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In vitro cytotoxicity of low Curie temperature Fe-ETM-Nb-B (ETM = Cr, Ti, Ta, Mn) biocompatible nanomaterials against cancer cells. Magnetic hyperthermia

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Our previous studies indicated that $Fe_{79.7-x}Cr_xNb_{0.3}B_{20}$ (x = 11.5÷13 at.%) rapidly quenched alloys show glassy structures in the as-quenched state and Curie temperatures (T_C) of 290÷330 K (17÷57^{0}C), depending on the Cr content (a lower Cr content results in higher T_C).

Lupu et al., IEEE T Magn **47**, 3791 (2011)
Lupu et al., INTERMAG 2012, Vancouver, Canada, May 2012, paper BB-02

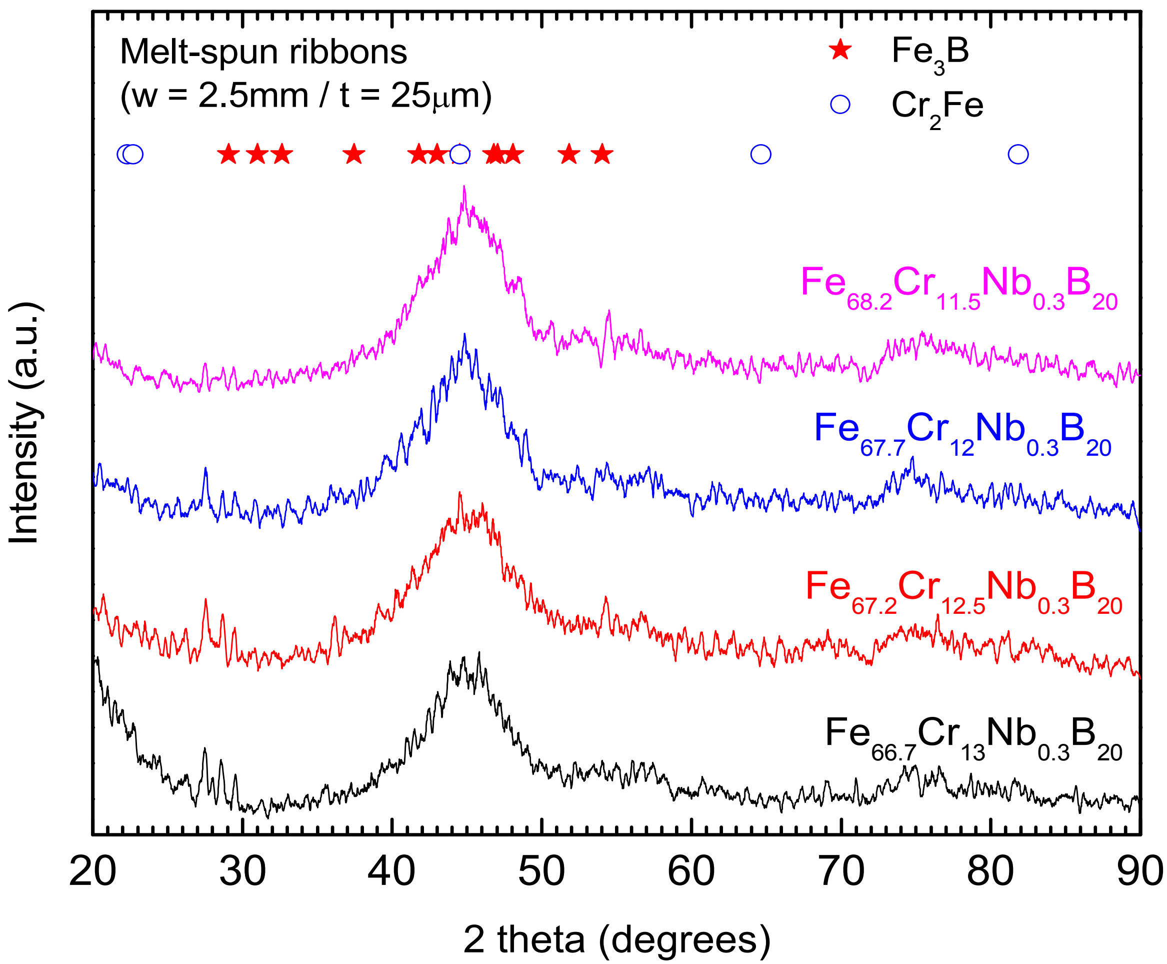
Advantage: The possibility to tailor the Curie temperature value as a function of the Cr content recommends these glassy alloys for sensing or biomedical applications (in particular, hyperthermia applications).

Requirement for hyperthermia applications: *submicron powders which can be easily targeted to the sick body organs by means of the external magnetic fields.*

J Phys D: Appl Phys **46**, 312001 (2013)
Int J Nanomed **8**, 2521 (2013)

Preparation of $Fe_{79.7-x}Cr_xNb_{0.3}B_{20}$ (x = 11.5÷13 at.%) submicron powders by high-energy ball milling from melt-spun ribbons (MSRs) precursors. ***The main challenge is to preserve the glassy structure existing in MSRs through the milling process, because only the glassy structure provides the low T_C.***

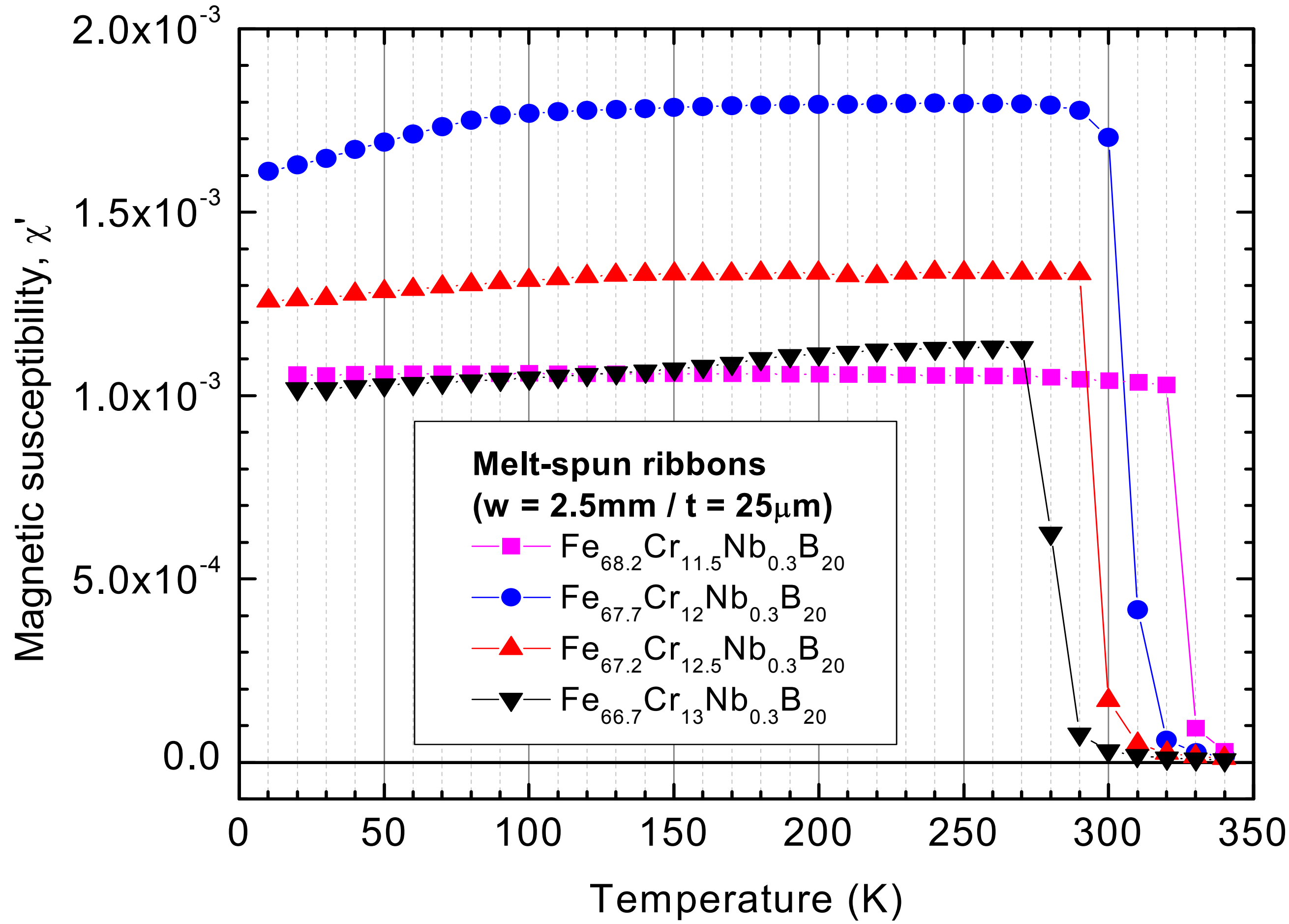
Microstructure / conventional XRD



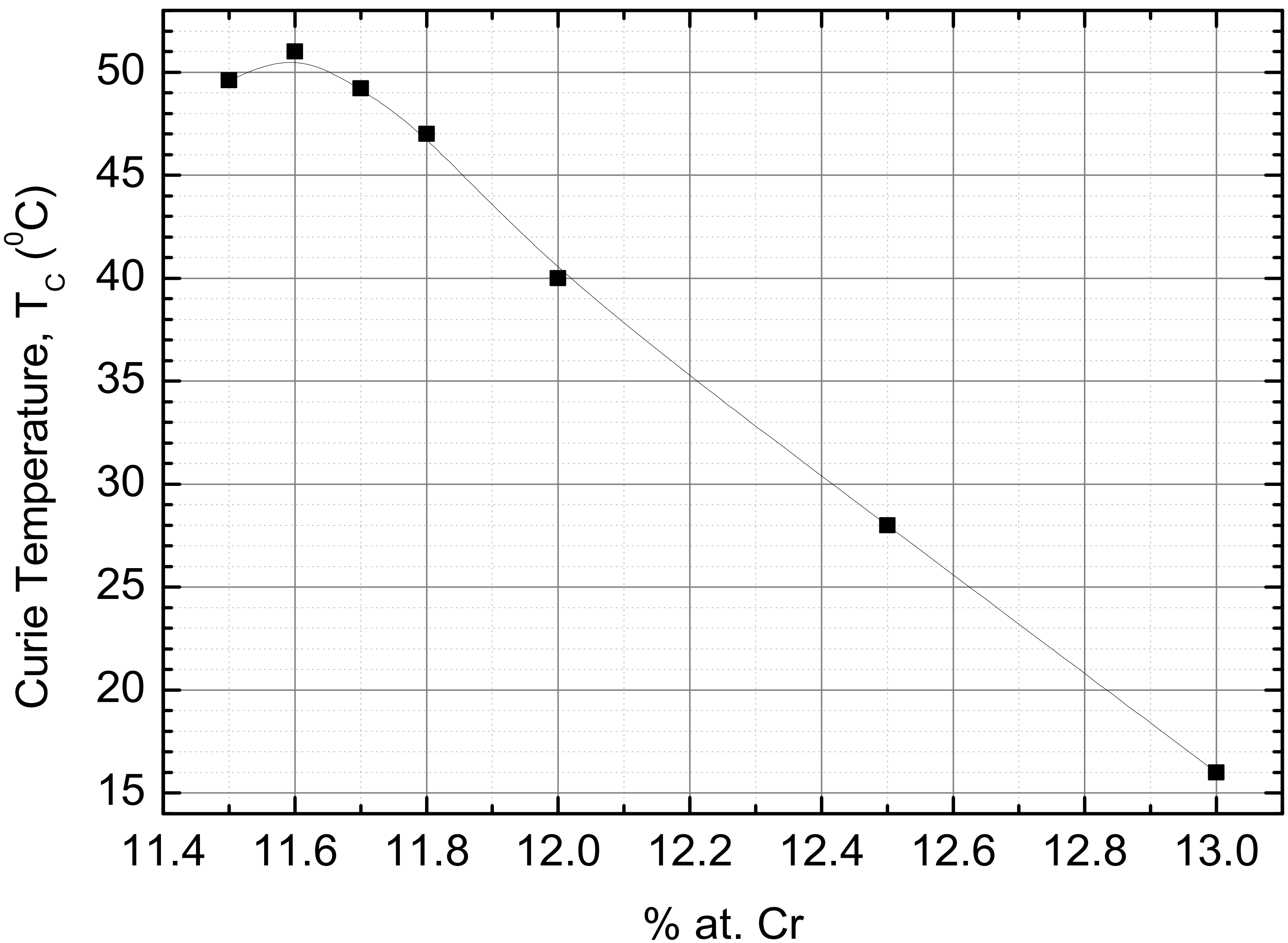
- B increases T_C and μ_{Fe}
- Cr is AF and reduces both T_C and μ_{Fe}
- *Similarities with binary Fe-Cr alloys with Cr >10 at.%* - phase separation occurs via self-diffusion and precipitation of Cr-rich clusters
  ***[J Phys Condens Matter 22**, 296001 (2010)]*
- **A glassy structure in the as-quenched state, consisting of very small Fe-Cr clusters (from several up to several tens of nm) embedded within the amorphous matrix.**

Magnetic properties vs. T

- T_C varies between 17 and 57^{0}C for melt-spun ribbons with Cr content varying between 11.5 and 13 at.%.



