Molecular Imprinting of Bovine Serum Albumin on Polypyrrolidone Magnetic Microparticles for Selective Recognition of Proteins

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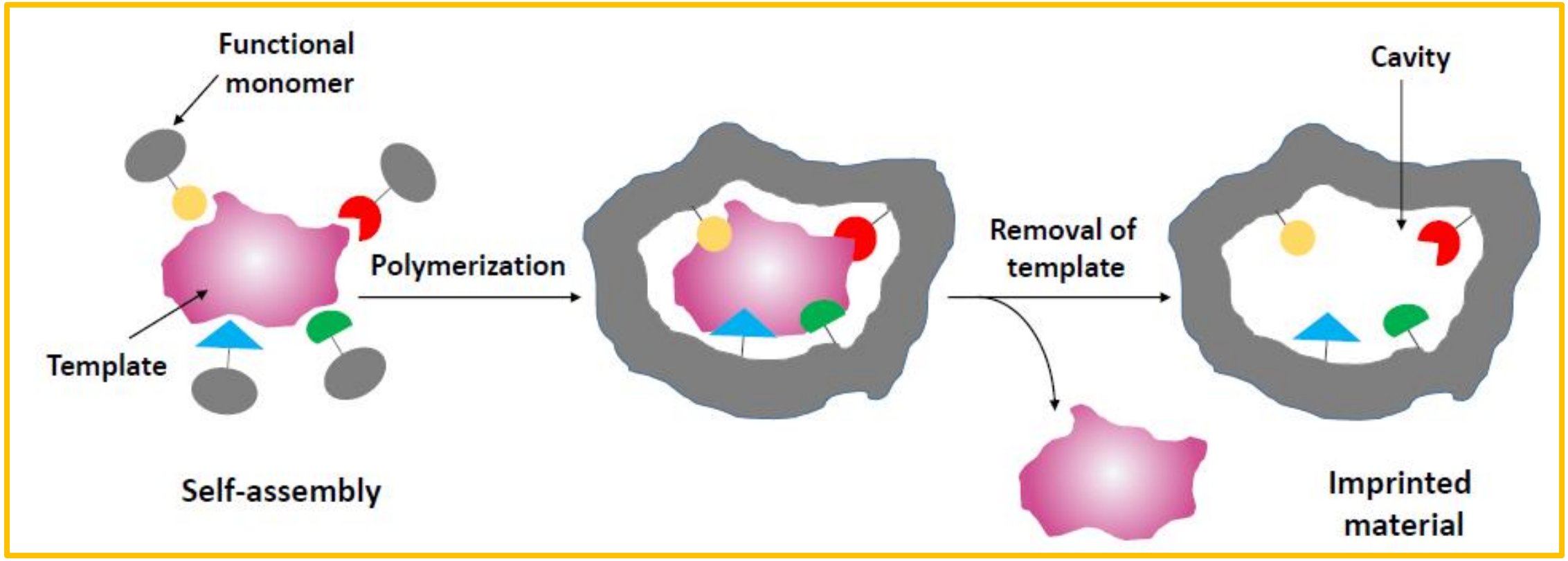
Outline

1. Molecular Imprinting – definition, components, trends
2. Microencapsulation by anionic polymerization of lactams
3. Microencapsulation + Molecular Imprinting of BSA
4. Results and Discussion
5. Conclusions

Molecular Imprinting Technology (MIT)

MIT - making **molecular locks** to match specific **molecular keys**, i.e., construction of specific recognitions sites in synthetic polymers.

MIT employs **templates, functional monomers, polymerization processes**, and methods for **selective elution** of the template.



Self assembly interactions: covalent, electrostatic/ionic, non-covalent, semi-covalent and coordinative

Template types in MIT:

Atoms, ions, molecules, macromolecules or ensembles of them, including microorganisms.

Main advantages of MIT:

Structure predictability, recognition specificity and application versatility.

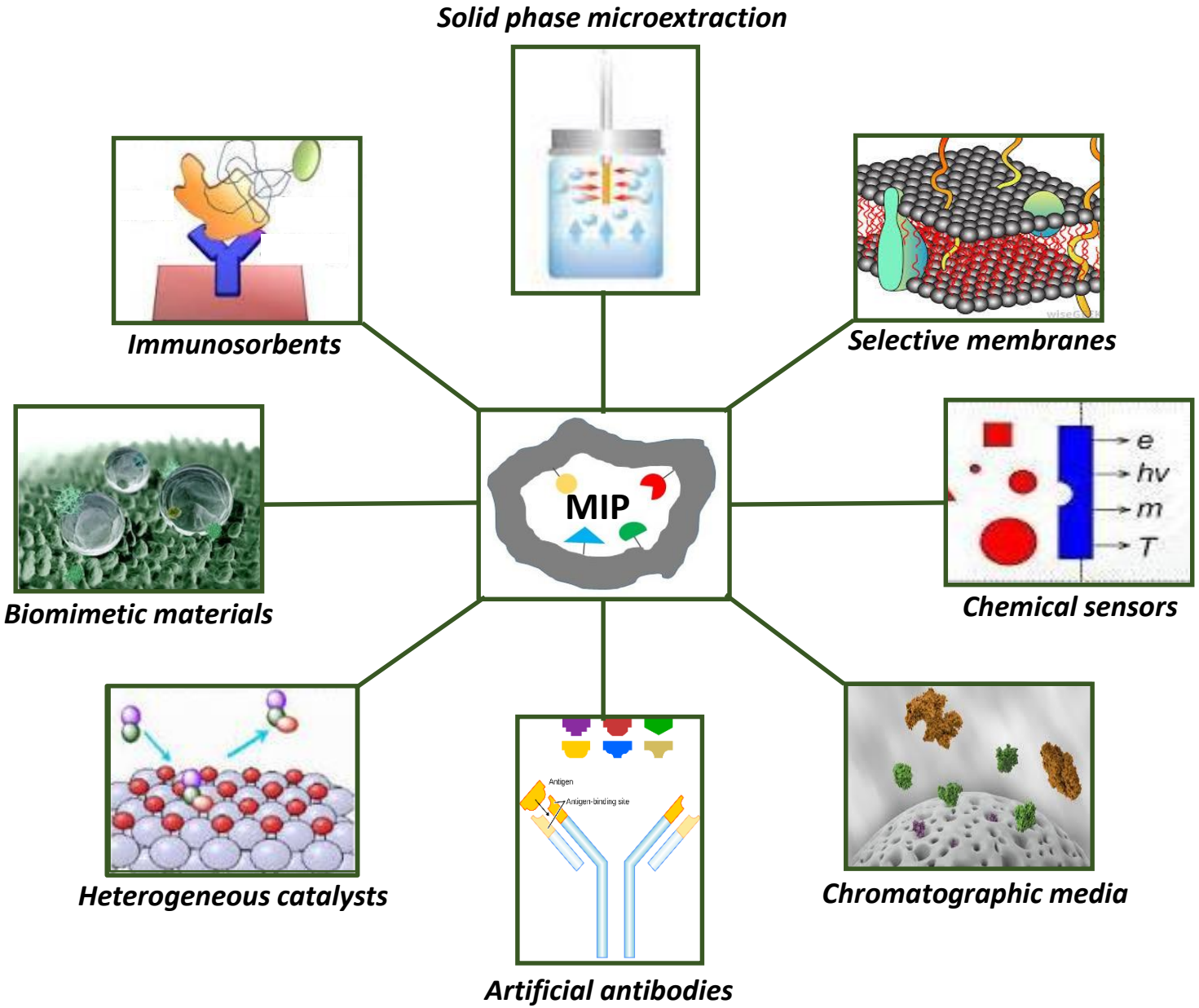
Main limitations of MIT:

Functional monomers - mostly vinyl or acrylics;
Polymerizations - mostly free radical with thermal/photochemical initiation.

Future Trends for MIT:

Use of new monomers and polymerization techniques;
Combination of MIT with other technologies;
Smart, stimuli-responsive molecularly imprinted polymers (MIP);
MIP with protein or enzyme templates.

Molecularly Imprinted Polymers (MIP) -- Applications



Main ideas of this work

1. Introducing a new group or functional monomers in MIT – lactams;

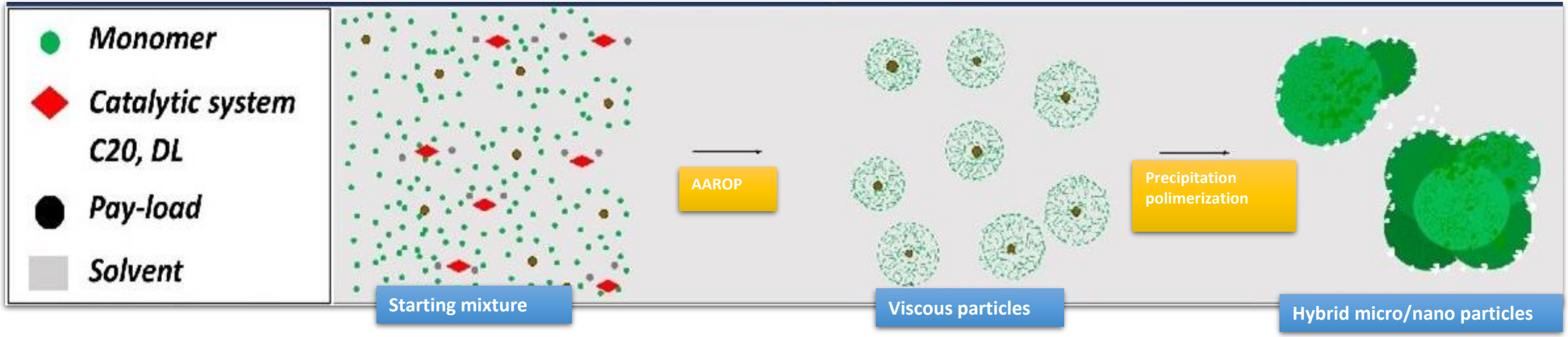
2. Introducing of activated anionic polymerization of lactams in MIT;

3. Combination of microencapsulation technology with MIT;

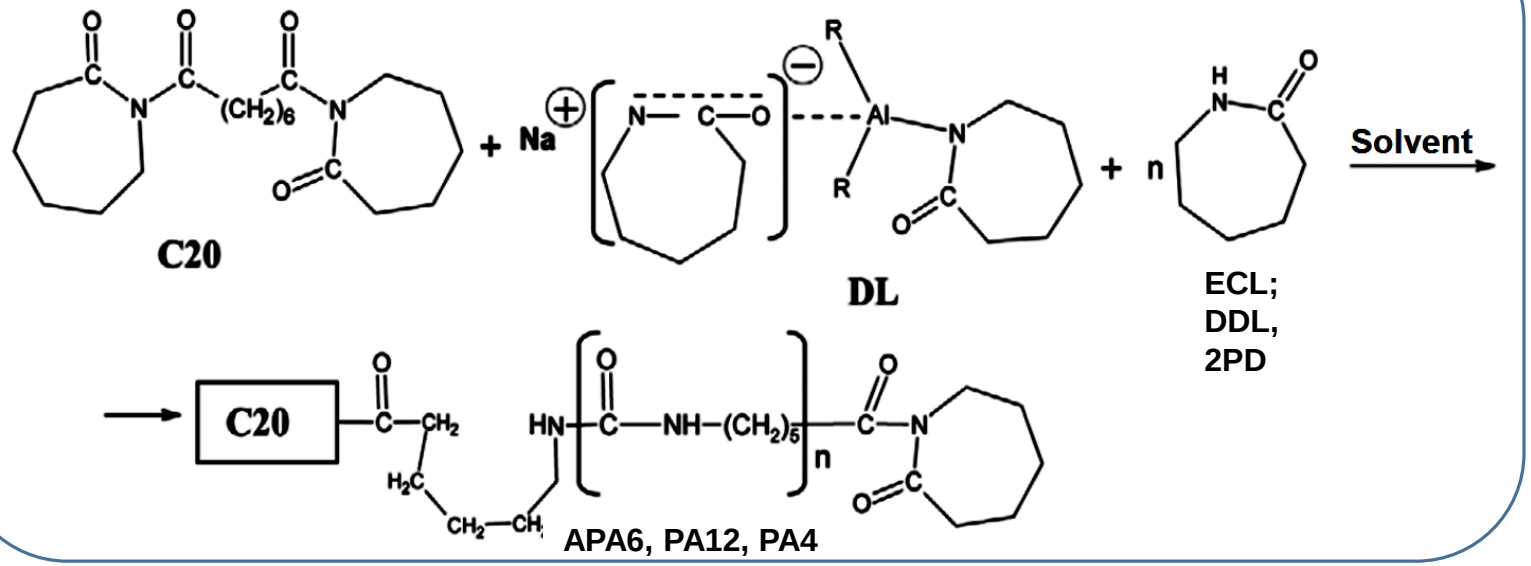
4. Molecular imprinting of model protein template;

5. Synthesis of smart MIP susceptible to external magnetic fields.

Microencapsulation via AAROP of lactams



The AAROP reaction

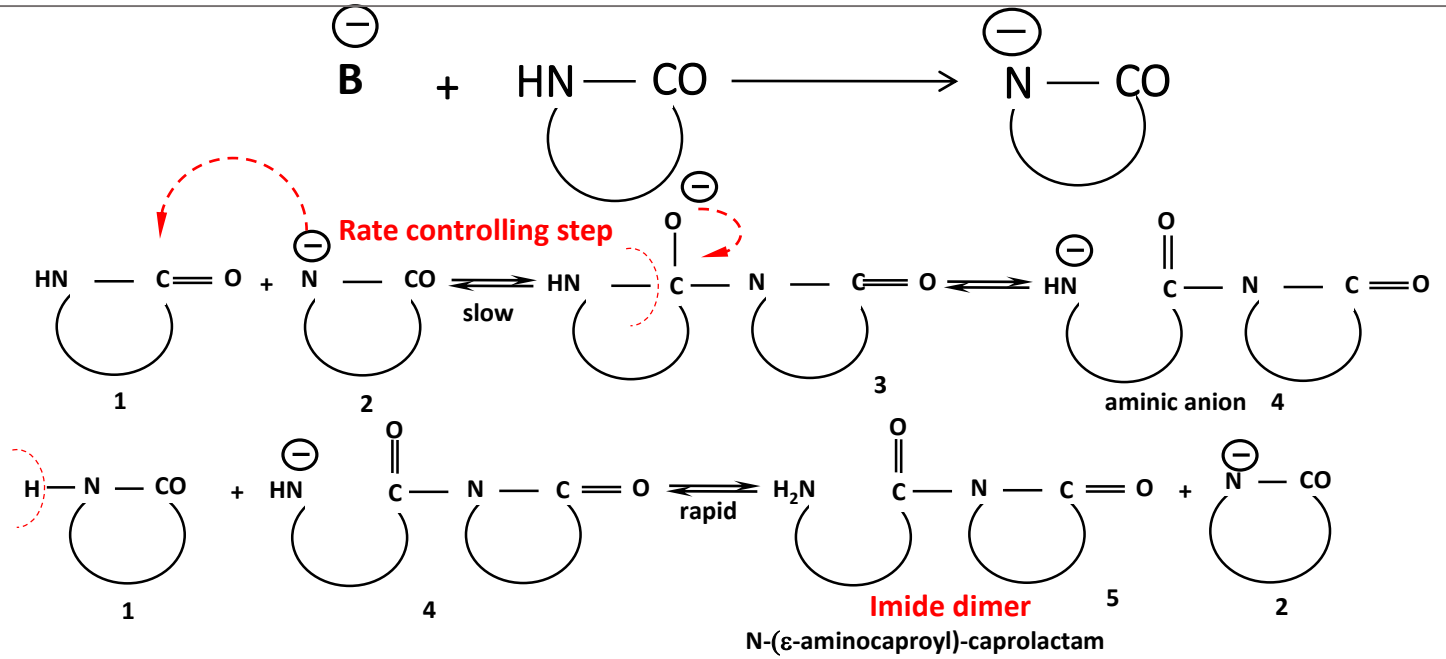


Different payloads neat or mixtures can be used:

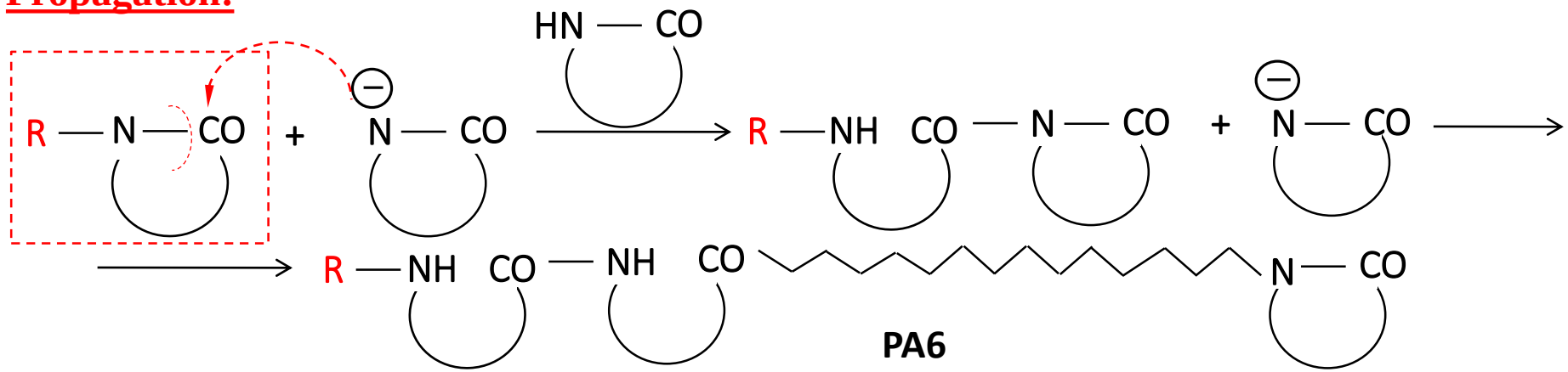
- Natural nanoclays -- CL15A, CL20A
- Carbon allotropes – CB, CNT, CNF, fullerenes
- Metals – Cu, Al, Mg, Ag, Zn, Ag
- Magnetic particles – Fe, Fe_3O_4

AROP of Lactams - mechanism

Initiation:



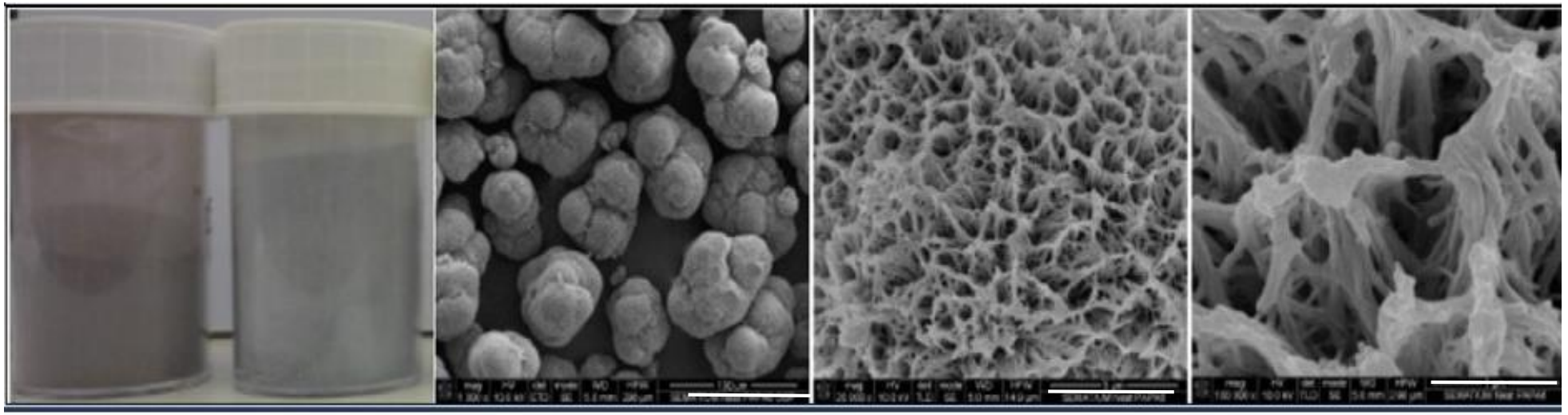
Propagation:

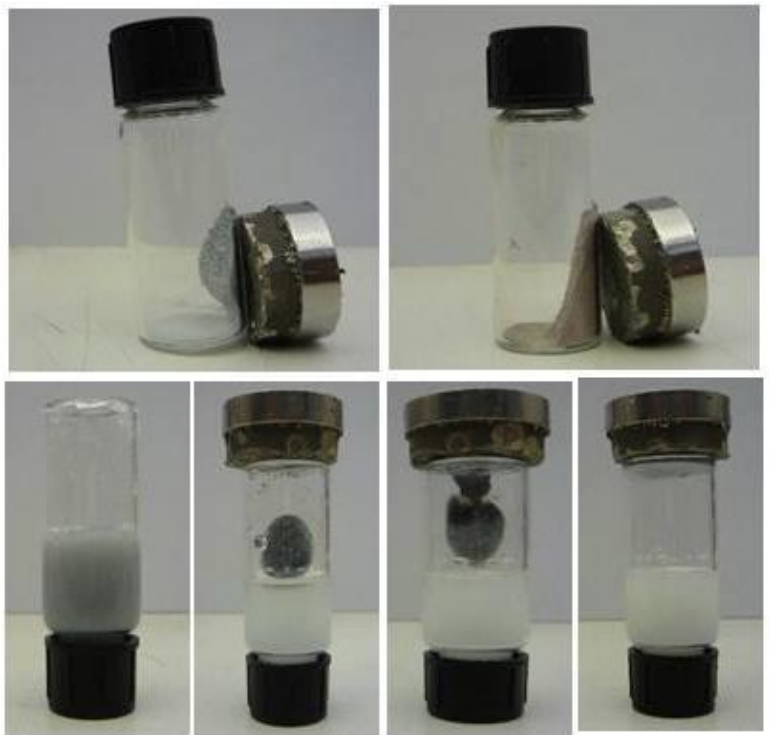


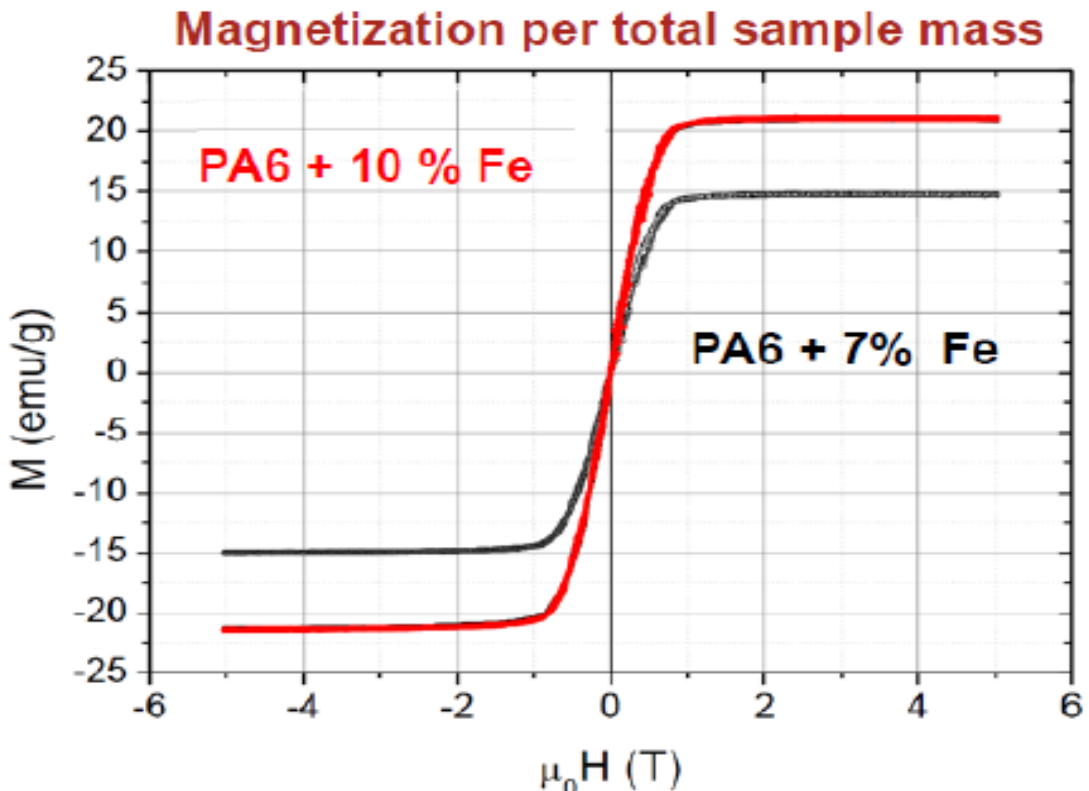
AAP Catalytic system used:

Initiator: DILACTAMATE® ; Activator: BRUGGOLEN® C 20

Morphology and magnetic properties of Microcapsules

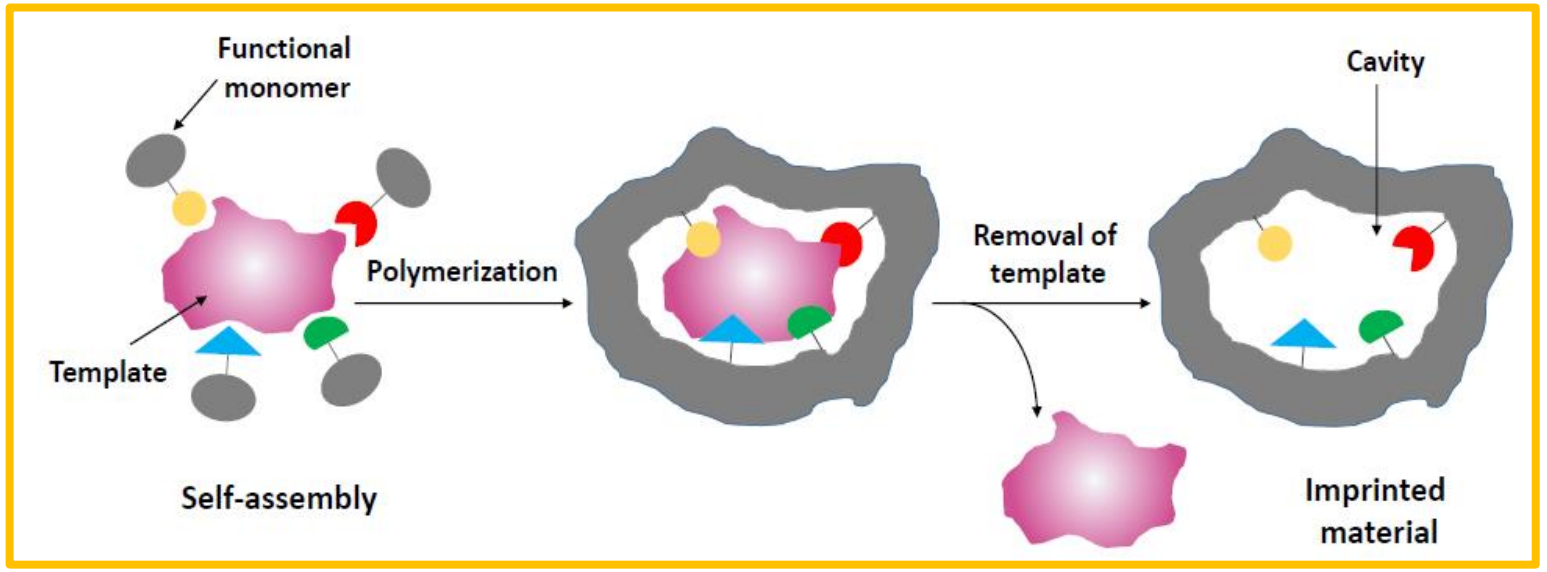






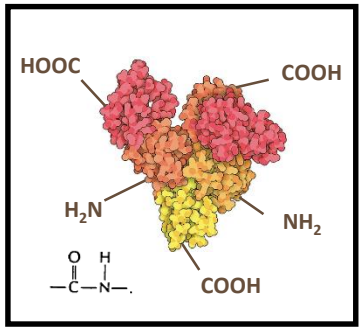
Molecularly Imprinted Polymers - MIP

Schematic representation of Non-covalent Molecular Imprinting

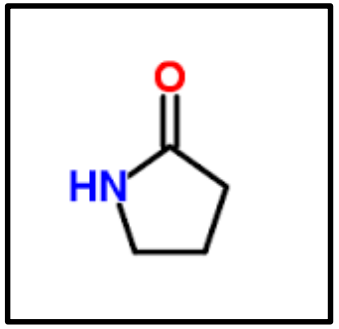


Materials used and processing

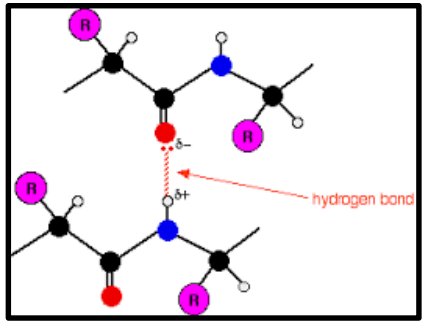
Template:
Bovine Serum Albumin (BSA)



Functional monomer:
2-pyrrolidone (PD)



PD:BSA Self-assembly
by **hydrogen bonds**:



Polymerization process Conditions:

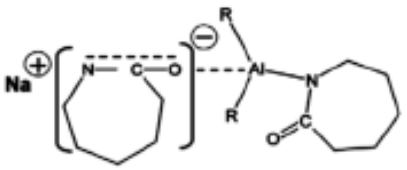
AAROP in the presence of magnetic nanoparticles:
Fe ; Fe3O4 – 1 wt.%
Catalytic system:
DL+C20
40ºC; no solvent.

MIP SAMPLES produced:

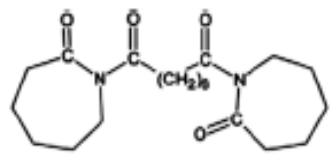
PPD@BSA
PPD-Fe-1%@BSA
PPD-Fe_3O_4- 1%@BSA
PPD-Fe_3O_4 -0.1%@BSA

and corresponding NIP:

PPD
PPD-Fe-1%
PPD-Fe_3O_4 -1%
PPD-Fe_3O_4 -0.1%



DL



C20

BSA structure

BSA

→ composed of **604 peptide units**;

→ molecular weight of **66462 g/mol**

→ consists of 55 – 65% α-helices, 21% β- sheet, and the rest are turns.

→ p*I* 4.6-5

BSA secondary structure highly depends on pH:

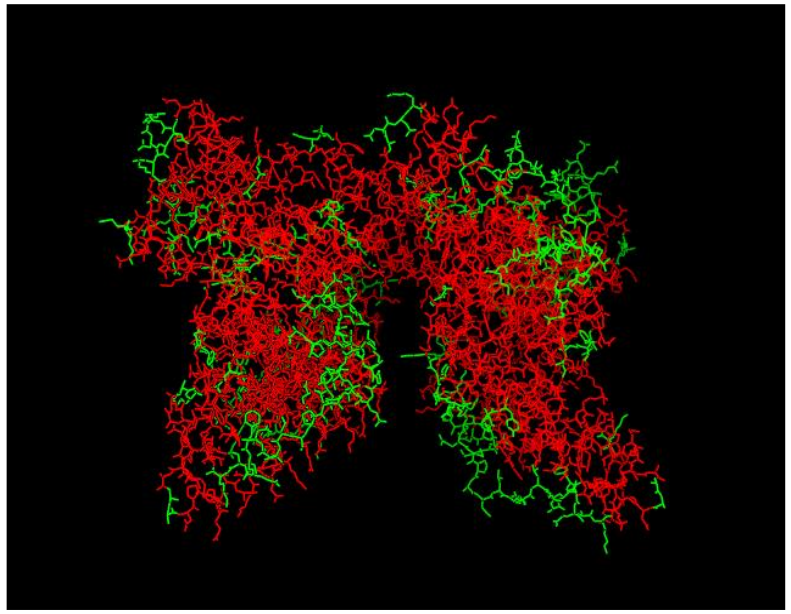
→ pH 4.3 to 8.0, this is the so-called **normal form**.

→ pH below 4.3 --- unfolding starts, **fast form** with 45% α-helix.

→ pH below 2.7 -- further unfolding to the **expanded form** with 35%

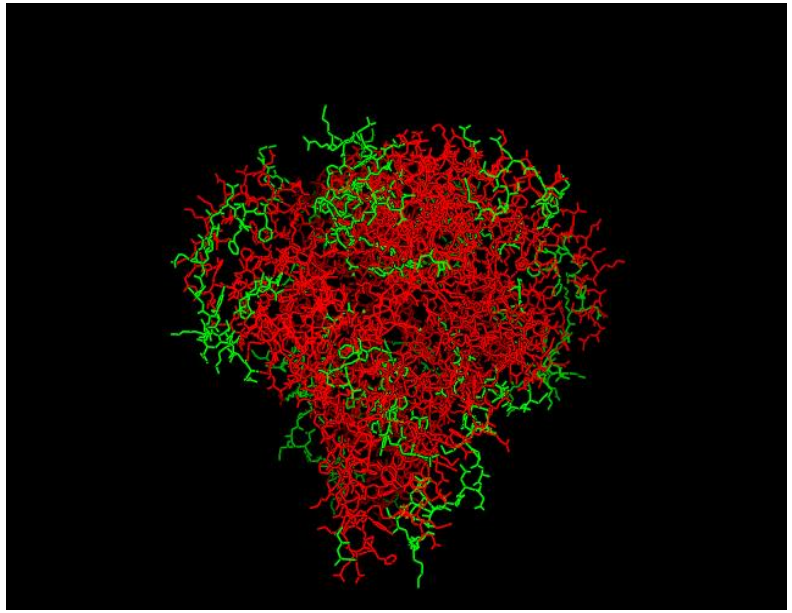
→ pH above 8 -- the molecule adopts the basic form which has 47% α-helix

Three dimension image of BSA molecule (normal conditions).
α-helix structure in red;
Loops colored in green.



