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Immobilization of a pH-low insertion peptide onto SiO_2/aminosilane-coated magnetite nanoparticles

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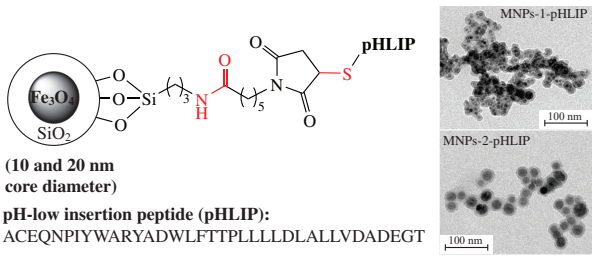
The covalent immobilization of a pH-low insertion peptide to aminosilane-coated magnetic nanoparticles with two core diameters (10 and 20 nm) was carried out. The materials obtained were characterized by TEM, XRD, vibrating sample magnetometry, DLS, IR spectroscopy, TGA and elemental analyses. The materials did not have significant cytotoxicity, and they can be used as MRI-guided nanocarriers for tumour targeting.



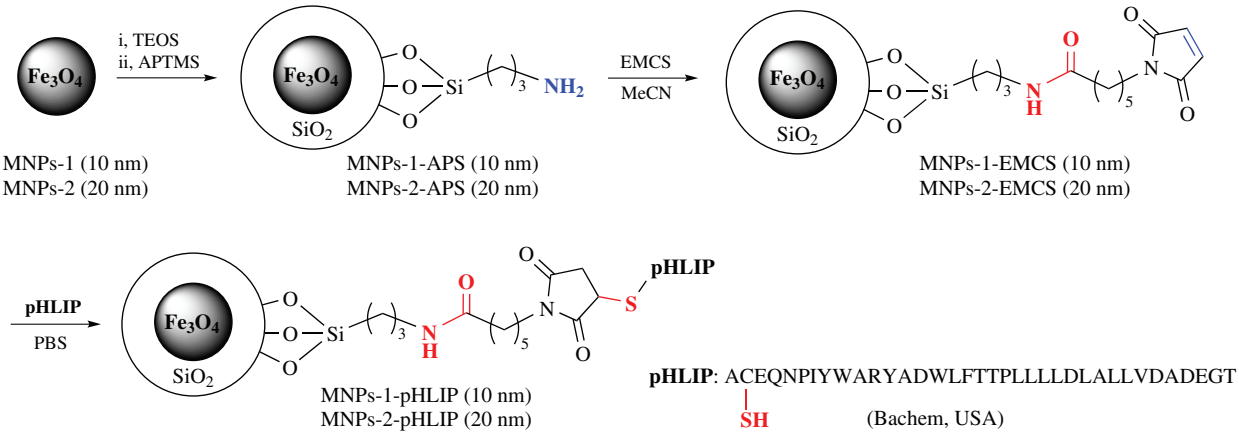
Magnetic nanoparticles (MNPs) are considered as a platform for the design of contrast agents for MRI and drug delivery systems.[1–4] Currently, Fe_3O_4-based MNPs are commercially available for medical use and the development of new materials (including those for clinical applications as MRI contrasts).[5] The pH-low insertion peptide (pHLIP),[6] which inserts into the cell membrane at pH above 7.0, is successfully used as a vector molecule for targeting drugs and contrast probes to solid tumours.[7,8] Only a few publications considered the immobilization of pHLIP on the surface of polymeric (with a Gd^{III} complex),[9] gold[10,11] and silica[12] nanoparticles for their targeting transport to the acidic tissues. Recently, we obtained pHLIP nanoconjugates of MNPs[13] and studied their biological activity.[14] Wei *et al.*[15] assembled MNP-pHLIP nanoclusters using poly-D-Lys and PEG derivatives. The MNP-pHLIP nanoconjugates demonstrated effective pH-responsive retention in cells and tumour tissues, and their accumulation can be controlled by MRI.[14]

The aim of this work was to synthesize new nanoconjugates based on silica-coated MNPs and pHLIP. For this purpose, we carried out a comparative chemical modification of Fe_3O_4 MNPs with two sorts of core size [obtained by the thermal decomposition of iron(III) oleate] by pHLIP and also a qualitative and quantitative evaluation of the peptide content on MNP surface and studied their cytotoxicity.

