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EVALUATING THE BINDING CAPACITY OF STREPTAVIDIN MAGNETIC PARTICLES USING HPLC: IMPLICATIONS FOR BIOTECHNOLOGY AND NANOMEDICINE

*Streptavidin-functionalized magnetic particles (SAMP) are an indispensable tool in biotechnology and biomedical research due to their unique physicochemical properties and highly specific biochemical interactions. These particles enhance biomolecule analysis, immunoassays, diagnostics, and purification processes. Streptavidin, known for its high affinity for biotin, acts as an ideal biocompatible linker, facilitating precise biochemical manipulations. This study aimed to synthesize and characterize SAMP and evaluate their binding activity toward biotin using high-performance liquid chromatography (HPLC). **Methods.** Recombinant streptavidin was produced in *E. coli*, and magnetic particles with an iron oxide core and a silica shell were functionalized with amino and carboxyl groups for streptavidin conjugation. The synthesis involved activating carboxyl groups with *N*-hydroxysuccinimide (NHS) and *N,N'*-dicyclohexylcarbodiimide (DCC), followed by covalent coupling with streptavidin. **Results.** The resulting SAMP exhibited a hydrodynamic diameter of approximately 1 μm and a polydispersity index of around 26%, indicating a uniform size distribution. A previously undescribed direct method for evaluating streptavidin-biotin interactions by HPLC was used to determine the activity of SAMP. This method proved to be reliable and overcomes the limitations of indirect methods, such as the spectrophotometric assay using 4-hydroxyazobenzene-2'-carboxylic acid (HABA) dye. The binding capacity was estimated to be approximately 0.21 μg of free biotin per mg of particles. Comparative analysis showed that SAMP prepared with in-house*

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streptavidin had higher activity (852 pmol/mg) compared to commercial streptavidin (683 pmol/mg). **Conclusios.** This research underscores the potential of SAMP for biomedical applications, including early diagnostics and personalized therapeutic approaches. The developed HPLC method offers a robust tool for assessing streptavidin-functionalized magnetic particle activity, advancing biotechnology and nanomedicine.

Keywords: streptavidin-functionalized magnetic particles, biotin-binding activity, HPLC, nanomedicine, biotechnology.

Streptavidin-functionalized magnetic microparticles are widely used in biotechnology and biomedical research due to their unique properties and specific interactions with biotin. These particles, composed of a magnetic core coated with a biocompatible layer, are easily manipulated by external magnetic fields, enabling rapid separation and concentration of biomolecules from complex biological matrices. This capability enhances experimental efficiency and reproducibility, eliminating the need for time-consuming methods like centrifugation or filtration (Safarik & Safarikova, 2004). Streptavidin, derived from *Streptomyces* bacteria, exhibits exceptionally high affinity for biotin, forming one of the strongest non-covalent interactions in nature. This interaction remains stable under various conditions, making it ideal for diverse biochemical applications (Suter, 2024). These microparticles are used in immunoassays, diagnostics, protein interaction studies, and nucleic acid purification (Zhang et al., 2007). They are particularly valuable in immunoaffinity purification and magnetic cell separation technologies (MACS) (Frenea-Robin & Marchalot, 2022; Xu et al., 2011).

In genetic research, these particles facilitate the capture and purification of biotinylated nucleic acids, improving the accuracy and purity of PCR and sequencing procedures (Paul et al., 2009; Horák et al., 2011). They also have potential in targeted drug delivery and diagnostic imaging, making them promising tools in nanomedicine (Hong, 2024). Despite their advantages, these particles face limitations such as reduced reusability after binding with biotinylated molecules and challenges in the presence of impurities (Holmberg et al., 2005). Ongoing advancements in functionalization technologies promise to expand

their applications, particularly in early diagnostics, personalized therapeutics, and fundamental biological research (Levenberg et al., 2023).