Side view

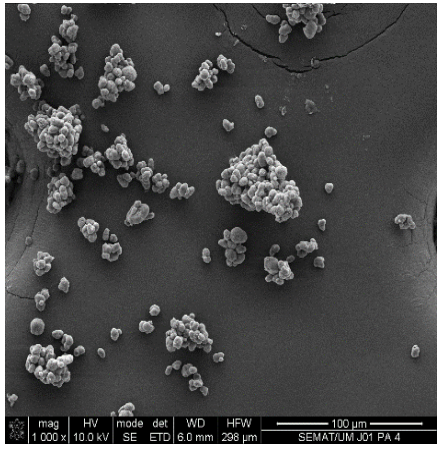
BSA has hearth shaped molecule **– equilateral triangle with side ~8 nm and depth ~3nm**

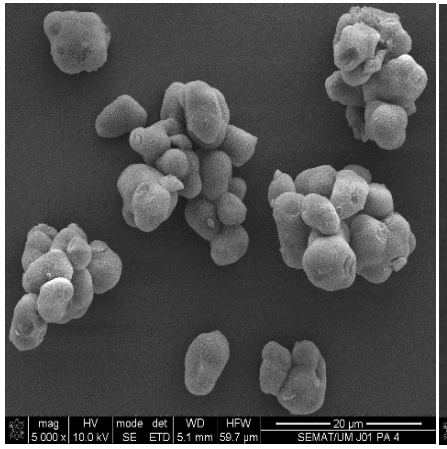


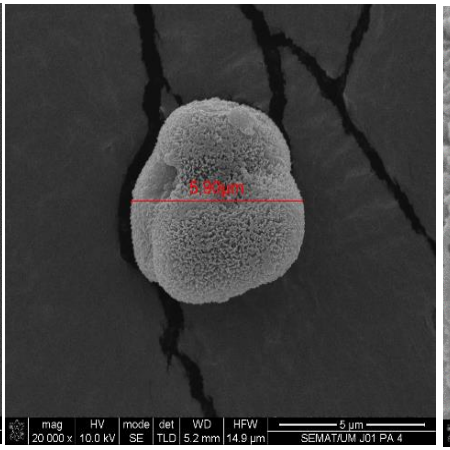
Front view

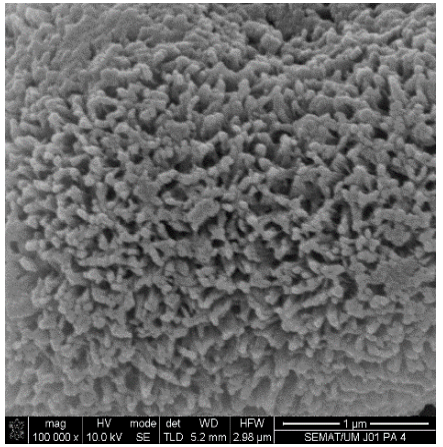
PPD@BSA MIP/NIP SEM characterization

PPD - NIP

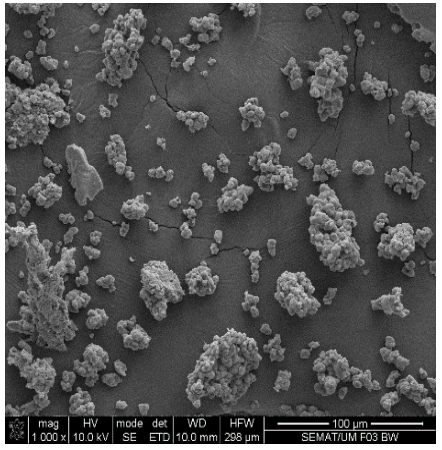


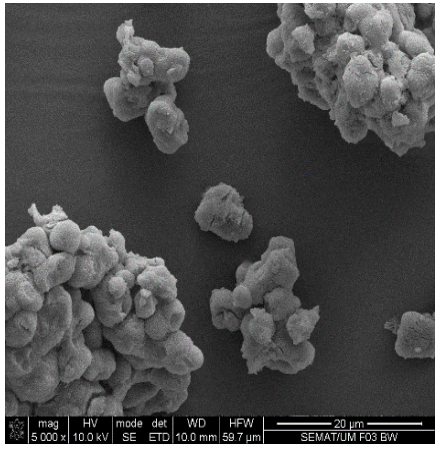


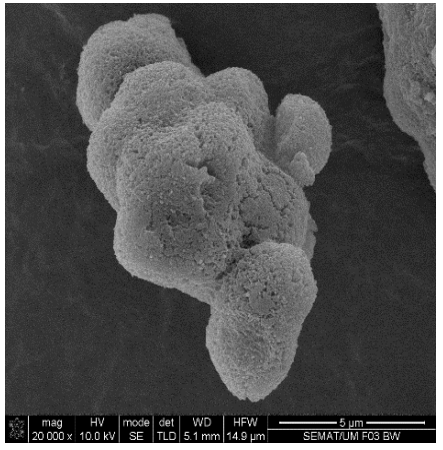


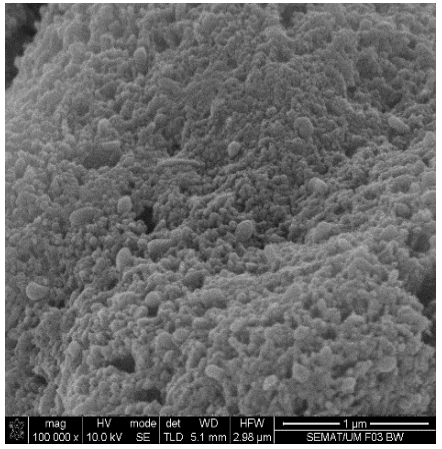


PPD @BSA MIP before removing the BSA template









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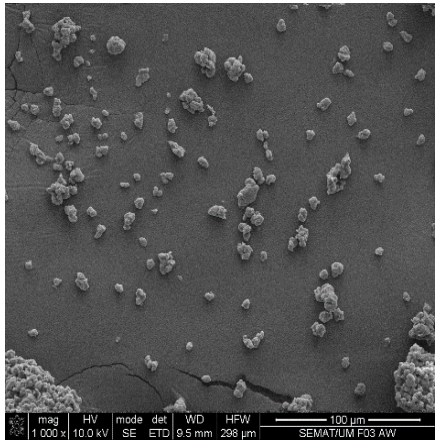
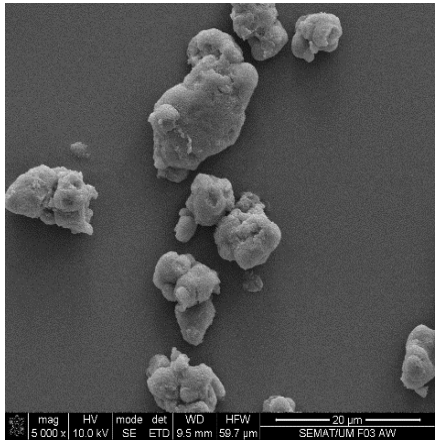
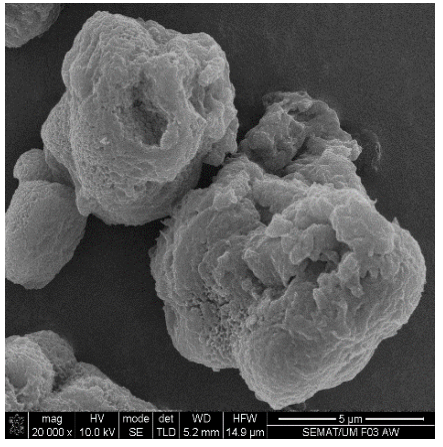
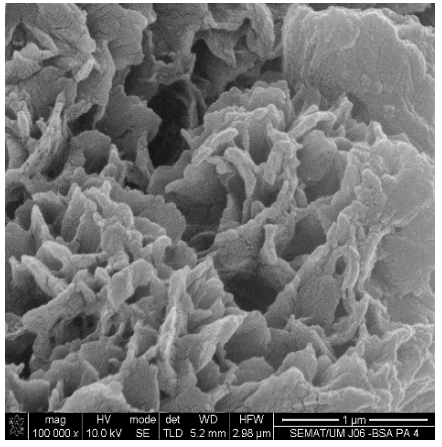


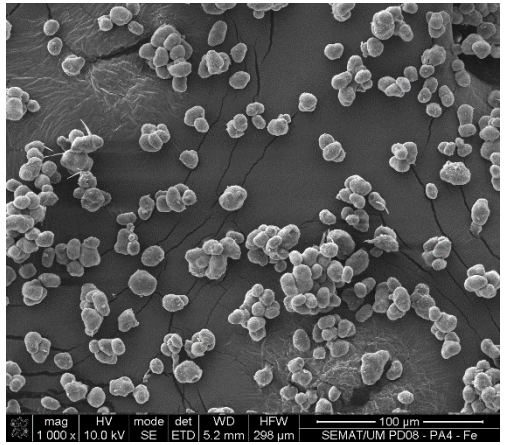


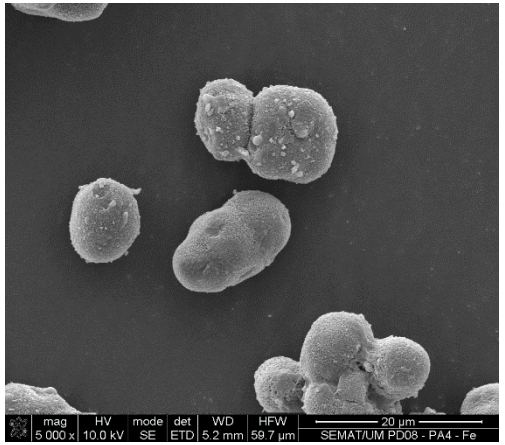


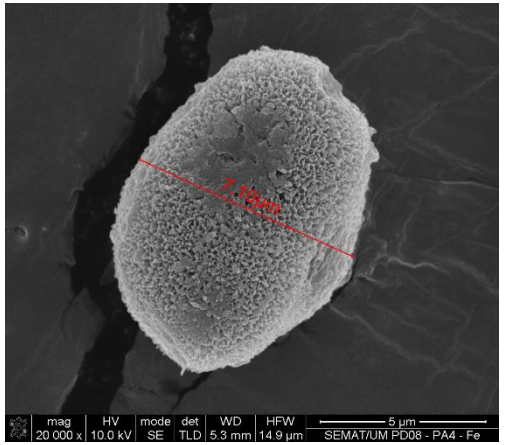


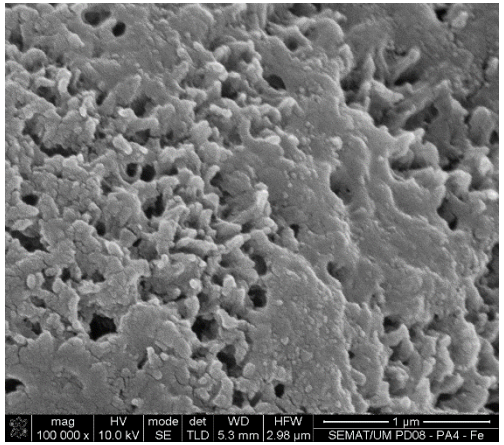
PPD-Fe 1% @ BSA NIP/MIP SEM characterization

