Szuperparamágneses vas-oxid nanorészecskék (SPIONs) teranosztikai célú fejlesztése

Tombácz Etelka

Vizes Kolloidok Kutatócsoport
Fizikai Kémiai és Anyagtudományi Tanszék
Szegedi Tudományegyetem

Biomedical applications of magnetic nanoparticles

The most important applications:

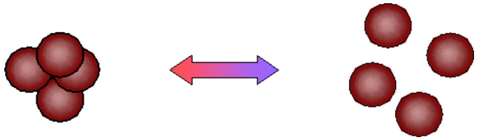
- **MRI contrast agent** – diagnosis
- magnetic **cell labeling and separation** - therapy
- **magnetic hyperthermia** - therapy
- **targeted drug delivery** - nanomedicine

Combination of therapy and diagnosis

theranostic agents

Criteria: these applications require the magnetic nanoparticles to be

non-toxic,
chemically stable,
uniform in size, and
well-dispersed in aqueous media.



Magnetic nanoparticles are of magnetite and maghemite dominantly, because living systems know what to do with them, **iron oxides are excreted via the liver** after the treatment.

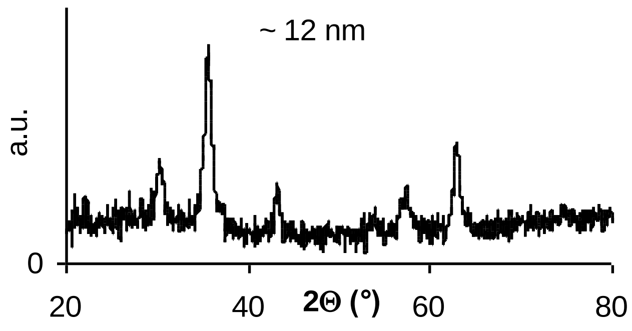
Particle aggregation must be excluded in magnetic field during application with reference to the **danger of embolism** in blood vessel.

Mágneses vas-oxid részecskék szintetizálása: méretfüggés a nano mérettartományban

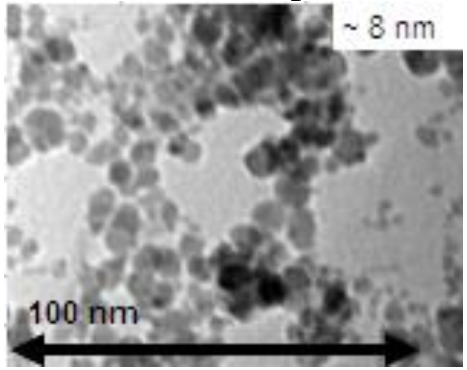
Ko-precipitáció ($Fe^{2+} + 2Fe^{3+} + 4OH^- \Rightarrow Fe_3O_4 + 4H^+$) +hidrotermális öregítés)

Ko-precipitációval előállított ~10 nm magnetit

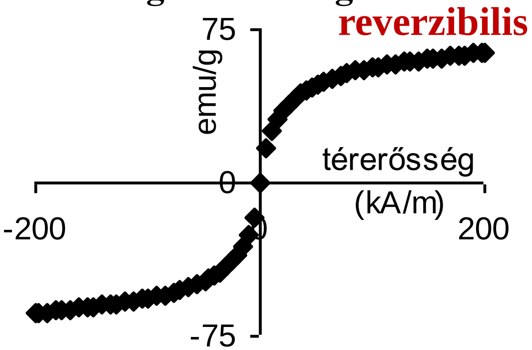
Röntgendiffraktogram



TEM kép



Magnetizációs görbe

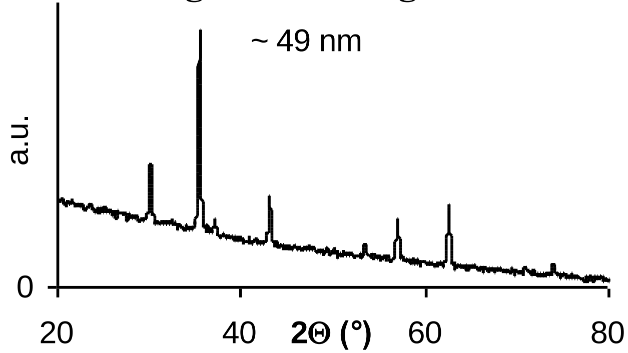


Szuperparamágneses viselkedés

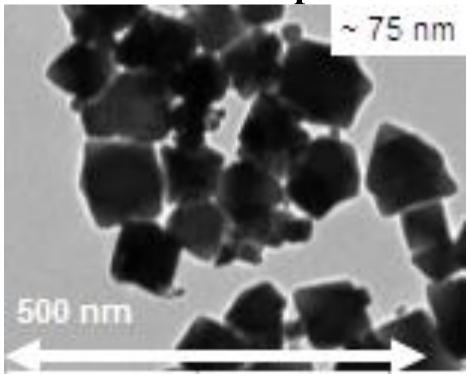
Oxidációs-precipitáció ($Fe(OH)_2$ zöld rozsda $\Rightarrow Fe_3O_4$ magnetit)

Oxidációs-precipitációval előállított ~75 nm magnetit

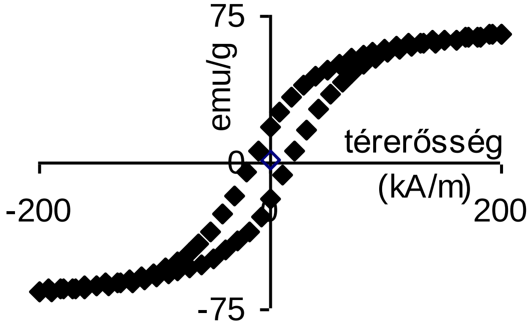
Röntgendiffraktogram



TEM kép



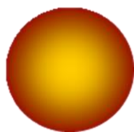
Magnetizációs görbe



Post-coating of SPIONs with polycarboxylates

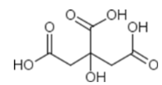
- via thermodynamically driven spontaneous multiple binding to ≡Fe-OH sites,
- providing combined electro-steric stabilization, hydrophilicity and
- free carboxylate sites for anchoring bioactive molecules via peptide bonding.

naked SPIONs:
preparation
purification
characterization

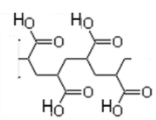


+

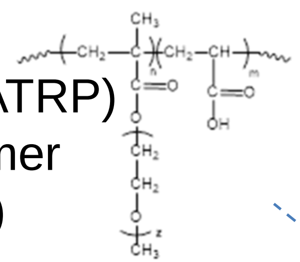
citric acid (CA)

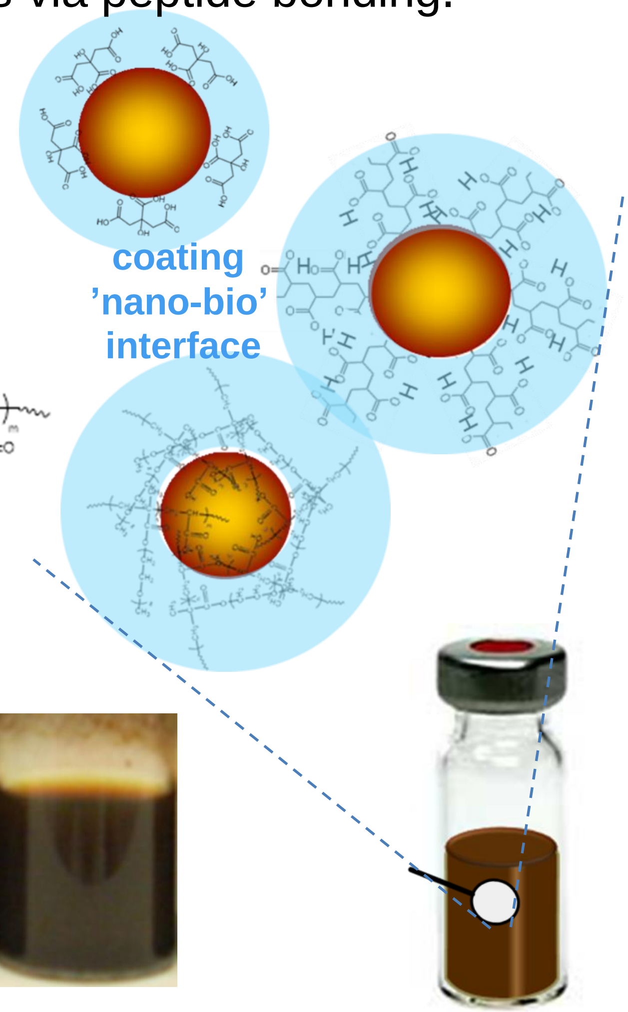


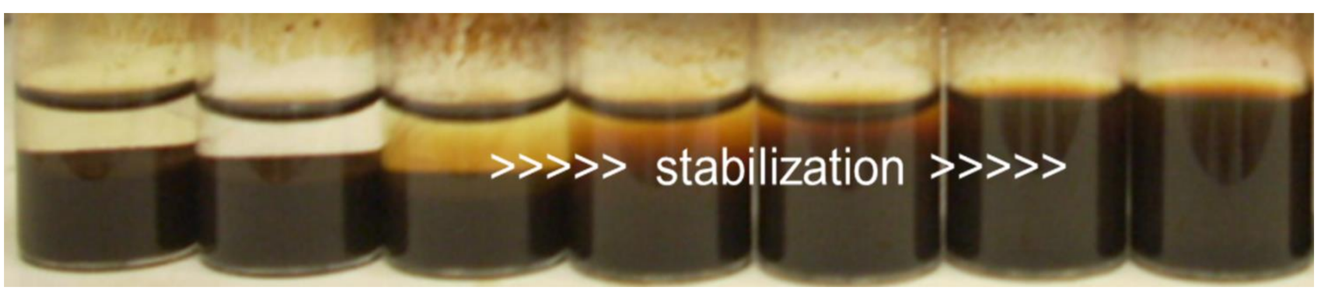
commercial
poly(acrylic acid)s
(PAA, PAM, etc.)