In microbiology, streptavidin-functionalized magnetic microparticles are used for efficient and specific binding of biotinylated molecules, aiding in microorganism identification, isolation, and study (Zhang et al., 2022; Shan et al., 2014). They accelerate microbial detection and enrichment processes, enhance diagnostic sensitivity, and facilitate research into microbial interactions and antibiotic resistance (Wang et al., 2020). Furthermore, they are instrumental in studying microbiomes and developing real-time biosensors for pathogen detection (Hnaiein et al., 2008).

This study **aims** to synthesize and characterize streptavidin-functionalized magnetic particles and evaluate their binding activity toward biotin using HPLC.

Materials and Methods. *Equipment used for magnetic particle synthesis and characterization.* For particle synthesis, we used an ultrasonic bath (KQ-200VDE), an overhead stirrer («Eurostar 20»), and reagents from Merck/Sigma-Aldrich. The particles were processed using an ultrasonic homogenizer (Scientz JY92-IIN), and separation was achieved using a neodymium magnet. The hydrodynamic diameter (HDD), polydispersity index (PDI), and zeta potential were determined using dynamic light scattering (DLS) with a «Litesizer™ 500» instrument (Anton Paar). HPLC was conducted on an Agilent 1260 series chromatograph equipped with a diode array absorbance detector and an Eclipse XDB-C18 (3.5 μm) 3 x 150 mm column (Agilent, USA). The mobile phase consisted of water, acetonitrile, and phosphoric acid (440 : 65 : 0.44 v/v/v) at a flow rate of 0.4 mL/min., 30 °C, and injection volume 1 μL

with needle washing. The buffers used were 10 mM phosphate buffer (PBS), 100 mM morpholinoethanesulfonic acid, pH 3.8 (MES), 100 mM carbonate-bicarbonate buffer, pH 9.6 (CB), and TRIS buffer, pH 8.0. For comparison, we used streptavidin from «Shanghai Macklin Biochemical, China» (Cat. No S817490) with an activity of 17 Unit/mg (1Unit binds 1mg D-biotin at pH 8.9) and NEBio Streptavidin Magnetic Beads (S1420, 1 μ m, binding capacity >1000 pmol/mg, «New England Biolabs», USA).

Obtaining recombinant streptavidin. The streptavidin gene was synthesized and cloned into the pET-24a plasmid vector, optimized for expression in *E. coli*. The recombinant plasmid was transformed into BL21(DE3) competent cells using heat shock transformation. Protein expression was induced by adding 0.5 mM IPTG, followed by incubation at 25 °C for 4 hours. Streptavidin, expressed as inclusion bodies, was solubilized in 6M urea and purified using Ni-NTA affinity chromatography. On-column refolding was performed by gradually decreasing the urea concentration.

SDS-PAGE analysis. The purity and activity of the purified streptavidin were assessed using SDS-PAGE. Samples were prepared in both boiled and non-boiled conditions. Boiled samples showed a monomeric streptavidin band at approximately 19 kDa, while non-boiled samples showed tetrameric streptavidin near 76 kDa (native conformation).

Synthesis of magnetic particles. The synthesis of iron oxide nanoparticles coated with silica gel (MPSiOH) has been described previously (Sereda et al., 2024). Briefly, magnetite nanoparticles (MNP) were synthesized by co-precipitation of iron (III)/(II) chlorides using concentrated ammonia solution. The resulting black precipitate was separated using a magnet, washed, and stabilized with citrate. Modification of MNP with silicon oxide was carried out in an ethanol solution of tetraethyl orthosilicate (TEOS) with the addition of a concentrated ammonia solution by homogenizing the mixture in an ultrasonic bath

and stirring at room temperature for 14 hours, washing with 0.1M HCl and water. The degree of coverage was measured by the increase in the weight of the dry residue. The density of the silicate shell can be controlled by the amount of TEOS taken into the reaction.