Fe 1% NIP

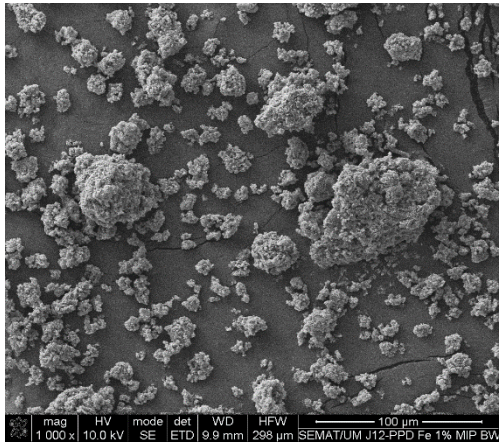


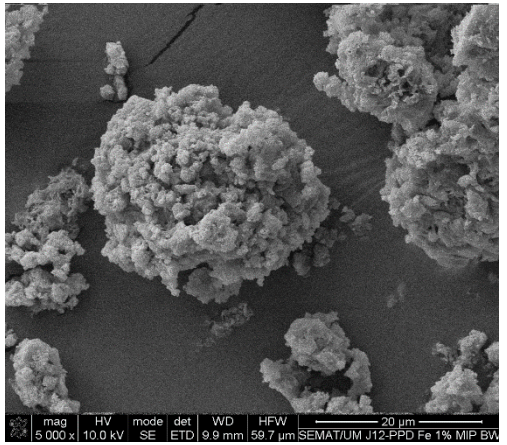


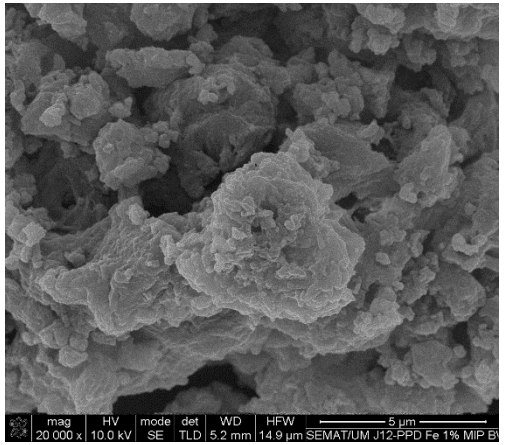


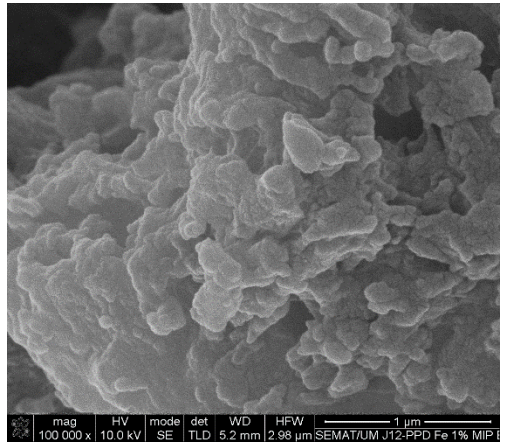


Fe - 1% MIP Before removing the BSA template

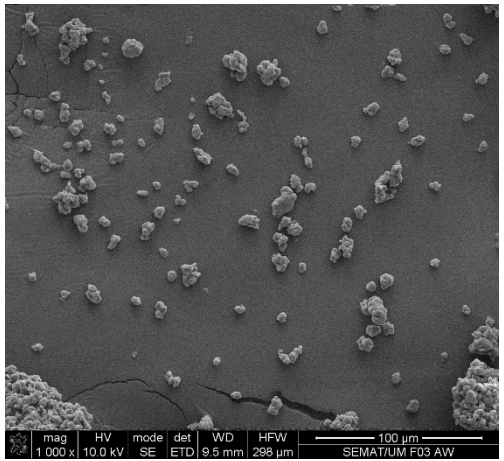


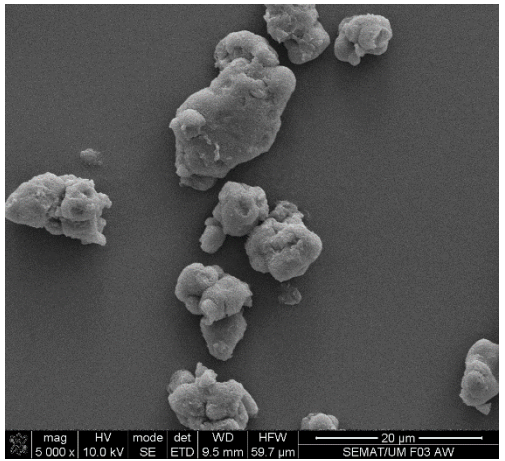


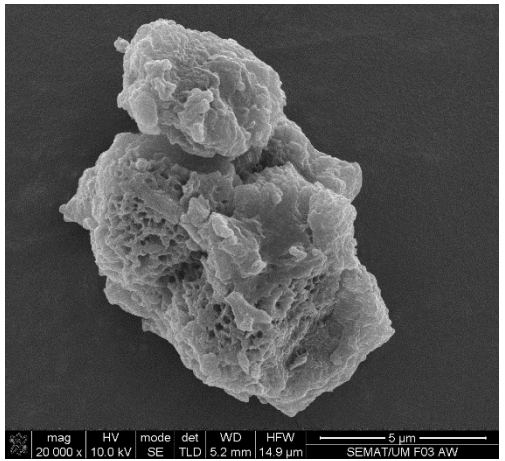


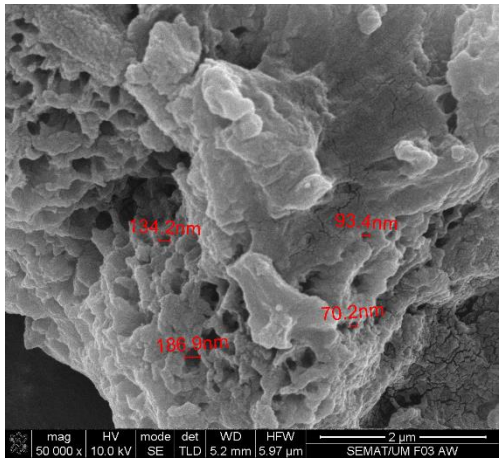


Fe - 1% MIP After removing the BSA template



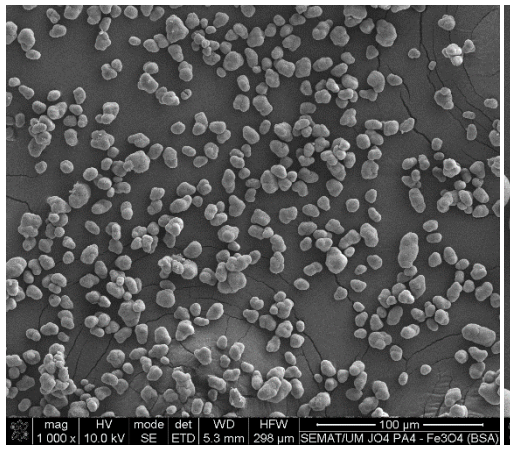


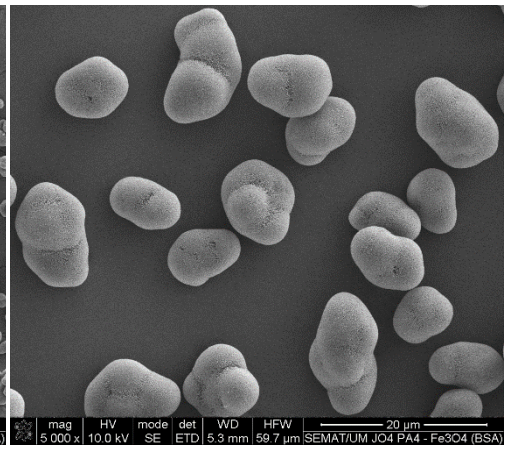


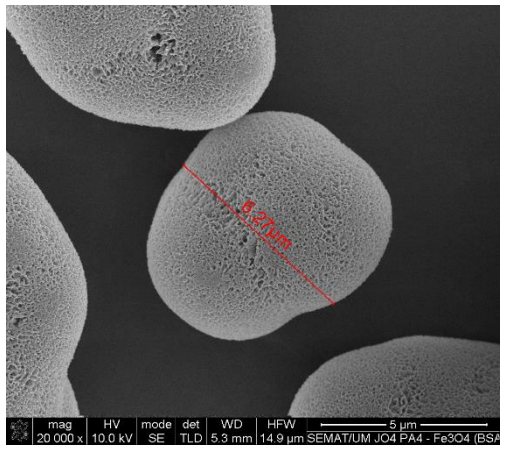


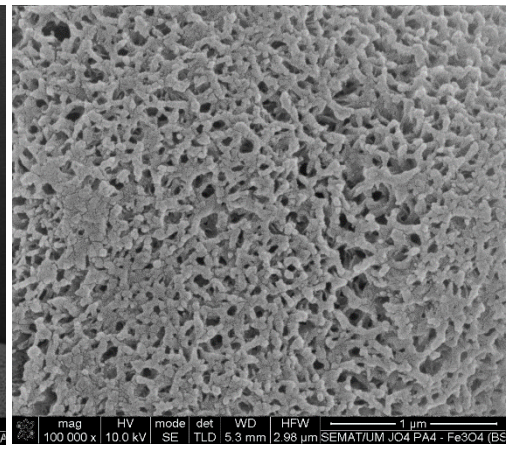
PPD-Fe_3O_4 1% @ BSA MIP/NIP SEM characterization

Fe_3O_4 -1% NIP

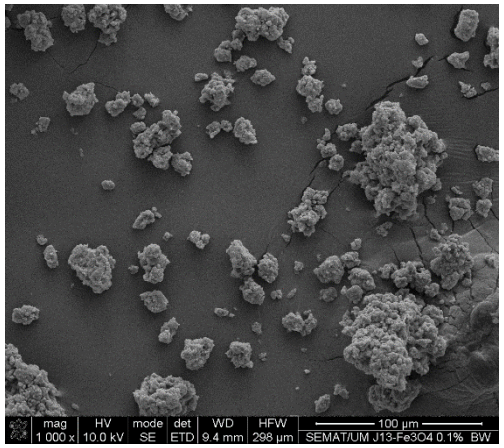


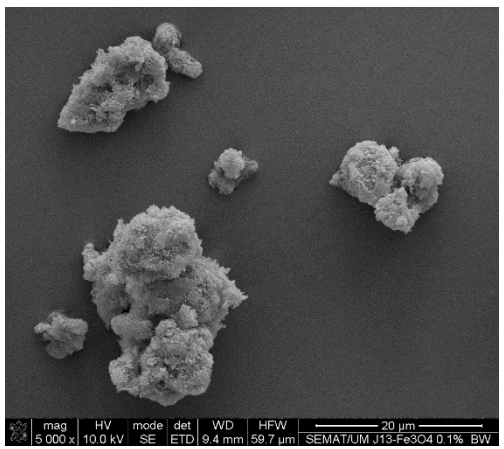


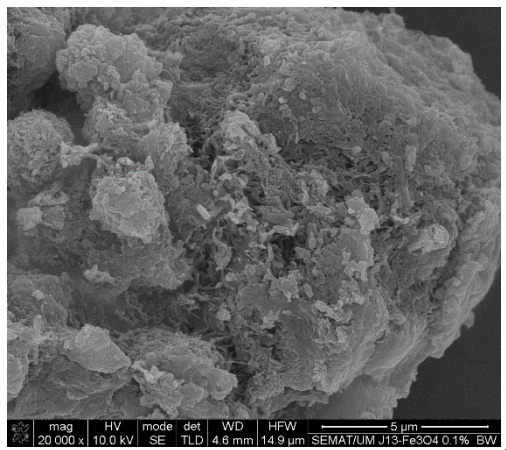


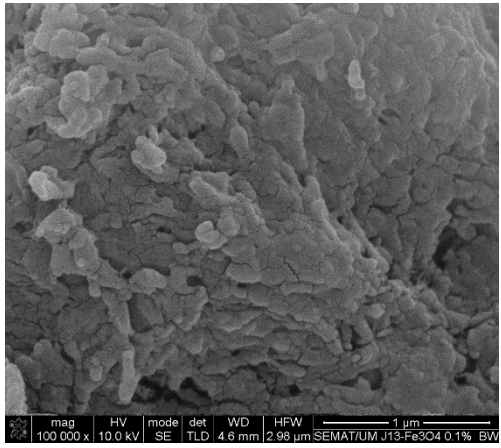


Fe_3O_4 - 1% MIP
Before removing
the BSA template

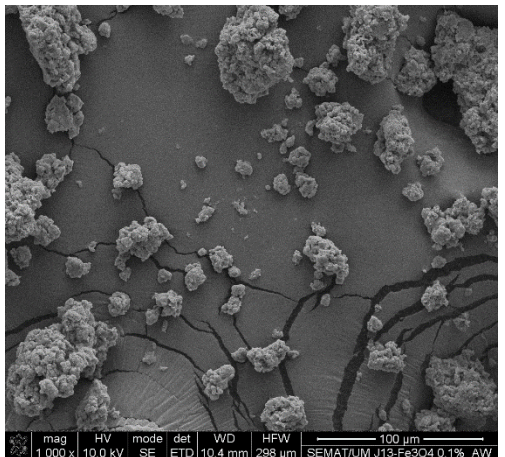


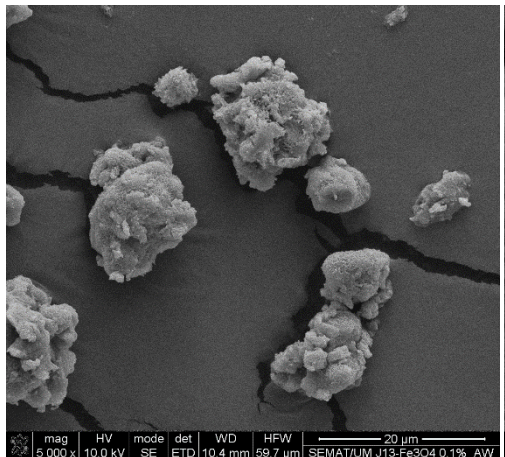


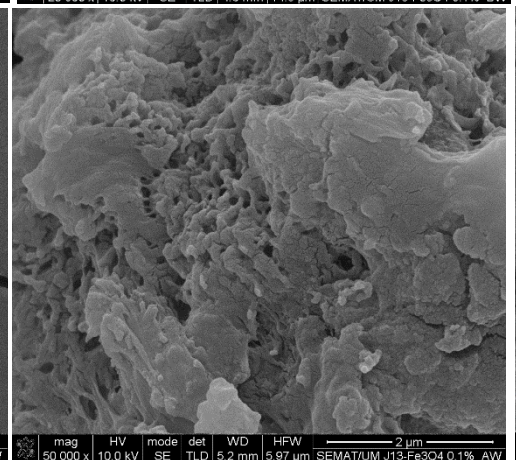


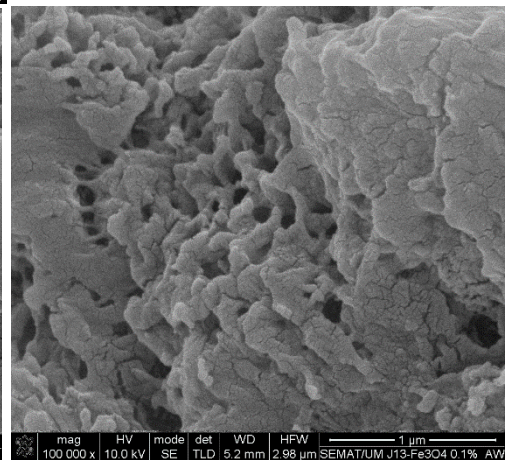


Fe_3O_4 - 1% MIP
After removing
the BSA template



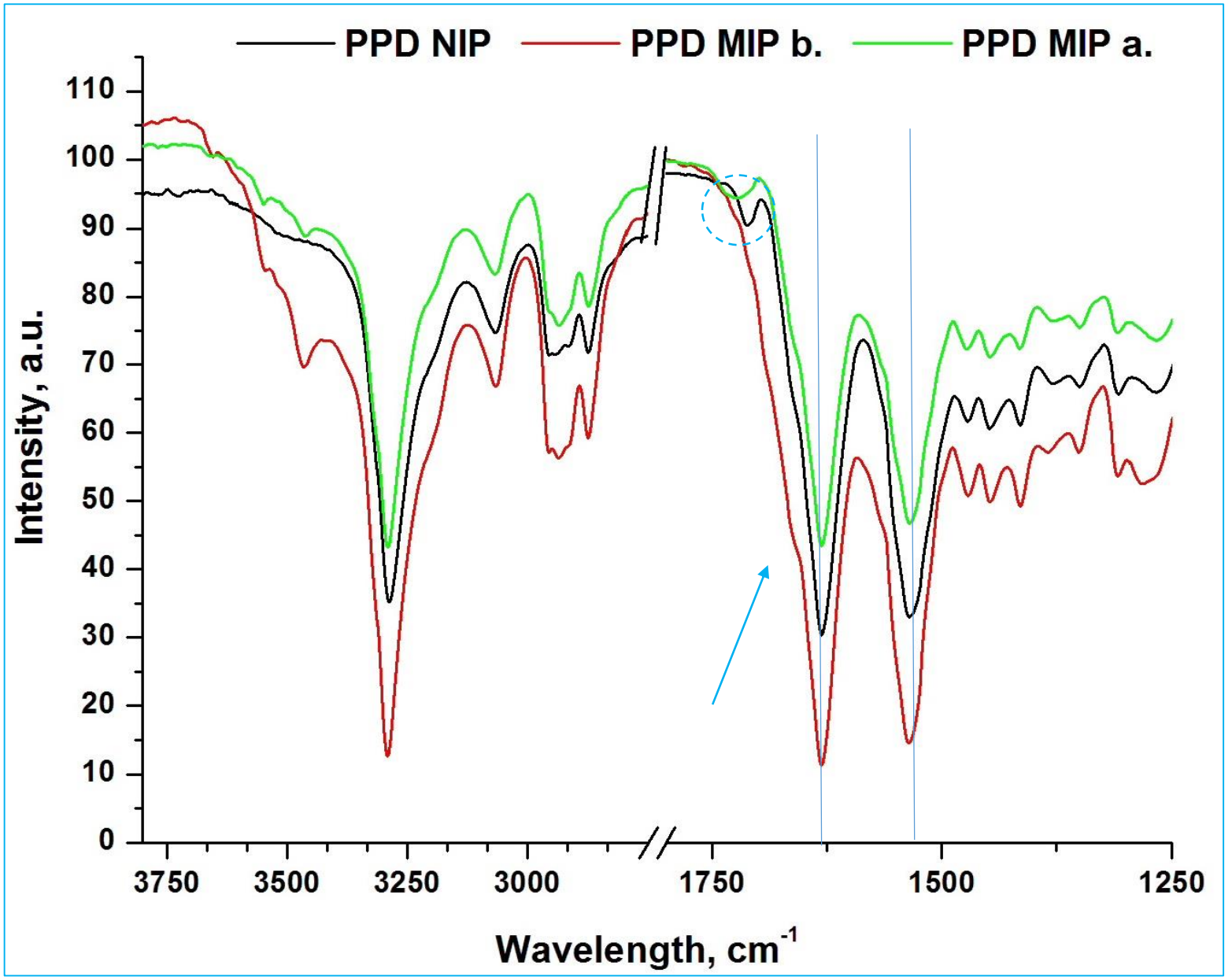






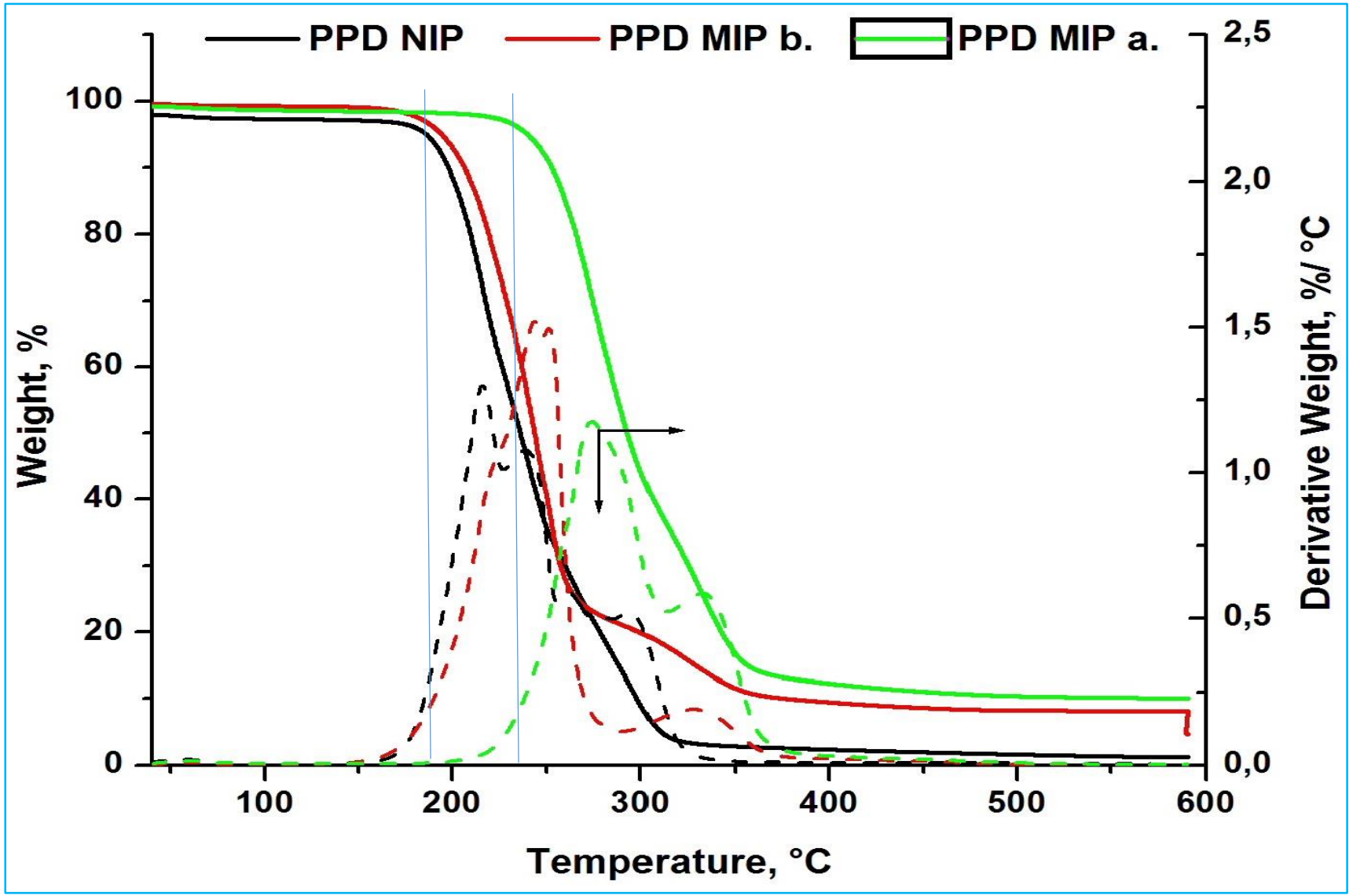
MIP/NIP characterization

FTIR (ATR)



