Silica-coated superparamagnetic iron oxide nanoparticles: New insights into the influence of coating thickness on the particle properties and lasioglossin binding

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Abstract
Silica-coated iron oxide nanoparticles are of enormous importance in biotechnology, nanomedicine, and catalysis. The study demonstrates the significant influence of the coating thickness on the particle properties. Though slow magnetophoresis, a thinner shell leads to higher surface areas, lower isoelectric points, and higher magnetizations. However, thick layers prevent oxidation and lead to stabilization. The coating thickness influenced the binding of the cationic peptide lasioglossin, with a maximal loading of 0.23 g g⁻¹ for the smallest particles. This knowledge can be used to specifically design particles for usage with a cationic tag system in biotechnology or drug delivery with antimicrobial peptides.

Introduction
Iron oxide nanoparticles (IONs) can be applied in various fields: in biotechnology, they can be used to purify proteins, peptides, and other molecules or to immobilize enzymes. In nanomedicine, they show a huge potential as contrast agents for imaging, hyperthermia, drug delivery, or cell therapy. The IONs are also in the focus of the research field of heterogeneous and homogeneous catalysis. Especially magnetite impresses through high biocompatibility and high magnetic saturation with superparamagnetic properties. Moreover, these particles can be produced through easy, fast, and cost-efficient synthesis methods with a small particle size distribution and a high specific surface area. Though bare IONs (BIONs) tend to agglomerate uncontrollably, oxidize over time, and undergo undesired interactions. Specific coatings can prevent these negative aspects and even improve the interaction with the desired target molecule. Commonly organic coatings such as dextran, alginate, polyvinyl alcohols, or inorganic silica coatings are used to stabilize the BIONs. ION@Silica are in the focus of research for multiple applications, from protein binding to nanomedicine (SI-Table 1). Even though various synthesis methods and applications have been analyzed before, often a full characterization regarding the core and shell size, magnetophoretic properties, agglomeration behavior, and surface properties are missing (SI-Table 2). The Stöber-method is mainly used to generate a silica coating. This process uses the slow hydrolysis and fast condensation of tetraethyl orthosilicate (TEOS) to form a shell structure around the BIONs in controlled growth. The inert silica layer stabilizes the iron oxide core by shielding magnetic dipole interactions, and the negative surface charge increases the electrostatic repulsion between particles. Moreover, the negative charge facilitates the possibility of electrostatic interaction with positively charged peptides. Antimicrobial peptides are generally short, positively charged and found in various microorganisms or animals. Examples are pexiganan, omignan, hLF₁₋₁₁, and lasioglossin. Especially the short cationic peptide Lasigloso-sin-III (LL, H-Val-Asn-Trp-Lys-Leu-Gly-Lys-Ile-Ile-Lys-Val-Lys-NH₂) from the bee Lasioglossum laticeps is an exciting molecule as it could be used as affinity peptide tag in biotechnology or as an anticancer and antimicrobial drug in drug delivery. The peptide interaction with surfaces depends highly on the peptide structure and conformation and the binding conditions like pH, temperature, and salt concentration. LL is an α-helical pentadecapeptide with a hydrophilic and a hydrophobic side. The five lysine residues give a strong positive character. Guo et al. have analyzed the interaction of fumed silica nanoparticles and l-lysine. They demonstrate a strong hydrogen bonding between the amine groups of lysine and the hydroxy groups on the silica surface. Especially at high salt concentrations the potential of the amino acid to form a monolayer on the silica surface rises.