T_C dependence on Cr content



By tailoring the Cr content it is possible to vary T_C from 15^0C to 50^0C.

Such a specific behavior is attained only when the samples exhibit a specific glassy structure.

Cr influence

- Among transition metals, Cr is the only one which is antiferromagnetic at room temperature ($T_N \approx 35^0$C).

- When it is alloyed with Fe, 3 types of magnetic interactions will co-exist:
  - the ferromagnetic ones between Fe-Fe atoms
  - the antiferromagnetic ones between Cr-Cr and
  - the antiferromagnetic ones between Fe-Cr atoms, respectively.

N. Kunitomi and S. Tsuge, *High Field Magnetism*, Ed. M. Date, North-Holland Publishing Company, 1983, pp. 87-96

- The macroscopic magnetic behavior of Fe-Cr binary alloys depends on the amount of Cr and the alloying temperature.

- The miscibility gap between bcc phases at low temperatures. Inside the miscibility gap, Fe-Cr alloys tend to decompose into a Cr-rich bcc phase (α′) and an Fe-rich bcc phase (α).

W. Pepperhoff and M. Acet in *Constitution and Magnetism of Iron and its Alloys*, Springer Verlag, 2001, pp. 94-98

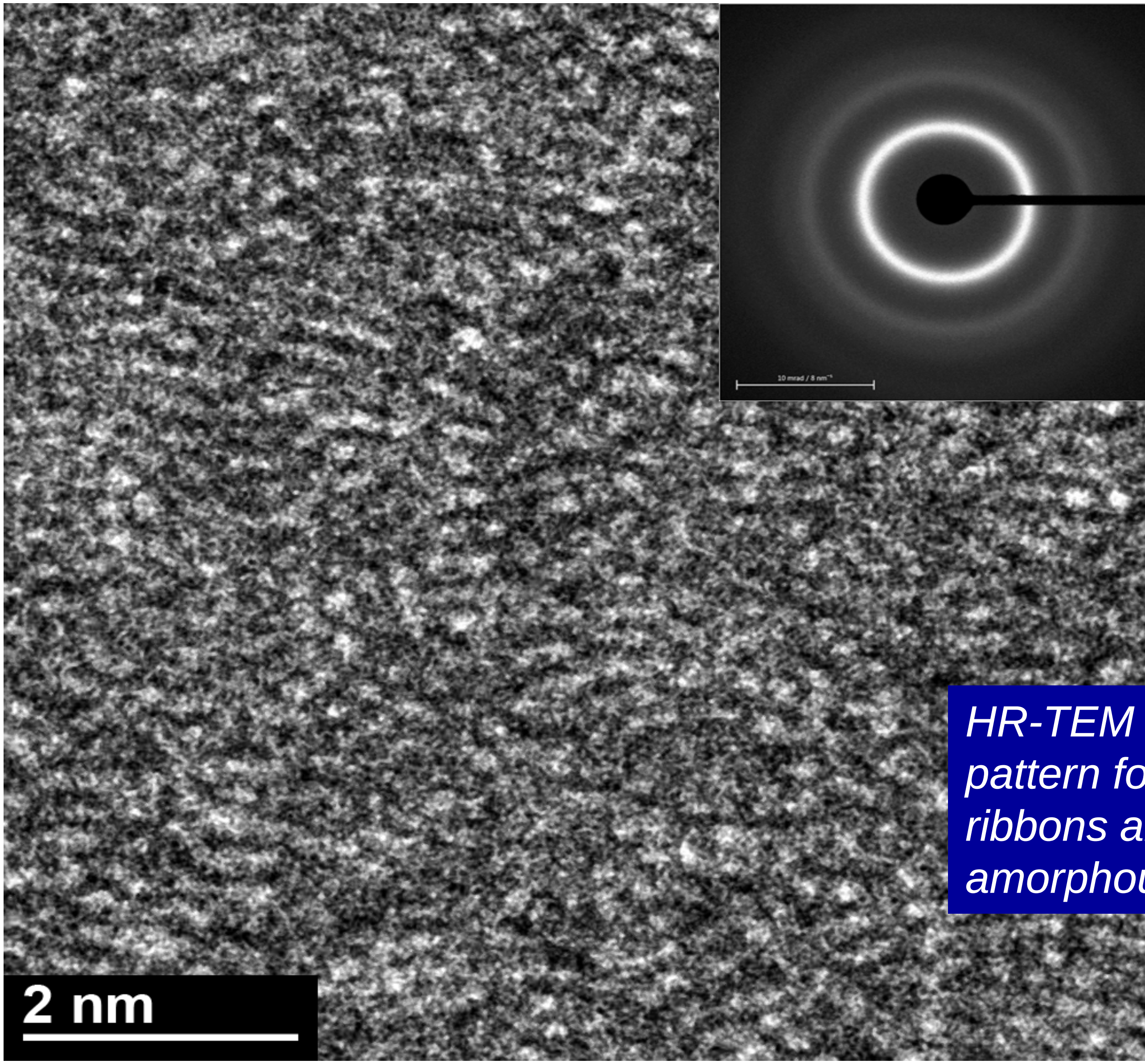
Cr influence

- Recent reports indicated the formation of the cluster-like structure for Cr contents above 10-12 at.%, for lower contents the alloy exhibiting short range order.

Phys. Rev. Lett. **53**, 687 (1984)
Phys. Rev. B **75**, 014208 (2007)
Phys. Rev. Lett. **99**, 237201 (2007)

- Considering the use of crystalline Fe-Cr in industrial steels for structural components in advanced nuclear energy installations and fusion reactors, in which Cr concentration is ranging from 2 to 20 at.%, but also the hyperthermia applications and sensitive magnetic temperature sensors involving Fe-Cr-Nb-B glassy alloys, it is extremely useful to understand the intrinsic mechanisms which determines the specific magnetic behavior of such alloys.

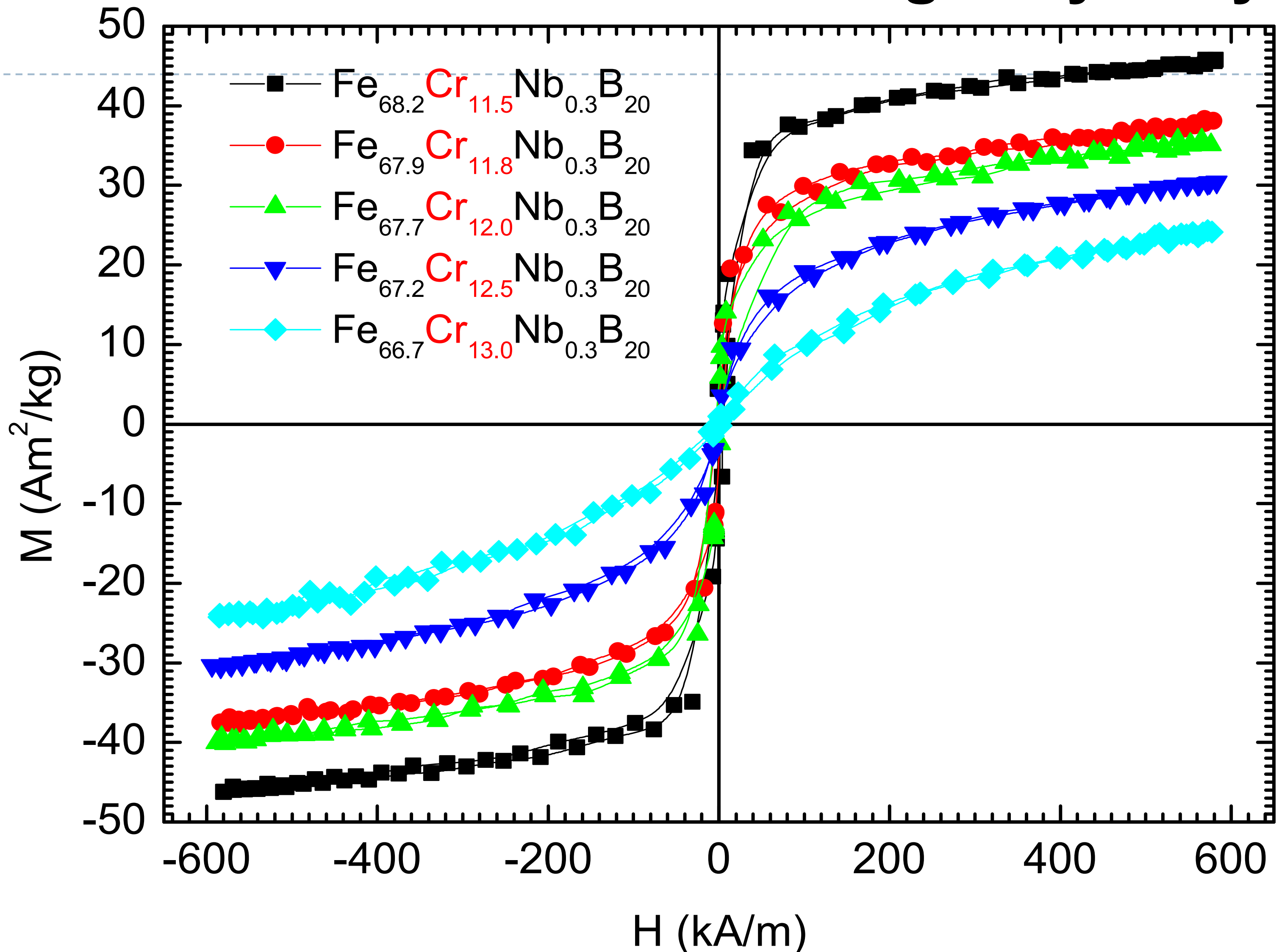
Microstructure



Chiriac et al., J Appl Phys (2015) accepted

HR-TEM image and electron diffraction pattern for $Fe_{67.7}Cr_{12}Nb_{0.3}B_{20}$ melt-spun ribbons and powders indicate a fully amorphous structure.

M-H curves of Fe-Cr-Nb-B glassy alloys



Superparamagnetic or Superferromagnetic?