Functionalization of MPSiOH with amino and carboxyl groups. About 200 mg of MPSiOH in 50% ethanol were treated with hard ultrasound (65%, 6 times x 5 sec); 0.55 mL of 3-aminopropyltriethoxysilane (APTES) and 0.25 mL of 25% ammonia were added, stirred in an ultrasonic bath for 15 min and left to stir overnight at 50 °C. The particles were magnetically washed successively with ethanol and water to neutral pH and several times with tetrahydrofuran (THF). Aminated MPs (MP-NH₂) were obtained. A solution of 0.1 g of succinic anhydride (SA) in 5 mL of THF was added, the mixture was stirred first in an ultrasonic bath for 2 min, then on an orbital shaker for 12 hours. The particles were magnetically washed with THF, water, and dioxane to yield carboxylated particles (MP-COOH).

Conjugation of streptavidin with MP-COOH. MP-COOH were washed with anhydrous dioxane, a solution of 0.26 g N-hydroxysuccinimide (NHS) in 4 mL anhydrous dioxane was added, then a solution of 0.42 g N,N'-dicyclohexylcarbodiimide (DCC) in 2 mL anhydrous dioxane was added drop-wise with stirring. After stirring for 2 h, particles were magnetically washed first with dioxane, then with methanol until dicyclohexylurea was completely removed (test on a watch glass until there was no dry residue). The resulting NHS-activated MPs could be stored in anhydrous dioxane in the refrigerator, or immediately used in the next reaction. They were magnetically washed with MES buffer to remove residual dioxane, mixed with MES buffer, and a solution of streptavidin (SAV) in PBS (pH 8) was added in a ratio of about 1 mg of protein per 70 mg of particles. CB buffer was added to pH 7 and stirred at 4 °C for 12 h. The presence of unbound SAV residues was controlled by the Bradford method. MPs

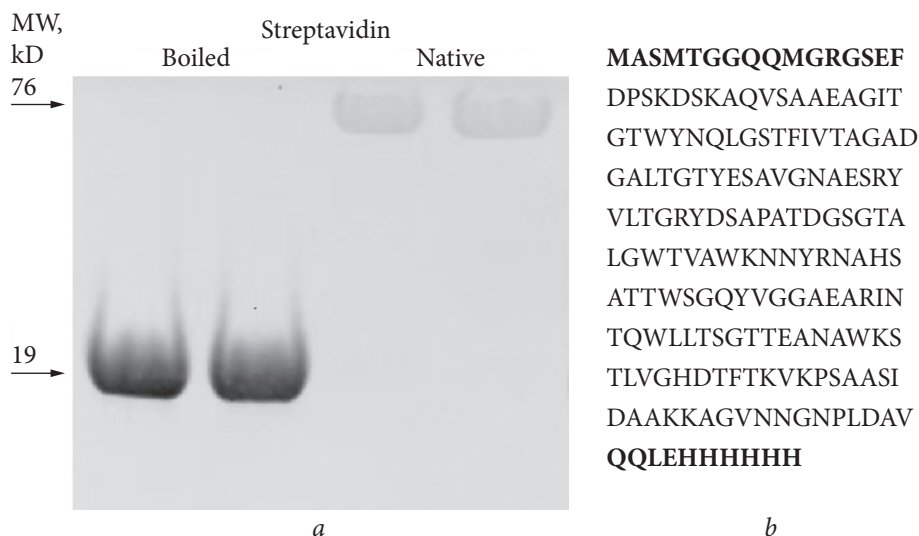


Fig. 1. Recombinant streptavidin characterization: **a)** SDS-PAGE of streptavidin monomer unit (boiled) and tetramer units (native); MW size is shown by arrows; **b)** Predicted streptavidin amino acid sequence with vector-derived amino acids and purification tag highlighted in bold

were magnetically washed several times with PBS (pH 8), kept for 4 h in a mixed buffer solution of 1 M TRIS with 0.5 M NaCl (1:1), then with 0.1 M TRIS and PBS (pH 6.8), and the volume of the MP suspension was brought to the desired concentration. The resulting streptavidin MPs (SAMP) were stored at 4 °C in PBS medium (pH 6.8) with the addition of bovine serum albumin BSA (0.1%), Tween 20 (0.05%) and sodium azide (0.02%).