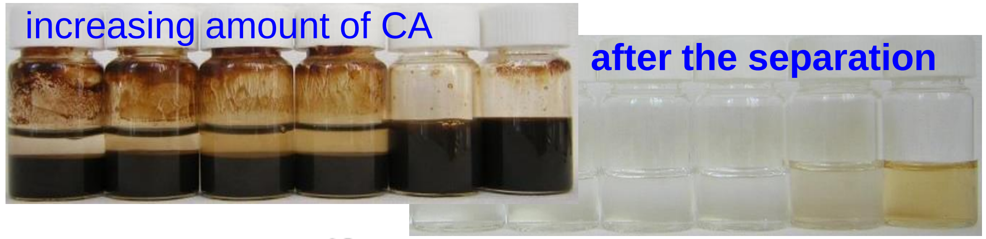
synthesized (via ATRP)
comb-like copolymer
P(PEGMA-co-AA)







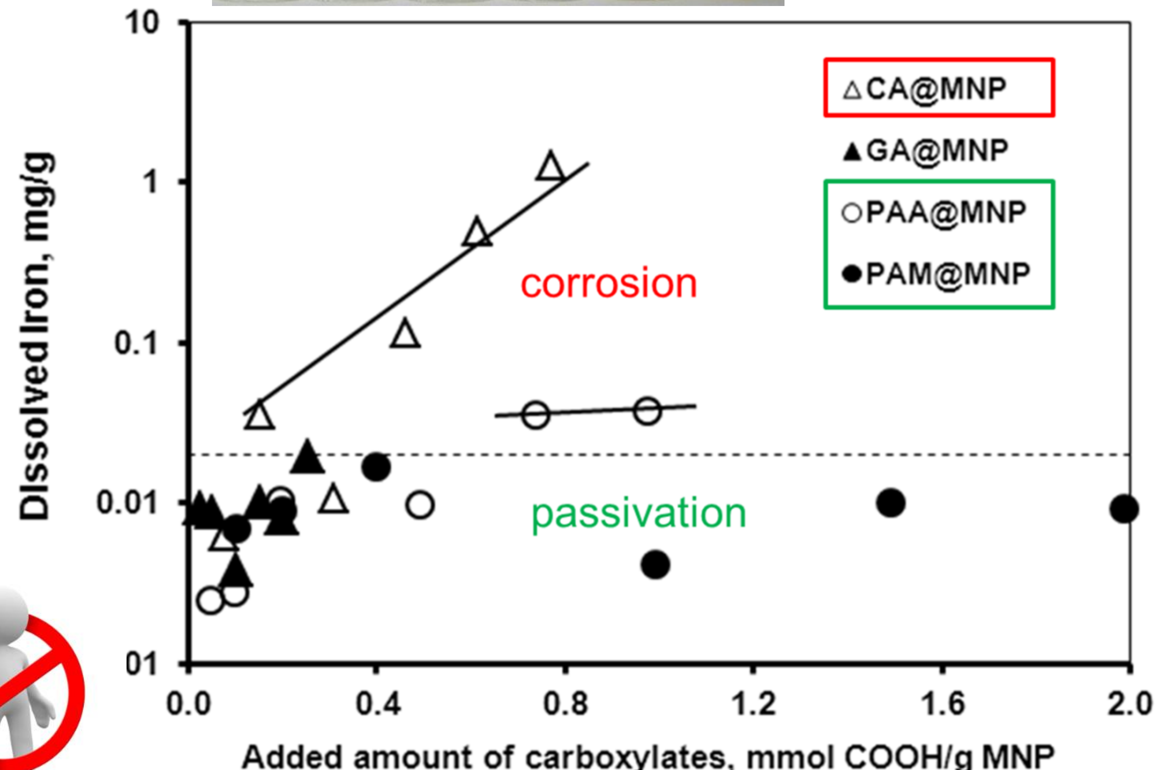
Characterization: chemical stability of carboxylated SPIONs



in the presence of citric acid (CA)
- iron oxide cores dissolve
- Fe(II)/Fe(III) ions leak into the medium

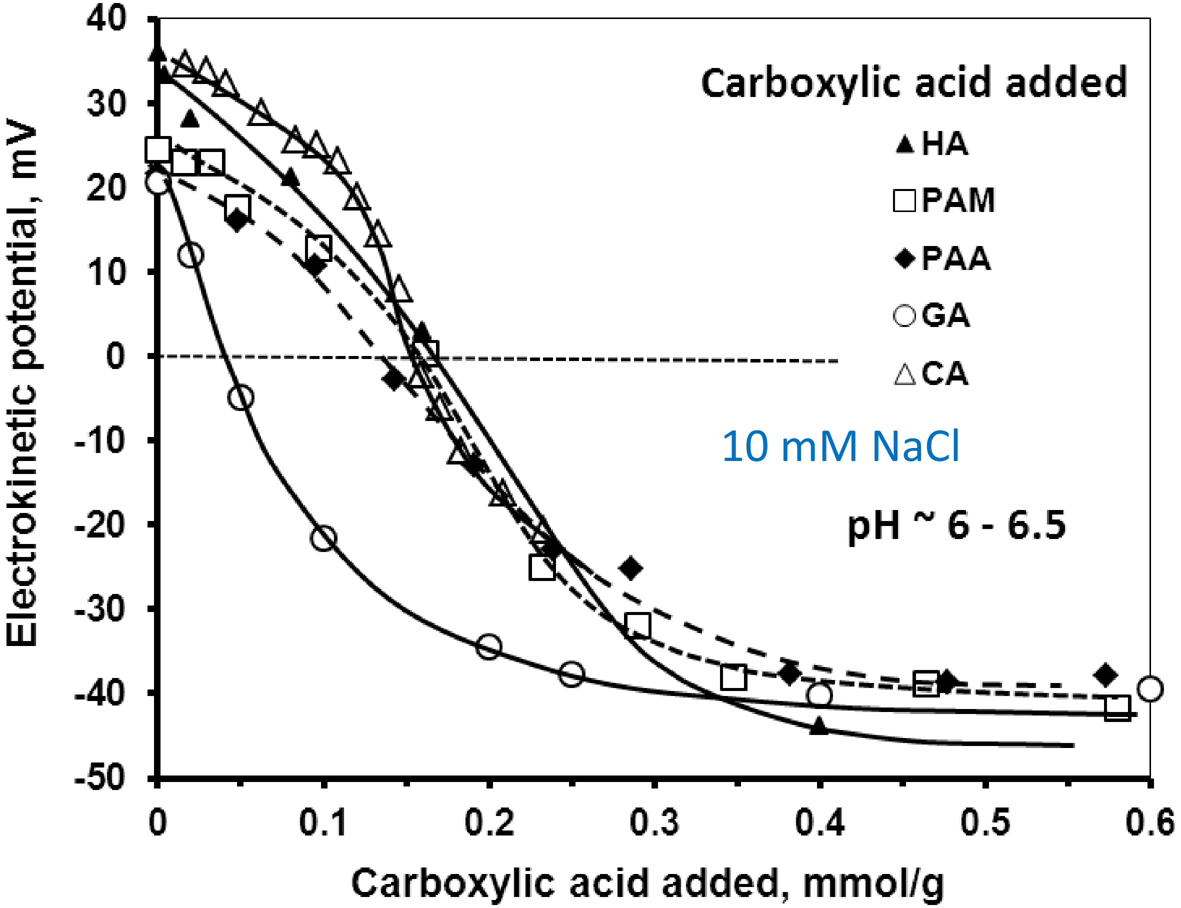
while polyelectrolyte coatings (PAA, PAM) prevent dissolution

Therefore **citrated SPIONs (CA@MNP)** **should be ruled out from in vitro/vivo tests and biomedical application.**



M.Szekeres, I.Y.Tóth, E.Illés, A.Hajdú, I.Zupkó, K.Farkas, G.Oszlánczi, L.Tiszlavicz, E.Tombácz, „**Magnetic Nanoparticles 2013**" *International Journal of Molecular Sciences*, 14, 14550-14574. 2013.

Characterization of particle charge: electrokinetic (ζ) potential

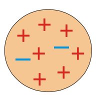


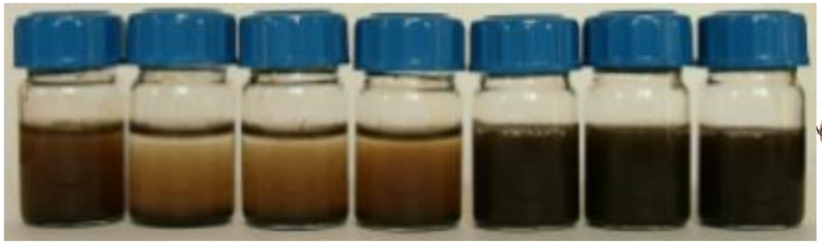
solution conditions:
pH, ionic strength,
buffer (quality and
concentration), etc.