To apply silica-coated IONs in biotechnology or nanomedicine with antimicrobial peptides, it is of central interest to improve the understanding of the peptide interaction that highly depends on the particle properties. In this work, different silica-coated IONs, synthesized with varying amounts of TEOS (0.98 Eq., 1.96 Eq., 3.91 Eq., and 7.82 Eq.) are analyzed regarding their particle composition with Fourier-transform infrared spectra (FT-IR), thermogravimetric analysis (TGA),
and X-ray diffraction (XRD). The influence on the thickness of the silica layer on the surface properties is analyzed by zeta potential and Brunauer, Emmett, Teller (BET) method. The particle size distribution is determined with transmission electron microscopy (TEM) and dynamic light scattering (DLS). With this, specifically, the influence of phosphate-buffered saline (PBS) on the agglomeration behavior is analyzed. The behavior of the IONs@TEOS in a magnetic field is investigated by the superconducting quantum interference device (SQUID) magnetometer and space and time-resolved extinction profiles (STEP) technology. The particle properties are highly dependent on the silica layer, influenced by the variation of TEOS equivalents. The knowledge about these differences is used to give a better understanding of the LL binding. The adsorption is analyzed in PBS buffer at different peptide concentrations, giving insights into the cationic peptide binding on the silica surface.

Materials and methods

Synthesis of IONs@TEOS

The BIONs are synthesized by co-precipitation analogously to a previous paper by Turrina et al.[4] The silica coating is prepared by the Massart process, similar to the prescription by Zanker et al.[12] Four mmol of bare IONs are dispersed in 180 mL of a citric acid solution (AppliChem GmbH, 2.00 g, 10.4 mmol). TEOS (0.98 mL, 3.89 mmol, 0.98 Eq.; ION@TEOS) is added in amounts of tetraethyl orthosilicate (Sigma-Aldrich, ION@TEOS) and hydroxylamine hydroxide (Sigma-Aldrich). Under a neutral pH is generated by titration with 25 wt% absolute EtOH (VWR, 2.72 L), bi-distilled water (720 mL), and nitrogen atmosphere, the particle suspension is mixed with tetramethylammonium hydroxide (Sigma-Aldrich). Under a nitrogen atmosphere, the particle suspension is mixed with bi-distilled water until the silica layer on the surface properties is analyzed by zeta potential and Brunauer, Emmett, Teller (BET) method. The particle size distribution is determined with transmission electron microscopy (TEM) and dynamic light scattering (DLS). With this, specifically, the influence of phosphate-buffered saline (PBS) on the agglomeration behavior is analyzed. The behavior of the IONs@TEOS in a magnetic field is investigated by the superconducting quantum interference device (SQUID) magnetometer and space and time-resolved extinction profiles (STEP) technology. The particle properties are highly dependent on the silica layer, influenced by the variation of TEOS equivalents. The knowledge about these differences is used to give a better understanding of the LL binding. The adsorption is analyzed in PBS buffer at different peptide concentrations, giving insights into the cationic peptide binding on the silica surface.

Characterization

The SQUID magnetometer MPMS XL-7 (Quantum Design) is used to measure the magnetization of the IONs. 10 mg of a sample are glued in the middle of a small plastic tube (Fixogum) and analyzed at 300 K and a magnetic field variation between ±50 kOe. The FT-IR spectra are generated by an Alpha II (Bruker Corporation, Billerica) with a platinum attenuated total reflection module. For each sample, 3 µL (>1.00 g L⁻¹) spectra between the wavenumber range of 4000–400 cm⁻¹ (24 scans) are measured. With the software OPUS8.1, the background is subtracted by the concave rubber band method. The spectra are normalized to the vibration corresponding to magnetite (~570 cm⁻¹). The BET method is used to determine the specific surface area of the IONs with the Gemini VII, Micromeritics Instrument Corp. (software Gemini VII version 2.00) at 77 K. The nitrogen adsorption isotherms are generated between 0.05 and 0.30 mPa. The diffractometer STOE Stadi-P Diffractometer with a molybdenum source (0.7093 Å, λ = 1) is used for flatbed XRD of freeze-dried IONs (Alpha 1–2 Lplus, Christ, –60°C overnight in vacuum). To determine the silica content, TGA is applied on STA 449C Jupiter of freeze-dried IONs (50 µL aluminum oxide jar) over a temperature range between 25 and 700°C (constant for 10 min). For TEM 10 µL of a 0.02 g L⁻¹ ION solution are dried on a carbon-coated copper grid that has been glow discharged. The device JEM JEOL 1400 plus is used for creating images in ×100k–120k magnification. The images are analyzed with the software ImageJ to measure at least 90 different particles in three other areas. The Zetasizer Ultra (Malvern Panalytical) is used to determine the zeta potential (flow cell, DTS1070, Malvern Instruments) and hydrodynamic diameters by DLS (Cuvetta STD UV clear side, CARTELL S.p.a.). A 1 g L⁻¹ solution (25°C) is used for all measurements. The isoelectric point is determined via Boltzmann fit. The LUMiReader (4532–123, LUM GmbH; for STEP measurements) is used to analyze the sedimentation rate of the IONs under a magnetic field (five stacked cylindrical neodymium boron ferrite magnets (d = 12 mm, h = 2 mm, N45, Webcraft GmbH, magnetization between 29.1 and 54.4 Am² kg⁻¹). The measurements take place at the wavelength 870 nm (profile: 700, 300; interval: 0 s, 1 s, angle 0°, light factor 1.00, temperature 25°C). The data is processed with the software PSA-Wizard (SEP view™, Analysis positions: 12.3 mm, 15.0 mm, 16.9 mm, and 18.7 mm).