The thermal decomposition of iron(III) compounds has certain advantages over a precipitation method due to the possibility to obtain nanoparticles with various sizes and shapes and a narrow size distribution.[16–18] In this work, the thermal decomposition synthesis of nanoparticles MNPs-1 and MNPs-2 with magnetite cores 10 and 20 nm in diameter [Figures 1(*a*),(*b*)] and their coating with SiO_2 and 3-aminopropylsilane (APS) (Scheme 1) were developed.† According to XRD data [Figures 1(*a*),(*b*), insert], the obtained nanoparticles had a magnetite structure (ICDD PDF-2 no. 00-019-0629); the average sizes of MNPs-1 and MNPs-2 crystallites were 7 and 14 nm, respectively, which are close to the TEM data. The sizes of MNPs-1-APS and MNPs-2-APS were 17–20 and 30–36 nm, respectively [Figures 1(*c*),(*d*)]. To preserve biological activity, pHLIP was conjugated using the thiol group of L-Cys.[10] For conjugation of pHLIP with nanoparticles, bifunctional cross-linkers containing the maleimide fragment are often used.[12–15] In this work, the immobilization of pHLIP was carried out using a 6-maleimidohexanoic acid *N*-hydroxysuccinimide ester (EMCS) linker. On the one hand, this linker binds to amino groups on the MNPs surface by formation of an amide bond and, on the other hand, to the thiol group of the peptide by addition to a double bond of the maleimide ring (see Scheme 1).†

Note that only two examples of producing pHLIP-containing magnetic nanoconjugates were published.[13–15] Unlike previously synthesized nanoconjugates,[13,14] new nanoparticles have a protective SiO_2 coating. The non-covalent binding of the peptide-containing conjugate to the surface of MNPs was also carried out.[15] Oppositely, the peptide molecules in MNPs-1-pHLIP and MNPs-2-pHLIP were covalently bound to the MNP surface. Thus, it can be assumed that the synthesized nanoconjugates are more resistant to undesirable core oxidation and pHLIP desorption in biological media compared to MNPs.[13,15] In addition, the presence of a mesoporous silica coating makes it possible to load drugs on the MNPs.[19] Thus, the nanoparticles

† For procedures and characteristics of the nanomaterials obtained, see Online Supplementary Materials.



Scheme 1 Covalent immobilization of pHLIP to Fe_3O_4 MNPs.

can be considered as promising materials for the development of targeting drug delivery systems.

According to the TEM data, after the immobilization of pHLIP, no significant changes in the morphology of the nanoparticles MNPs-1-pHLIP and MNPs-2-pHLIP were observed (16–20 and 30–36 nm, respectively) [Figures 1(*e*),(*f*)]. The specific magnetization (M_s) of MNPs after modification decreased significantly (17 and 24 emu g^{-1} for pHLIP-modified MNPs), probably, because of the formation of a non-magnetic silica shell on magnetic cores [Figure 1(*g*)]. According to the DLS data [Figure 1(*h*)], the average hydrodynamic diameters (D_h) of MNPs-1-APS and MNPs-2-APS were 28 and 51 nm, and the ζ-potentials were 38 and 40 mV, respectively. For MNPs-1-pHLIP and MNPs-2-pHLIP, D_h increased to 122 and 142 nm, respectively. The ζ-potential of MNPs after CTAB removal from MNP surface and the immobilization of the peptide became slightly negative (–11.9 and –9.2 mV for MNPs-1-pHLIP and MNPs-2-pHLIP, respectively) and was similar to the ζ-potential of pHLIP-containing MNPs.[11–15] The particle size distribution is unimodal. This can indicate the presence of individual particles and their small dynamic agglomerates in colloidal solutions.

The coating of MNPs with SiO_2, APS and pHLIP was confirmed by FTIR spectroscopy data. The presence of absorption bands at 570 cm^{-1} (ν Fe–O) and 1050 cm^{-1} (ν Si–O) in the IR spectra regions was attributed to a magnetite core and a silica shell in the nanoparticles, respectively. After MNP modification with pHLIP, in FTIR spectra a slight shift, change in the shape and intensity of the absorption bands relative to the bands of original peptide were observed: 2937 (ν CH_2), 1637 (C=O, amide I) and 1534 cm^{-1} (N–C=O, amide II).