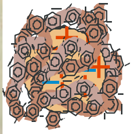
Effect of organic acids

quantitative comparision for surface charge neutralization

Magnetite





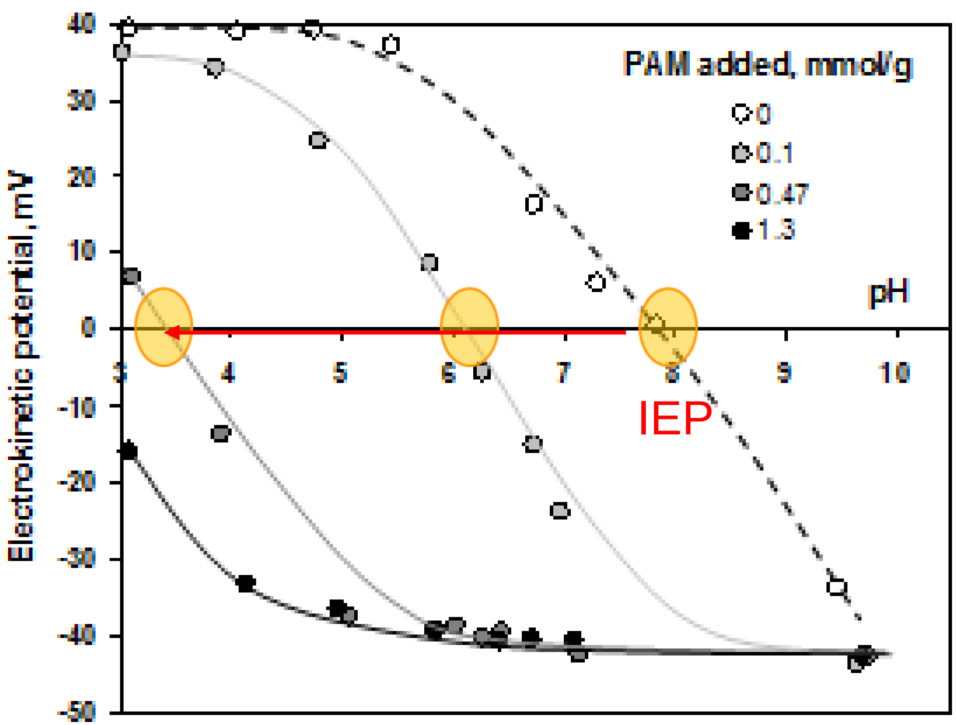


overcharging, if the amount of organic polyacids exceeds the amount of oppositely charged sites (~0.05 mmol/g) on magnetite

Increasing amount of HA ⇒ charge neutralization ⇒ charge reversal
destabilization restabilization

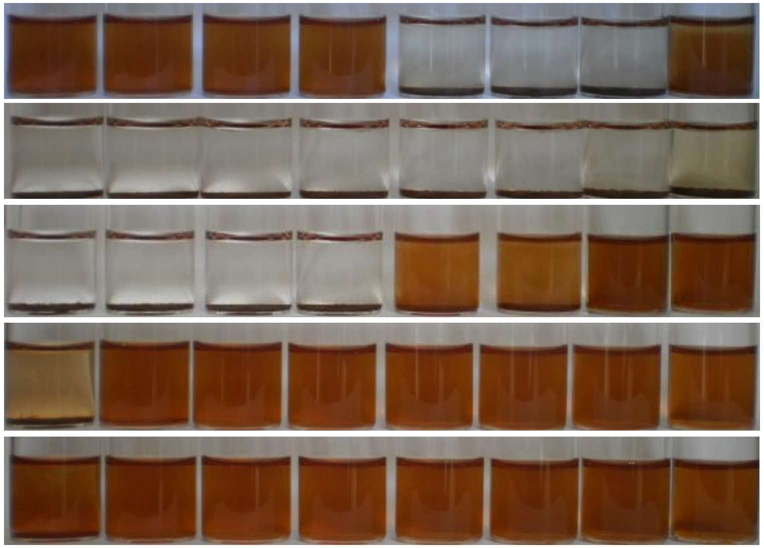
E. Tombácz, E. Illés, A. Hajdú, I.Y.Tóth, R.A. Bauer, D. Nesztor, M. Szekeres, I. Zupkó, L. Vékás, PPChE, 58, 3-10, 2014.

pH-dependent colloidal stability

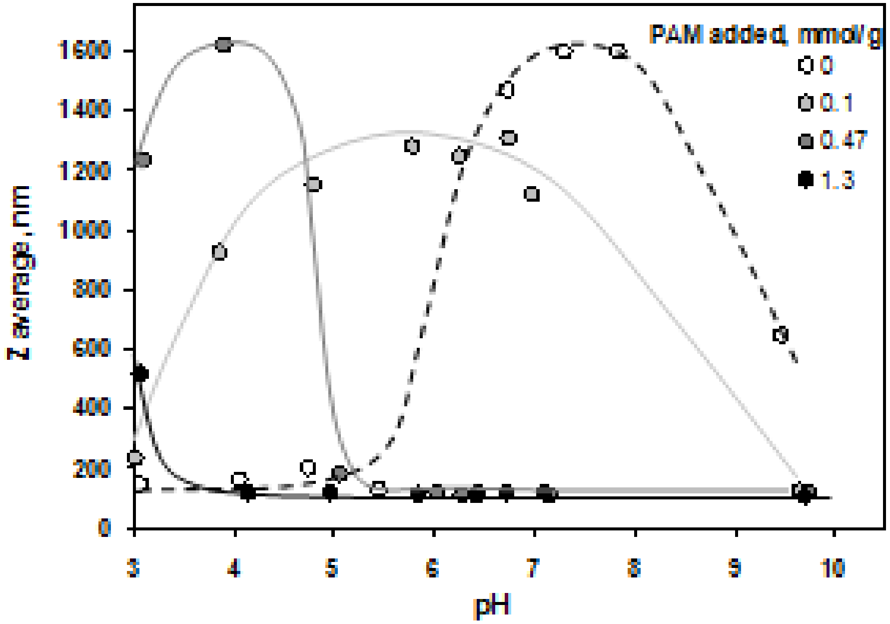


>>> increasing pH >>>

<<< increasing loading <<<



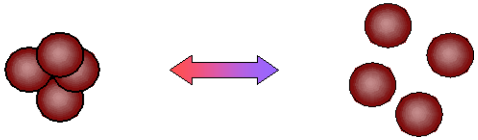
- the isoelectric point (IEP) shifts with increasing loading of any polycarboxylate
- low amount ⇨ aggregation
- amount high enough ⇨ stabilization over broad range of pH~3-10



I.Y.Tóth, E.Illés, R.A. Bauer, D.Nesztor, I.Zupkó, M.Szekeres, E.Tombácz:, Langmuir, 28(2012), 16638–16646..

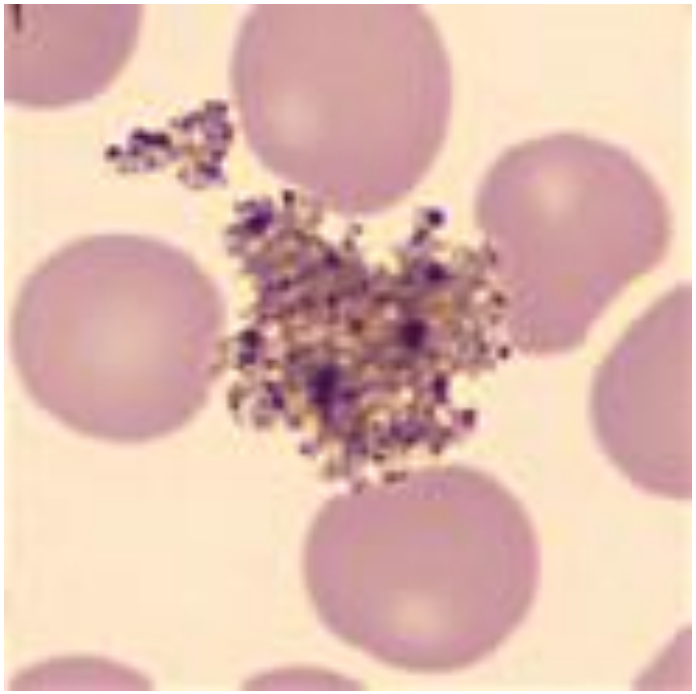
I.Y. Tóth, E..Illés, M. Szekeres, E.Tombácz, *Journal of Magnetism and Magnetic Materials*, 380:168-174 (2015)