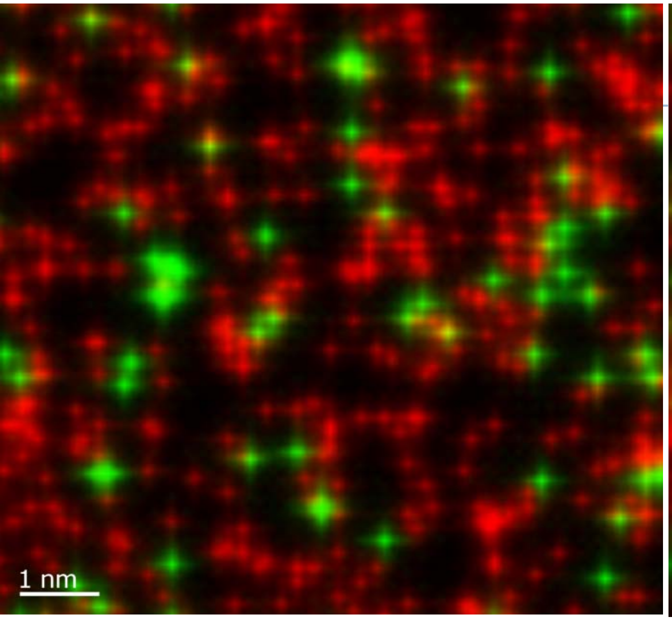
Superparamagnetism vs. superferromagnetism

Superparamagnetism is a form of magnetism, which appears in small ferromagnetic or ferrimagnetic nanoparticles.
In sufficiently small nanoparticles, magnetization can randomly flip direction under the influence of temperature. The typical time between two flips is called the Néel relaxation time.
In the absence of an external magnetic field, when the time used to measure the magnetization of the nanoparticles is much longer than the *Néel relaxation time*, their magnetization appears to be in average zero: they are said to be in the superparamagnetic state. In this state, an external magnetic field is able to magnetize the nanoparticles, similarly to a paramagnet. However, their magnetic susceptibility is much larger than the one of paramagnets.

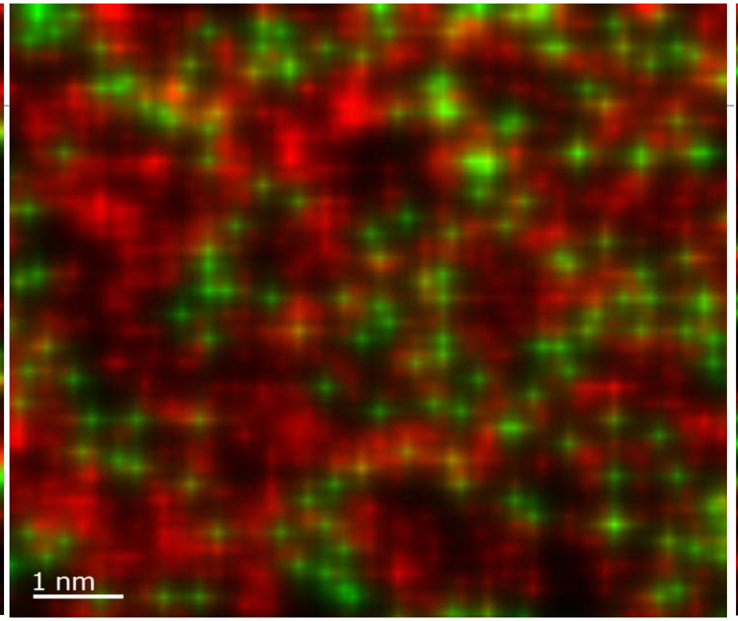
vs.

Superferromagnetism is the magnetism of an ensemble of magnetically interacting super-moment-bearing material particles that would be superparamagnetic if they were not interacting. Nanoparticles of iron oxides, such as ferryhydrite (nominally $FeOOH$), often cluster and interact magnetically. These interactions change the magnetic behavior of the nanoparticles (both above and below their blocking temperatures) and lead to an ordered low-temperature phase with non-randomly oriented particle super-moments.

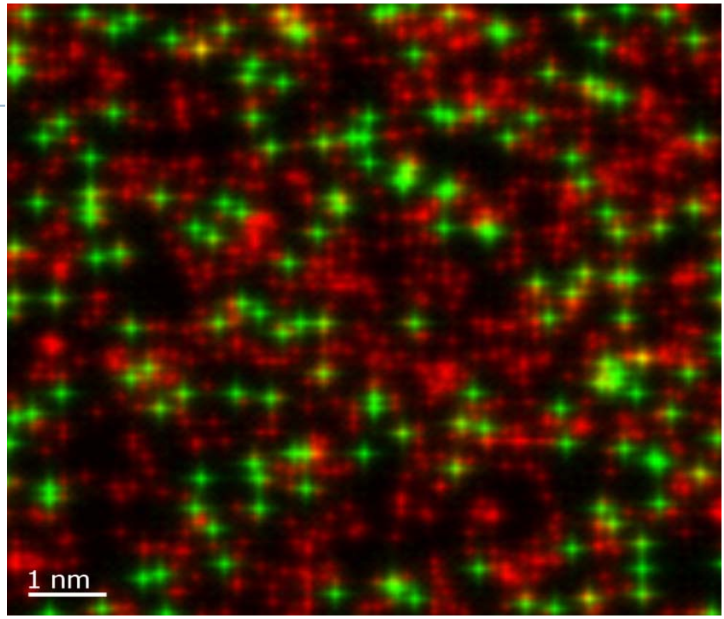
Specific cluster structure → superferromagnetism



11.5 at.% Cr / T_C = 49.6^{0}C



12 at.% Cr; T_C = 40^{0}C



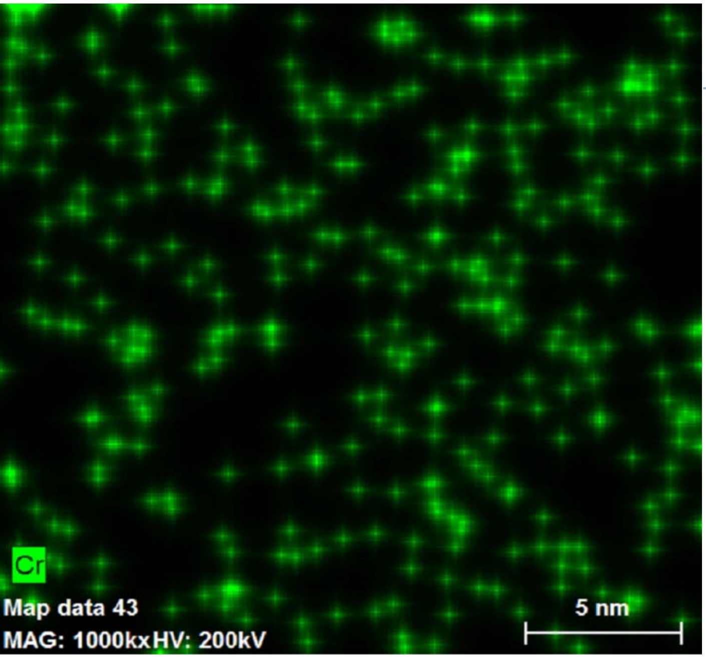
13 at.% Cr; T_C = 16^{0}C

Red – Fe / Green - Cr

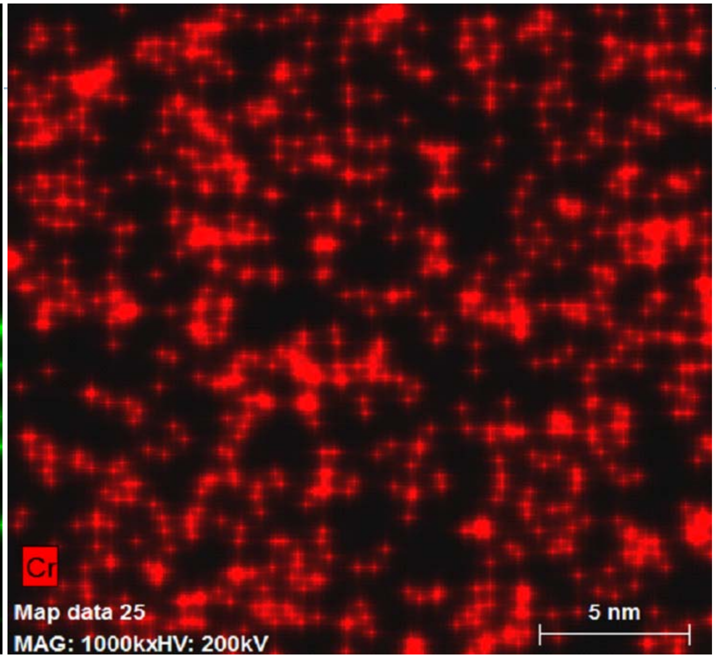
Chiriac et al., J Appl Phys (2015) accepted

Clear indications have been found of Fe and Cr clusters of approximately 1 nm to 2-3 nm in size with the increase of Cr content from 11.5 to 13 at.%.The Fe and Cr atoms clearly segregate the atomic scale to form nanometer sized clusters, influencing strongly the macroscopic magnetic behavior. One can notice the agglomeration of a few Fe atoms and Cr atoms independently. Ferromagnetic Fe clusters shrink once the content of Cr in the melt-spun ribbons increases and are becoming comparable as size with antiferromagnetic Cr clusters.

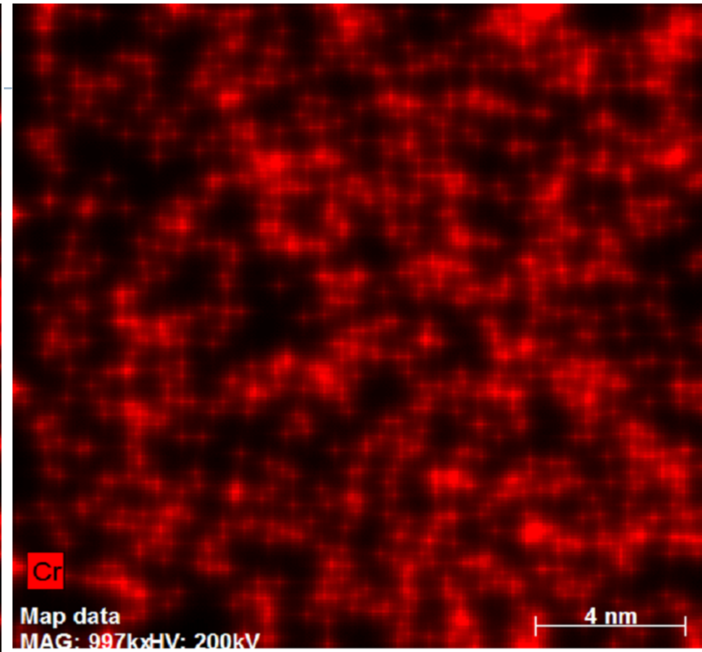
Specific cluster structure → superferromagnetism



11.5 at.% Cr / T_C = 49.6^{0}C



12 at.% Cr; T_C = 40^{0}C

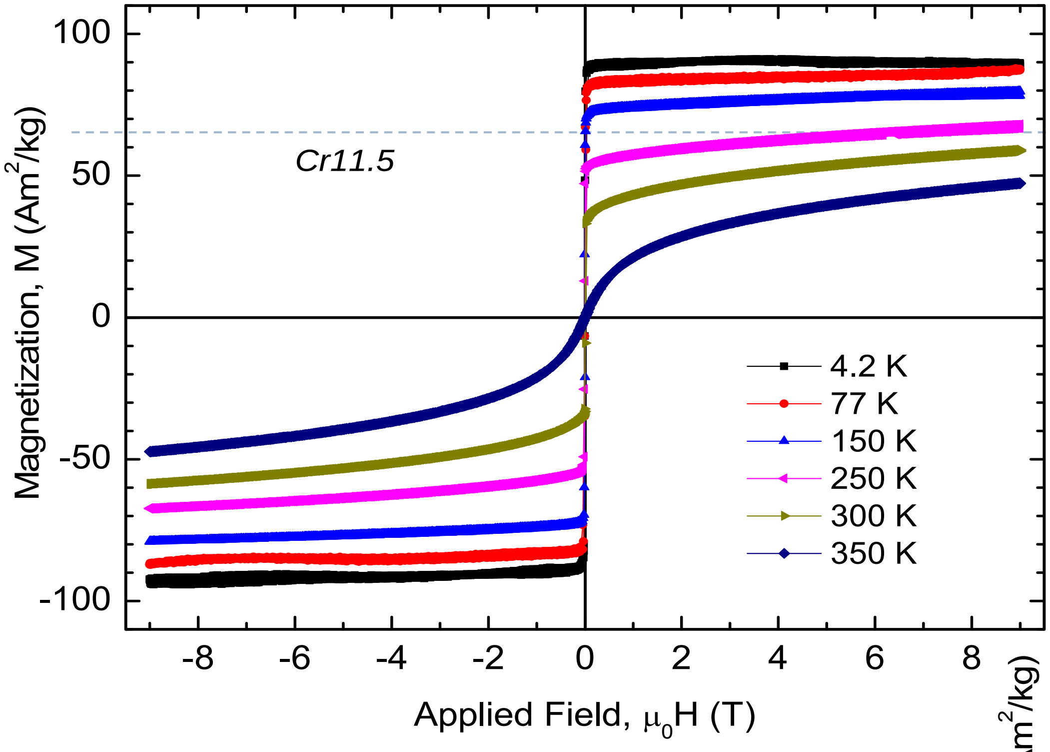


13 at.% Cr; T_C = 16^{0}C

The inter-cluster interactions are much stronger for lower contents of Cr, the microstructure is less uniform and T_C^{system} increases from 290 K for 13% at. Cr to 330 K for 11.5 at.% Cr. The whole system transforms to a ferromagnetic state through interactions between the clusters.

