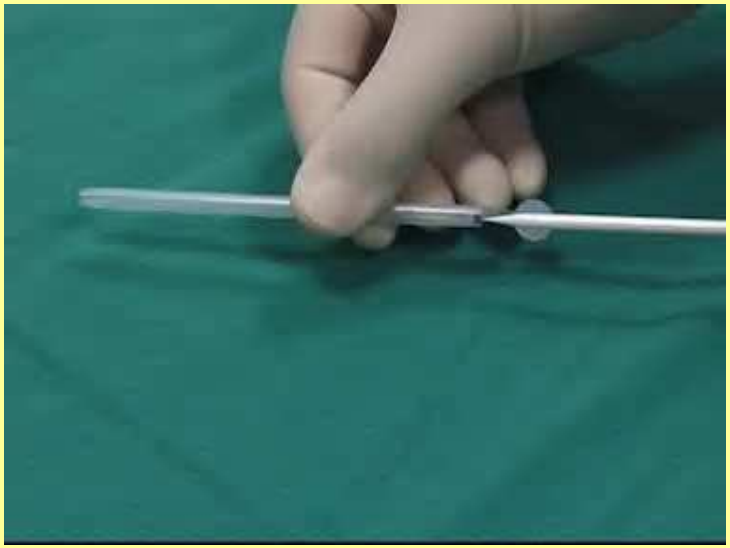
Surgical model: Percutaneus insertion of a permanent magnet

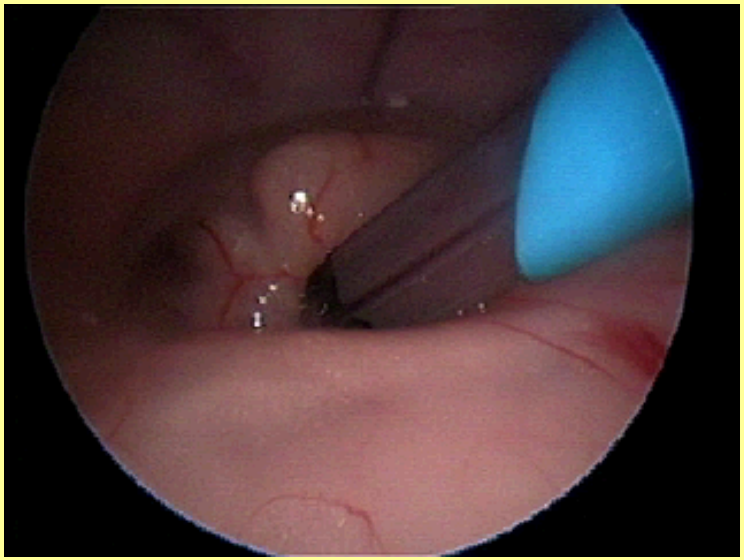




The rabbits were submitted to general anaesthesia with endotracheal intubation.

A laparoscopic optic was introduced by using a 5 mm trocar.

Optical Microscope Image of the permanent magnet coated with gold.



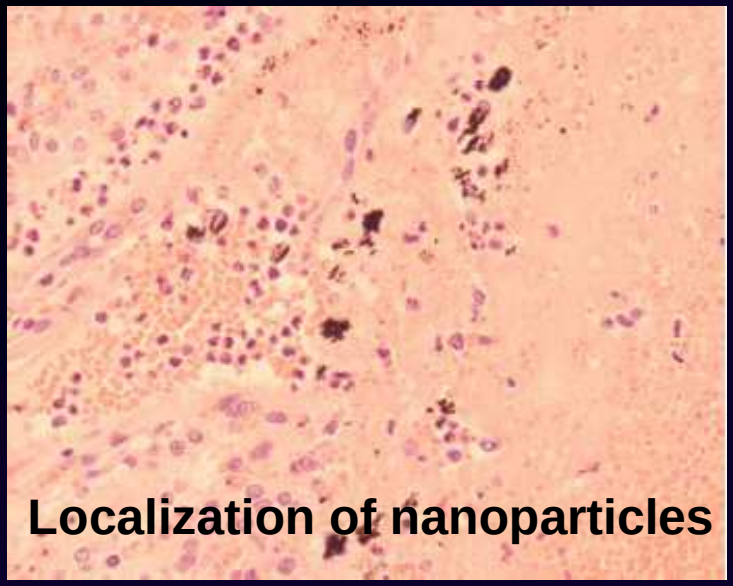
Under endoscopic control, the permanent magnet was implanted in the lower pole of the righ kidney