Peptide binding

The electrostatic binding of LL (Genscript, Netherlands) is tested in triplicates. 250 µL of a 2.00 g L⁻¹ ION@TEOS solution in 50 mM PBS at pH 7.4 is mixed (1:1) with 250 µL LL concentration (4.00 g L⁻¹, 2.00 g L⁻¹, 1.00 g L⁻¹, 0.50 g L⁻¹, 0.20 g L⁻¹, 0.10 g L⁻¹, 0.05 g L⁻¹, 0.00 g L⁻¹) in 50 mM PBS at pH 7.4. The suspensions are incubated at 25°C (1000 rpm) for 1 h. After magnetic decantation, the clear supernatant is removed and measured at 280 nm (Tecan, Magellan-Data 7). The particles are resuspended in a fresh buffer for the washing steps and incubated for 10 min (25°C, 1000 rpm). Each supernatant is analyzed, and the LL content is calculated via a calibration line.[4]

Results and discussion

Particle characterization

The silica-coated IONs are synthesized with four different equivalents of TEOS (0.98 Eq., 1.96 Eq., 3.91 Eq., and 7.82 Eq.) to generate four different thicknesses of coating. All IONs@TEOS and BIONs, for comparison, are analyzed regarding their composition, particle size, surface properties, and magnetization. The different coating properties influence
the adsorption of the cationic peptide LL. The BIONs used as base material show comparable characteristics to previous measurements of BIONs (Figs. 1 and 2). The diameter is 8.25 nm determined with TEM and 9.00 nm calculated with the Scherrer equation of XRD data (Table I, SI-Table 4), the BET surface is 103 m² g⁻¹ (Table I), and the magnetization is 64.9 emu g⁻¹ [Fig. 1(d)].

![Graphs and images](image_url)

Figure 1. Comparison of different IONs@TEOS with BIONs by (a) IR spectra normalized on the magnetite peak (~ 570 nm), (b) size distribution by number at 25°C in water for a 1 g L⁻¹ solution at pH 7, and (c) in 50 mM PBS buffer (pH 7.4), (d) magnetization fitted with LangevinMod, (e) cumulative speed distribution in contact with a magnetic field in water, and (f) TGA curves until 700°C.