Particle aggregation



Behavior in non-uniform magnetic field

in the blood



Smear from whole blood mixed with SPIONs

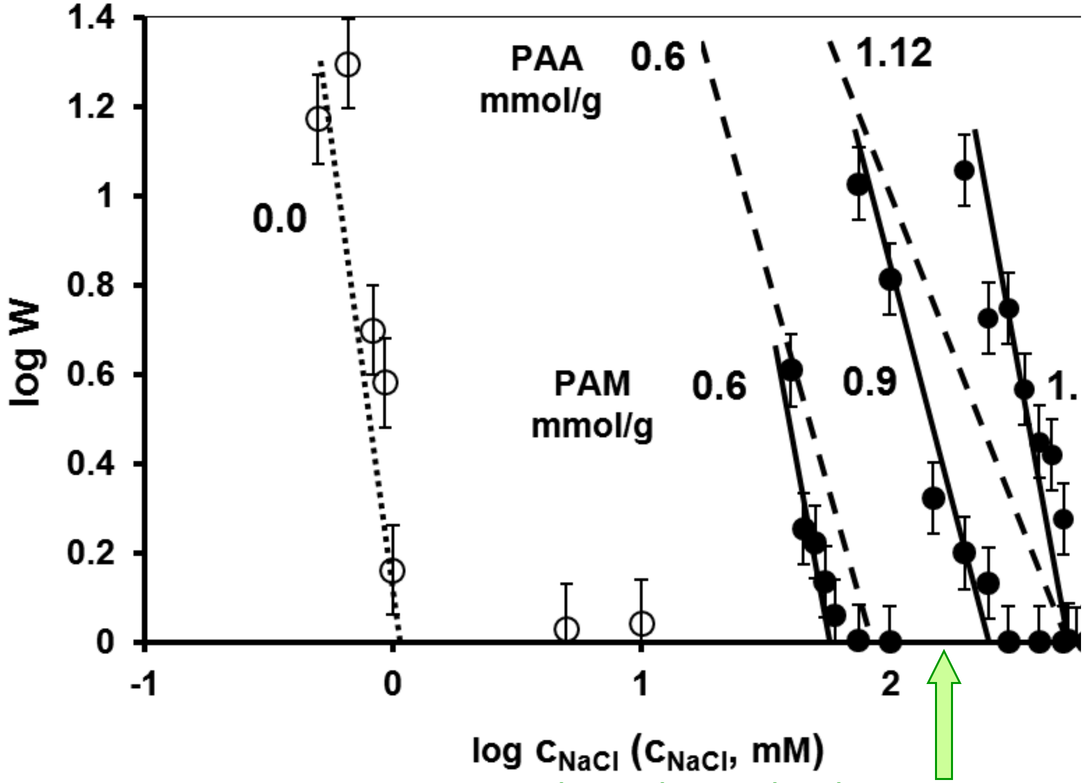
Photo of the Month November 2010

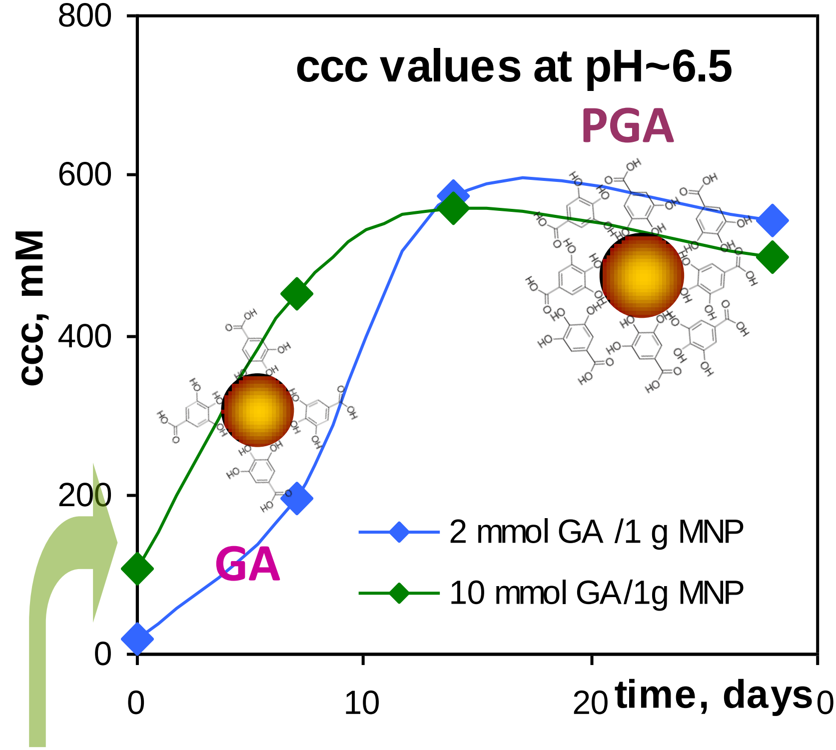
http://magneticmicrosphere.com/index.php?potm_date=11.2010

Well coated superparamagnetic nanoparticles make beautiful ferrofluids (right), while less stable ones (left) agglomerate in high salt concentrations (e.g., blood!) under the influence of an applied magnetic field. This movie is from Prof Etelka Tombacz at the University of Szeged in Hungary (2010).

Resistance against electrolytes
⇒ critical coagulation electrolyte concentration (ccc)

Stability plot: log w vs. log c





Physiological salt concentration **150 mM**

Enhanced stabilization due to thick, overcharged protective layers

A.Hajdú, M.Szekeres, I.Y.Tóth, R.A.Bauer, J.Mihály, I.Zupkó, E.Tombácz, *Colloids and Surfaces B: Biointerfaces* 94 (2012) 242.
I.Y.Tóth, E.Illés, R.A. Bauer, D.Nesztor, I.Zupkó, M.Szekeres, E.Tombácz:, *Langmuir*, 28(2012), 16638–16646..

I.Y.Tóth , M.Szekeres, R.Turcu, S.Sáringer, E.Illés, D.Nesztor, E.Tombácz, *Langmuir* 30 (2014) 15451-15461:

Resistance against electrolytes
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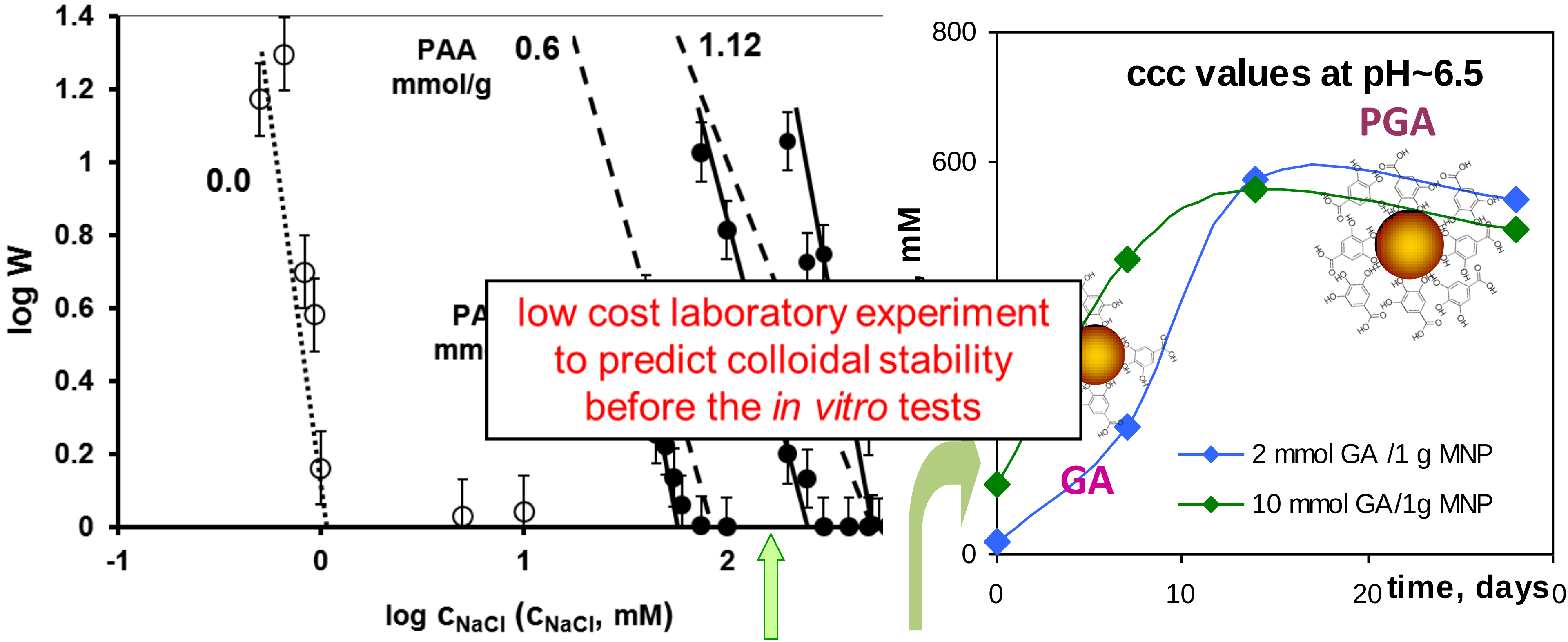

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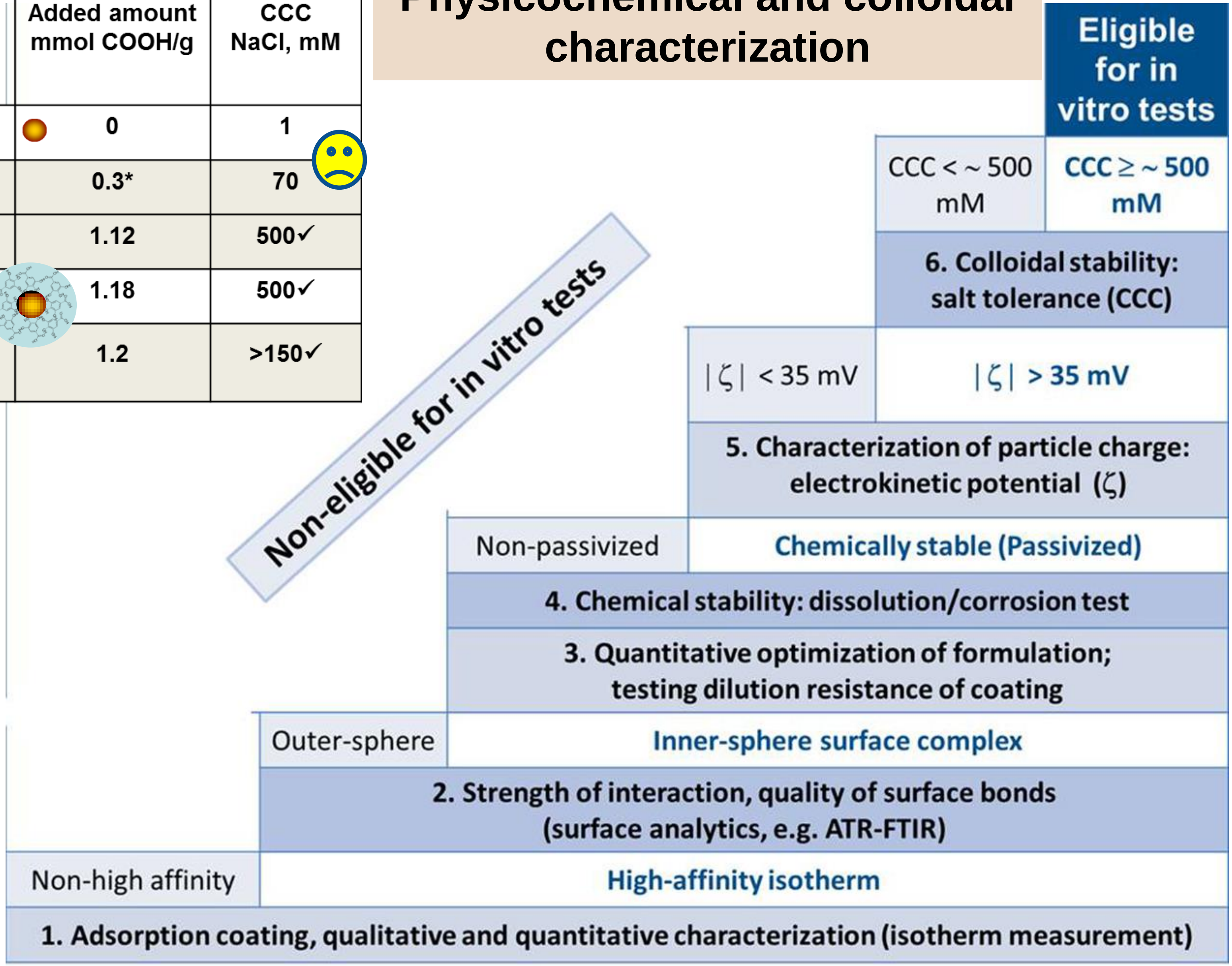
I.Y.Tóth, E.Illés, R.A. Bauer, D.Nesztor, I.Zupkó, M.Szekeres, E.Tombácz:, *Langmuir*, 28(48), 16638
.