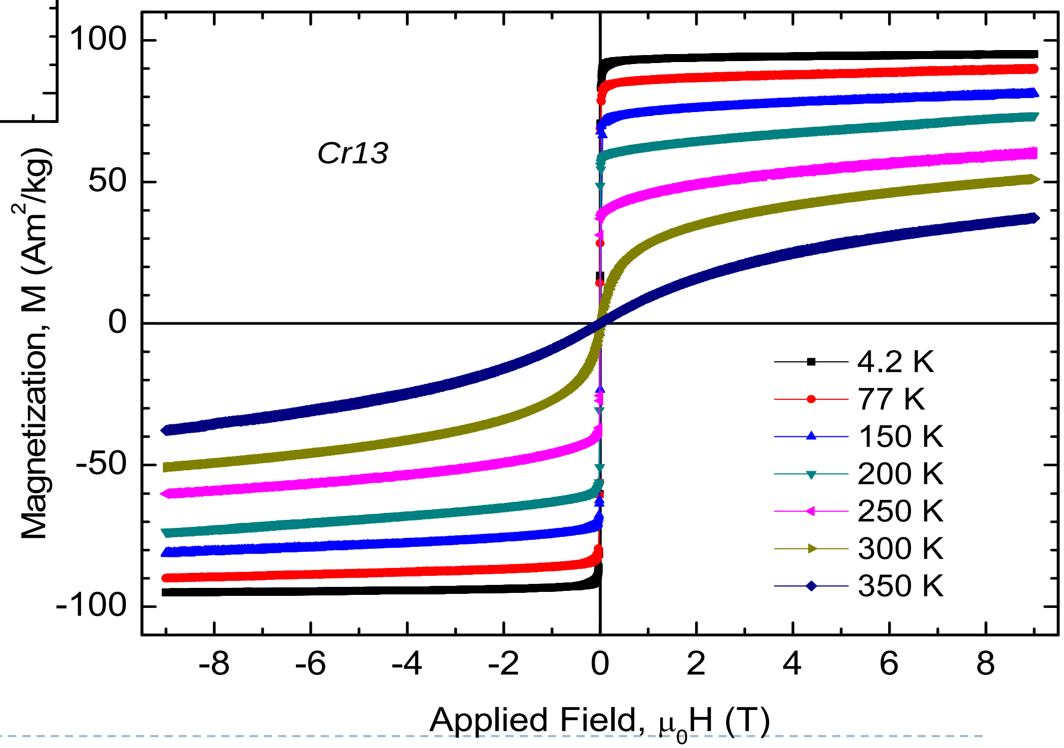
Chiriac et al., J Appl Phys (2015) accepted

Specific cluster structure → superferromagnetism



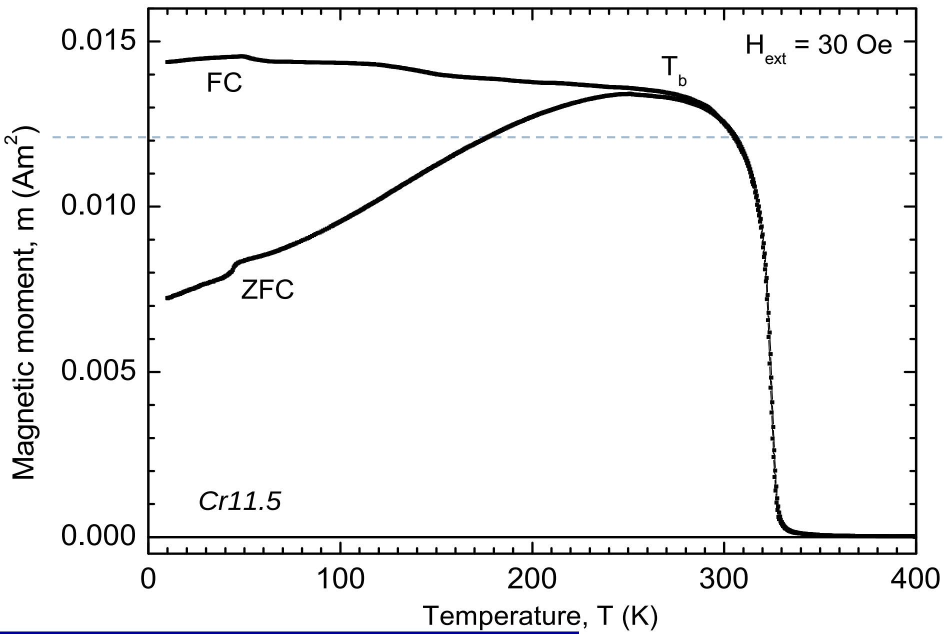
The hysteresis loops at 350 K for $Cr_{11.5}$ and Cr_{12} ribbons as well as the one at 300 K for Cr_{13} ribbons exhibit a similar behavior, characteristic to a system consisting of very small ferromagnetic clusters, whose magnetization can randomly flip direction under the influence of temperature.

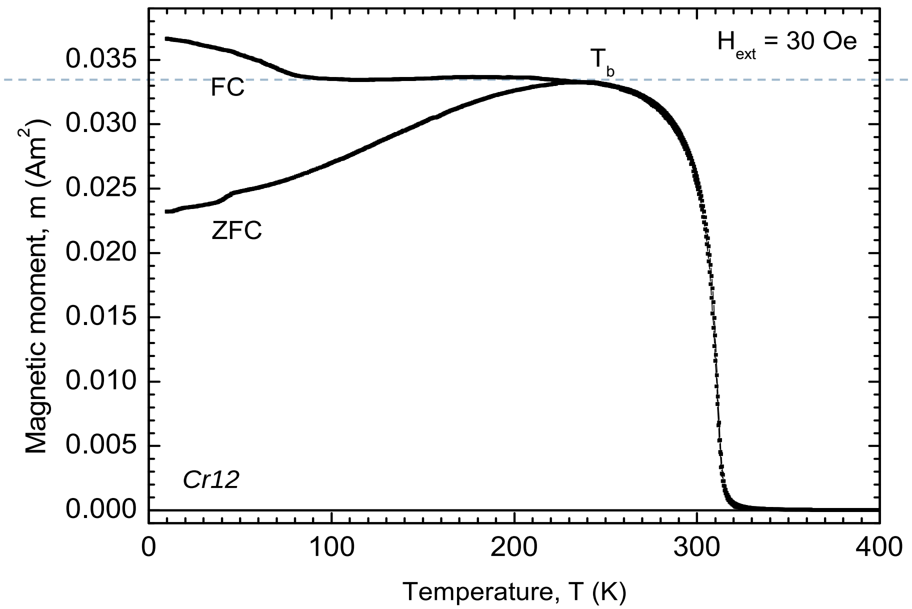
However, all the investigated ribbons are behaving ferromagnetically, at least at temperatures below 250 K, which is evidenced also on ZFC-FC curves as blocking temperature, T_b.



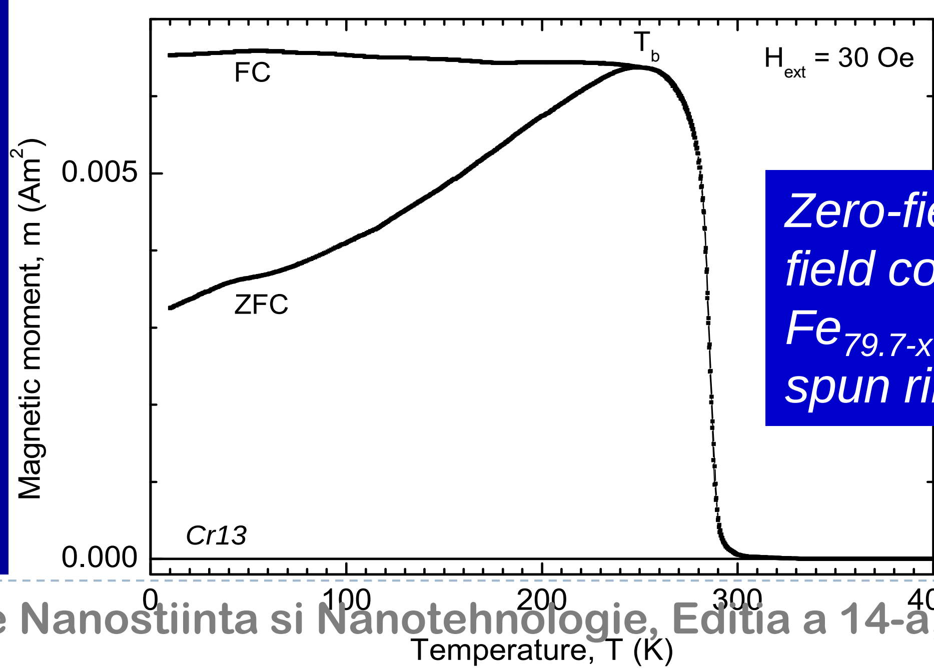
Chiriac et al., J Appl Phys (2015) accepted

Specific cluster structure → superferromagnetism





The presence of the cusp around 250 K, regardless of the Cr content, is suggesting the presence of a non-uniform structure, with respect to cluster size or cluster interactions, similar to the previously reported behavior for spin-glass-like systems.