![Graphs and images](image_url)

Figure 2. XRD diffractograms (a) and TEM images of (b) BIONs (100 k), (c) ION@TEOS0.98 (100 k), (d) ION@TEOS1.96 (100 k), (e) ION@TEOS3.91 (120 k), and (f) ION@TEOS7.82 (120 k).
Furthermore, via IR spectroscopy, the typical Fe–O stretching vibration at ~570 cm\(^{-1}\) is visible [Fig. 1(a)], and the XRD diffractogram shows the iron oxide reflections ([220], (311), (400), (511), and (440)) exemplary for magnetite or maghemite [Fig. 2(a)].\[14-16\]

### Particle composition

The compositions of the IONs@TEOS are determined with IR, TGA, and XRD measurements. The successful silica coating is examined with FT-IR spectroscopy [Fig. 1(a)]. In addition to the magnetite peak at 570 cm\(^{-1}\), all IONs@TEOS show the Si–O rocking vibration at 452 cm\(^{-1}\), the Si–O bending vibrations at 794 cm\(^{-1}\), the Si–OH bond at 949 cm\(^{-1}\), and the Si–O–Si stretching vibrations at 1058 cm\(^{-1}\).[17] The intensity of the typical silica peaks rises with the higher used TEOS amounts, showing the thickest coating for ION@TEOS7.82. The thermal stability based on TGA gives information about the silica amount of each particle [Fig. 1(e)]. The BIONs exhibit high thermal stability with an overall mass loss of 2.52%. This behavior is based on the melting point of magnetite, which is 1538°C.[18] To calculate the amount of bound silica, the difference between the residual weight of each particle and the weight loss during TGA is used. The IEP of BIONs is used from a previous publication by Turrina et al.[4]

### Surface properties

The surface properties are determined by zeta potential and BET measurements (Table 1, SI-Fig. 2). The specific surface area of ION@TEOS0.89 of 115 m\(^2\) g\(^{-1}\) (ION@TEOS0.98) is comparable to BIONs of around 100 m\(^2\) g\(^{-1}\).[4] Here, the effect of silica coating is comparably thin and does not influence the surface significantly. However, rising TEOS equivalents, which means thicker silica coating, lead to a decrease of the BET surface to 23.2 m\(^2\) g\(^{-1}\) (ION@TEOS7.82). This effect is similar to previous studies by Mendonca et al.[22] The particles size consequently lowers the specific surface area. This effect highly influences the amount of free reactive groups on the particle surface. The silica surface exists of siloxane groups (Si–O–Si) that can form through dissociative chemisorption silicon groups (Si–OH) that can create depending on the pH deprotonated hydroxy groups (Si–O\(^-\)).[23] Since the surface area of the smaller IONs@TEOS is bigger than the thicker ones, the ION@TEOS0.98 have the most deprotonated hydroxy groups compared to the other particles. Zeta potential measurements determine the IEP electric point. It shows comparable values for ION@TEOS7.82 and ION@TEOS3.91 at around pH 4.20, while the thinner coatings lead to lower IEP until 1.87 for ION@TEOS0.98 (Fig. 1 Table, SI Fig. 1). These values fit into the range of previously synthesized magnetic silica particles that had an IEP between 1.0 and 4.50.[24,25] The IEP of BIONs lies between 7.00 and 8.40 depending on the oxidation state from magnetite to maghemite.[4,13] The curious behavior that lower TEOS equivalents lead to lower IEP could be explained by the fact that these particles have a higher specific surface area and smaller agglomerates, with more silanol groups on the surface that can be ionized. This trend is a sign of a consistent silica coating, not showing the magnetite surface.

### Particle size distribution

The particle size is influenced by the thickness of the coating and the agglomeration behavior. Therefore, all IONs@TEOS are analyzed by TEM and DLS. TEM images of the particles

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**Table 1.** The particle’s diameter and hydrodynamic diameter measured with TEM, XRD, and DLS, the IEP, BET area, and the weight loss during TGA.