I.Y.Tóth , M.Szekeres, R.Turcu, S.Sáringer, E.Illés, D.Nesztor, E.Tombácz, *Langmuir* 30 (2014) 15451-15461:

Physicochemical and colloidal characterization

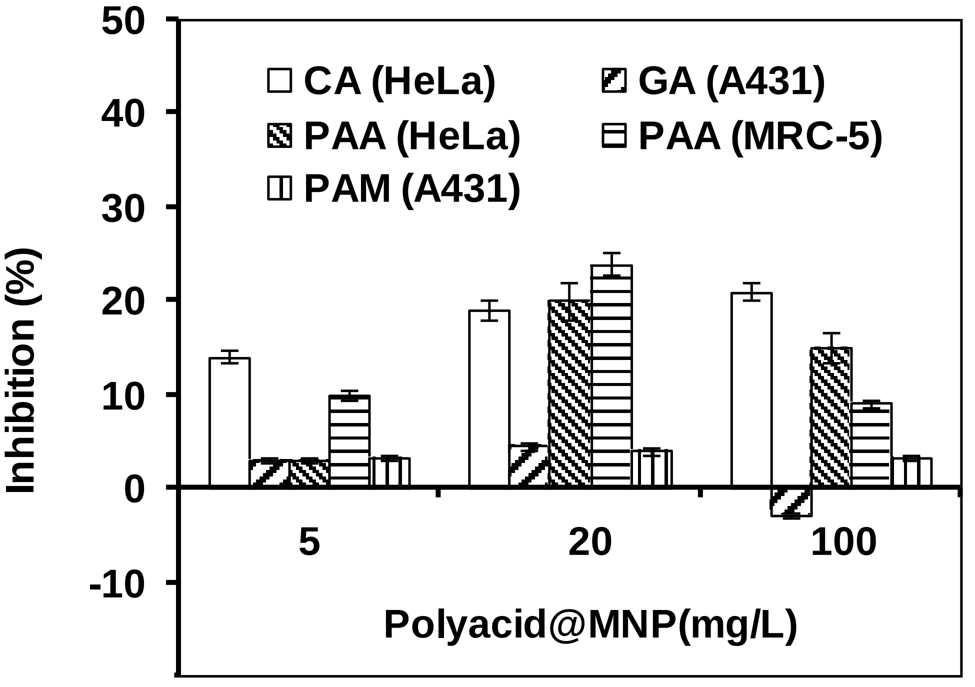
Polyacids@MNP	Added amount mmol COOH/g	Approx. CCC NaCl, mM
Naked MNP	0	1
CA@MNP	0.3*	70
PAA@MNP	1.12	500✓
PAM@MNP	1.18	500✓
PEGMA-AA@MNP	1.2	>150✓



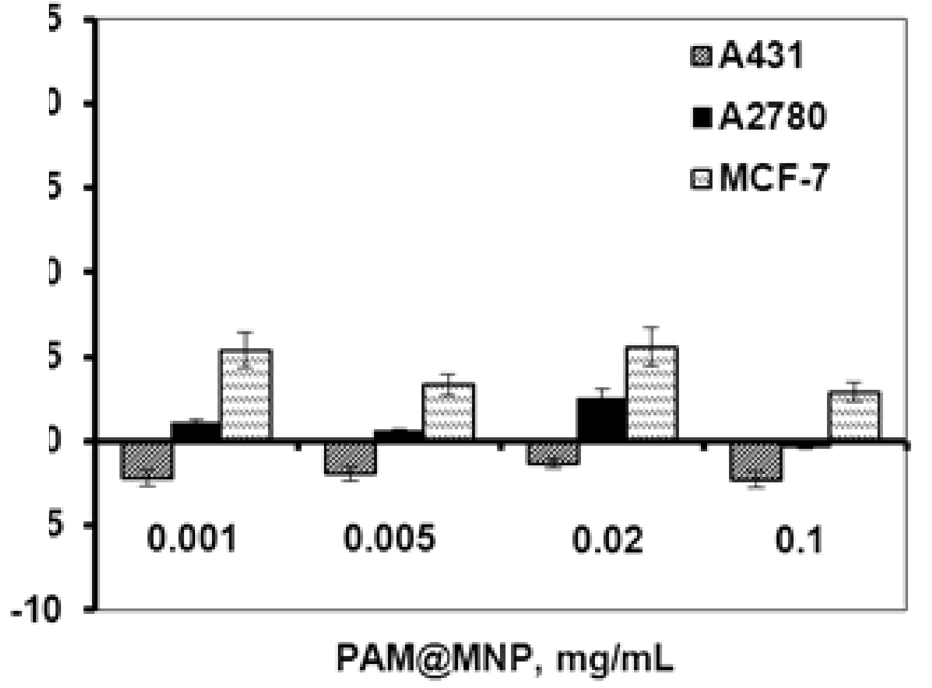
M.Szekeres, I.Y.Tóth, E.Illés, A.Hajdú, I.Zupkó, K.Farkas, G.Oszlánczi, L.Tiszlavicz, E.Tombácz, „**Magnetic Nanoparticles 2013**" *International Journal of Molecular Sciences*, 14, 14550-14574. 2013.

Biocompatibility of polyacid coated MNPs

Cell viability experiments (MTT assays)



Cell inhibition of the CA, GA, PAA and PAM-coated MNPs, added in identical concentrations to human cell cultures HeLa, MRC-5 and A431.



Cell inhibition of the PAM-coated MNPs, added in identical concentrations to human cell cultures MRF-7, A2780 and A431.

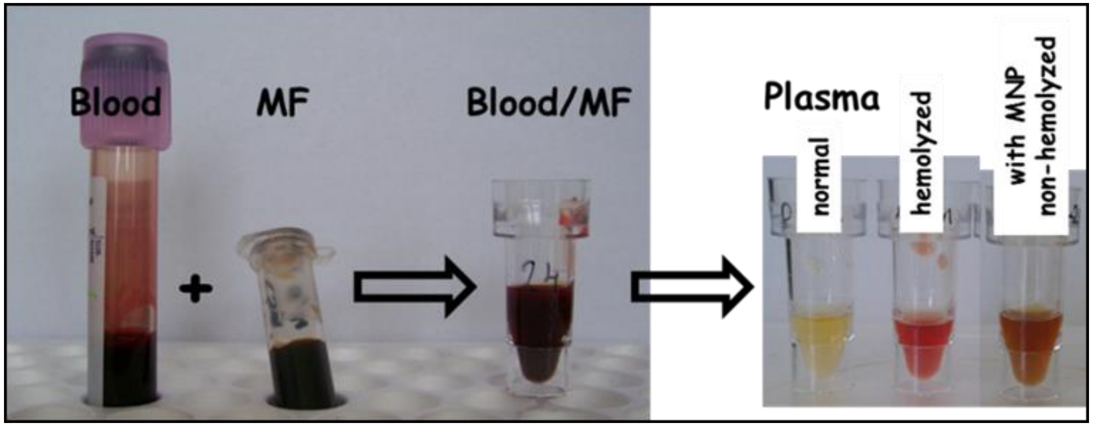
E. Tombácz et al. Colloidal stability of carboxylated iron oxide nanomagnets for biomedical use, *PPCE*, 2014, 58, 3-10.

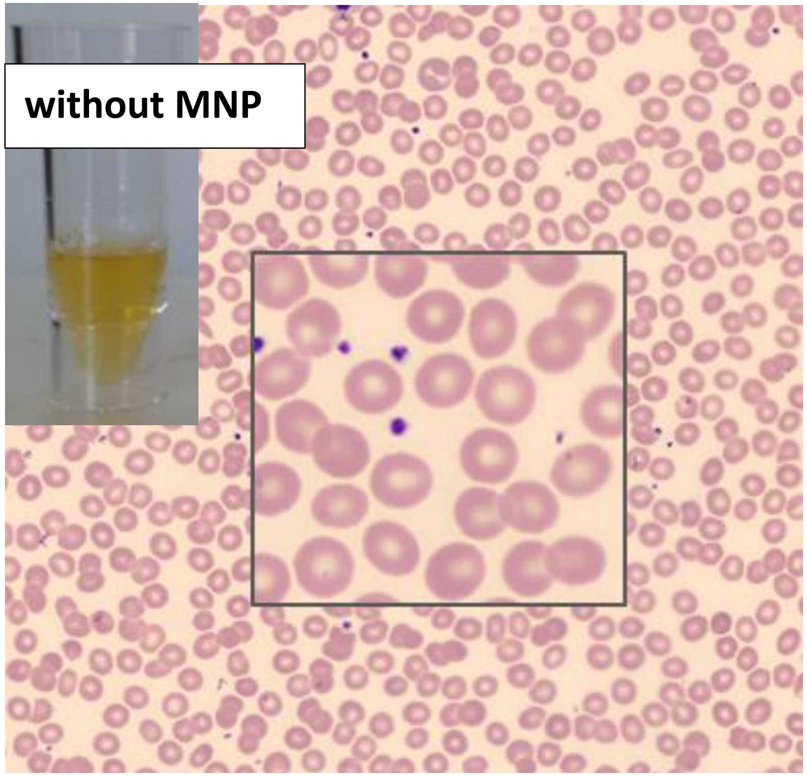
I.Y. Tóth, E. Illés, R. A. Bauer, D. Nesztor, M. Szekeres, I. Zupkó, E.Tombácz: Designed Polyelectrolyte Shell on Magnetite Nanocore for Dilution-Resistant Biocompatible Magnetic Fluids, *Langmuir*, 2012, 28 (48), 16638–16646.