The amount of pHLIP on the nanoparticle surface was calculated in this work (note that there are no data on the pHLIP content of nanomaterials in refs. 9–12, 14). The peptide amount of nanoconjugates may play a crucial role in nanoparticle–cell interactions. The number of organic molecules on the MNP surface was calculated using elemental analysis data† (wt% of carbon) by analogy with previous publications.[4,13,20] The peptide content of pHLIP-modified MNPs was calculated by subtracting the carbon mass fraction for the EMCS-modified MNPs from the carbon mass fraction of MNPs modified with pHLIP. Thus, MNPs-1-pHLIP and MNPs-2-pHLIP contained 4.62 and 4.01 μmol of peptide per gram of MNPs, respectively. It was close to pHLIP concentration in MNPs[13] (6.7 μmol g^{-1}). The difference in pHLIP contents can be related to the surface area of MNPs. As smaller MNPs possess a larger surface area, the peptide conjugation is more efficient.

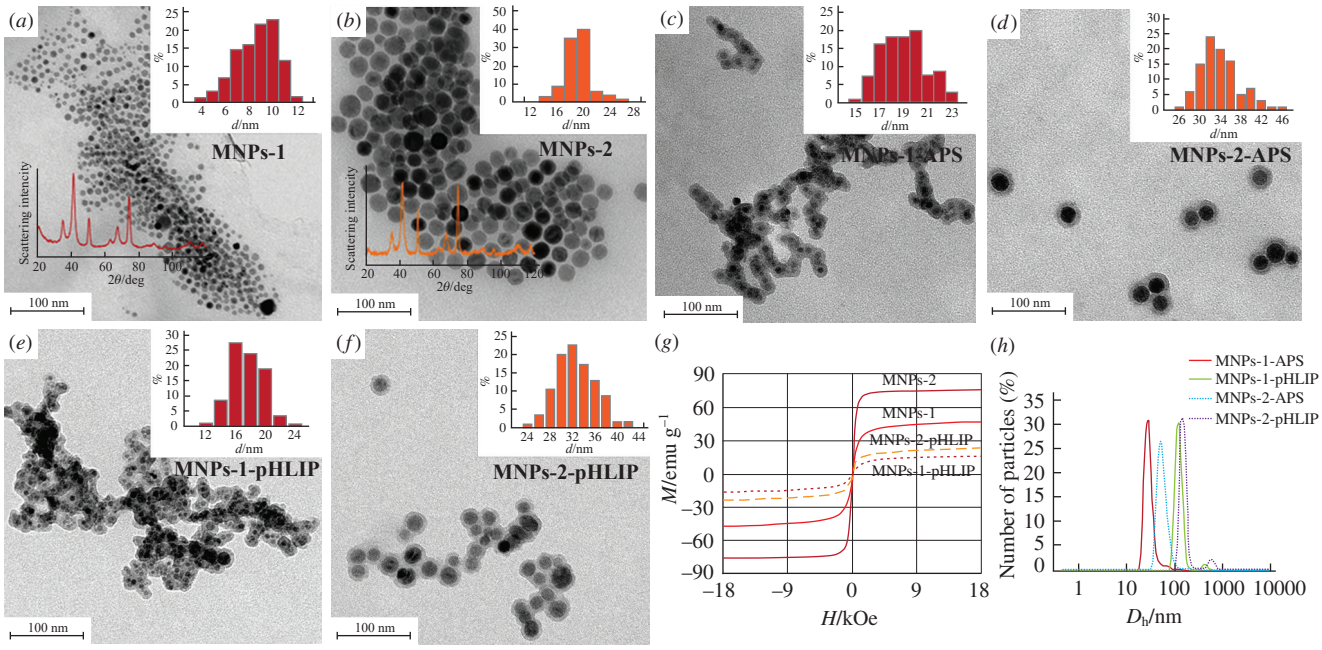


Figure 1 (*a*)–(*f*) TEM images (on the top inserts – size distribution, on the bottom inserts in *a*,*b* – XRD patterns), (*g*) magnetic hysteresis loops and (*h*) DLS data of APS- and pHLIP-modified MNPs.