Determination of SAMP activity by HPLC. The method involves quantitatively determining the concentration of D-biotin in the initial solution and after the addition of SAMP. The activity of SAMP toward biotin is determined by the reduction in the biotin peak area on the chromatograms of the test solution. Three solutions were chromatographed. Reference solution: 1 µg/mL biotin solution in PBS (pH 6.8). Test solution: 100 µL of SAMP particle suspension was decanted magnetically; 200 µL of biotin reference solution was added, kept under gentle stirring for 15 min, magnetically precipitated, and the settled liquid was filtered through a syringe filter with a pore size of 0.22 µm. Control solution: prepared

in the same way as the test solution, only aminated particles MP-NH₂ were added to the biotin reference solution. The prepared solutions were chromatographed sequentially at least 3 times, and the amount of bound biotin was calculated from the difference in the biotin area in the chromatograms of the reference and test biotin solutions. The recalculation of the activity of particles toward biotin was carried out based on the ratio: 1 µg = 4000 pmol of biotin.

Results. Protein expression and purification. Streptavidin was expressed in BL21(DE3) cells, forming inclusion bodies. The protein was solubilized in 6M urea and purified using Ni-NTA affinity chromatography. On-column refolding yielded native streptavidin, which was eluted and analyzed. The protein consists of 183 amino acids, with a molecular mass of 19.2 kDa and a theoretical isoelectric point (pI) of 6.43. SDS-PAGE analysis revealed a single band at approximately 19 kDa for boiled streptavidin samples, confirming the presence of monomeric streptavidin. Non-boiled samples showed a band near 76 kDa, indicating the formation of tetrameric streptavidin (Fig. 1 a, b).

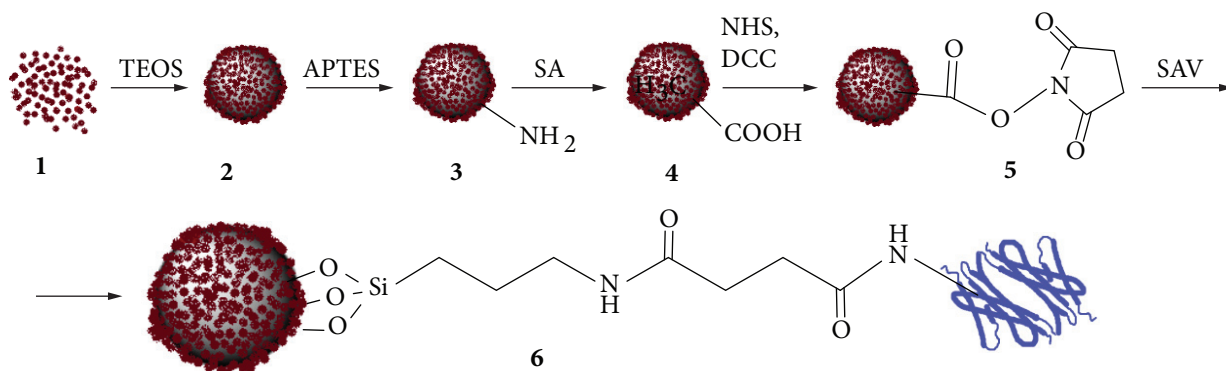


Fig. 2. SAMP synthesis scheme: 1 — Fe_3O_4 nanoparticles; 2 — $\text{Fe}_3\text{O}_4@SiO_2$ (MP-SiOH); 3 — MP-NH₂; 4 — MP-COOH; 5 — NHS-activated ester; 6 — SAMP (final product)

Synthesis and characterization of SAMP. The synthesis of MNP was carried out using a standard method involving the action of ammonia on a mixture of ferrous and ferric salts. The resulting black-brown nanoparticles had a hydrodynamic diameter of approximately 100 nm, a polydispersity index of 8-17%, and a zeta potential ranging from -45 to -48 mV.