| Particles         | TEM (nm)   | XRD Scherrer (nm) | DLS (nm) | IEP      | BET (m\(^2\) g\(^{-1}\)) | TGA silica amount (%) |
|-------------------|------------|-------------------|----------|----------|---------------------------|-----------------------|
| BIONs            | 8.25 ± 0.16 | 9.00 ± 1.77       | 153 ± 21.5 | 7.98 [4] | 103 ± 0.36                |                       |
| ION@TEOS0.98     | 8.67 ± 0.36 | 8.83 ± 1.03       | 88.2 ± 22.7 | 1.87     | 115 ± 0.65                | 1.38                  |
| ION@TEOS1.96     | 12.0 ± 0.13 | 8.96 ± 0.87       | 125 ± 17.8 | 3.77     | 50.6 ± 0.77               | 6.74                  |
| ION@TEOS3.91     | 22.7 ± 0.07 | 8.99 ± 0.82       | 223 ± 4.33 | 4.20     | 27.9 ± 0.38               | 10.7                  |
| ION@TEOS7.82     | 31.3 ± 3.92 | 7.68 ± 0.91       | 329 ± 19.9 | 4.17     | 23.2 ± 0.13               | 12.3                  |

The IEP of BIONs is used from a previous publication by Turrina et al.[4]
clearly show a core–shell structure for ION@TEOS1.96 until 7.82 [Fig. 2(d–f)]. Therefore, the iron oxide core is visible as a dark crystal surrounded by lighter silica. Only for ION@TEOS0.98 [Fig. 2(c)], the silica shell is not visible because it is too thin. The size difference with a diameter of 8.67 nm to BIONs (8.25 nm) is only 0.42 nm (Fig. 1, Table 1). Higher equivalents of TEOS lead to a particle size between 12.0 and 31.3 nm. That means the ION@TEOS1.96 have a 3.75 nm, the ION@TEOS3.91 a 14.5 nm, and the ION@TEOS7.82 a 23.1 nm thick silica shell.

It is well known that the TEM diameters vary from the hydrodynamic diameters depending on the medium and pH. In water at pH 7 (around the IEP), the BIONs tend to agglomerate. Therefore, DLS measurements show a hydrodynamic diameter of around 150 nm. [4] The IONs@TEOS diameters between 88.2 and 329 nm are measured, rising with the silica thickness. If the TEM diameter is used to calculate the number of particles for an average agglomerate, this leads to a number of 18.5 BIONs. For all IONs@TEOS, this value lowers to only ~10.2 particles. Therefore, all IONs@TEOS show the same amount of stabilization toward the BIONs, indicating a comparable continuous silica surface. That different TEOS equivalents form uniform homogenous silica coatings has already been shown by Lu et al. [25] In 50 mM PBS, agglomeration behavior is very different in comparison to deionized water. As shown in other publications, BIONs possess high hydrodynamic diameters of more than one micrometer in PBS buffer. [4] This effect can be explained by free potassium and sodium ions that could interact with the free hydroxy groups on the BION surface and therefore balance the surface loading. [26] Figure 1(c) shows that this behavior is highly dependent on the particle concentration. High BIONs concentrations between 0.07 and 0.70 g L⁻¹ show a comparable diameter of around 2 μm. While for lower concentrations such as 0.05 g L⁻¹ or 0.03 g L⁻¹, the particles form smaller agglomerates, smaller than 1 μm (518 nm and 428 nm). Even for the lowest concentration, the aggregation is 2.85 times higher than in water, with an average amount of 51.9 nm. The silica coating shows an apparent stabilization leading to less agglomeration with a thicker layer. ION@TEOS1.96 and ION@TEOS3.91 show a similar behavior at the concentrations 0.3 to 0.7 g L⁻¹ with an average diameter of 1327 nm. ION@TEOS7.82 builds the smallest agglomerates at these particle concentrations of around 657 nm. This is a factor of only 2.00 times as high as in water. The lowest ION@TEOS7.82 concentrations of 0.03 g L⁻¹ even show comparable agglomeration to water with a hydrodynamic diameter of 331 nm. In summary, the particle size increases with a thicker silica coating, and the silica coating leads to the formation of smaller agglomerates in water and PBS buffer.