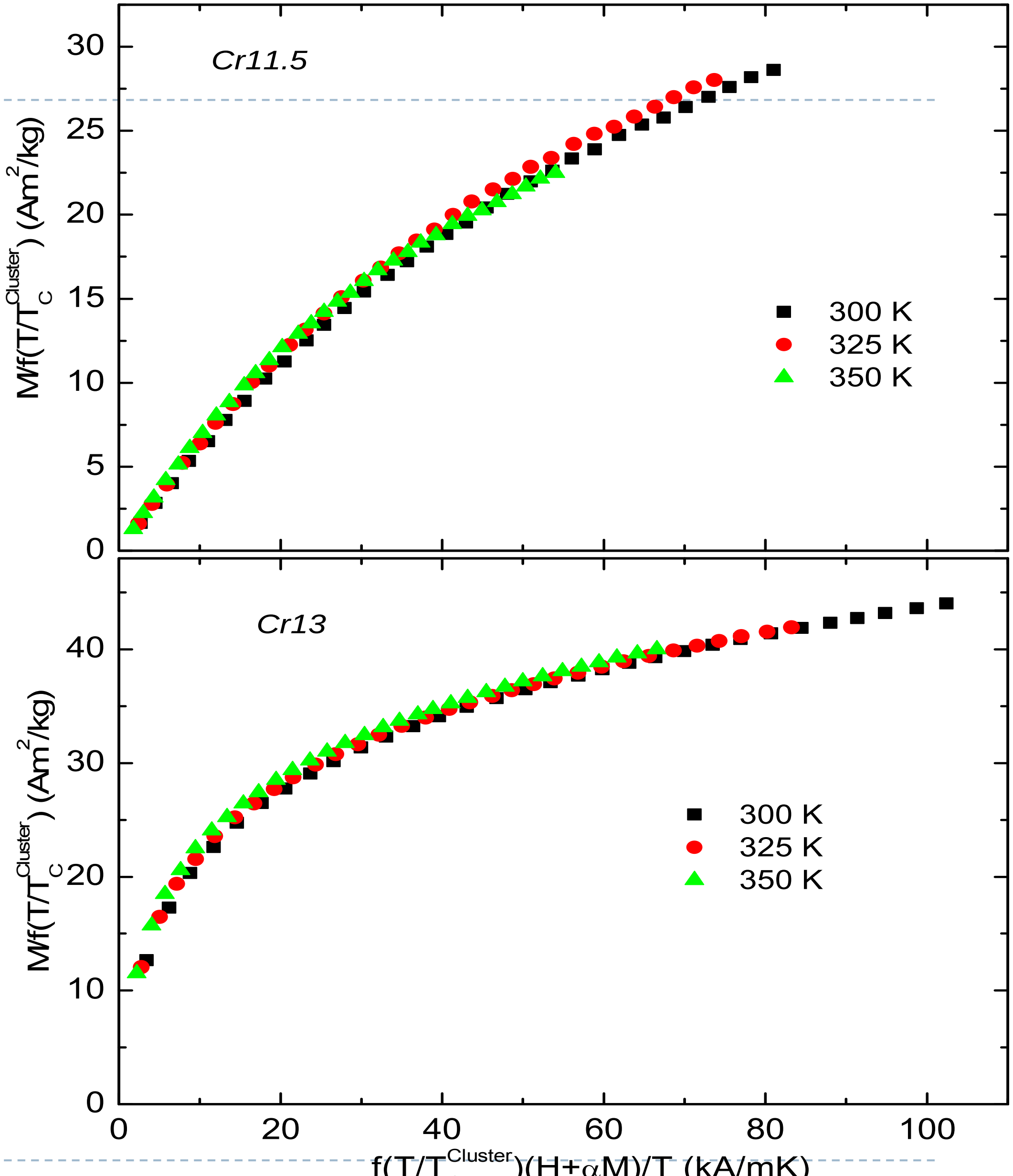


Chiriac et al.,
J Appl Phys (2015) accepted

Zero-field cooled (ZFC) and field cooled (FC) curves for $Fe_{79.7-x}Cr_xNb_{0.3}B_{20}$ glassy melt-spun ribbons.

Specific cluster structure → superferromagnetism

Mater Trans, JIM **39**, 102 (1998)
J Phys Condens Matter **12**, 4253 (2000)



The Langevin function fitting curves $M(T,H)/f(T/T_C^{Cluster})$ versus $[f(T/T_C^{Cluster})(H+\alpha M)]/T$ for $Fe_{68.2}Cr_{11.5}Nb_{0.3}B_{20}$ and $Fe_{66.7}Cr_{13}Nb_{0.3}B_{20}$ glassy melt-spun ribbons indicate that above T_b the ribbons behave as superferromagnetic systems

Chiriac et al., J Appl Phys (2015) accepted

Specific cluster structure → superferromagnetism

Parameters derived from the analysis of the magnetization data for glassy $Fe_{79.7-x}Cr_xNb_{0.3}B_{20}$ melt-spun ribbons

Parameter	x = 11.5	x = 12	x = 13
Curie temperature of the cluster ($T_C^{Cluster}$) (K)	652	589	486
Molecular field coefficient (α)	320	265	195
Curie temperature of the system (T_C^{system}) (K)	330	320	290

Chiriac et al., J Appl Phys (2015) accepted

Conclusions (1)

- By increasing the Cr content in the melt-spun ribbons the T_C^{system} decreases.
- In the meantime the Curie temperature in the cluster, $T_C^{Cluster}$ decreases by decreasing the Fe content in the ribbons, indicating that the interaction between Fe atoms in the cluster is weakened.
- Considering also that the molecular field coefficient, α, is 1230 for bcc-Fe, whilst for $Fe_{79.7-x}Cr_xNb_{0.3}B_{20}$ melt-spun ribbons is less than one quarter, one can conclude that the interaction between clusters for the ribbons is much weaker than the interaction between Fe spins in bcc-Fe due to the presence of antiferromagnetic Cr, which frustrates the ferromagnetic Fe-Fe and Fe-Cr interactions.
- As Fe concentration increases the average size of clusters increases, and, consequently, the number of clusters, N, decreases because the clusters combine in each other.

Submicron powders preparation

- $Fe_{79.7-x}Cr_xNb_{0.3}B_{20}$ (x = 11.5÷13 at.%) precursor glassy melt-spun ribbons have been prepared by rapid quenching from the melt, and subsequently annealed in vacuum at temperatures of 250 to 450^{0}C to reduce the ductility in order to ease the preparation of powders by high-energy ball milling;
- pieces of annealed ribbons have been cut, introduced into 2 hardened steel vials (to avoid powders' contamination) and milled in dry (air, Ar, He) or/and wet environment (water, distilled water, oleic acid (OA) as surfactant and n-heptane as solvent);
- balls-to-powder weight ratio = 50:1;
- $v_{milling}$ = 550 rpm;
- $t_{milling}$ = 1÷90 h
- the powders produced by using OA as surfactant have been washed several times with n-heptane to remove the excess surfactant and then dried at moderate temperatures (40-50^{0}C) to evaporate the solvents.

Chiriac et al., J Appl Phys **115**, 17B520 (2014)

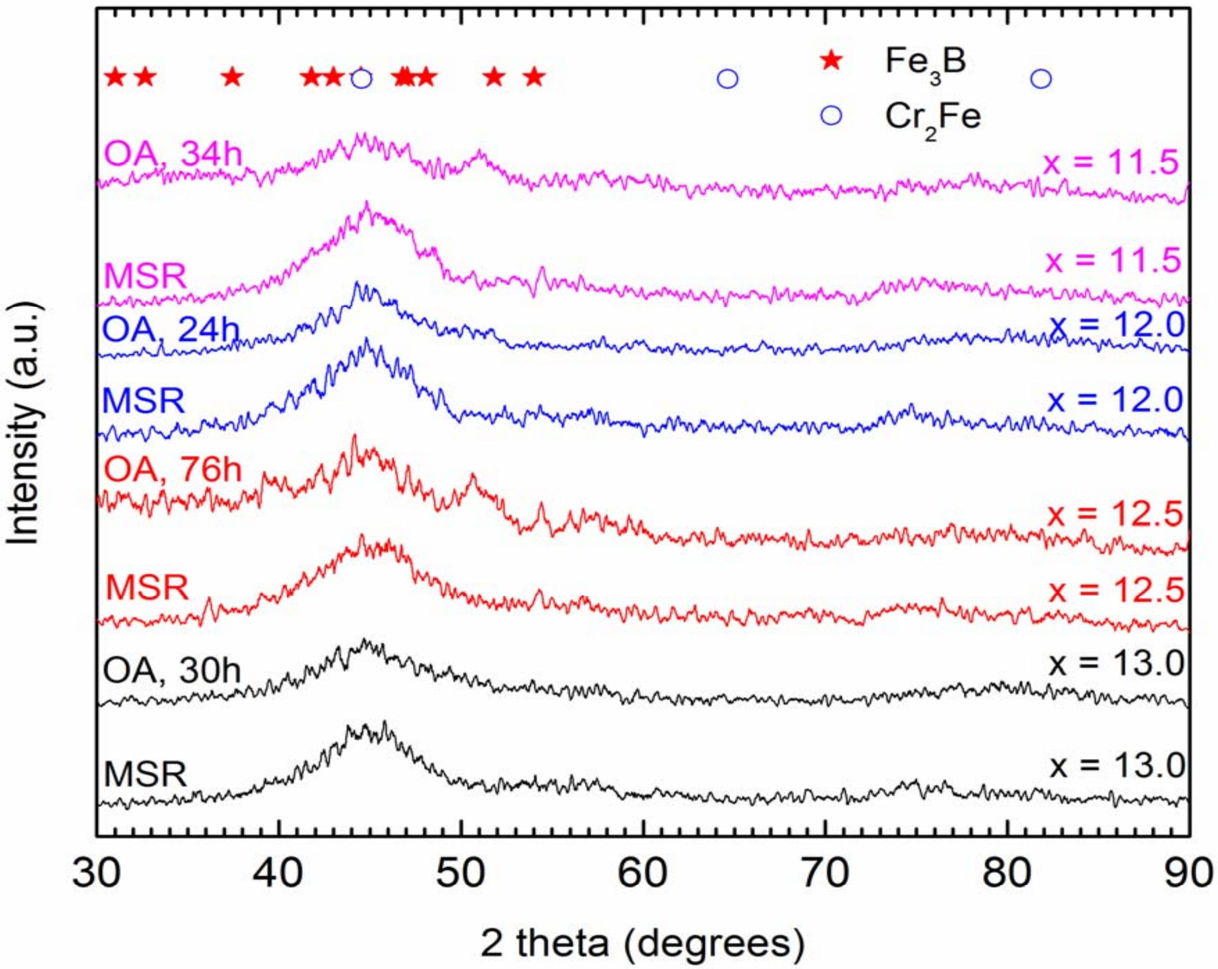
Influence of milling conditions

The size and morphology/microstructure/magnetic properties of powders are strongly dependent on the milling conditions:

- precursor melt-spun ribbons or powder/balls ratio;
- type of vials (stainless steel is preferred to avoid contamination);
- the energy of milling – moderate milling speeds are preferred;
- wet/dry milling;
- with or without surfactants;
- intermediary sieving is helpful in obtaining powders with similar sizes;
- milling atmosphere (air is the worst, Ar or He preferred).

Chiriac et al., J Appl Phys **115**, 17B520 (2014)