To coat the magnetite nanoparticles with a silicate shell, we used a ratio of 6 mL TEOS per 1 g of MNP, resulting in MPSiOH particles (2, Fig. 2) with a silica content of approximately 65%. These dark brown particles were strongly attracted to a magnet, had a size of about 1 μm and a polydispersity index of around 30% (Sereda et al., 2024). Surface modification of the particles was performed sequentially by synthesizing aminated particles (3), carboxylated particles (4), obtaining NHS-activated esters from the carboxyl groups (5), and conjugating streptavidin (Fig. 2).

The results of amination and carboxylation of MP-SiOH were monitored by measuring the hydrodynamic diameter and zeta potential of the particle surface using DLS. As expected, the zeta potential became positive upon amination and reverted to negative upon the introduction of carboxyl groups. The HDD of the particles remained relatively unchanged, averaging around 1 μm (Table 1).

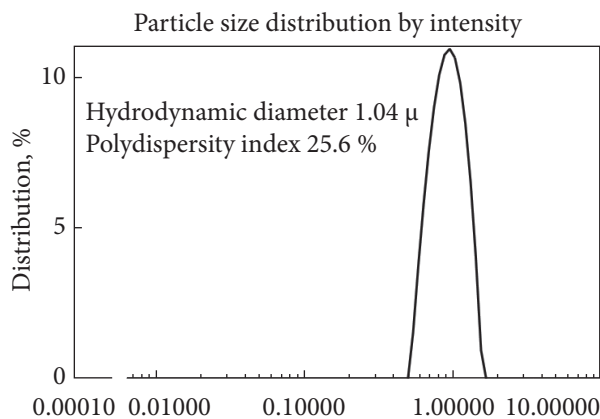


Fig. 3. DLS characteristic of SAMP

Table 1. Data of the magnetic particles study using the DLS method

MP	Zeta potential, mV	Hydrodynamic diameter (HDD), μm	Polydispersity index (PDI), %
-SiOH (2)	- 34 ÷ - 37	0.8 — 0.9	20-27
-NH ₂ (3)	+ 20 ÷ + 23	1.0 — 1.3	17-22
-COOH (4)	- 35 ÷ - 37	0.9 — 1.1	25-30

The final product, SAMP (6, Fig. 2) exhibited a dark brown color, strong magnetic attraction, a size of approximately 1 μm as determined by DLS, and a polydispersity index of around 26% (Fig. 3).

Binding activity of SAMP. The binding activity of SAMP toward biotin was determined us-