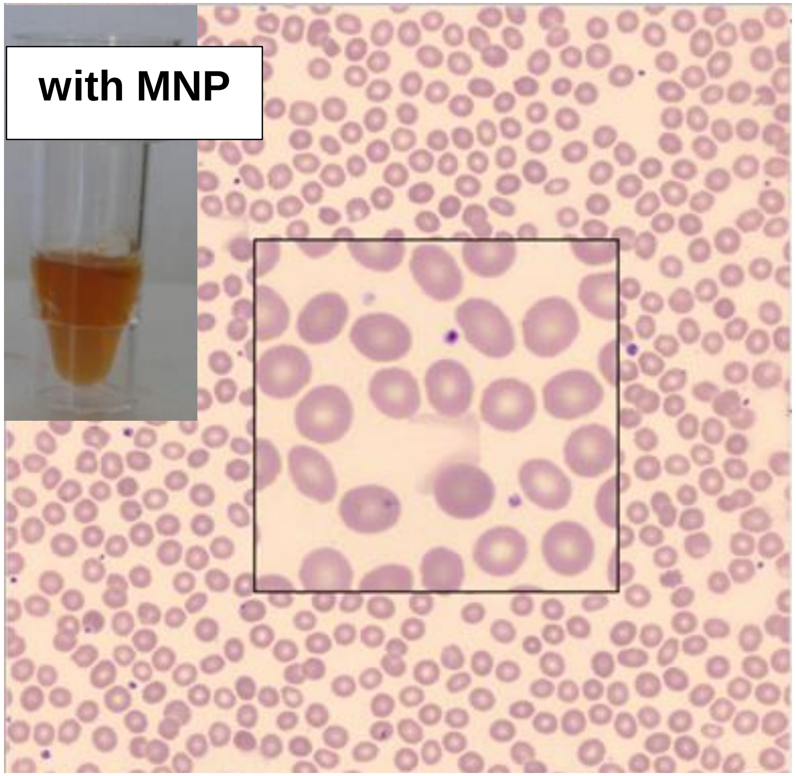
Hemocompatibility tests for P(PEGMA-AA)@MNP

Peripheral blood smears

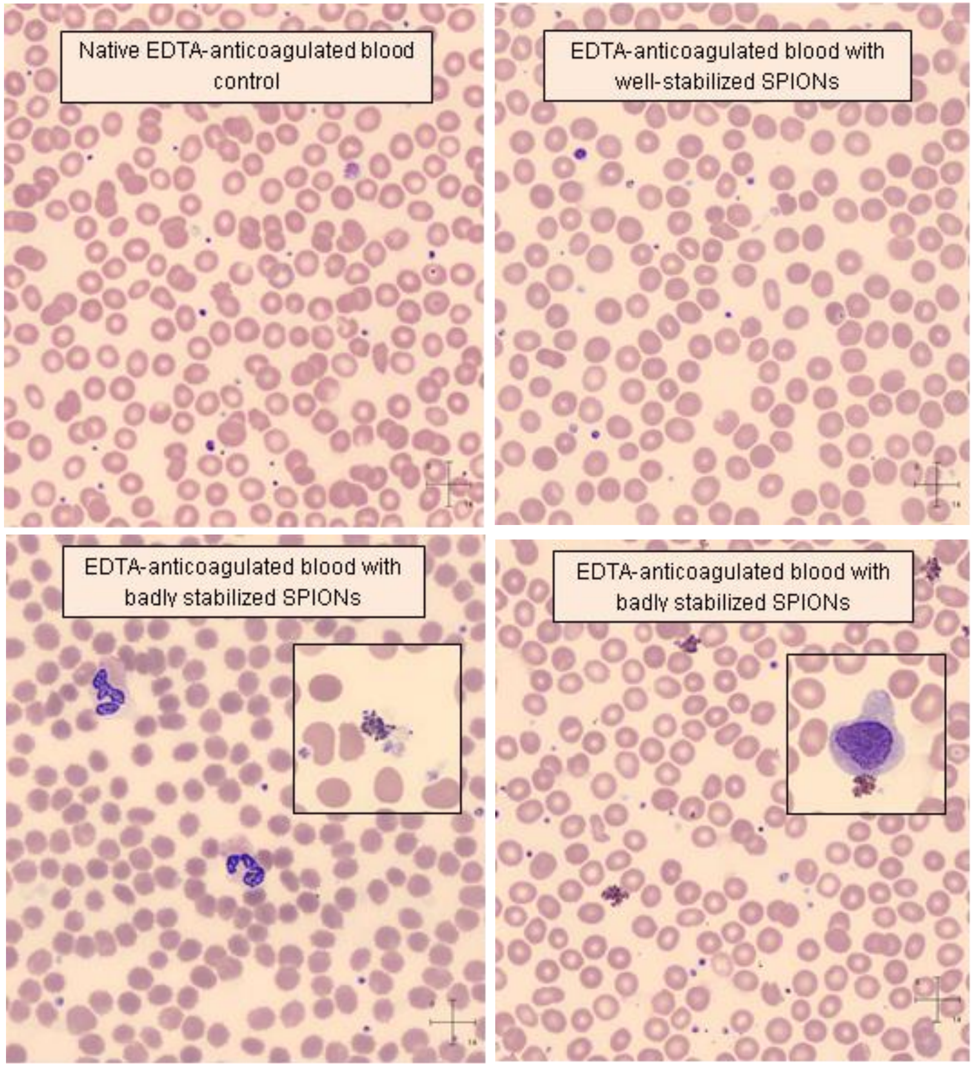
- no hemolytic activity
- no sign for thrombocyte aggregation



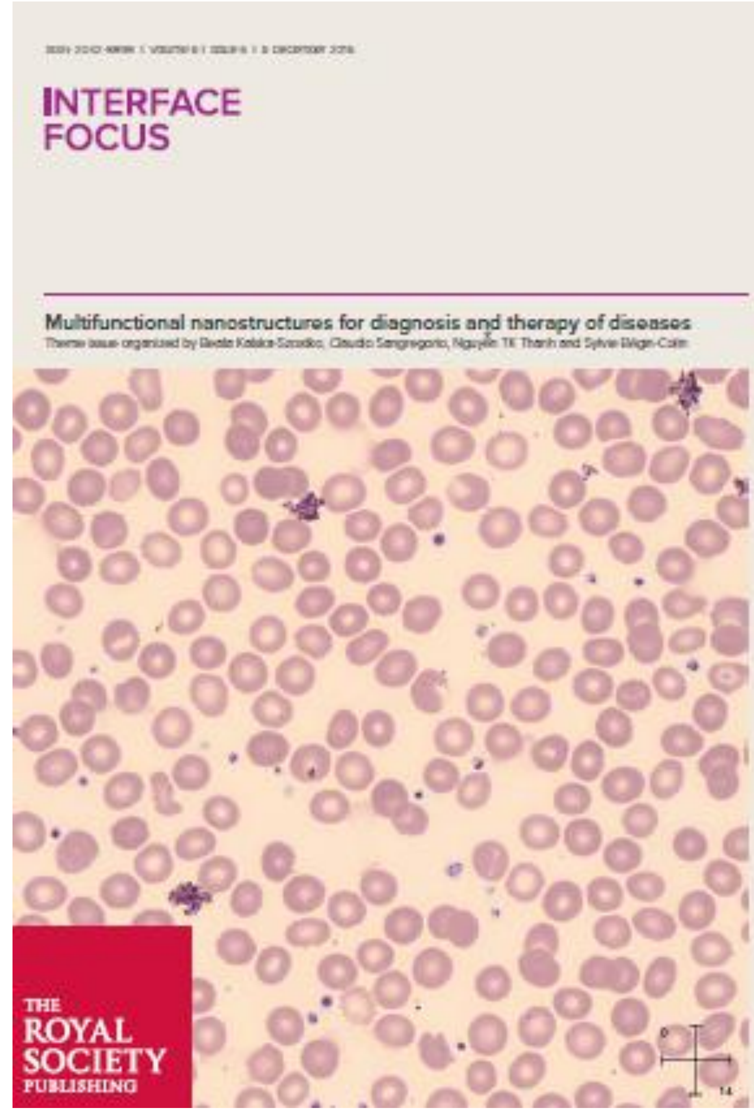




E. Illés, E. Tombácz, M. Szekeres, I.Y. Tóth, Á. Szabó, B. Iván: Novel carboxylated PEG-coating on magnetite nanoparticles designed for biomedical applications, *Journal of Magnetism and Magnetic Materials*, 380: 132-139 (2015)

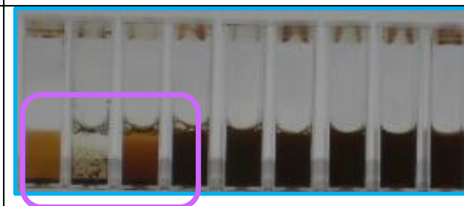
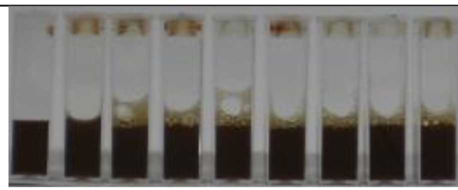
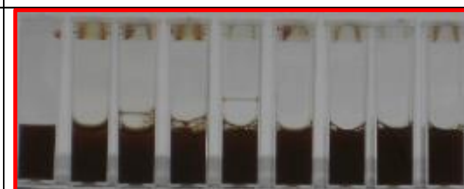


Tombácz E, Farkas K, Földesi I, Szekeres M, Illés E, Tóth IY, Nesztor D, Szabo T. (2016) Polyelectrolyte coating on superparamagnetic iron oxide nanoparticles as interface between magnetic core and biorelevant media, *Interface Focus* 6: 20160068. doi:10.1098/rsfs.2016.0068



Cover image
Haemocompatibility test (blood smear) of superparamagnetic iron oxide nanoparticles (SPIONs). (Image courtesy of Katalin Farkas and Imre Földesi (Department of Laboratory Medicine, University of Szeged).)

Interaction of carboxylated SPIONs with human plasma

	>>> human plasma in increasing concentration >>>	
mixing with	**after 2 hours**	**after 21 hours**
CA@MNP		
PAM@MNP		
PEGMA-AA@MNP		

citrated SPIONs aggregation

The top PEG layer can not hinder interactions with proteins.