**Behavior in a magnetic field**

The magnetization of all particles is determined by SQUID measurements, and further magnetic sedimentation is analyzed in water by STEP technology. As XRD experiments have shown, all particles, BIONs, and IONs@TEOS, have the same iron oxide core. It is typical for magnetite to show a superparamagnetic behavior, which means that the particles have no hysteresis and are only magnetic in the existence of an external magnetic field. [27] The BIONs and IONs@TEOS show the characteristic sigmoidal curve [Fig. 1(d)]. Though, with a thinner coating, the ION@TEOS and especially the BIONs experience a higher slope from the ideal curve shape simulated by the Langevin function, indicating the magnetic field of around ±20 kOe. If magnetite oxidizes to maghemite, the paramagnetic content rises, explaining the curve variation. [28] That effect means that the increasing thickness of the silica coating leads to preventing the magnetite core from oxidation. The coating shields the magnetic core; thus, the magnetization decreases from ION@TEOS0.98 from a maximal magnetization of 66 emu g⁻¹, comparable to the BIONs, to 11.8 emu g⁻¹ for ION@TEOS7.82. In solution, the magnetic behavior is not only influenced by the magnetization. The STEP technology is used to analyze the sedimentation under magnetophoresis in water. Figure 1(e) shows the cumulative speed distribution dependent on the sedimentation speed. Contacted with a magnetic field, the BIONs sediment the fastest. The comparably high magnetization and agglomeration can explain this behavior. The ION@TEOS7.82, ION@TEOS3.91, and ION@TEOS1.96 have a similar sedimentation speed distribution, which is notably lower than the one of the BIONs. ION@TEOS0.98 is sedimenting even slower, ~291 times slower than the BIONs (SI-Table 2). The measured sedimentation speed is contrary to the magnetization of the silica-coated particles. In water, the IONs@TEOS form agglomerates with the same amount of particles, so this factor can be neglected. However, the sedimentation behavior of the BIONs and the IONs@TEOS is influenced by a significant difference: The surface is covered in silanol groups that lead to electrostatic stabilization. The particles are repelled by each other because of their dissociating behavior. [29] The amount of silanol groups rises with a higher specific surface area. This explains the slowest sedimentation of ION@TEOS0.98 in the magnetic field. All in all, the IONs@TEOS have a core–shell structure. The rising thickness of the silica coating leads to bigger particles and, therefore, lower specific surface area, less magnetization, higher IEP, and better electrostatic repulsion. The silica shell prevents the particles from oxidation and leads to a better stabilization in water and PBS buffer. All these characteristics majorly influence the applicability of the different IONs@TEOS.