Microstructure evolution vs. Cr content

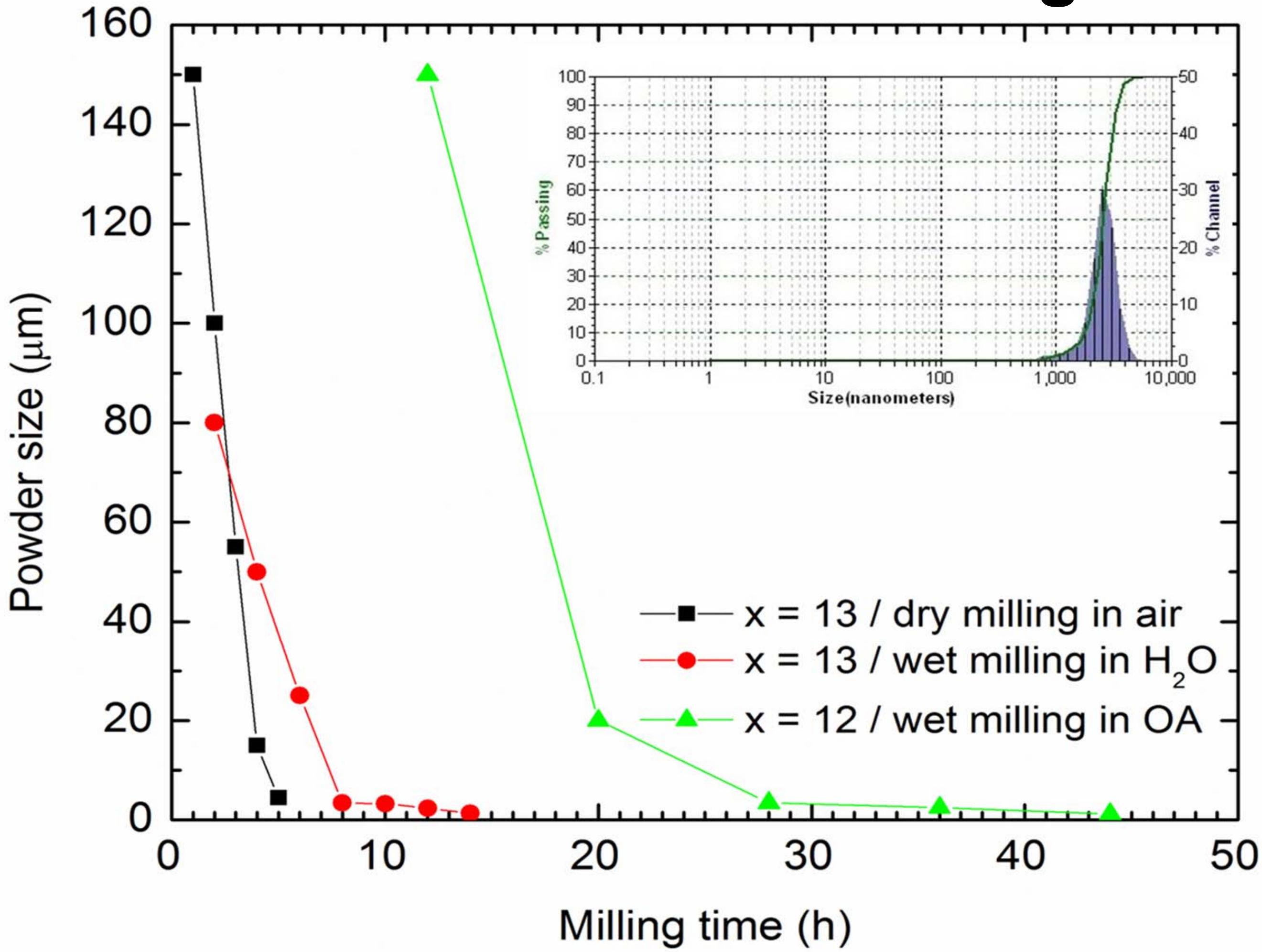


A glassy structure in the as-quenched state**, consisting of* **very weak magnetic Fe-Cr clusters** (the inner part is rich in ferromagnetic Fe, whilst the outer shell is rich in antiferromagnetic Cr) **surrounded by the Fe_3B-based residual matrix with enhanced T_C** – ***the necessary condition to have low T_C.

The deterioration of the glassy structure leads to T_c of 770-790^{0}C.

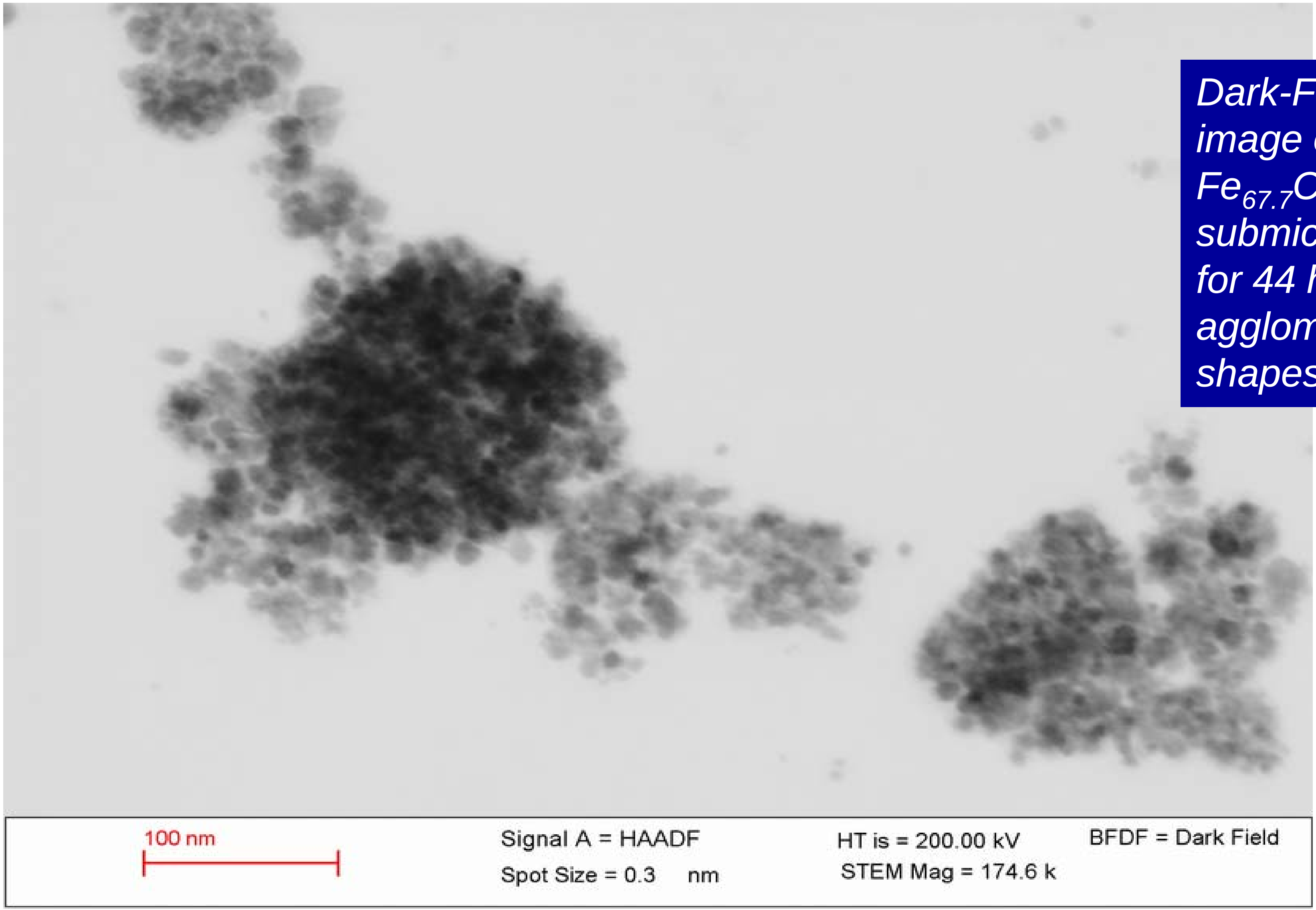
$Fe_{79.7-x}Cr_xNb_{0.3}B_{20}$ (x = 11.5÷13 at.%) melt-spun ribbons (MSR) and submicron powders prepared by wet milling in oleic acid (OA) and n-heptane for different milling times.

Powders size evolution vs. milling conditions



The dependence of size on milling conditions for $Fe_{79.7-x}Cr_xNb_{0.3}B_{20}$ powders prepared by high-energy ball milling from glassy precursors. In the Inset is shown the size distribution of $Fe_{67.7}Cr_{12}Nb_{0.3}B_{20}$ powders, milled in oleic acid for 44 h and determined by dynamic light scattering.

STEM image of submicron powders



Dark-Field HR-STEM image of $Fe_{67.7}Cr_{12}Nb_{0.3}B_{20}$ submicron powders milled for 44 h in OA indicates agglomerated irregular shapes of 25÷40 nm

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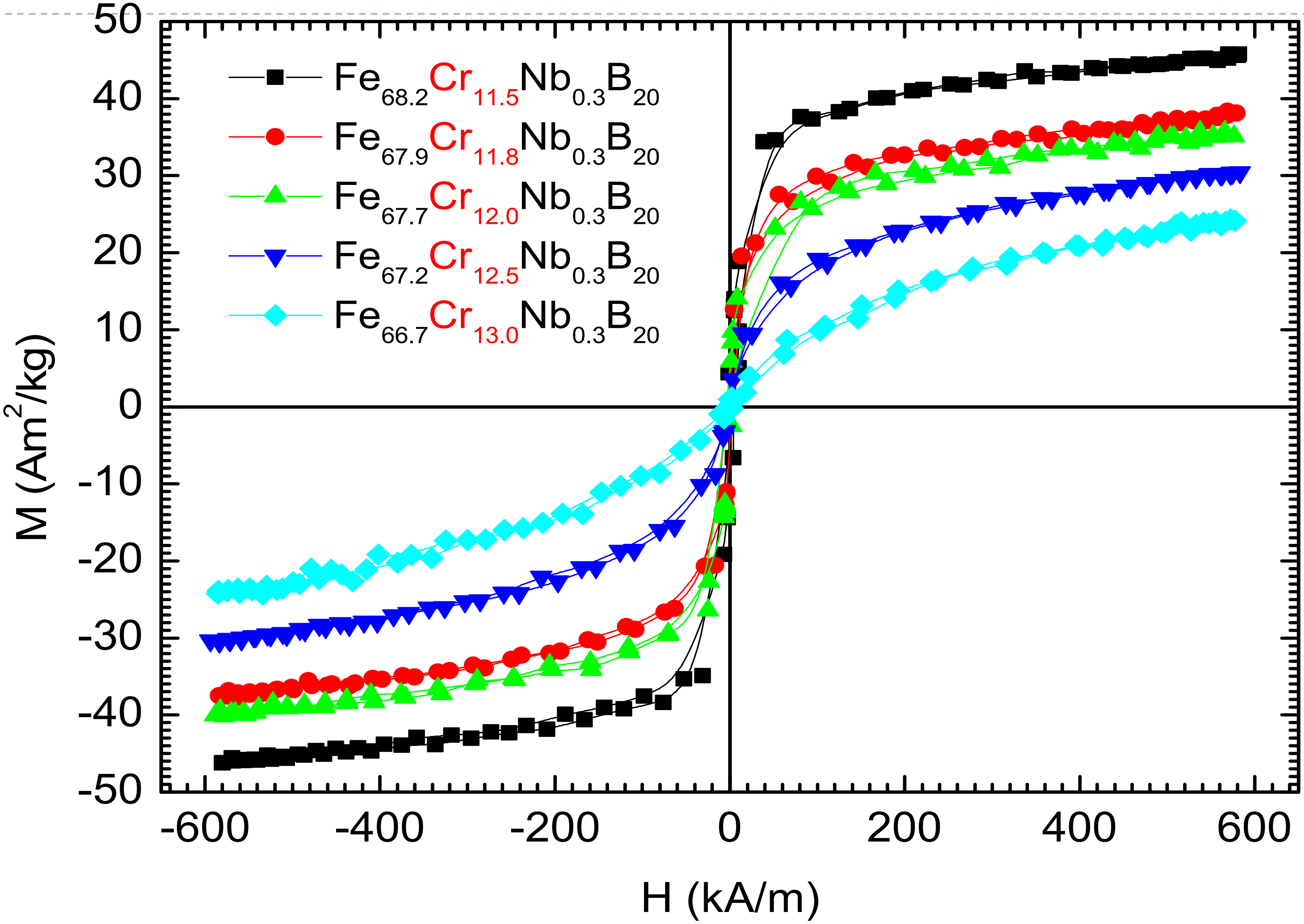
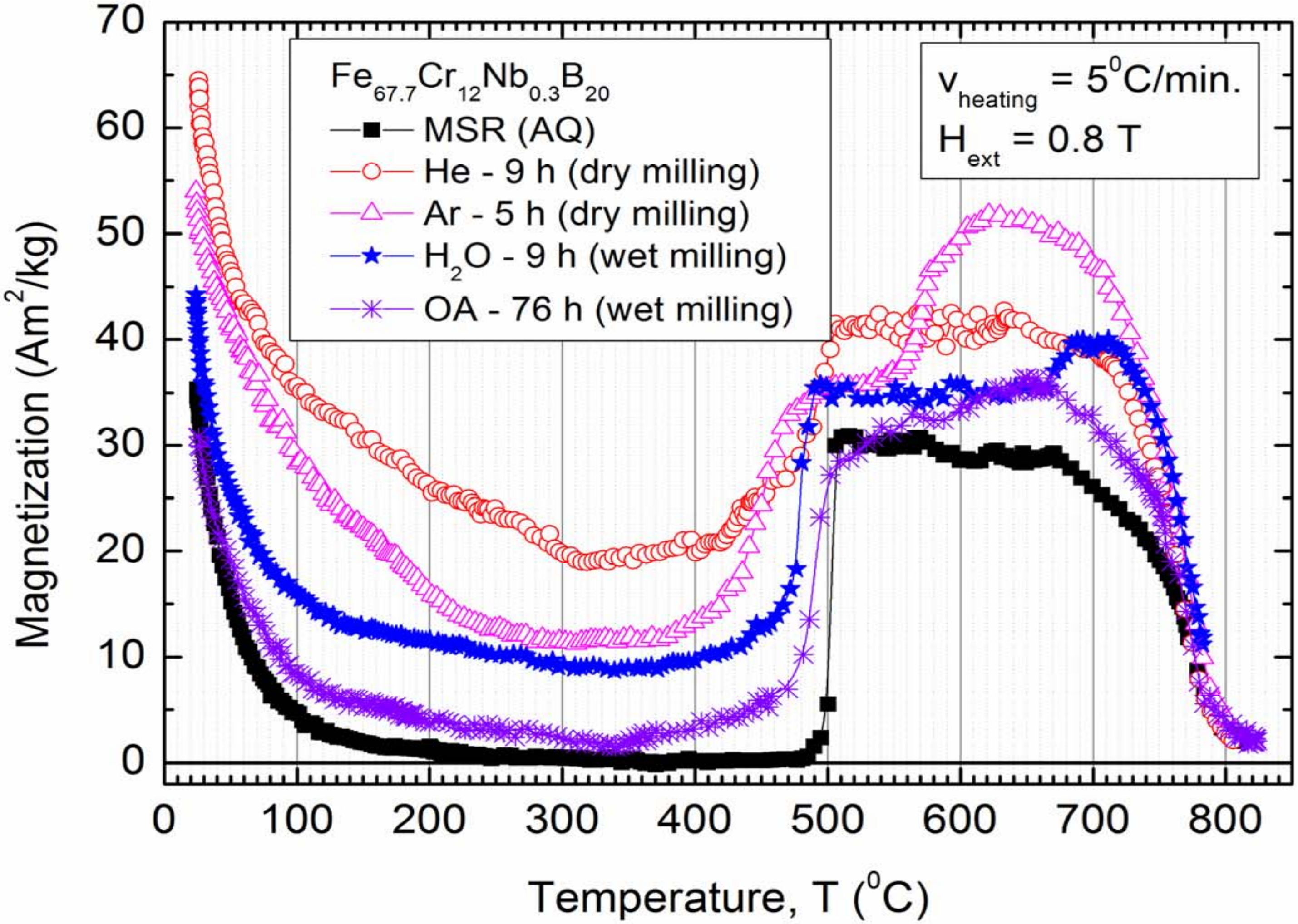
M-H curves of Fe-Cr-Nb-B submicron powders