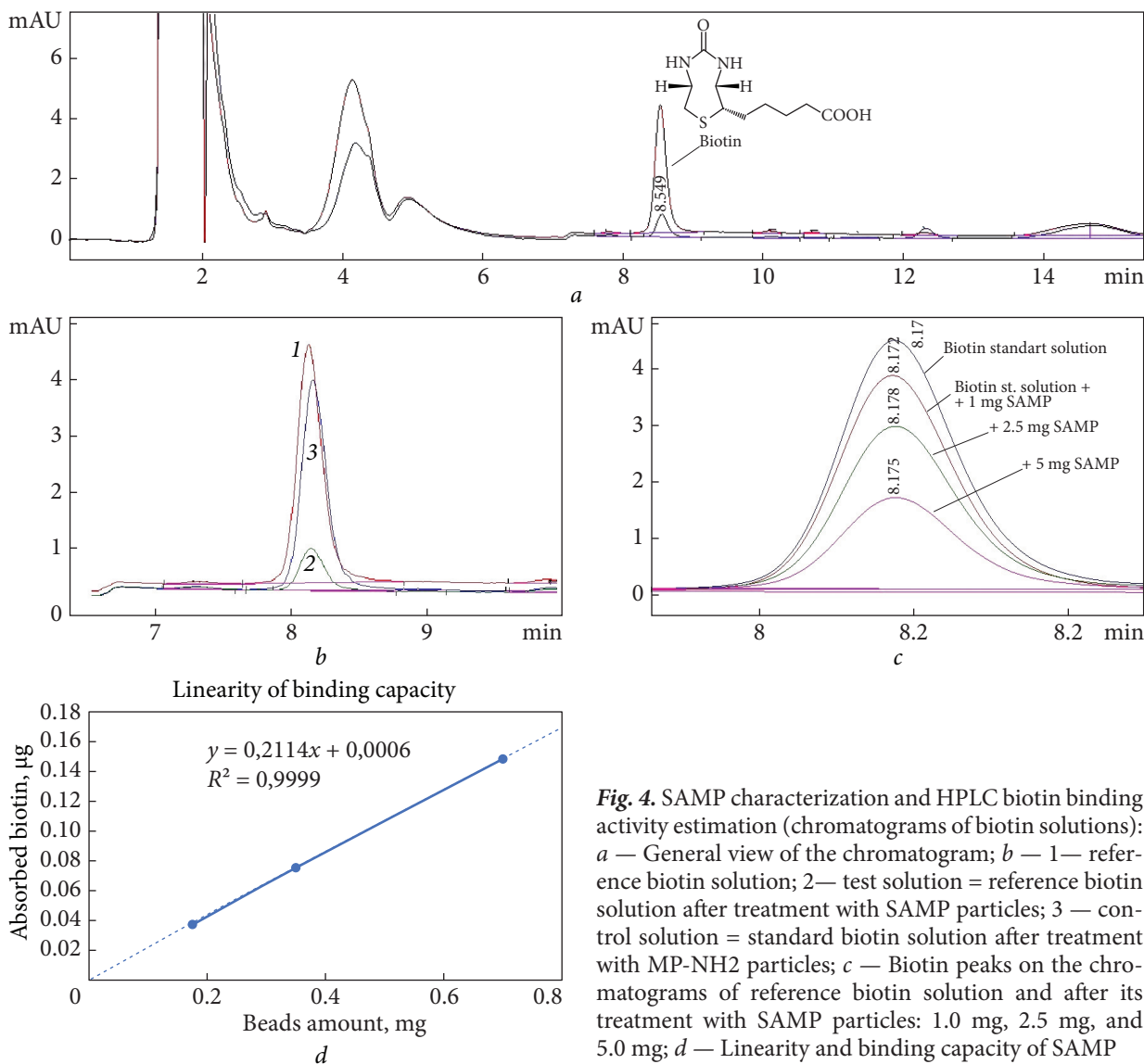


Fig. 4. SAMP characterization and HPLC biotin binding activity estimation (chromatograms of biotin solutions): *a* — General view of the chromatogram; *b* — 1— reference biotin solution; 2— test solution = reference biotin solution after treatment with SAMP particles; 3 — control solution = standard biotin solution after treatment with MP-NH2 particles; *c* — Biotin peaks on the chromatograms of reference biotin solution and after its treatment with SAMP particles: 1.0 mg, 2.5 mg, and 5.0 mg; *d* — Linearity and binding capacity of SAMP

ing HPLC. Under the optimized conditions, the biotin peak eluted at approximately 8.5 minutes and was well-separated from accompanying impurities (Fig. 4, *a*). As shown in Fig. 4, *b*, the biotin peak area decreased after the exposure to solutions containing SAMP particles. To control for non-specific binding, the biotin solution was treated with MP-NH2 particles under the same conditions. The resulting chromatograms showed only a slight reduction in the biotin peak area, within the experimental error, indicating

specific affinity interaction between SAMP and biotin, with no significant non-specific absorption by the particles.