Bioferrofluid

125 mg of our nanoparticles were suspended in 100 ml of **gelafundine**

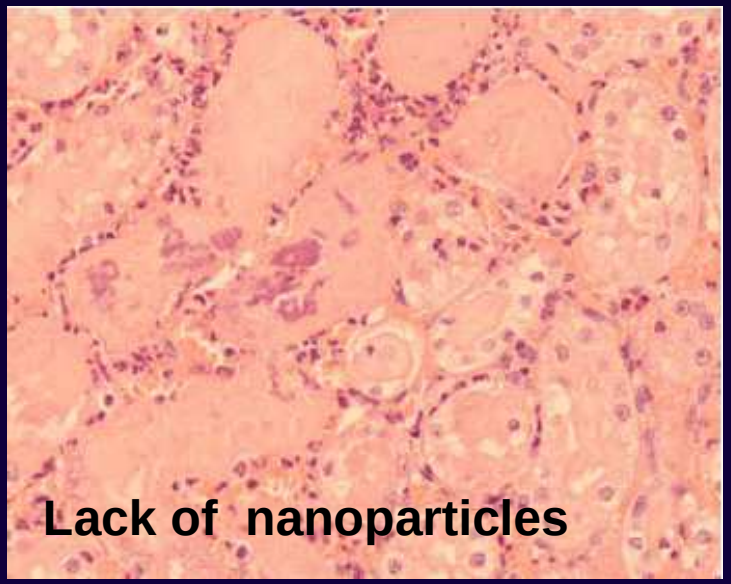
1 ml of the fluid were injected intravenously in the marginal ear vein of the rabbit.