MIP/NIP characterization

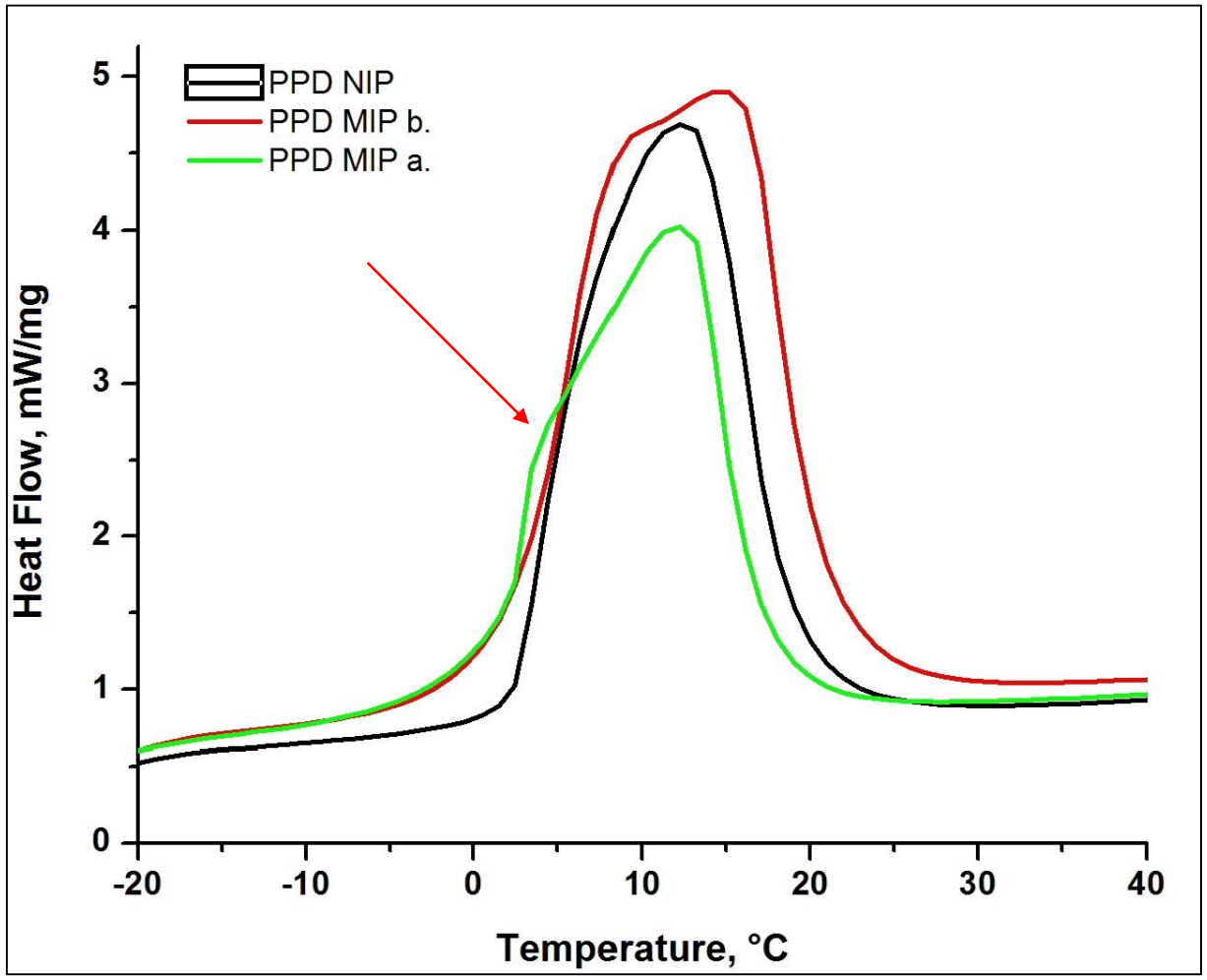
TGA



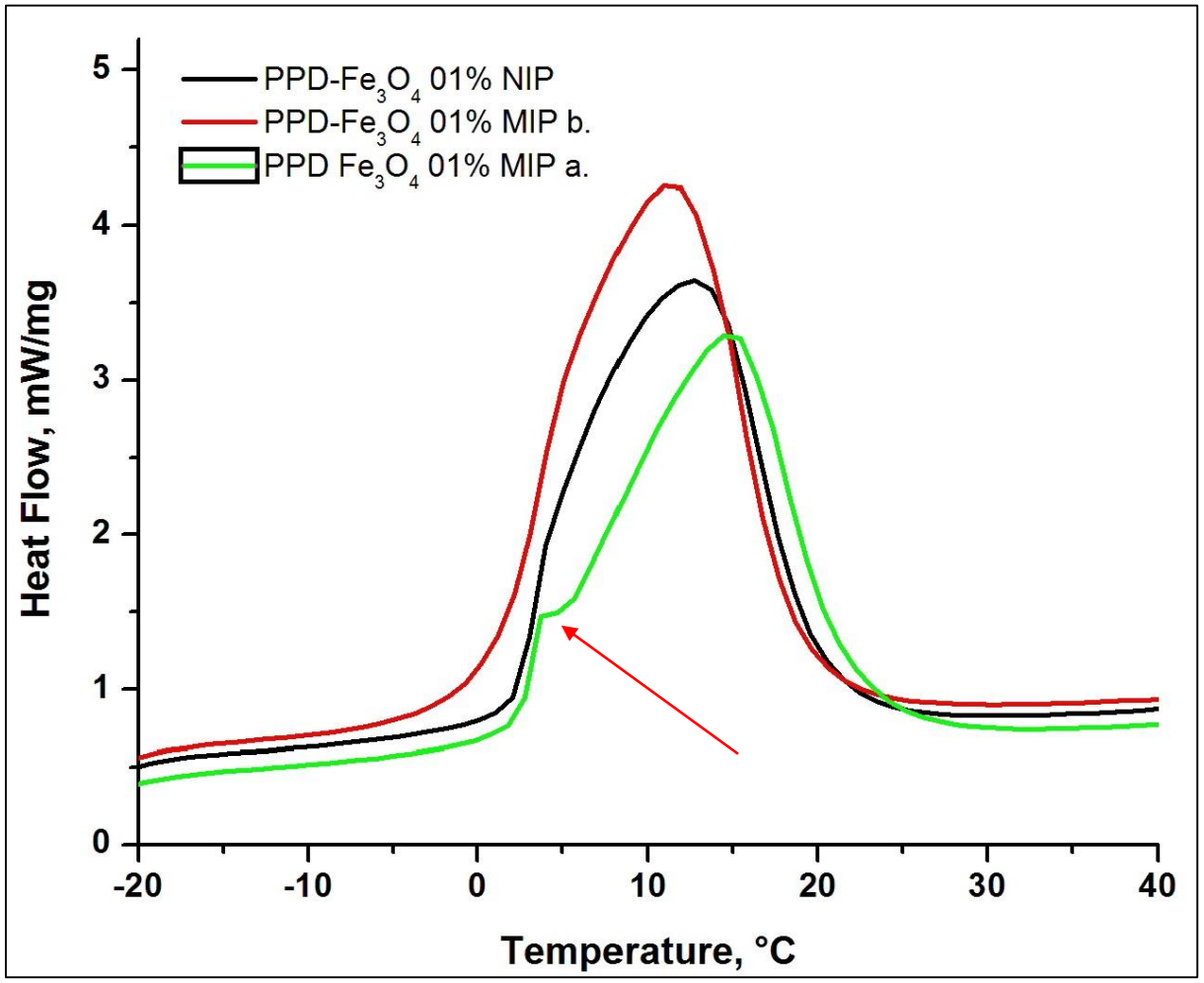
$T^{id}_{MIP} > T^{id}_{NIP}$

Low Temperature DSC analysis of in-pore constrained water

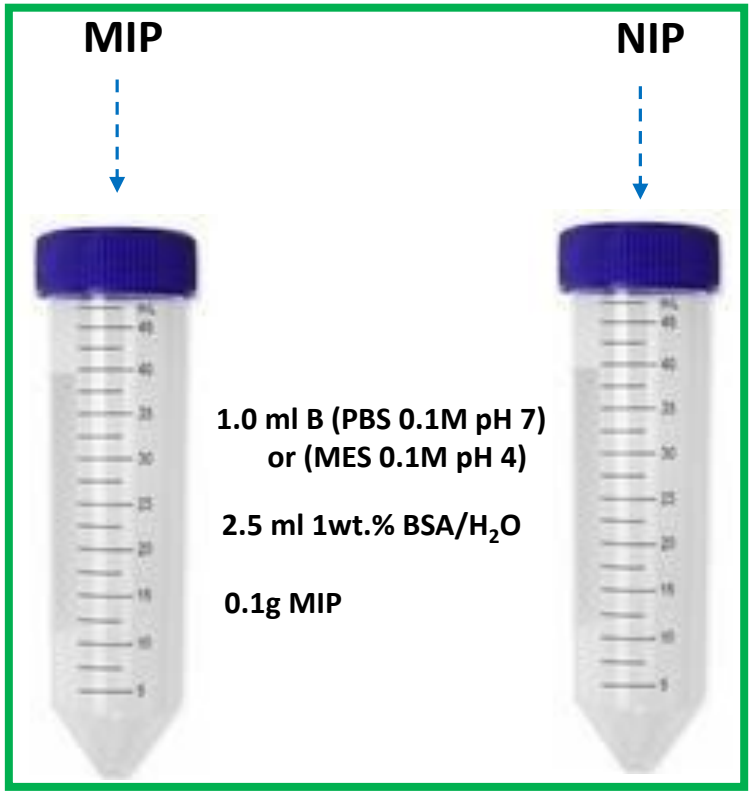
PPD NIP/MIP

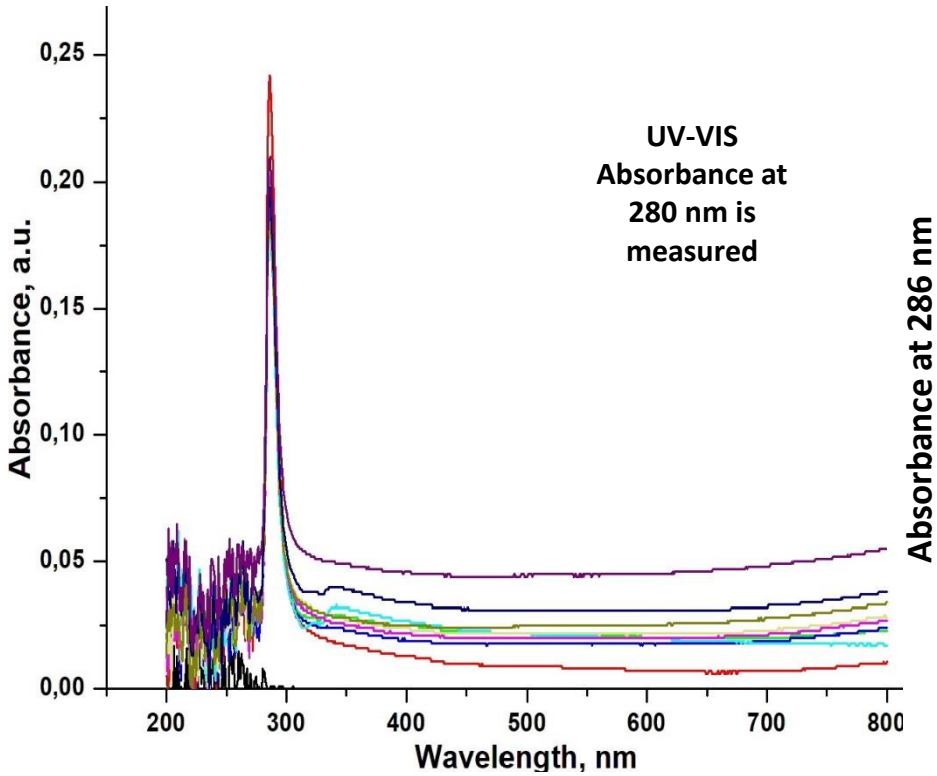


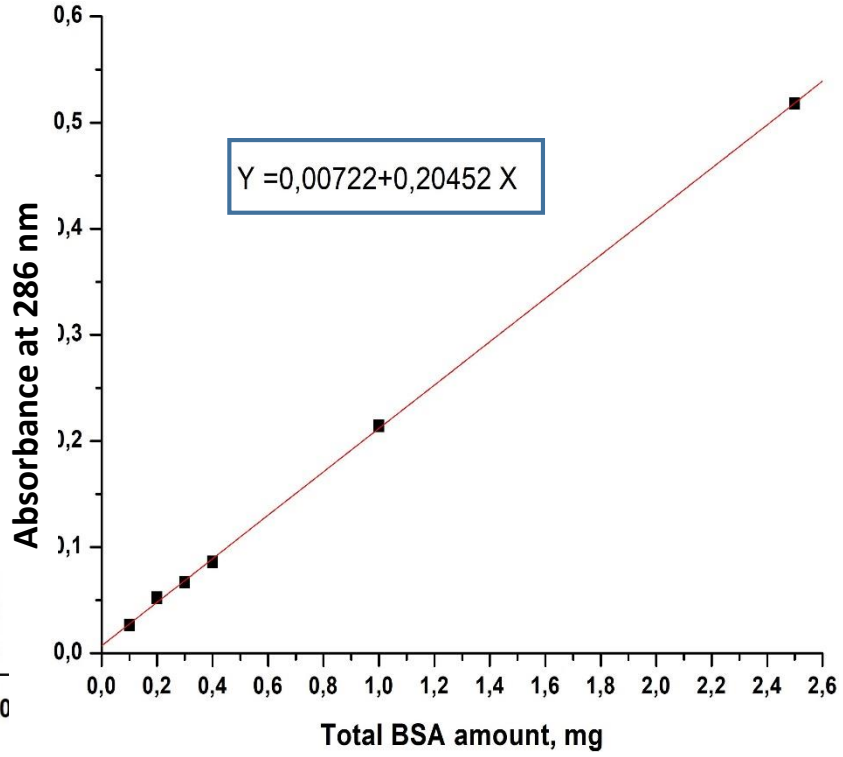
PPD-Fe_3O_4 NIP/MIP



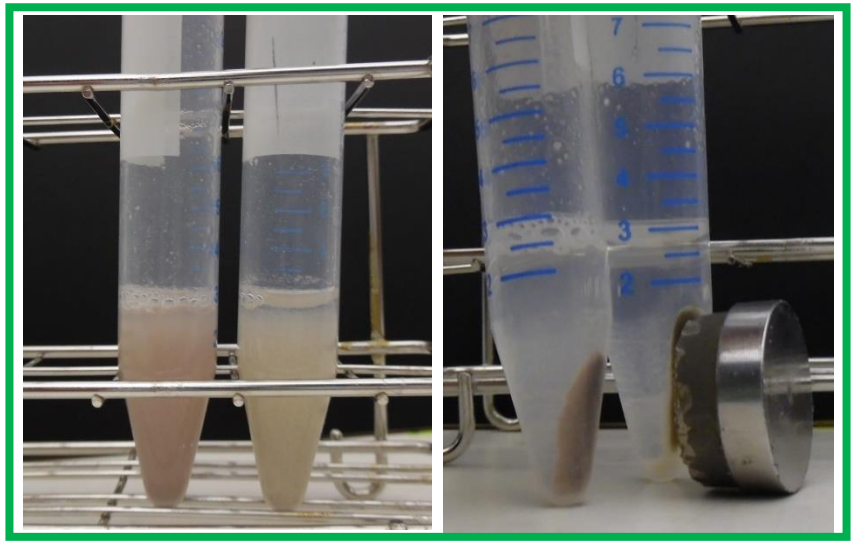
BSA Adsorption kinetics of MIP/NIP samples