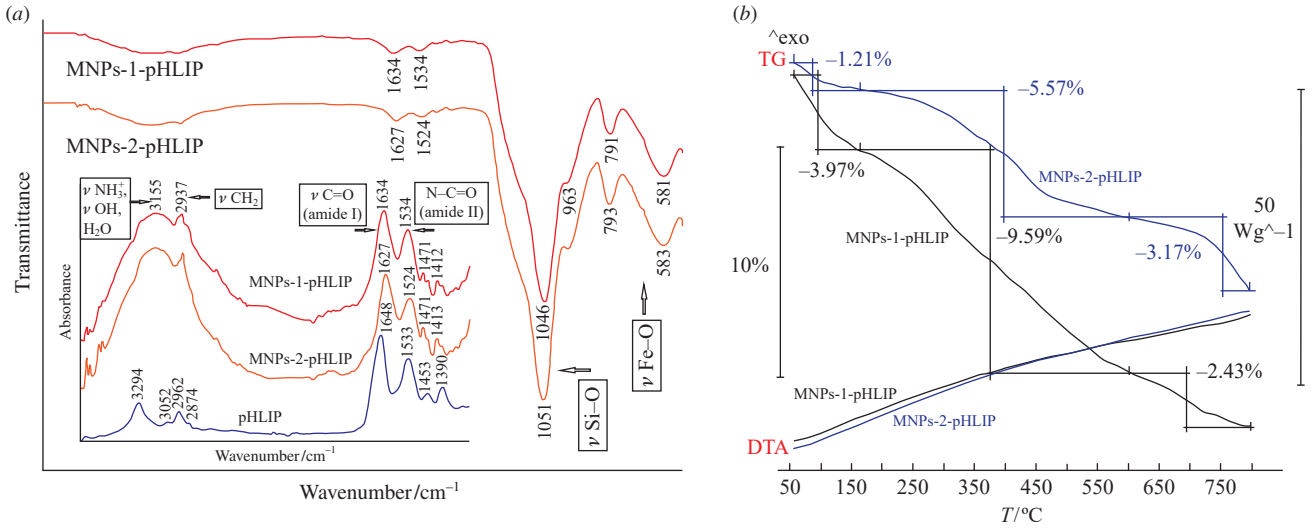


Figure 2 (*a*) FTIR spectra of pHLIP and pHLIP-modified MNPs (insert: at enlarged fragment of FTIR spectra in a range of 3800–1250 cm^{-1}). (*b*) TGA and DTA curves of modified MNPs.

According to TGA data [Figure 2(*b*)], the thermal destruction of nanoconjugates includes a stage of weight loss due to the removal of physically adsorbed water (up to 160 °C) and two-stage organic coating decomposition without significant energy effects. At the second stage (160–600 °C), the thermal decomposition of organic coating takes place. The next stage of weight loss can be explained by the carbonization of organic molecules and the oxidation of a Fe_3O_4 core to Fe_2O_3. Since the change in mass at the third stage can result not only from the decomposition of the organic coating, it is more rational to use only data on the weight loss at the second step of TG curves to characterize the synthesized nanoconjugates. MNPs-1-pHLIP contained at least 9.59 wt% organic coating (total quantity of APS, EMCS and pHLIP) and MNPs-2-pHLIP, 5.57 wt%. The peptide fractions in MNPs-1-pHLIP and MNPs-2-pHLIP calculated based on the elemental analysis data were up to 1.9 and 1.6 wt%, respectively.

According to the MTT assay[21] (Figure 3), modified MNPs at concentrations up to 100 mg dm^{-3} did not show a significant cytotoxic effect on the test cells (for details, see Online Supplementary Materials).

Thus, new nanoconjugates based on two size sort Fe_3O_4 MNPs and pHLIP have been synthesized. The surface modification of MNPs by the peptide proceeds slightly more efficiently for the smaller nanoparticles (4.62 and 4.01 μmol g^{-1} for MNPs-1-pHLIP and MNPs-2-pHLIP, respectively). The resulting materials are nontoxic and possess high magnetic and MRI contrast properties. Due to the presence of mesoporous silica coatings suitable for drug loading, they can be considered as promising materials for the further development of drug delivery systems for tumour therapy.

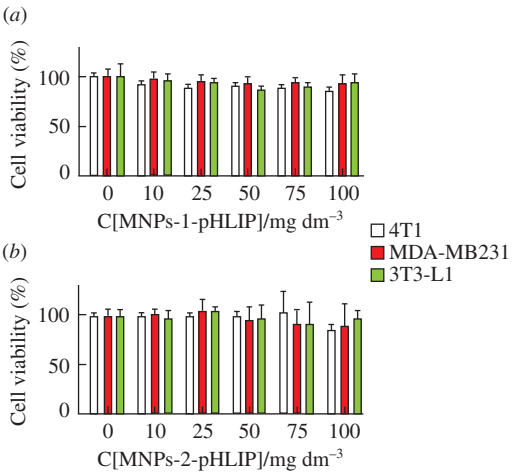


Figure 3 MTT assay of cell viability for pHLIP-modified MNPs.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.11.008.

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