In coll. Veterinarian Clinical Hospital



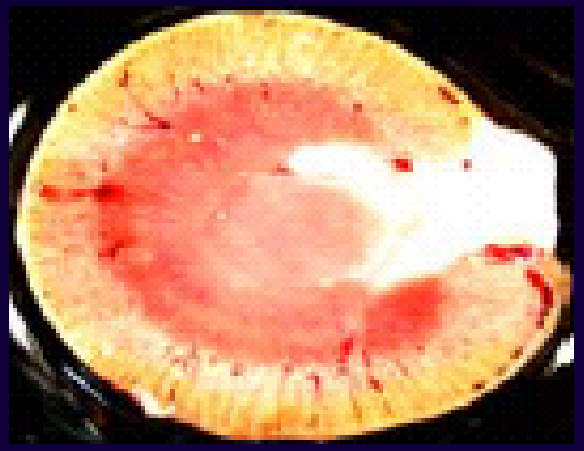
Localization of nanoparticles

Magnet implant in the left kidney

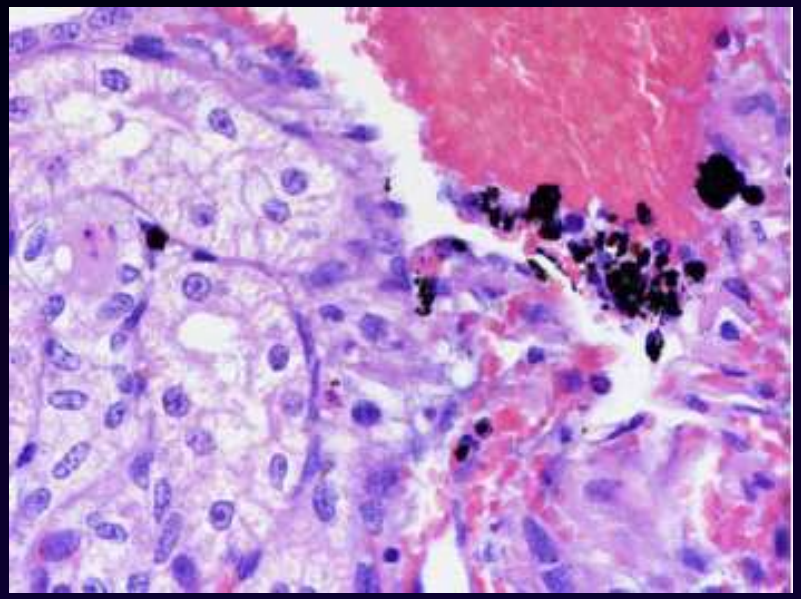