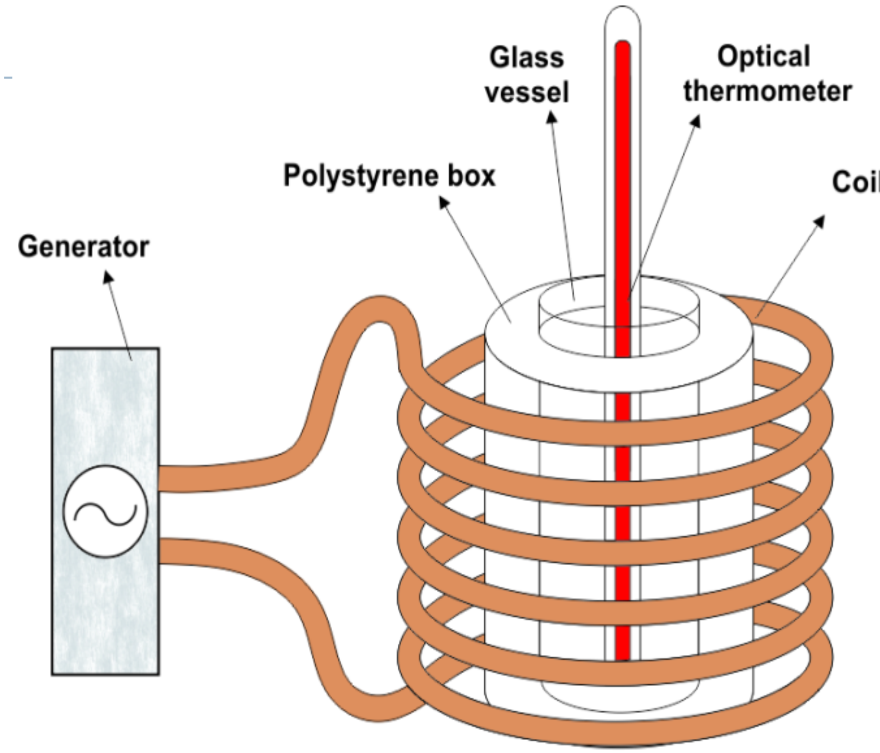
M(T) vs. milling conditions



M(T) variation for $Fe_{67.7}Cr_{12}Nb_{0.3}B_{20}$ glassy melt-spun ribbons and submicron powders milled in different conditions for different milling times. The transition temperature is strongly dependent on the microstructure of the milled submicron powders.

Hyperthermia setup

- The heating efficiency of Fe-Cr-Nb-B ball-milled powders was investigated in an a.c. field of 350 mT (f = 153 kHz) created by a home-made magnetic-induction hyperthermia unit, consisting of a HF generator (HFG3 IGBT from Eldec) and a specially designed temperature measurement system adapted to HF working conditions (Optical Thermometer from Optocon).
- The testing chamber was completely isolated relative to the external environment, to avoid the heating exchange and provide the adiabatic conditions for the heating efficiency tests.
- The concentration of the tested solutions was of 75 mg/mL magnetic powders in a mixture of water and styrene glycol.



A controlled constant temperature in the range 42-47^{0}C range is the most important parameter for in-vivo magnetic hyperthermia applications.

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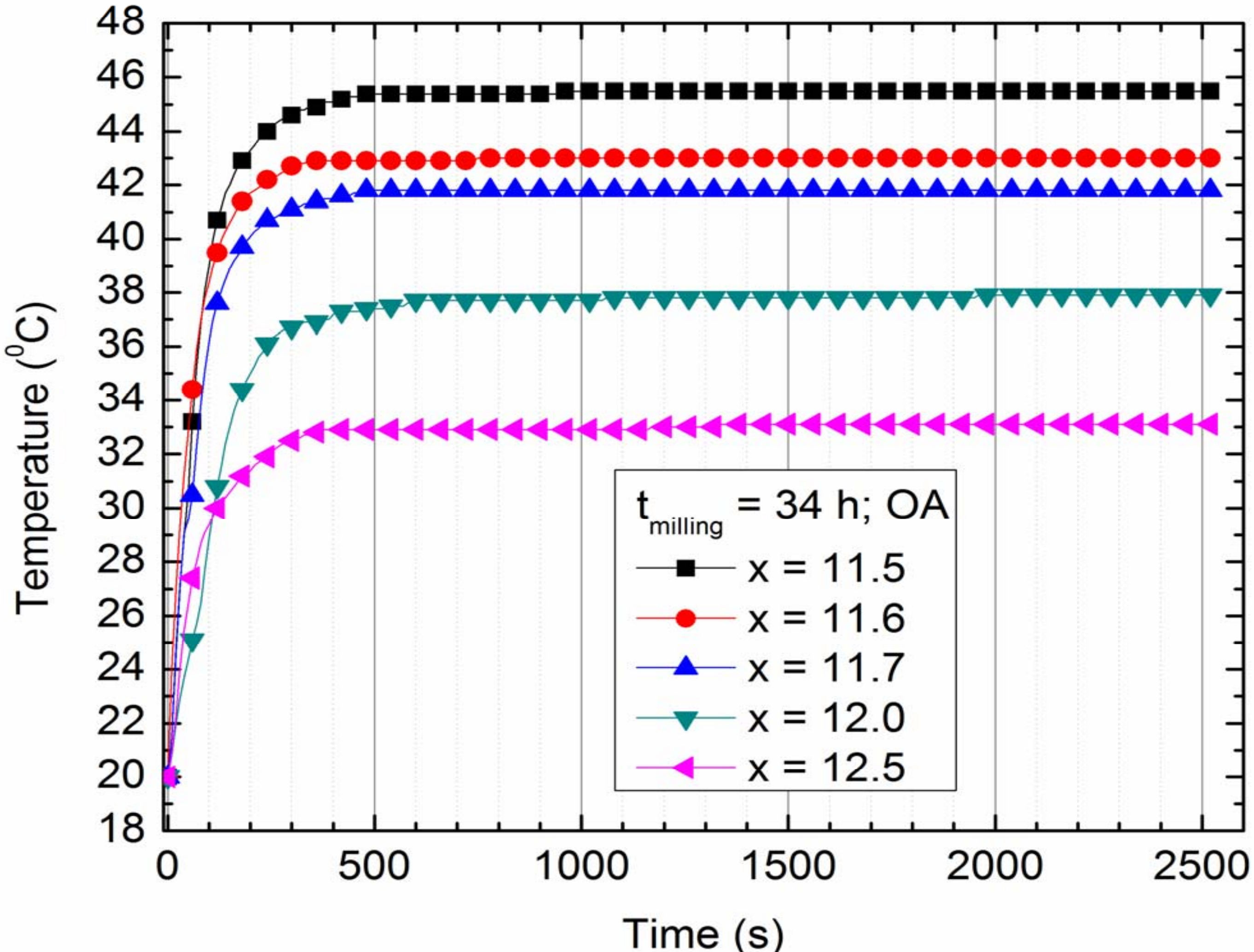
Hyperthermia applications



The heating efficiency curves of $Fe_{79.7-x}Cr_xNb_{0.3}B_{20}$ (x = 11.5÷12.5 at.%) submicron powders vs. time and Cr content.