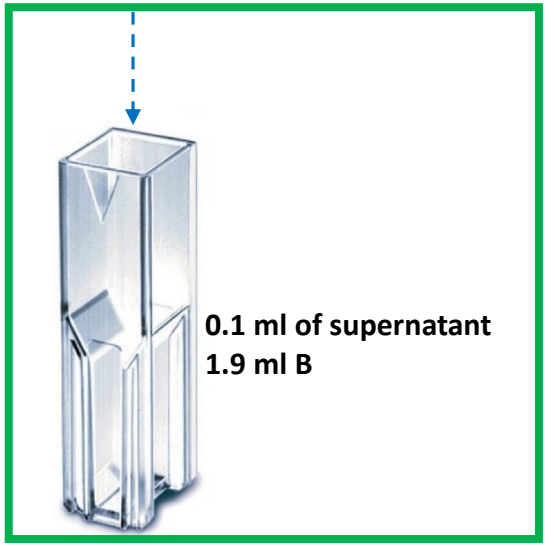




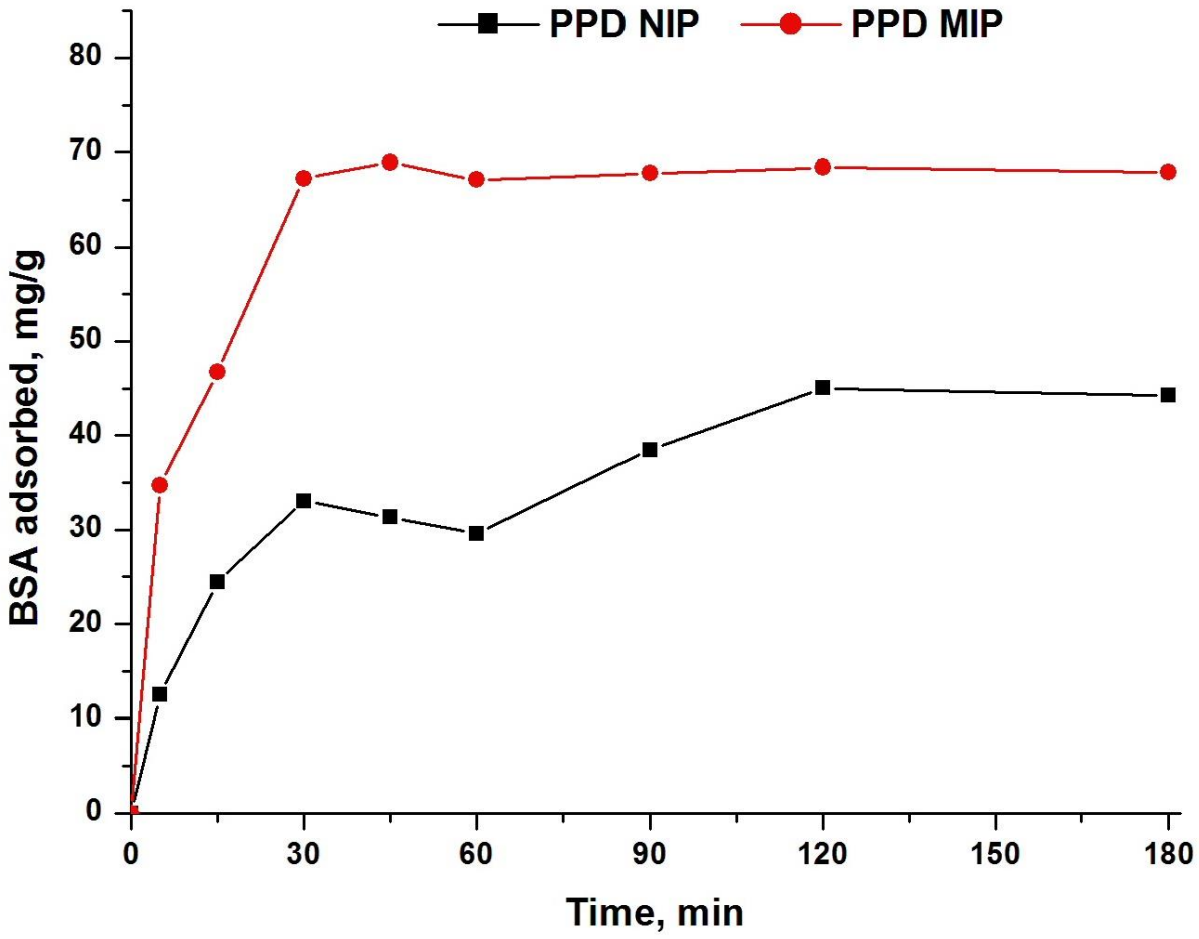


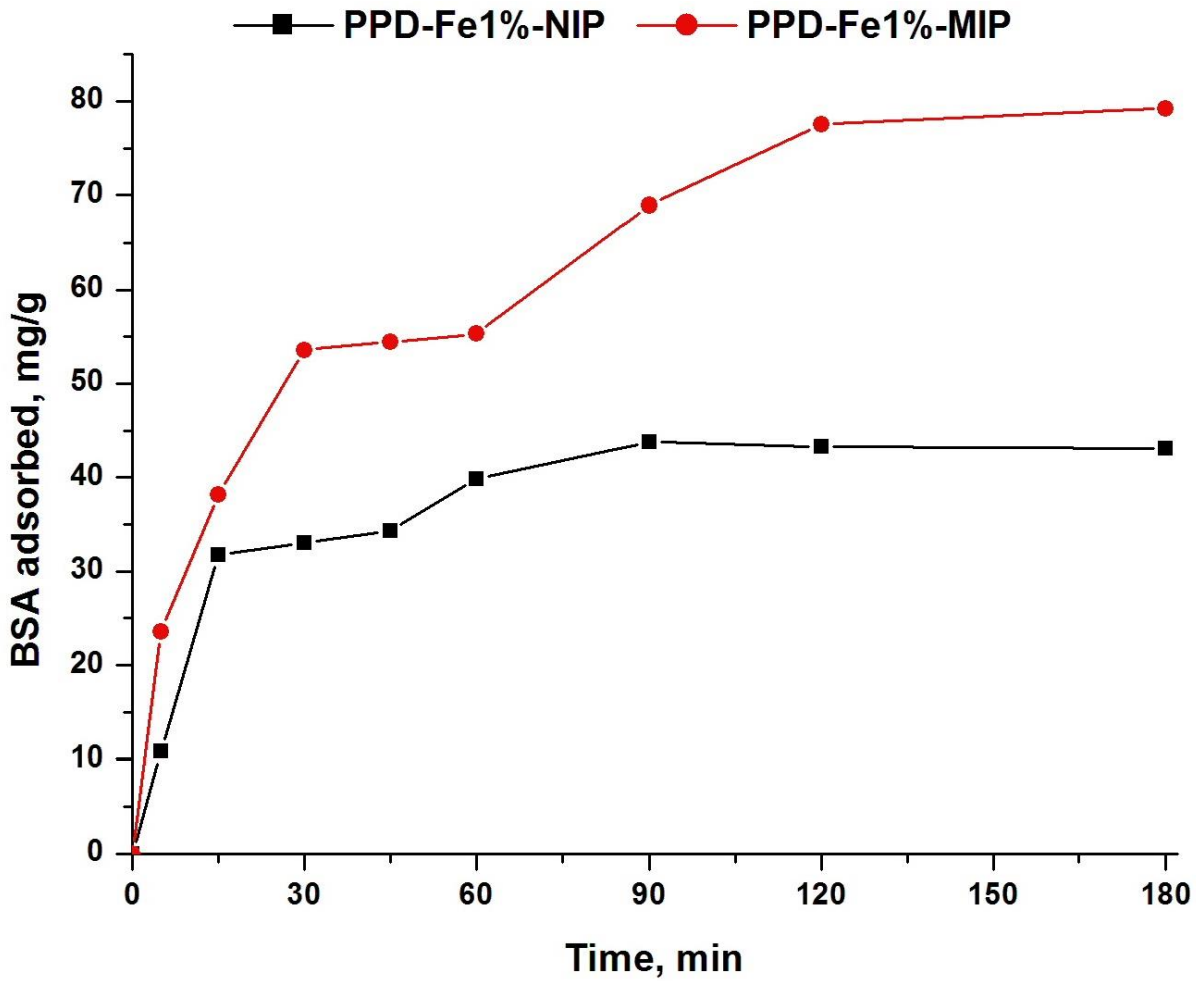
Samples are removed after
0, 15, 30, 45, 60, 90, 120,
180 min incubation at
37ºC





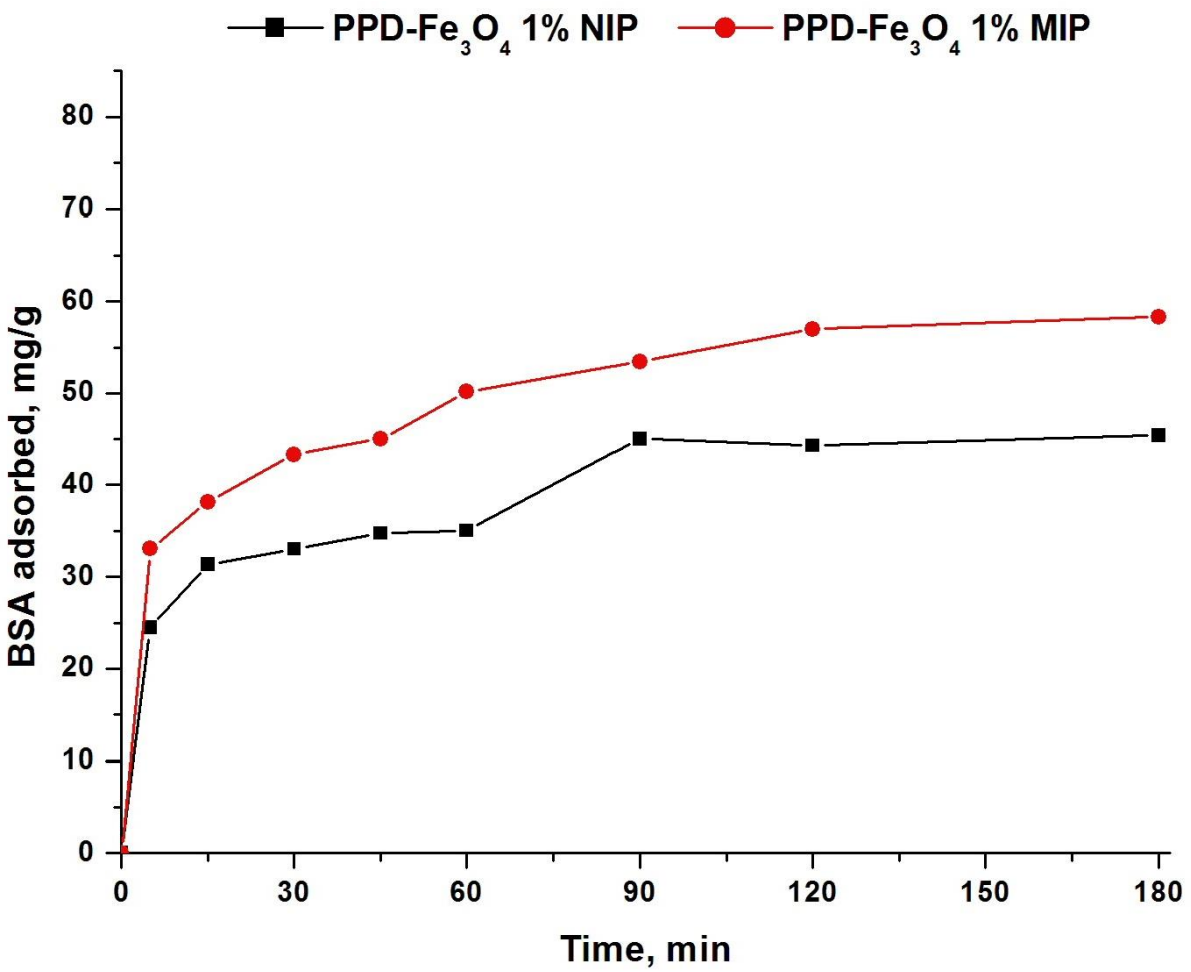
PPD & PPD-Fe 1% MIP/NIP – Adsorption capacity Q, in MES Buffer

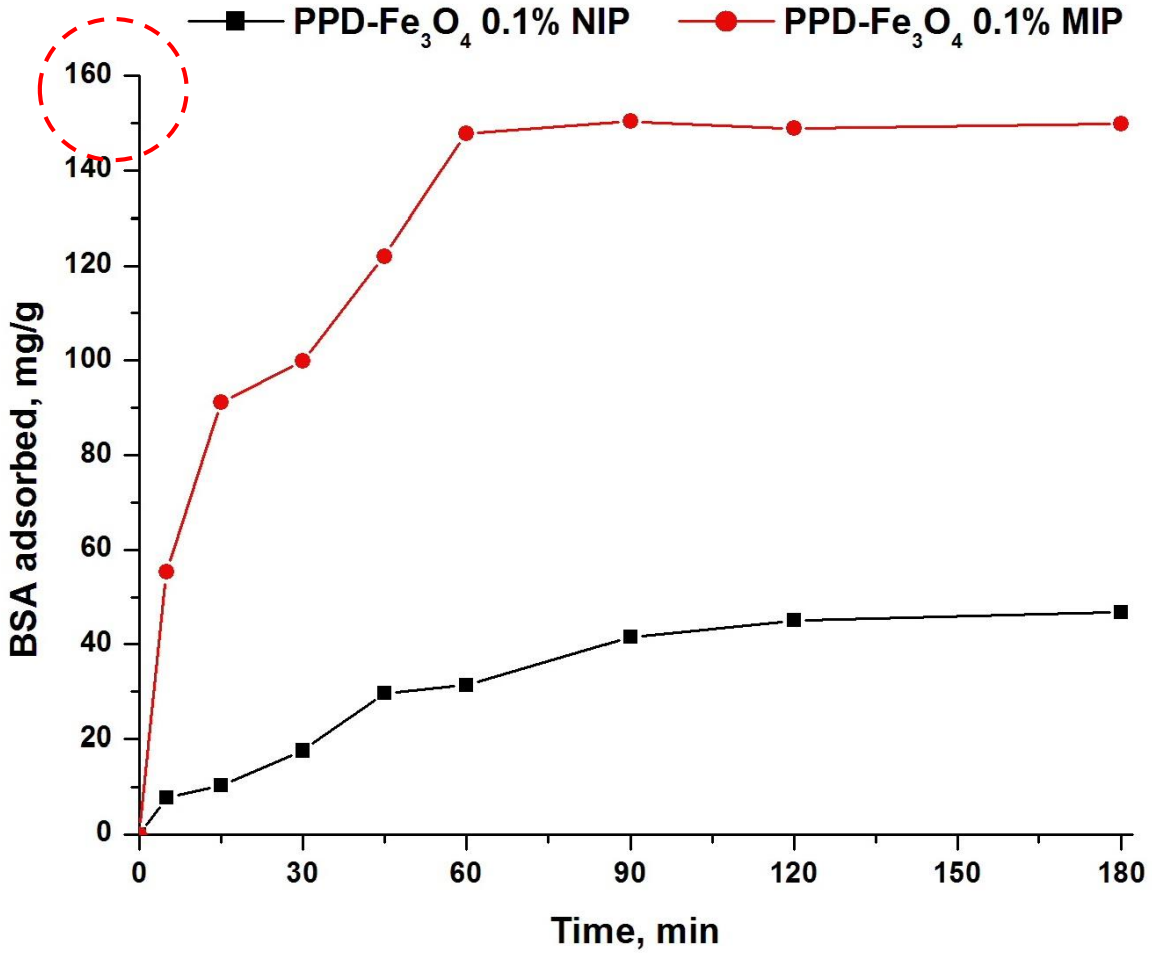




Conditions: 0,1 g NIP/MIP; 1wt.% BSA; 3h incubation time; 37°C; 0.1 M pH 4.1 (MES buffer)