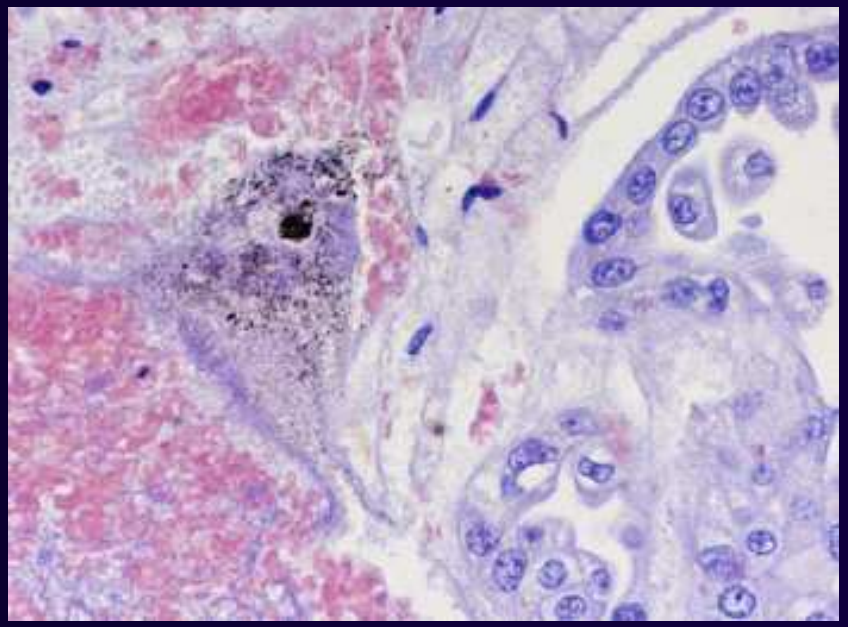
Lack of nanoparticles

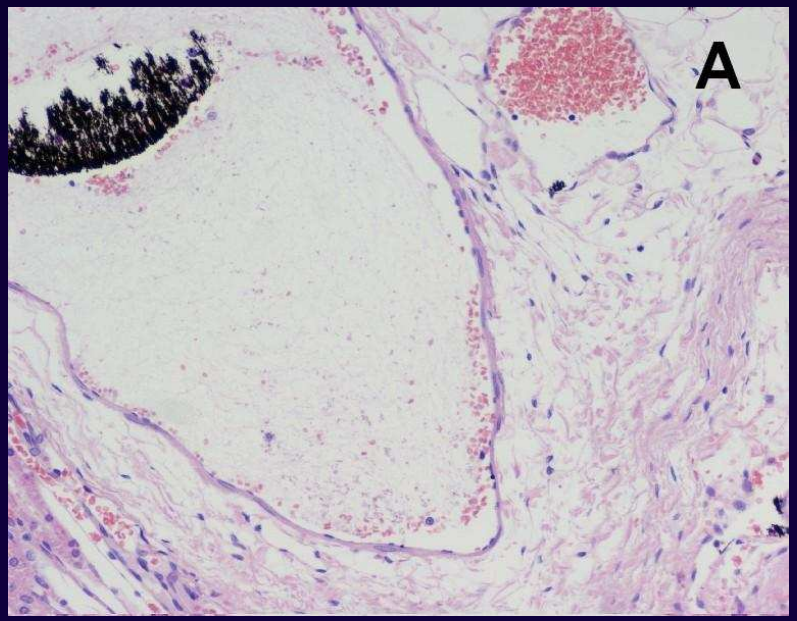
Right kydney witout magnetic implant



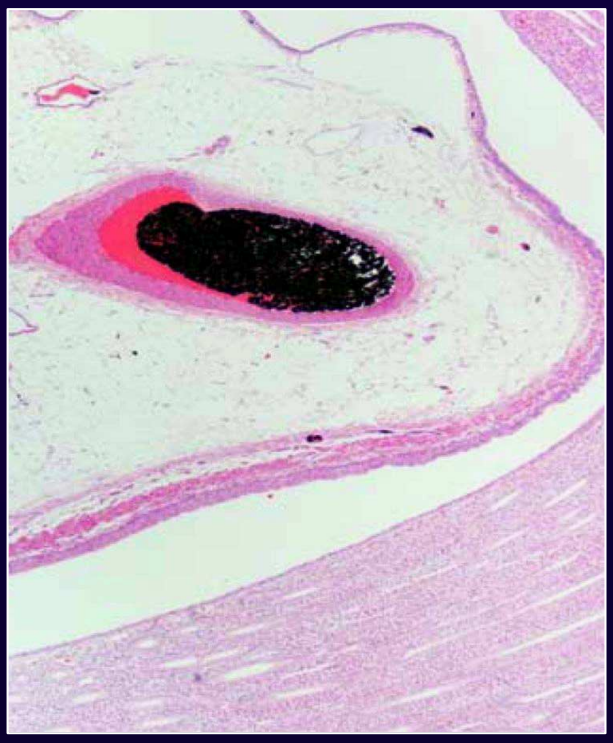
Kidney with magnetic implant:

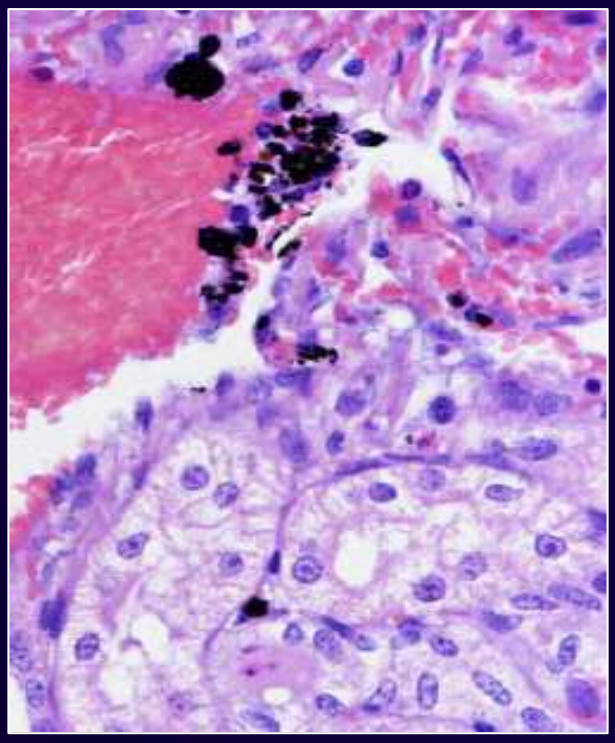


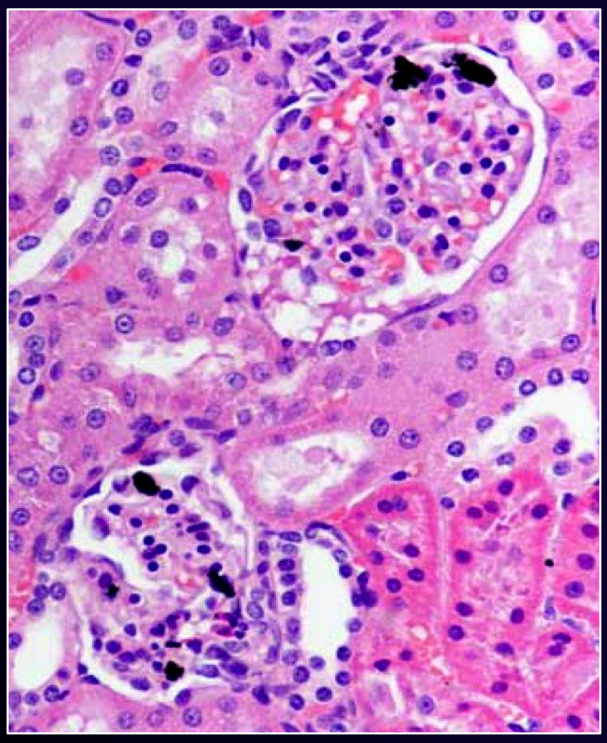


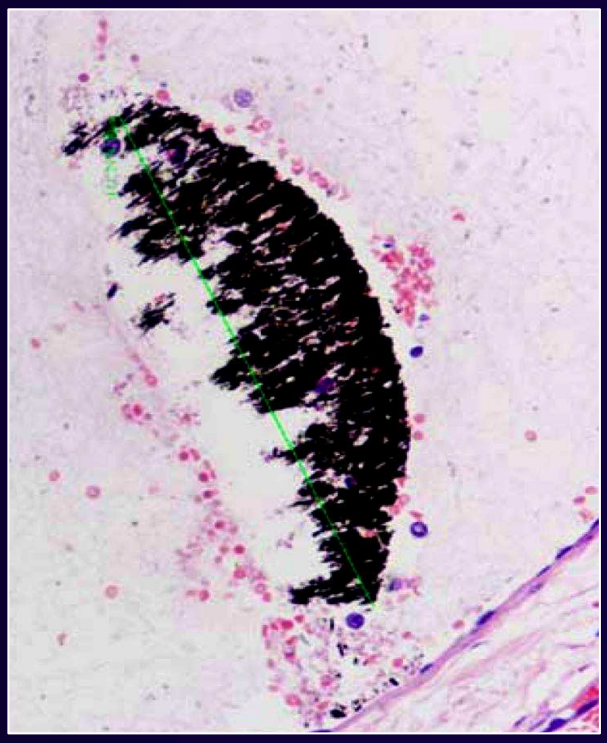


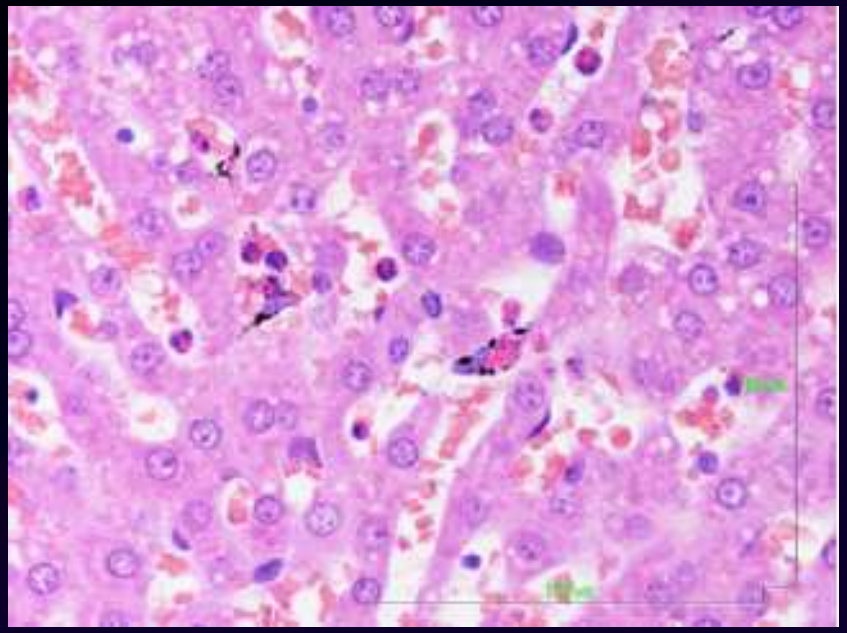
The advantage of this method is the huge field gradient obtained (30 T/m)