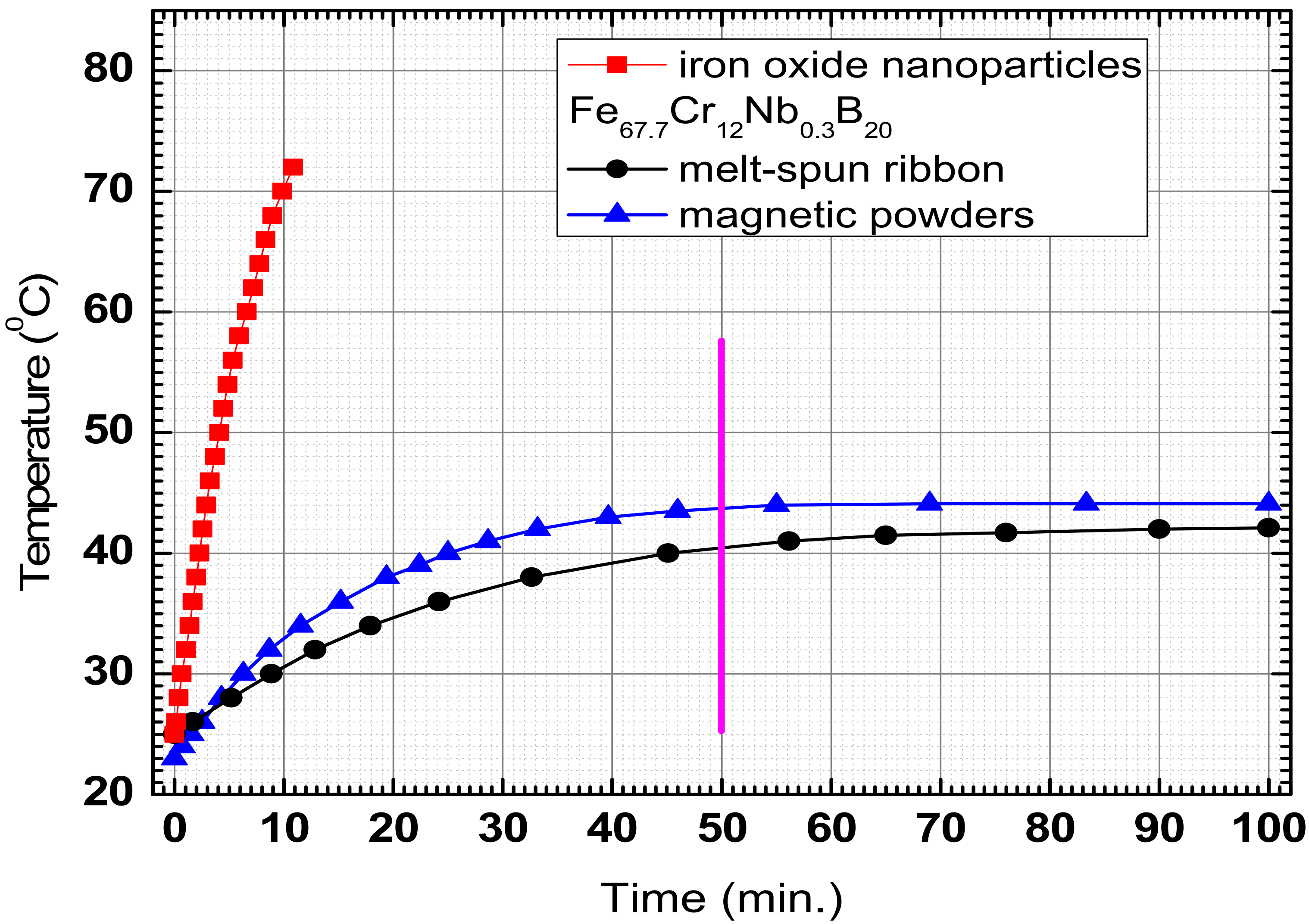
Chiriac et al., J Appl Phys **115**, 17B520 (2014)

Hyperthermia applications

- The equilibrium temperatures are in agreement with the Curie temperatures, sustaining the hypothesis that the glassy structure is preserved in submicron powders milled in oleic acid for moderate periods of time.
- By changing of the Cr content by a single percent (from 12.5 down to 11.5 at.%), it is possible to tune the heating efficiency between 33 and 46^{0}C.
- **The smaller the Cr content, the larger the heating efficiency temperature is.**
- **This specific behavior is a clear evidence for the suitability to using Fe-Cr-Nb-B submicron powders for hyperthermia applications, since it allows keeping unchanged the temperature in a desired area for a given period of time.**
- **The equilibrium temperature can be tuned in a narrow range either by modifying the Cr content, i.e. the content of antiferromagnetic element, or by controlling very strictly the milling conditions.**
- **It is worthy to note that by achieving the equilibrium temperature it is possible to heat the sick organ at a desired temperature, independent of the supplied power.**

Chiriac et al., J Appl Phys **115**, 17B520 (2014)

Hyperthermia applications



In vitro Cytotoxicity Studies

Cell culture preparation

❑ For „in vitro" experiments, a Human Osteosarcoma cell line (Eppelheim, Germany) was used.

❑ After thawing cells in a thermostated water bath (37^0C), they were washed with complete culture medium supplemented with 10% fetal bovine serum, 2% L-glutamine and 1% antibiotic. The cells were settled by centrifugation, re-suspended in 10 ml complete medium, and sub-cultivated in 25 cm^2 flasks.

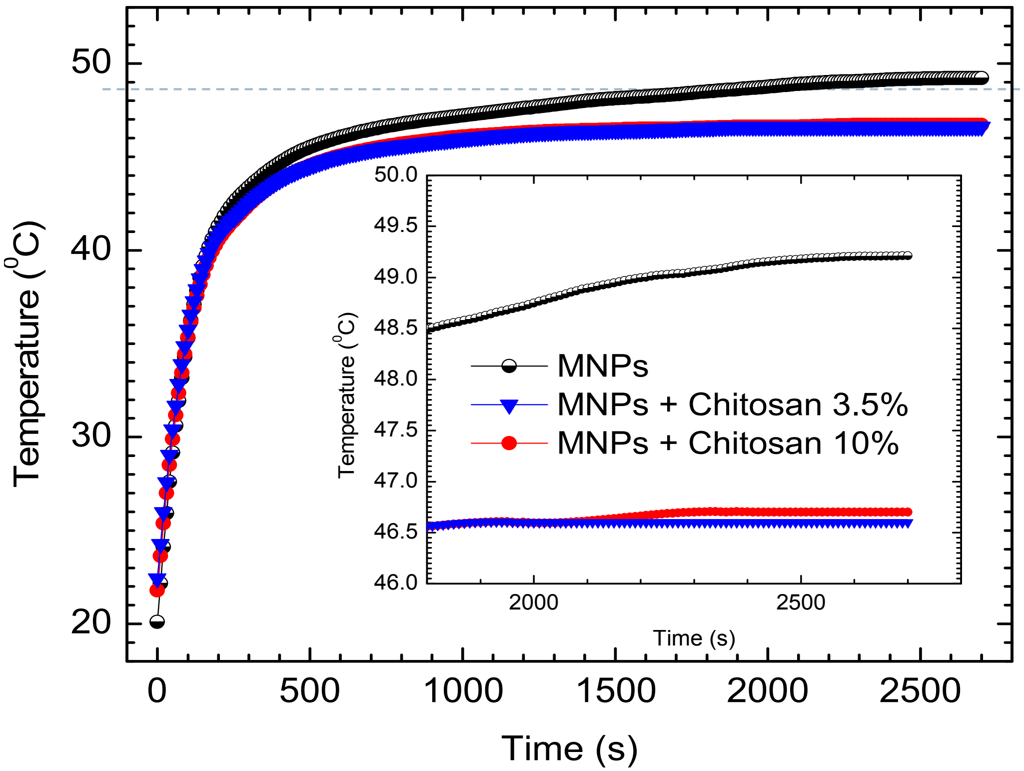
❑ After 48 h, the cells were detached from the flask by using trypsin-EDTA solution.

❑ Following addition of complete medium, the suspension was centrifugated, and the resulted pellet was re-suspended in 1 ml complete medium.

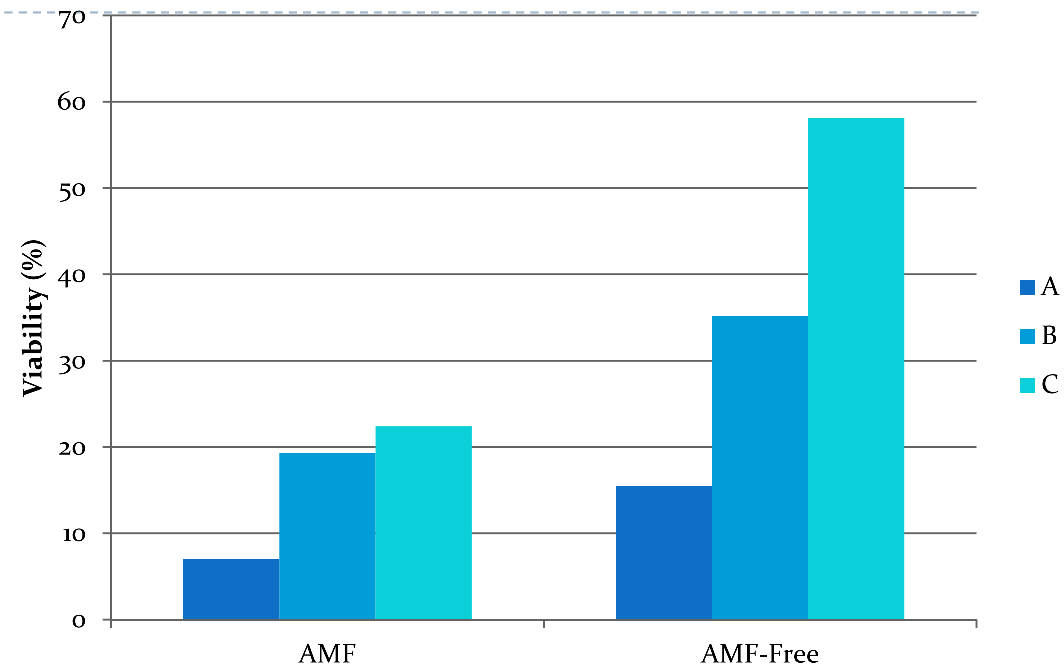
❑ Finally, the cells were seeded into 4 flasks and incubated at 37^0C, 5% CO_2, and 95% humidity. A density of $5x10^6$ cells/ml, translated into a complete cell confluence, was established after 3 days of incubation.

Chiriac et al, J Magn Magn Mater **380**, 13 (2015)

Cytotoxicity in AC Magnetic Fields



350 mT (f = 153 kHz)



Sample A – MNPs coated with chitosan 3.5%
Sample B – MNPs coated with chitosan 10%
Sample C – MNPs uncoated

Cell viability after AMF exposure as compared with AMF-free conditions for 5 mg/ml concentration of MNPs.

The uncoated $Fe_{68.2}Cr_{11.5}Nb_{0.3}B_{20}$ MNPs show a good biocompatibility in the cancerous cell culture, regardless of the testing conditions.
In high frequency alternating magnetic fields, the MNPs-mediated heating seems to be a determining factor for cell viability decrease in the tested cell culture model.

In vitro Cytotoxicity Studies

- The specific absorption rate varies from 215 W/g for uncoated MNPs to about 190 W/g for chitosan-coated ones, and an equilibrium temperature of 46^{0}C is reached when chitosan-coated MNPs are subjected to AMF.

	FeCrNbB uncoated	FeCrNbB + 3.5% chitosan	FeCrNbB + 10% chitosan
SAR (W/g)	**215**	**189**	**192**

- The uncoated $Fe_{68.2}Cr_{11.5}Nb_{0.3}B_{20}$ MNPs prove a good biocompatibility and low citotoxicity in all testing conditions.
- The chitosan-coated MNPs induce strong tumoricidal effects when a cell-particle simultaneous co-incubation approach is used.
- In high frequency AMF, the particle-mediated heat treatment is the critical cause for decreasing *in vitro* the viability of a cancer cell line.

Chiriac et al., J Appl Phys **115**, 17B520 (2014)
Chiriac et al, J Magn Magn Mater **380**, 13 (2015)

Conclusions (2)

- $Fe_{79.7-x}Cr_xNb_{0.3}B_{20}$ (x = 11.5÷13 at.%) submicron powders have been prepared by high-energy ball milling from glassy melt-spun ribbons precursors, with T_C ranging from 16 to 50^0C, depending on Cr content.
- The Curie temperature of the submicron powders is strongly dependent on the milling conditions.
- Even when they are prepared by wet milling in oleic acid as surfactant and n-heptane as solvent, the powders agglomerates. However, their individual sizes goes down to 25÷40 nm after more than 40 h of milling.
- The measured heating efficiency in an a.c. high frequency field indicates equilibrium temperatures of 33÷46^0C, depending on the Cr content.
- **Fe-Cr-Nb-B MNPs are suitable for self-regulating magnetic hyperthermia**, compared with Fe-oxides (mainly Fe_3O_4) which work indeed up to moderate temperatures (below 47^0C), but the capacity to retain the temperature in the range of 41-46^0C requires a very rigorous control of the power of the high frequency generator.

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Gabi Ababei, Mihaela Lostun – ribbons and submicron powders preparation
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Gabi Ababei – FMR measurements
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Camelia Danceanu, Daniel Herea, Luminita Labusca (M.D.) – hyperthermia and toxicity tests

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Thank you for your kind attention!