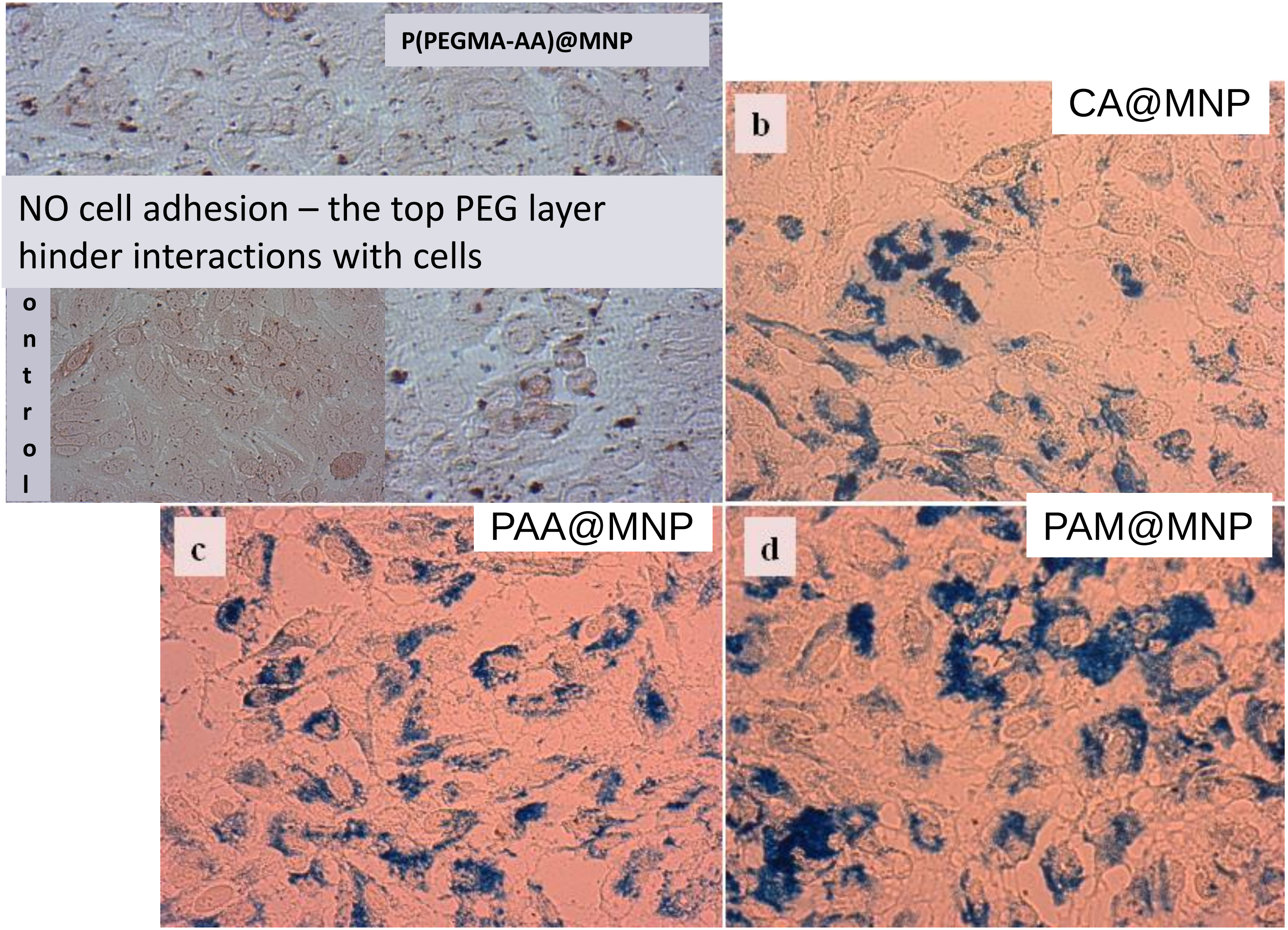
no difference between carboxylated and PEG-carboxylated SPIONs

Materials	Protein adsorption	Cell adhesion
Hydrophilic materials		
PEG-based materials		
PS-g-PEGMA and PMMA-g-PEGMA [92]	Yes	No
PEG-poly(phosphonate) terpolymer [93]	Yes	No
PLL-g-PEG [94,95]	Yes	Yes
PEGMA [96,97]	Yes	No
$PPEG_xLys$ [98]	Yes	No
POEGMA [99–102]	Yes	Yes
PEO-PU-PEO [61,103–105]	Yes	No
PEO-PPO-PEO [106]	Yes	No
PEO [33]	Yes	No

M. Szekeres, I.Y. Tóth, R. Turcu, E.Tombácz, The effect of polycarboxylate shell of magnetite nanoparticles on protein corona formation in blood plasma, *Journal of Magnetism and Magnetic Materials* DOI: 10.1016/j.jmmm.2016.11.017

S. Chen et al. **Surface hydration: Principles and applications toward low-fouling/nonfouling biomaterials,** Polymer 51 (2010) 5283-5293.

Prussian blue staining of HeLa cells (**a**) incubated with 7.35 mg/mL concentration dispersions of CA@MNP (**b**), PAA@MNP (**c**), and PAM@MNP (**d**)



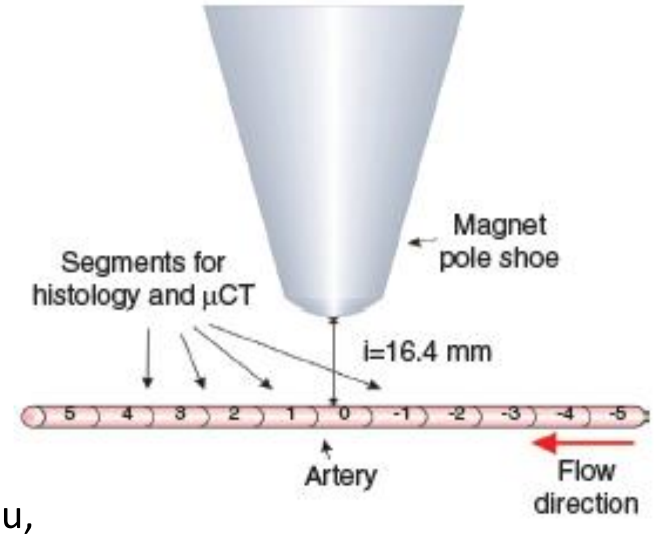
M. Szekeres, I.Y. Tóth, E. Illés, A. Hajdú, I. Zupkó, K. Farkas, G. Oszlánczi, L. Tiszlavicz, E. Tombácz:, *International Journal of Molecular Sciences,* 14, 14550-14574. 2013. ; Illés E. et al. submitted

Grafting proteins to SPIONs via carboxylate anchoring groups

Magnetic targeting local application of drug

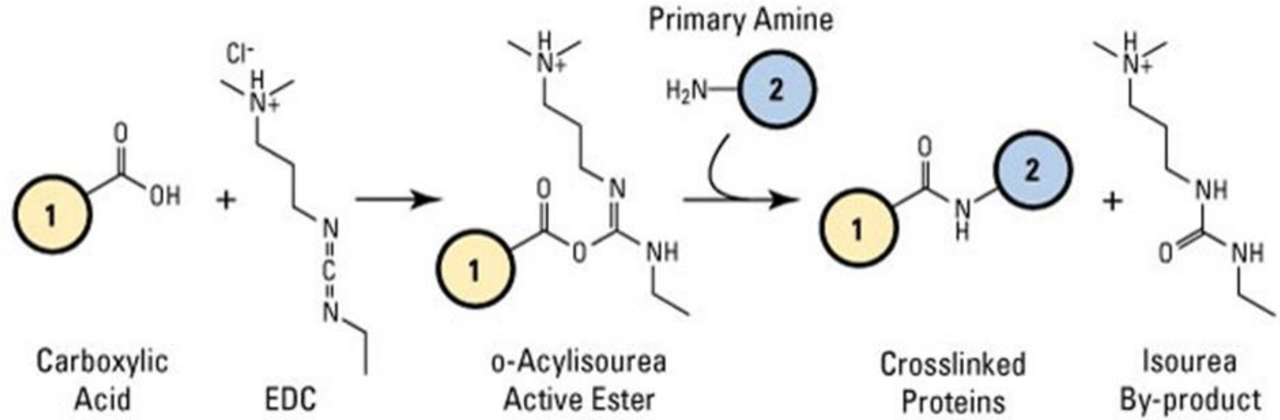
Recombinant tissue plasminogen activator (**tPA**, Actilyse®) was coupled to polyacrylic acid-co-maleic acid coated SPIONs (**PAM@MNP**) using an amino-reactive activated ester reaction (**EDC/NHS** – carbodiimide/N-hydroxysuccinimide **activator**).
Covalent linkage significantly **improves the reactivity and long term stability** of the conjugated **SPION-tPA system** compared to simple adsorption.

RP Friedrich, J Zaloga, E Schreiber, IY Tóth, E Tombácz, S Lyer, C Alexiou, *Nanoscale Research Letters* (2016) 11:297



in SEON group of **Prof. Christoph Alexiou** (University Hospital Erlangen, Section for Experimental Oncology and Nanomedicine, Erlangen, Germany)

Reaction scheme



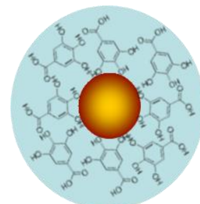
Carbodiimide Crosslinker Chemistry, ThermoFisher, https://www.thermofisher.com/