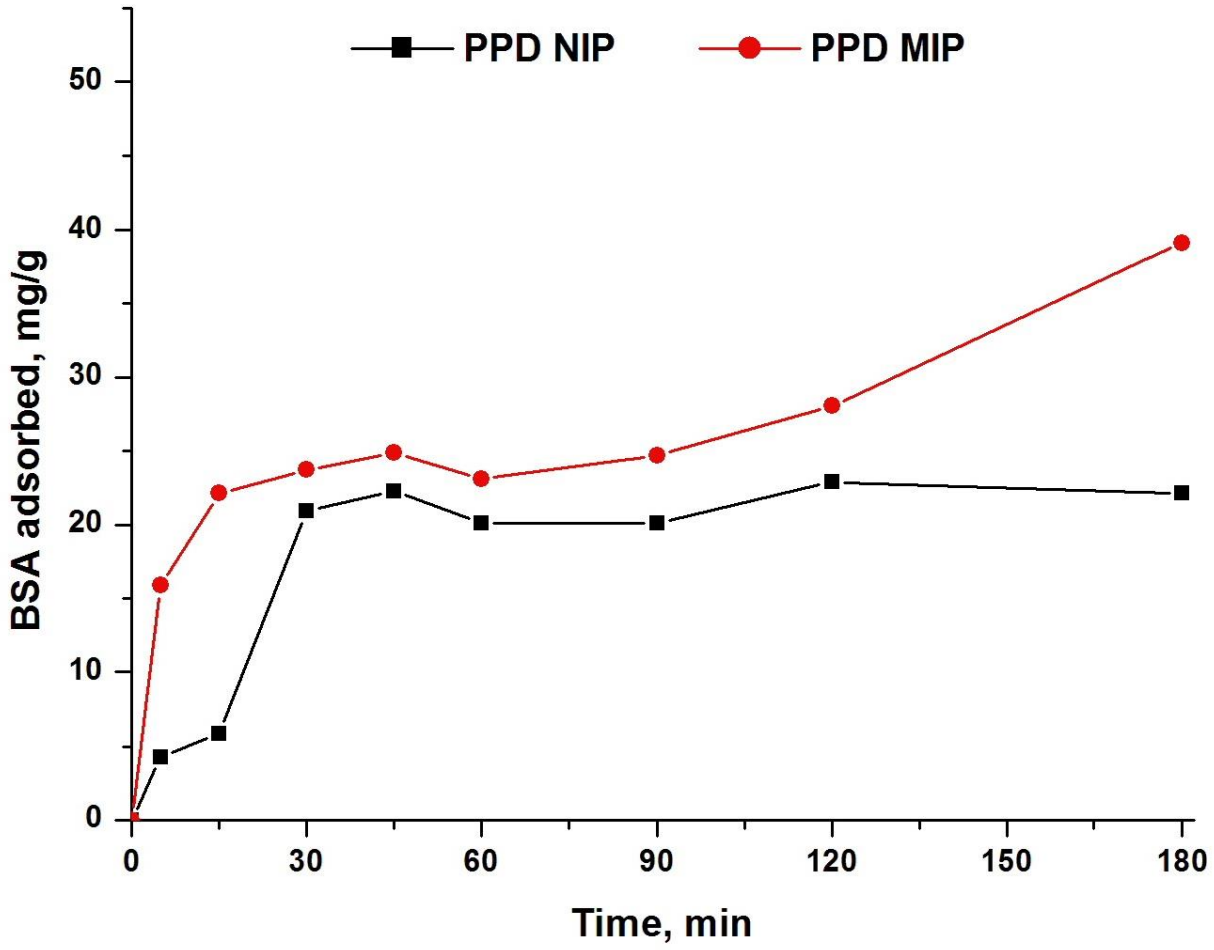
PPD-Fe_3O_4 MIP/NIP – Adsorption capacity Q, in MES Buffer

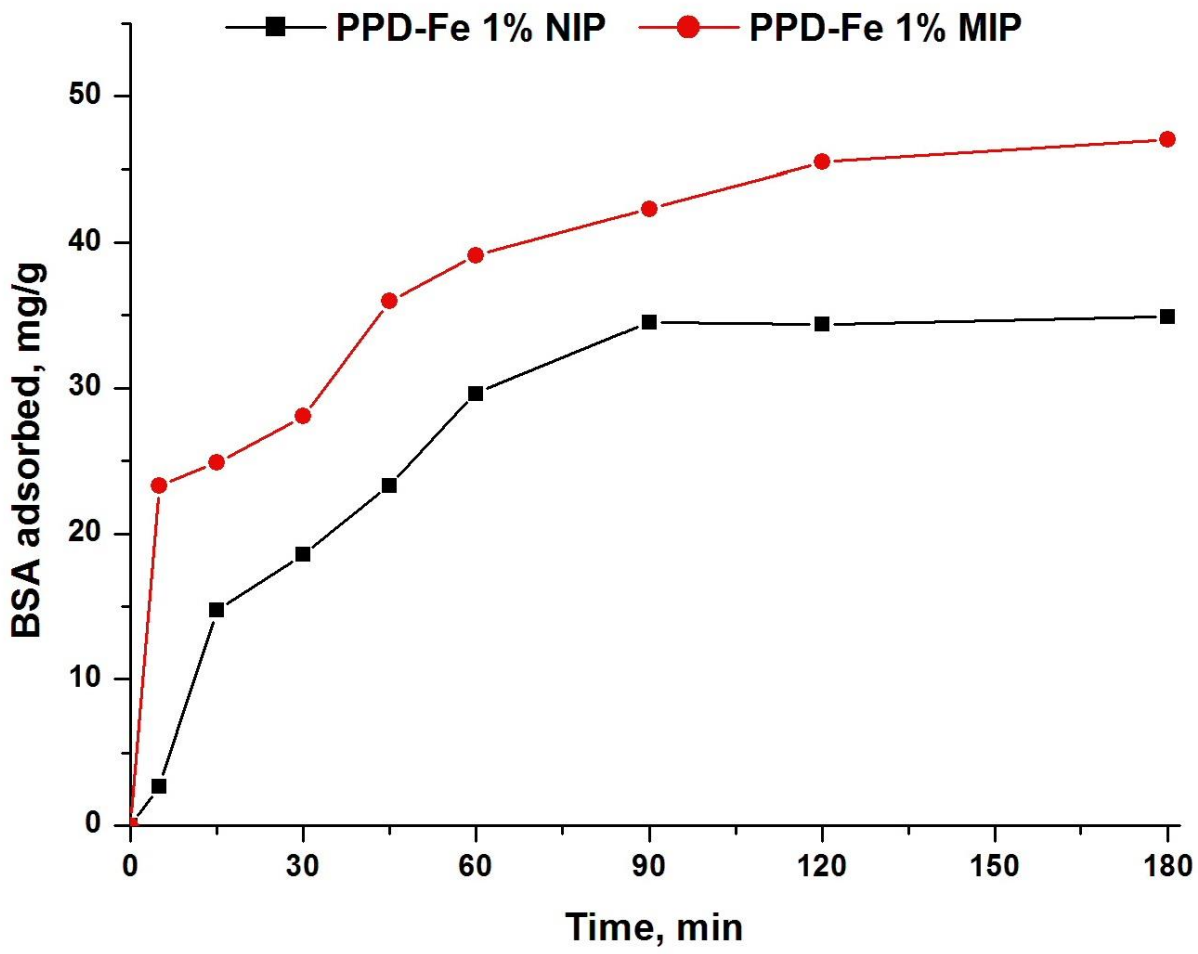




Conditions: 0,1 g NIP/MIP; 1wt.% BSA; 3h incubation time; 37°C; 0.1 M pH 4.1 (MES buffer)

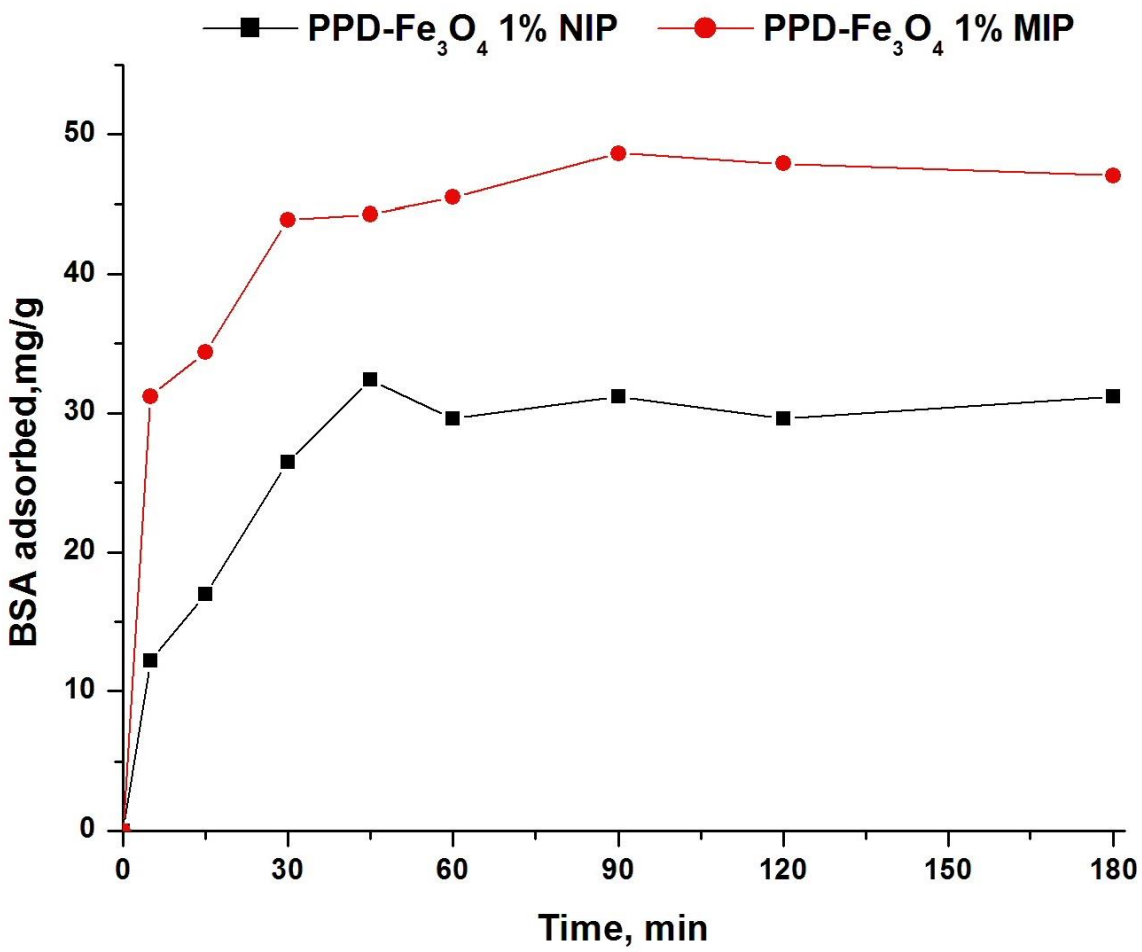
PPD&PPD-Fe 1% MIP/NIP - Adsorption capacity Q, in Phosphate Buffer

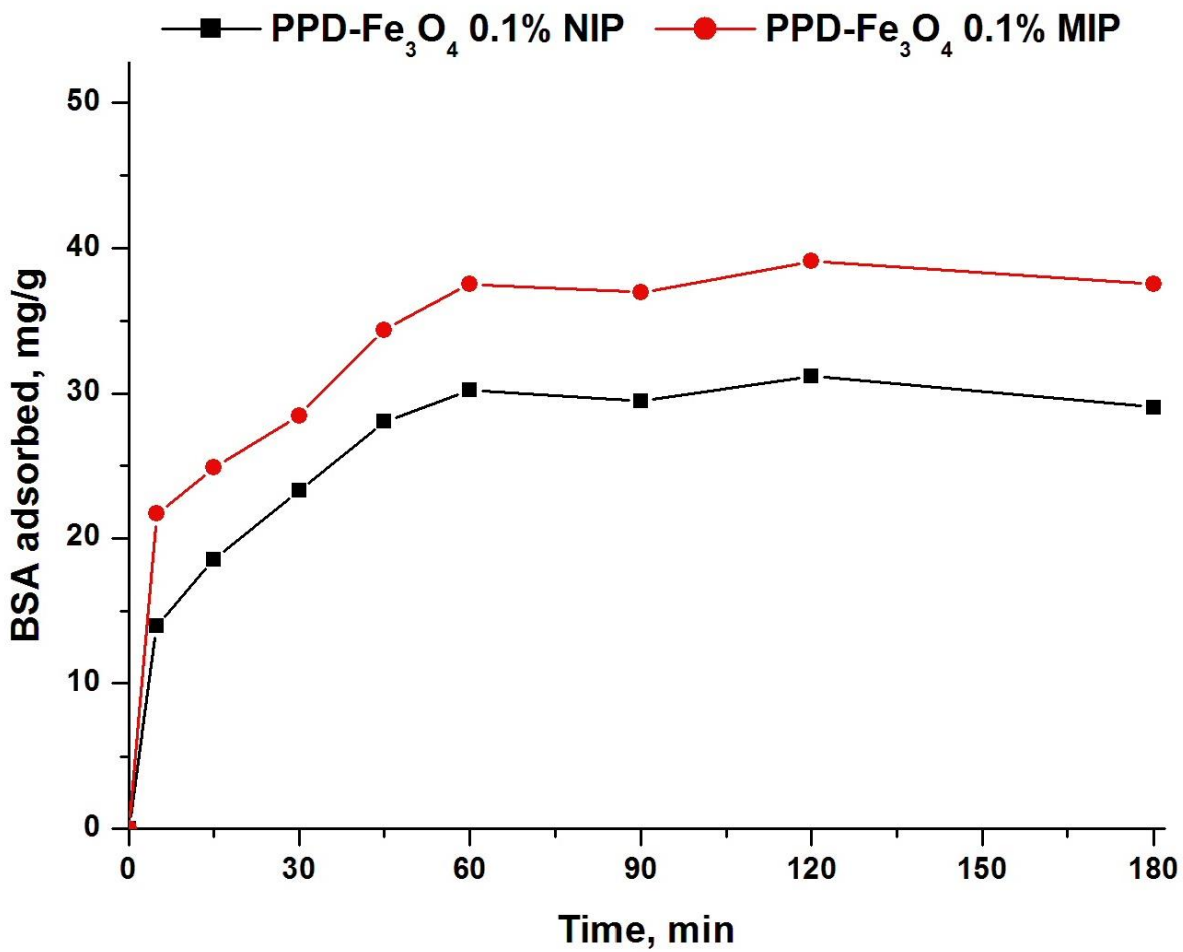




Conditions: 0,1 g NIP/MIP; 1wt.% BSA; 3h incubation time; 37°C; 0.1 M pH 7.2 (Phosphate buffer)