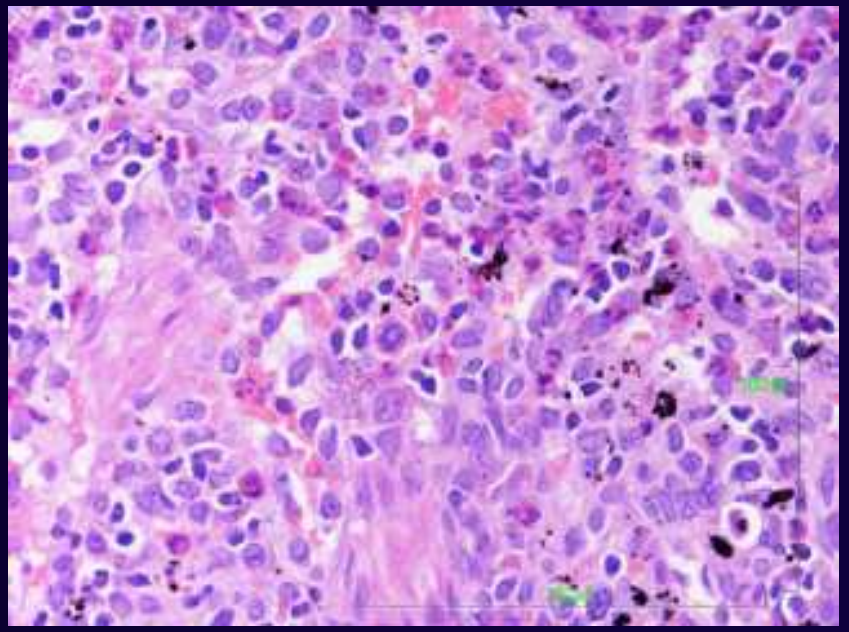




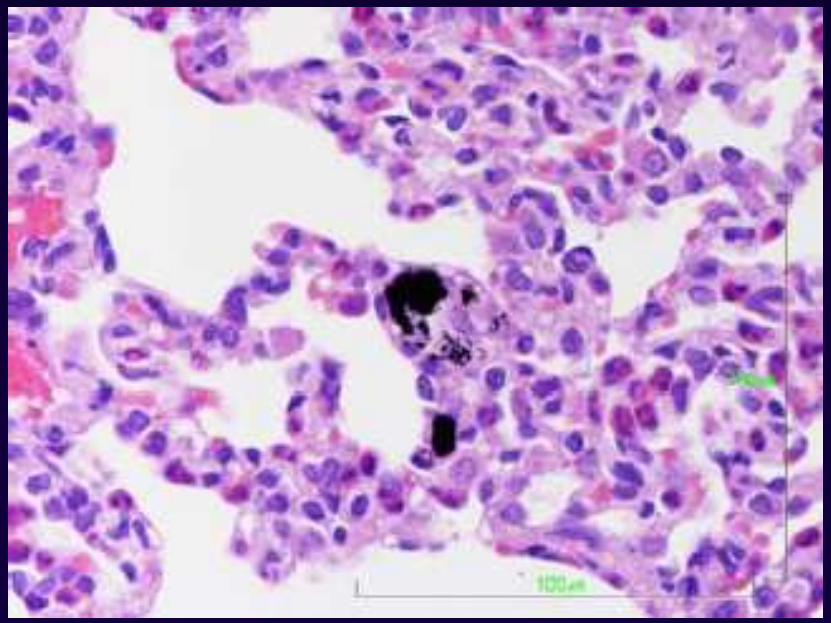




Liver Capture in Kuffer cells



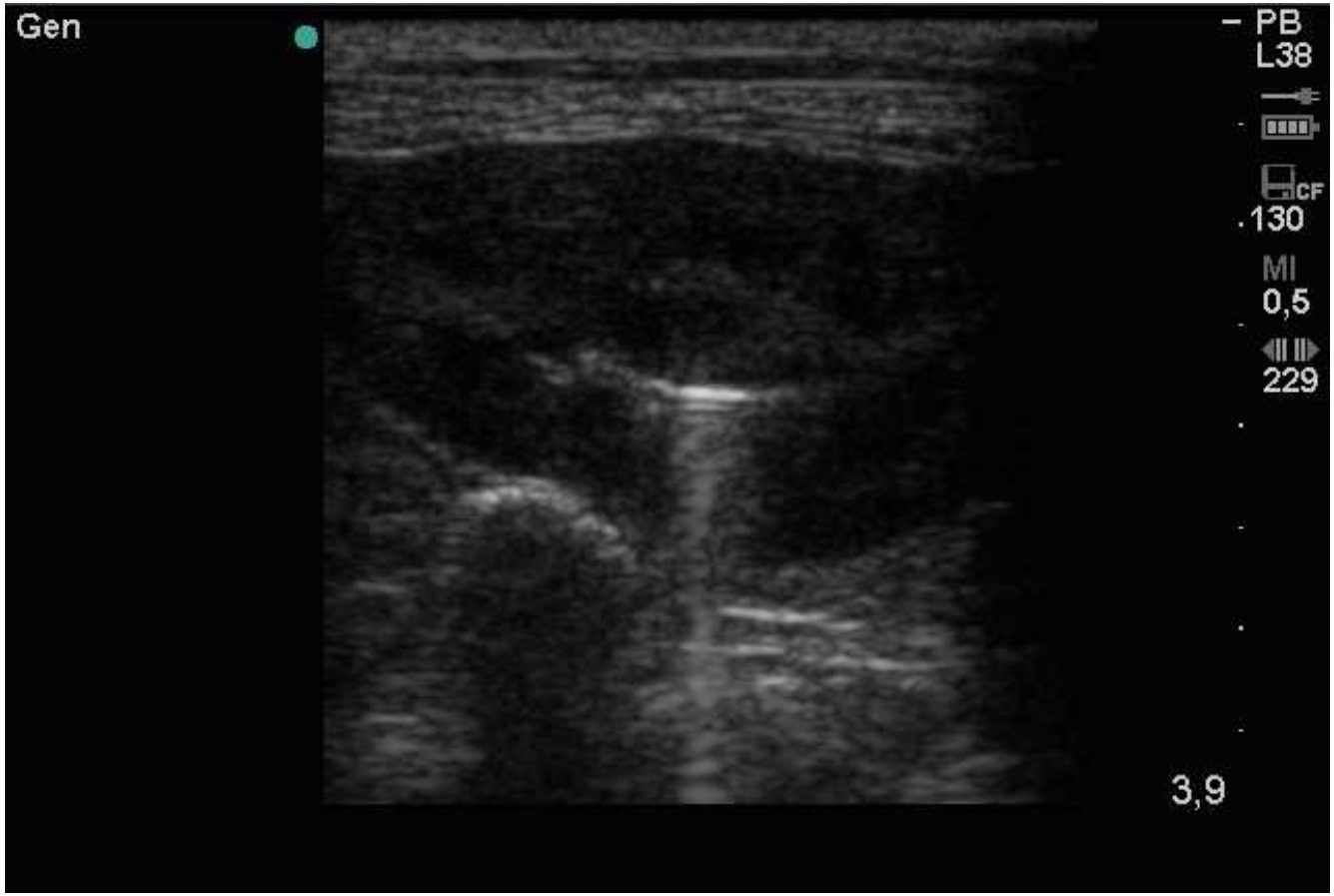
Nanoparticles in spleen

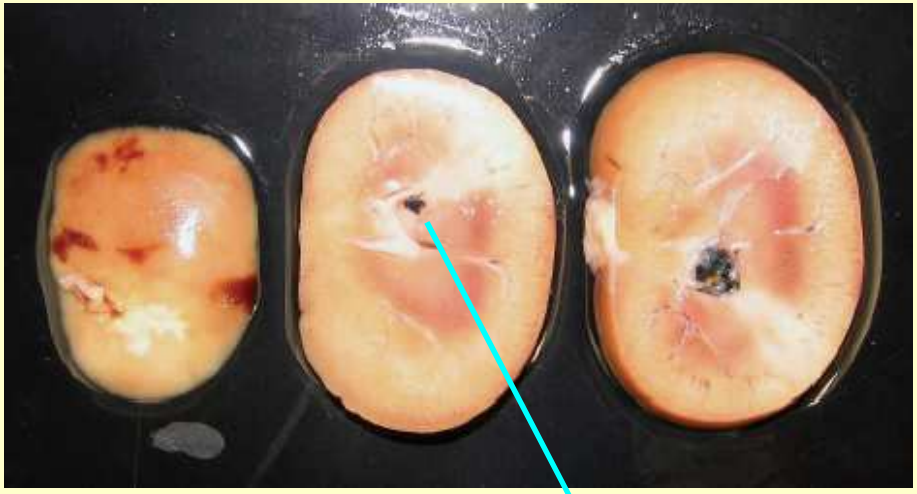