**Performance of ION@TEOS for electrostatic peptide binding**

All IONs@TEOS are predominantly negatively charged at pH 7.4 regarding their IEPs. The peptide LL has five lysine residues which mainly influence its cationic character. Between its two pK values of pK₁ 2.2 and pK₂ 9.2, lysine is in the form H₂Lys⁺. [30] Previous binding experiments of LL with BIONs have shown the best electrostatic interaction in 50 mM PBS buffer (pH 7.4), due to its pH stabilization, increase in ionic strength, and phosphate ion linkage between the mainly
positive charged BION surface and the LL. As a result, 0.55 g g⁻¹ of LL was adsorbed, but each washing step led to a loss of an average of 35% of the bound peptide. For example, only 0.23 g g⁻¹ was left after three washing steps [4] Adsorption experiments of all IONs@TEOS in 50 mM PBS buffer at pH 7.4 are shown in Fig. 3. The loading of LL for all four particles rises with higher starting concentrations of LL up to a specific maximal loading. For ION@TEOS1.96, the effect is less visible due to the high standard deviation of the last point. As thicker the coating gets as sooner the maximal loading is reached. For example, the ION@TEOS0.98 [Fig. 3(a)] reach a constant LL loading from a peptide starting concentration of 0.5 g L⁻¹, while ION@TEOS7.82 [Fig. 3(d)] experiences the saturation already at 0.1 g L⁻¹ of the peptide. The maximal loading decreases from 0.28 g g⁻¹ at an equilibrium concentration of 1.70 g L⁻¹ of ION@TEOS0.98 to 0.10 g g⁻¹ at 1.89 g L⁻¹ of ION@TEOS7.82 with the increasing silica thickness. The higher specific surface area can explain this trend, and with this, the higher number of reactive silanol groups on the surface for the ION@TEOS with a thinner silica layer. The stabilizing effect of silica on the agglomeration in PBS does not outweigh this behavior. On the contrary, to the BIONs, the bound LL is not washed away continuously with every washing step. [4] The first washing step removes LL bound to the electrochemical double layer, while the peptide loading stays constant after a second washing step for all four IONs@TEOS. That behavior demonstrates that LL is bound stronger to the silica surface than to the bare iron oxide surface because of the different IEPs. Furthermore, the five lysines of LL can form hydrogen bonds with the silanol groups. [11] In Fig. 3(e) the ION@TEOS are compared after the second washing step. The highest reached peptide loading is 0.23 g g⁻¹ for ION@TEOS0.98, 0.21 g g⁻¹ for ION@TEOS1.96, 0.12 g g⁻¹ for ION@TEOS3.91, and 0.07 g g⁻¹ for ION@TEOS7.82 after washing. The loading of ION@TEOS0.98 is comparable to the one of BIONs after three washing steps. Still, it shows a more robust and more efficient electrostatic binding because the loading will stay constant with further washing steps.[4]

**Conclusion**

Four different silica-coated nanoparticles have been successfully synthesized. All ION@TEOS have a core–shell character. The characterization of the particles gave new insights into the agglomeration behavior, magnetophoresis, and surface properties depending on the diameter of the silica layer. The thickness of the silica layer influences the particle composition, size, agglomeration, magnetization, and surface properties. ION@TEOS0.98, the particles with the thinnest coating, showed the smallest particle size, highest specific surface area, lowest IEP, and highest magnetization.

Figure 3. Adsorption isotherms of the electrostatic interaction of LL and (a) ION@TEOS0.98, (b) ION@TEOS1.96, (c) ION@TEOS3.91, and (d) ION@TEOS7.82. (e) Comparison of the LL loading depending on the particle species and the peptide starting concentration.
However, these particles also experienced the slowest sedimentation speed in a magnetic field. The silica coating, in general, can prevent the oxidation of the magnetite core and leads to the formation of smaller agglomerates in water and PBS buffer. Significantly, the ION@TEOS7.82 showed the lowest aggregation in buffer due to its thick silica coating. The stabilization and protection of silica make the ION@TEOS an exciting material for multiple applications in biotechnology and nanomedicine.

All four particles bind the cationic peptide LL by strong electrostatic interactions and hydrogen bonds. The peptide loading stays constant after one washing step. The highest LL loading of 0.23 g g⁻¹ was reached with ION@TEOS0.98. The silica-coated IONs are a promising material for adsorbing cationic, antimicrobial peptides in general and specific to LL, which could also be used as a tag. These facts give them a huge potential for application in nanomedicine and biotechnology. Depending on their potential applications, the IONs@TEOS can be specifically designed and synthesized with different coating thicknesses. In combination with LL or other short positively charged drugs, IONs@TEOS could be used in cancer therapy as a magnetically controlled drug delivery system.

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Author contributions
CT: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing—original draft, visualization; AO: investigation, data curation, visualization; MM: investigation, data curation, SB: resources, funding acquisition, writing—review & editing, supervision; SS: conceptualization, investigation, data curation, writing—review & editing, supervision; MM: conceptualization, methodology, validation, functionalization, and application; CT: conceptualization, methodology, validation, functionalization, and application.

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Data availability
The datasets generated and/or analyzed during this study are available from the corresponding author on reasonable request.

Declarations
Conflict of interest
The authors declare no conflict of interest.

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Supplementary Information
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