PPD-Fe_3O_4 MIP/NIP - Adsorption capacity Q, in Phosphate Buffer

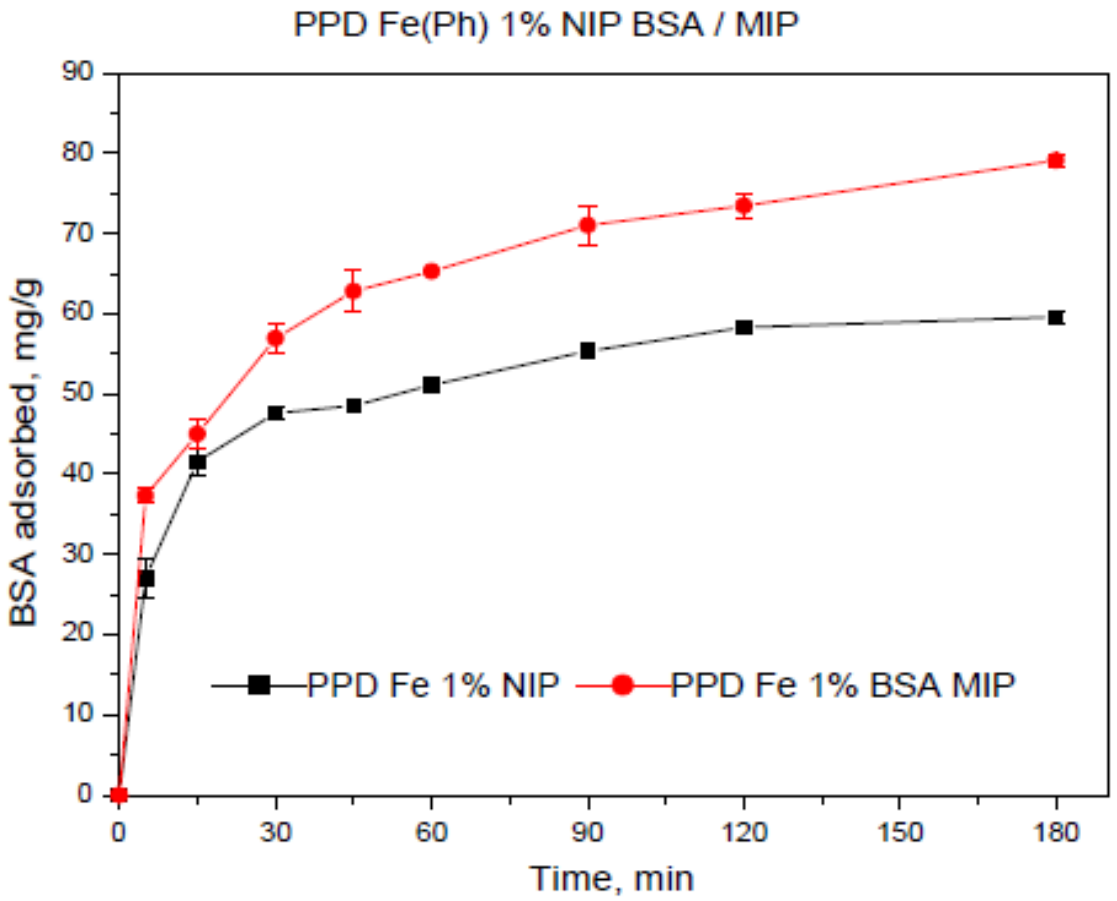




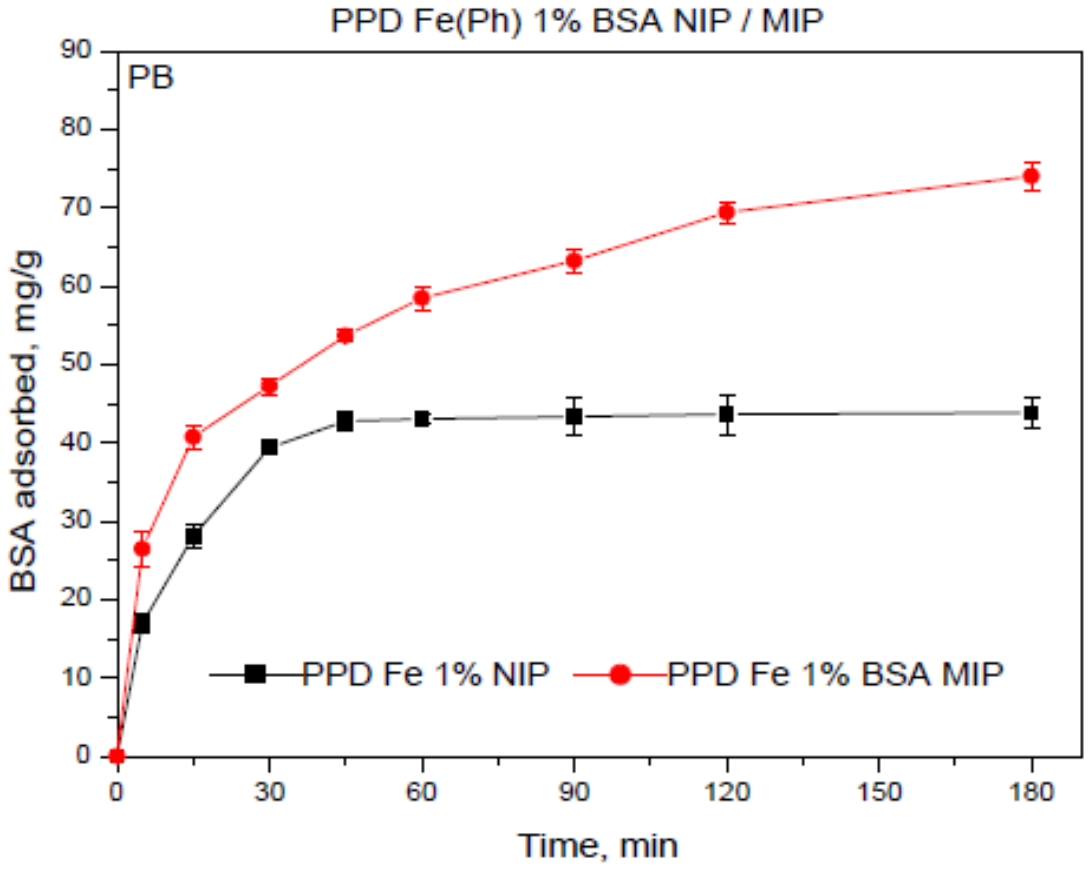
Conditions: 0,1 g NIP/MIP; 1wt.% BSA; 3h incubation time; 37°C; 0.1 M pH 7.2 (Phosphate buffer)

PPD-$Fe_{(PO4)}$ MIP/NIP - Adsorption capacity Q

MES buffer 0.1 M pH 4.1



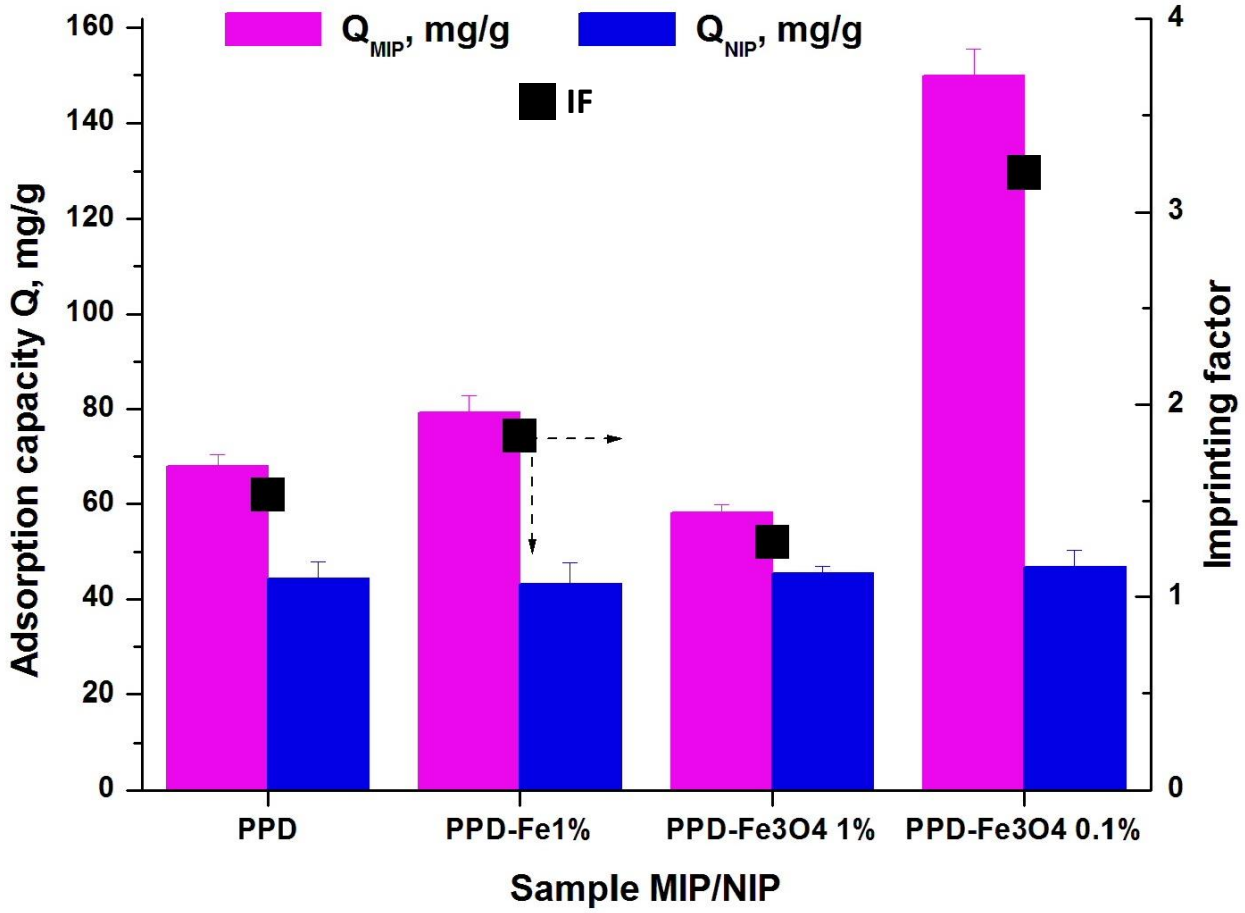
Phosphate buffer 0.1 M pH 7.2



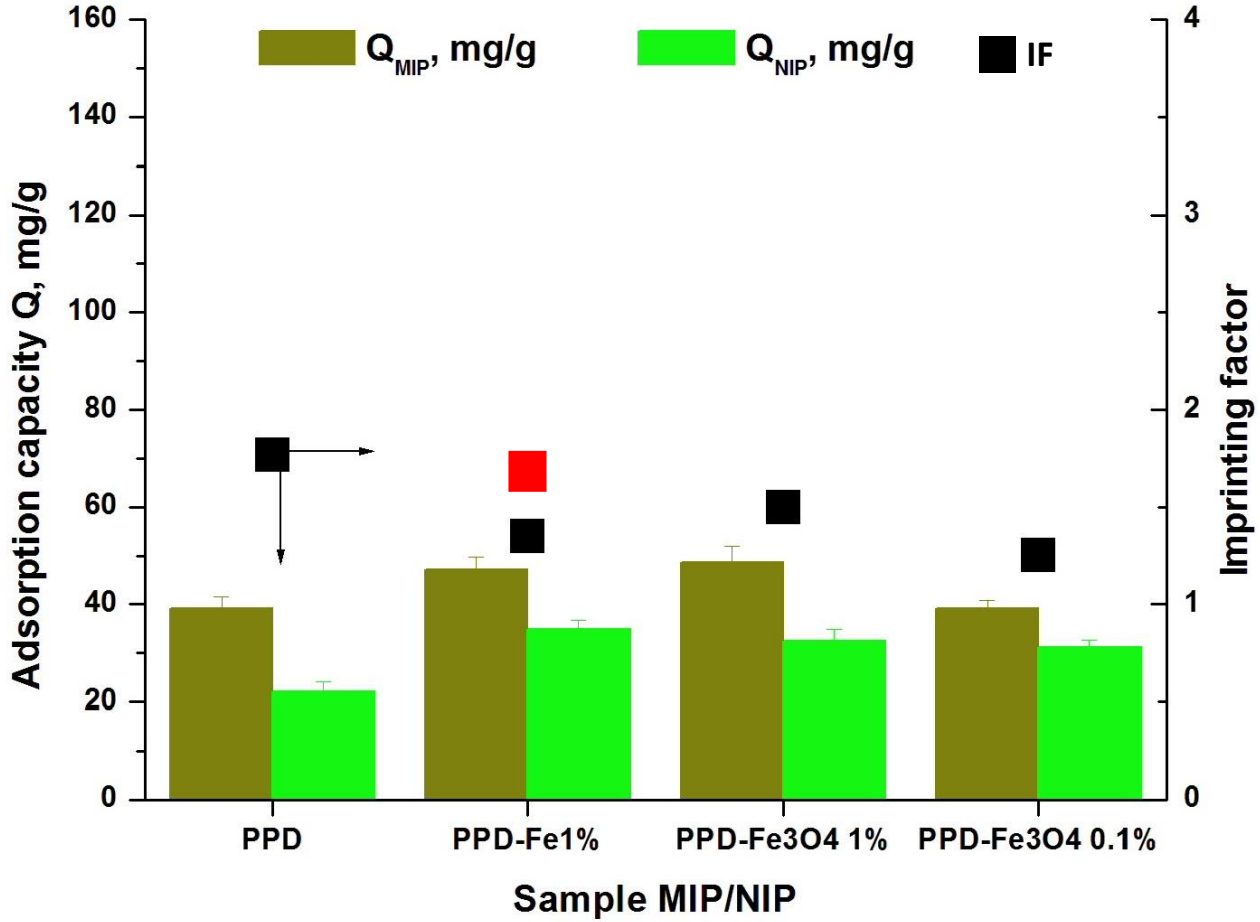
Conditions: 0,1 g NIP/MIP; 1wt.% BSA; 3h incubation time; 37°C

Evaluation of Imprinting Factor IF of PPD-MIP samples

In pH 4,1 (MES buffer)



In pH 7.2 (Phosphate buffer)



$$IF = Q_{MIP} / Q_{NIP}$$

Conclusions

- Novel BSA molecularly imprinted magnetic responsive particles based on PA4 (2-polypyrrolidone) are synthesized via activated anionic polymerization;
- The MIP particles are with controlled shape and sizes of 10-50 µm; the maximum dimensions of the imprinted cavities falls between 70-200 nm;
- All MIP samples showed superior adsorption capacity toward the template BSA protein, as compared to the respective NIP samples, the improvement factor varying between 30 and 320%;
- The adsorption capacity Q toward template protein is pH dependent, i.e., much stronger in acidic than in basic media.
- Q can also depend on the surface treatment of the magnetic particles.

Acknowledgements

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COMPETE 2020

PORTUGAL 2020

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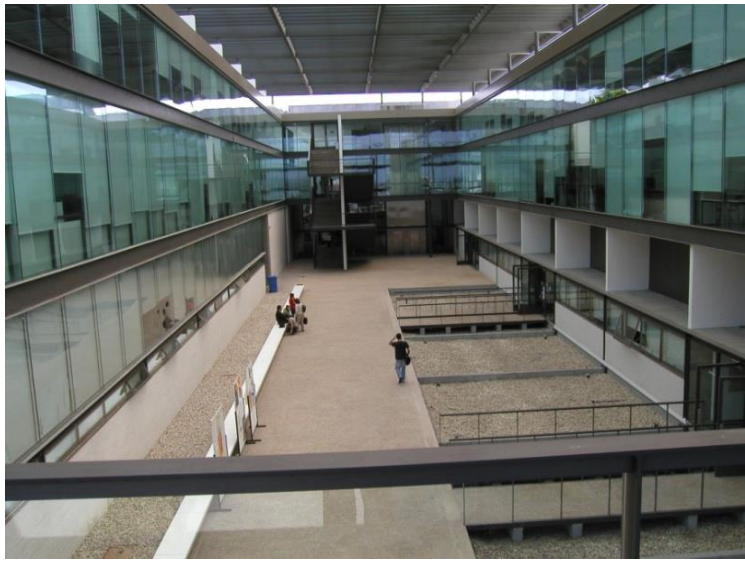
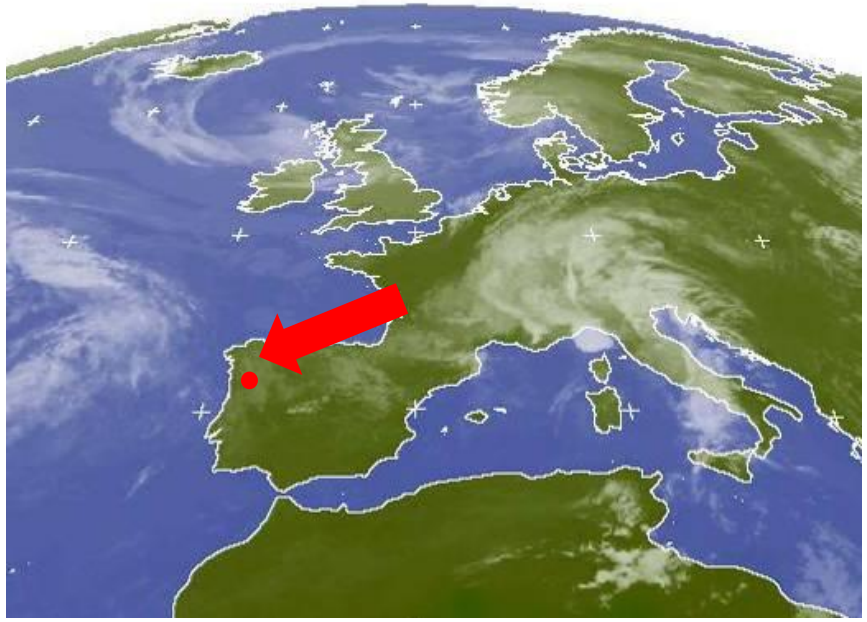
Joana Rompante
Joana Braz
Filipa D. Oliveira
Shafagh Tochidi

University of Minho



Thank you!

University of Minho localization



University of Minho

Founded:1974; Localization: Braga & Guimarães;
Number of students: ca. 21.000 (incl. Post-graduation)

School of Architecture

School of Engineering

School of Natural Sciences

Law School

School of Economics and Management

School of Health Sciences

Psychology School

Higher School of Nursing

School of Social Sciences

Higher Institute of Education

School of Philology

School of Engineering

Department of Bioengineering
Department of Civil Construction
Department of Industrial Electronics
Department of Mechanical Engineering
Department of Polymer Engineering (DEP)
Department of Textile Engineering
Department of Informatics
Department of Production Systems
Department of Informational Systems