To verify the linearity of the developed method, varying amounts of SAMP were added to the reference biotin solution, and the reduction in the biotin peak area was measured on the resulting chromatograms. In the investigated concentration range of 0.18—0.7 mg SAMP per 0.2 µg biotin, a linear relationship between the signal and concentration was observed (Fig. 4, *c*, *d*).

We applied our developed method to assess the activity of various SAMP series that we had produced, which were tested using HPLC. Comparing particles synthesized under the same conditions but using different streptavidin sources revealed the advantage of our streptavidin production method over the commercially available Macklin product (MP4, MP3, Table 2). The particles based on our home-made streptavidin exhibited a higher activity (852 pMol/mg) compared to those based on commercial streptavidin (683 pMol/mg). In cases where the same streptavidin was used, various synthesis conditions were tested, and optimal conditions were selected (MP1, MP2, MP3, Table 2).

Discussion. The successful expression and purification of recombinant streptavidin from a synthetic gene demonstrate the feasibility of using gene synthesis and plasmid cloning for protein production. The formation of inclusion bodies and subsequent solubilization in urea enabled efficient purification and refolding of the protein. SDS-PAGE results confirmed the purity and activity of streptavidin with the expected molecular mass observed for both monomeric and tetrameric forms. The ability to refold streptavidin on-column and recover it in its native tetrameric form is crucial for its biotin-binding activity and potential applications.

Core-shell particles based on magnetite and a silica oxide shell (MP-SiOH) are among the most popular in biomedical research and applications due to their good biocompatibility, low cost, and versatility. The silicate shell protects the core from oxidation and provides physico-chemical stability of the particles. Thanks to the well-developed organosilicon chemistry toolkit, the particle surface can be easily modified with various functional groups and conjugated with different ligands (Zeleňáková et al., 2024).

Based on the physical characteristics obtained by us earlier and data from other authors (Lee et al., 2018; Natarov et al., 2018), the MP-SiOH can be characterized as spherelike agglomerates about 1 μm in size of partially oxidized magnetite nanoparticles ($\text{Fe}_3\text{O}_4/\gamma\text{Fe}_2\text{O}_3$) coated with silica. In solution, the particles easily aggregate and show a size of 1–4 μm . These particles have been successfully used for nucleic acid isolation and in this work, for further surface modification.

We performed covalent coupling of SAV to the particles in 4 stages (Fig. 2). Despite the multistep process, the synthesis is easy to perform due to the magnetic properties of the particles. The stages of washing and separation are carried out by bringing a magnet to the wall of the reaction vessel and collecting the mother liquor with an automatic pipette-dispenser («one-pot

Table 2. Results of HPLC determination of the activity of particles of different series

Particles		SAV source	Conjugation method SAV + MP	Absorbed biotin	
Origin	mg/ml			pMol/mg	pMol/mL
Own MP4 #185	7	Own	NHS-ethers, own method, var.3	852.1	5965
Own MP3 #169	7	Macklin	NHS-ethers, own method, var.3	683.0	4781
Own MP1 #173_1	7	Macklin	NHS-ethers, own method, var.1	538.2	3767
Own MP2 #173_2	7	Macklin	NHS-ethers, own method, var.2	482.6	3378
NEBio Streptavidin Magnetic Beads	4	—	—	1208.6	4834

synthesis»). Amination to obtain aminated particles MP-NH₂ (Fig. 2, 3) was carried out using APTES on the silanol groups of the particle shell in a 50% alcohol solution, rather than in water as performed by the authors in (Schiestel et al., 2004). In our opinion, under these conditions, amination proceeds more efficiently due to better homogenization of the suspension. In the next stage of acylation of the amino groups with succinic anhydride (SA), the particle surface is functionalized with carboxyl groups. It was noted that the introduction of amino and carboxyl groups into the silicate shell of MP-SiOH increases the stability of the particles against aggregation. Data obtained from HDD determination by DLS over time showed more stable results for MP-NH₂ and MP-COOH than for the original MP-SiOH.