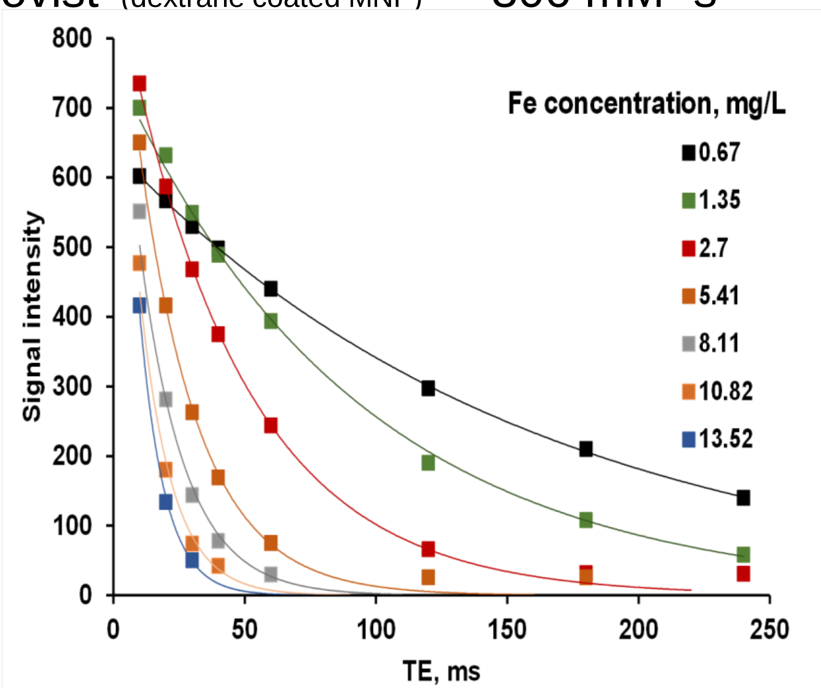
Potential for theranostic application

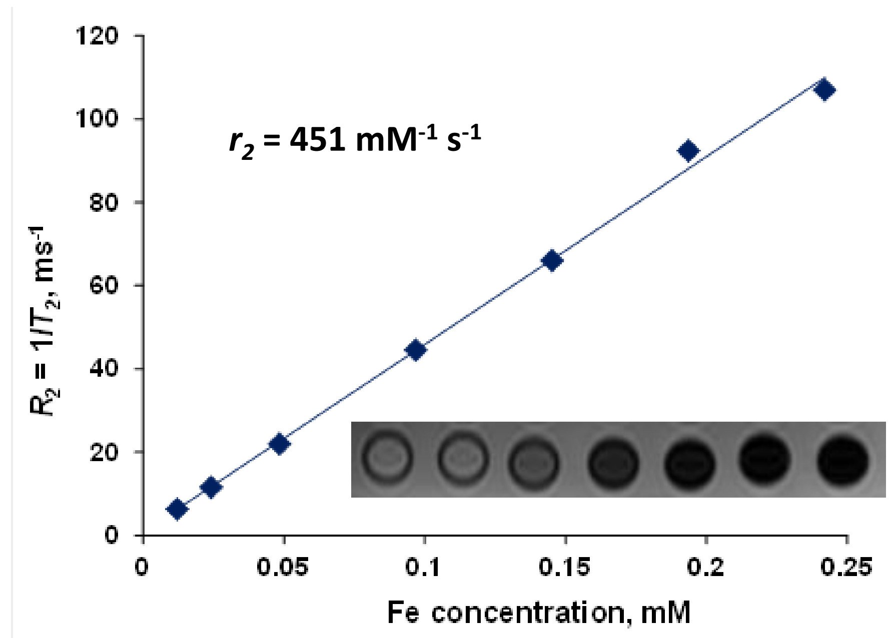
GE Excite HD (1,5 T, EUROMEDIC DIAGNOSTICS SZEGED KFT.)

Magnetic Resonance Imaging (MRI) – venous admin

Coated SPIONs	r_2 relaxivities
CA@MNP	156 $mM^{-1}s^{-1}$
PAA@MNP	**396** $mM^{-1}s^{-1}$
PAM@MNP	**440** $mM^{-1}s^{-1}$
PEGMA-AA@MNP	**451** $mM^{-1}s^{-1}$
Resovist (dextrane coated MNP)	306 $mM^{-1}s^{-1}$







Jedlovszky-Hajdú, E. Tombácz, I. Bányai, M. Babos, A. Palkó, *Journal of Magnetism and Magnetic Materials*, 2012, 324, 3173-3180;
Szekeres M, Illés E, Janko C, Farkas K, Tóth IY, Nesztor D, Zupkó I, Földesi I, Alexiou C, Tombácz E, *J Nanomed Nanotechnol.* 2015, 6: 252.;
E. Illés, E. Tombácz, M. Szekeres, I.Y. Tóth, Á. Szabó, B. Iván, *Journal of Magnetism and Magnetic Materials*, 380: 132-139 (2015)

Potential for theranostic application

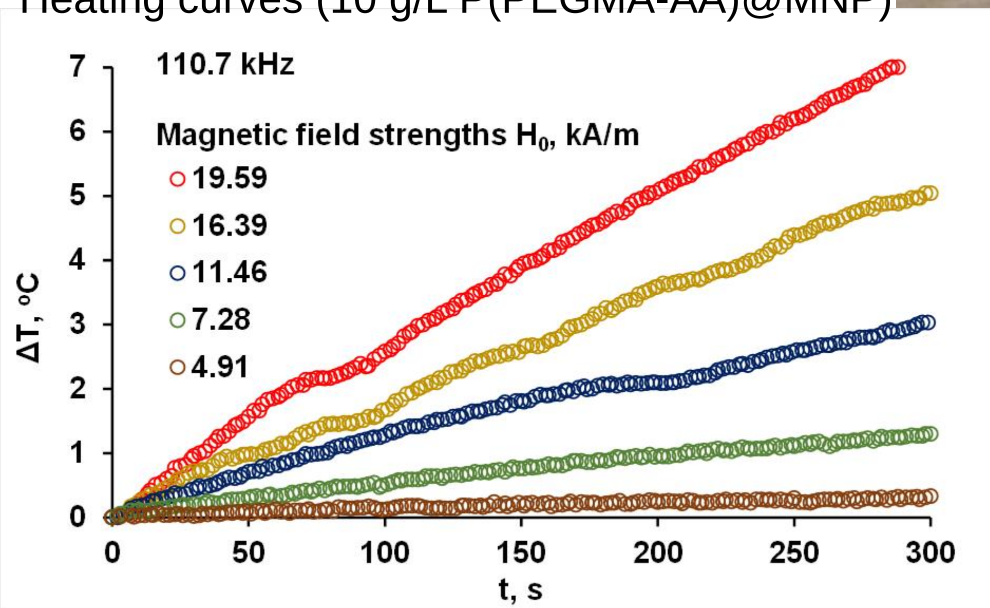
Magnetic hyperthermia

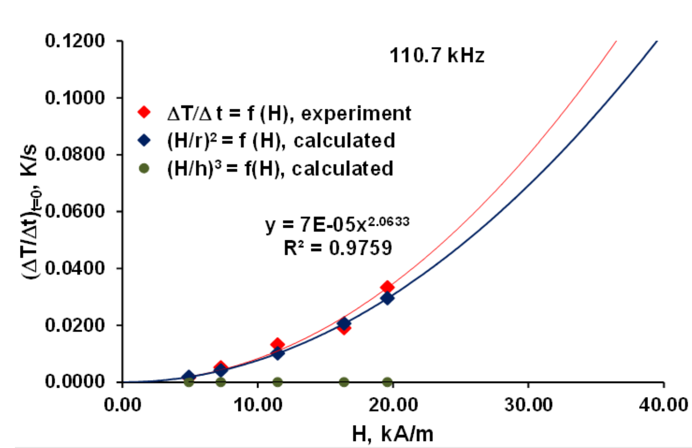
AC magnetic field applied
– heat release measured

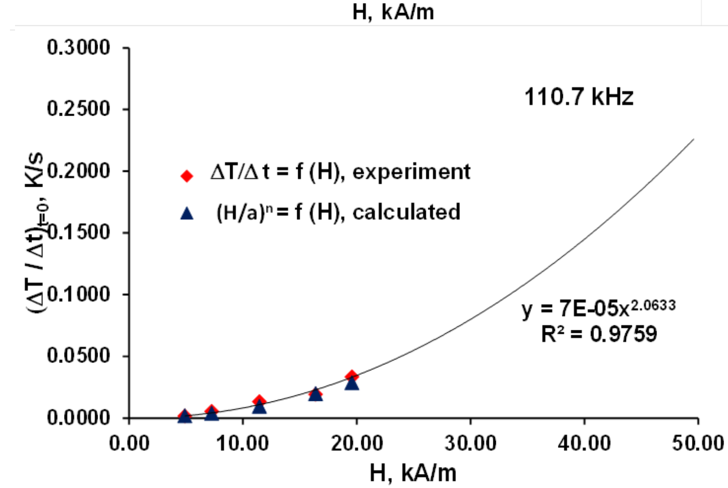
RADIOMAG (COST TD1402)

http://www.cost-radiomag.eu/

Heating curves (10 g/L P(PEGMA-AA)@MNP)







SAR (specific absorption rate) **-** no significant effect of coating on SAR

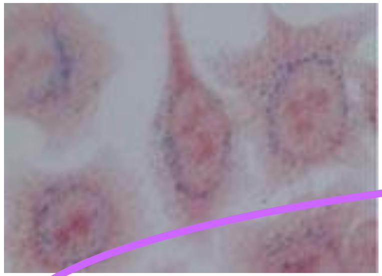
14.2 - 2.2 W/g at 110.7 kHz (H_0=19.6-4.9 kA/m)

17.4 - 8.72 W/g at 329 kHz (H_0=13.1-5.5 kA/m)

Summary

~~CA~~
PAA
PAM
PEGMA-*co*-AA

The optimization of carboxylated/PEGylated coating on SPION cores results in chemically and colloidally stable products with hydrophilic, negatively charged, non-fouling surface and anchoring sites for biofunctions.



Teranostics - prospects

HeLa

MRI

r2 relaxivities	$mM^{-1}s^{-1}$
PAA@MNP	396
PAM@MNP	440
PEGMA-AA@MNP	451
Resovist	306

Hyperthermia

SAR: ~14-17 W/g
(110 - 330 kHz)

Magnetic targeting

proteins
chemotherapeutics

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Thanks

my colleagues

Márta Szekeres,
Erzsébet Illés,
Ildikó Tóth,
Daniel Nesztor and
Tamás Szabó
for the excellent work, and

L. Vekas Magnetic Fluid Laboratory,Romanian Academy-Timisoara, Romania
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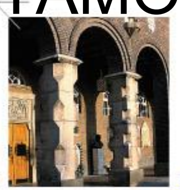
I. Zupkó Department of Pharmacodynamics and Biopharmacy, (USz) Szeged, Hungary
C. Alexiou Section of Experimental Oncology and Nanomedicine, Erlangen, Germany
for collaboration

and grant OTKA (NK 84014)
TÁMOP-4.2.2.A-11/1/KONV-2012-0047
for financial support,



and you for kind attention!

Búcsúzik a Vizes Kolloidok Kutatócsoport

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