Reduced concentration of nanoparticles in lung

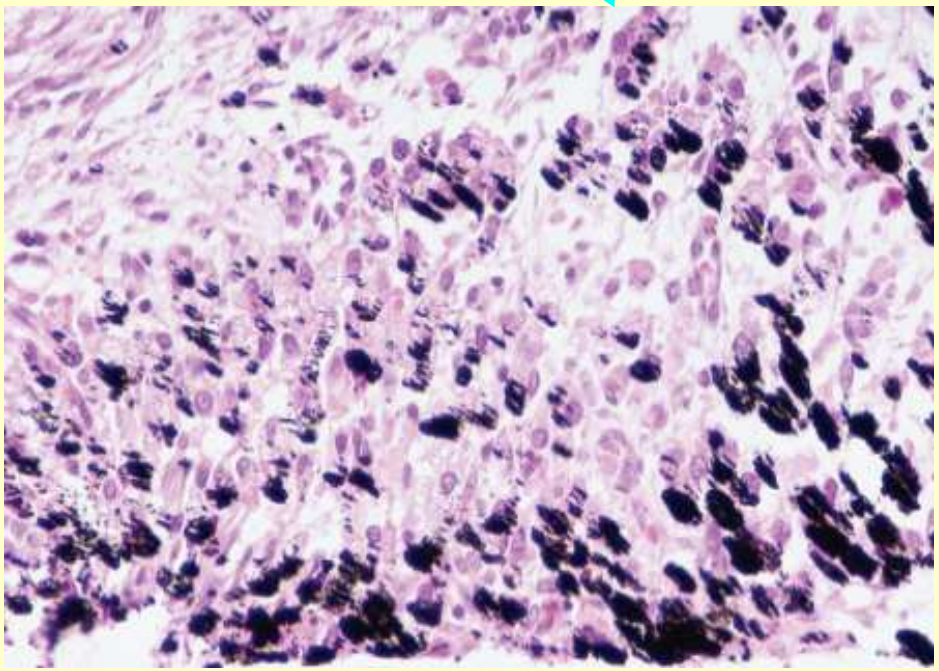
Macrophagy capture in organs

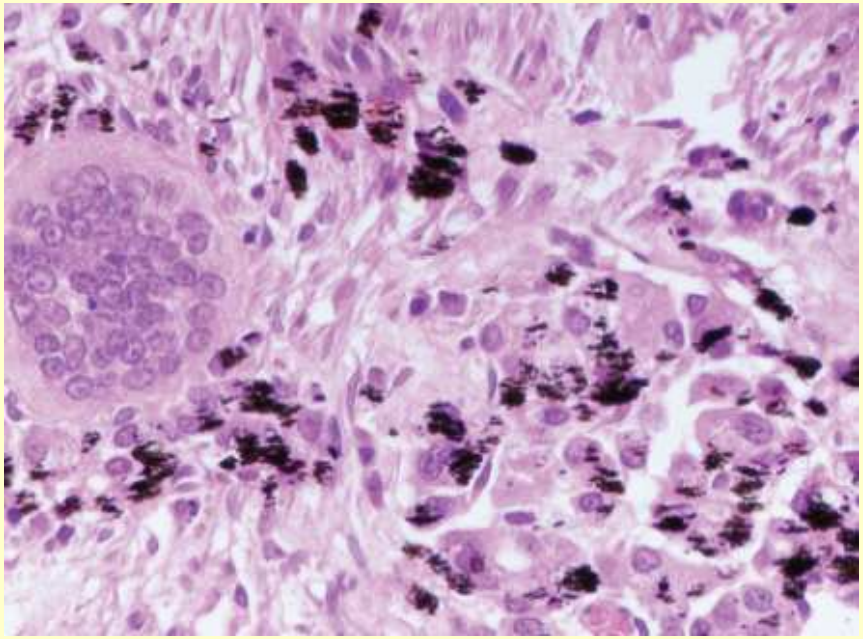
The ultrasound scan displays the image of a gold coated magnet inserted inside a kidney. After the injection magnetic nanoparticles remain attracted to the magnet, the amount of metallic then increases and the echo of the ultrasound is broadened





Ecoguided puncture for drug delivery to avoid macrophages capture






I HOPE YOU UNDERSTOOD
EVERY THING