As a result of sequential amination and carboxylation of MP-SiOH with APTES and succinic anhydride, carboxyl groups are removed from the particle surface by a distance of an 8-atom spacer arm (Fig. 2), making them more accessible for ligand conjugation due to reduced steric hindrance by the matrix. The resulting MP-COOH (Fig. 2, 4) is a convenient product for attaching various molecules by standard bioconjugation methods through the carrier's carboxyl group and the protein's amino group. Conjugation can be performed either in one step using the «Single-Step EDC Coupling Protocol» (Hermanson, 2013), or, for example, through the formation of intermediate NHS esters. The reaction of N-hydroxysuccinimide esters with a primary amino group forming a covalent bond is one of the most common methods for bioconjugation. The reaction proceeds under physiological conditions and with high specificity (Wong & Jameson, 2011). We obtained particles with an activated ester group (Fig. 2, 5) through reacting them with NHS and water-insoluble DCC in anhydrous medium and, after purification, introduced them into the SAV coupling reaction. We did not find references to such a method of obtaining NHS esters of MNPs in the literature. The advantages of this method,

in our opinion, are i) the use of cheap DCC, ii) the reaction is carried out in an anhydrous medium, which guarantees complete esterification of carboxyl groups on the particle surface, and iii) the obtained activated ester can be stored in anhydrous dioxane in the cold and used as needed.

There are many methods for quantitatively assessing the interaction of SAV with biotin, and streptavidin MP in particular (Dai et al., 2014). The most significant is the spectrophotometric method, based on the interaction of SAV with the dye HABA to form a yellow-colored complex. As a result of the affinity interaction of added biotin (or biotinylated molecules) with SAV, the dye is displaced, and the decrease in color intensity is used to evaluate the biotin-binding activity (Green, 1970). The disadvantage of this method is that it is an indirect method of analysis. HABA has a dissociation constant of 2.5 times that of biotin (Mukherjee et al., 2024) and may not fill all the binding sites of SAV, thus distorting the results.

Methods involving the use of fluorescent labels (biotin-4-fluorescein) and biotinylated enzymes (such as peroxidase, phosphatase, or galactosidase) require special fluorescent readers (Mukherjee et al., 2024), and the enzyme activity is greatly influenced by many factors, such as pH, temperature, and reaction time, which reduces the accuracy of the analysis (Dorgan et al., 1999). To determine the binding constant (K_A) of SAMP-biotin, other approaches were used, such as capillary electrophoresis, isothermal titration calorimetry, surface plasmon resonance, surface-enhanced Raman scattering, etc., however, these methods are complex and are of narrower scientific interest (Dai et al., 2014; Bouzas-Ramos et al., 2018).

The advantage of our proposed method is that, unlike the others, it allows the determination of the interaction of immobilized SAV with biotin molecules, rather than biotin modified with other molecules, which can influence the formation of this complex. For example, as shown in (Dorgan

et al., 1999), using large molecules of biotinylated probe to assess biotin-binding activity gives lower biotin binding values due to steric hindrance.

Biotin is a B-group vitamin, so many methods, including chromatographic ones, have been developed for its quantitative determination in biological samples (Carling & Turner, 2019). Biotin has no chromophore groups and does not absorb UV light at wavelengths above 210 nm. Nevertheless, modern HPLC methods with UV detection allow accurate measurements at 200 nm, for example, in multicomponent vitamin preparations (Ekpe & Hazen, 1998). The use of the HPLC method for evaluating biotin-SAV interactions was not found in the literature, but our studies have shown the successful application of this approach to assess SAMP activity. The method has good reproducibility and linearity of the peak area dependence on concentration (Fig. 4d). To ensure specificity, it is necessary to select appropriate chromatographic conditions for separating the biotin peak from accompanying peaks in each specific case (chromatographic column, buffers, solvents, etc.). The disadvantage of the method is its low sensitivity due to the low absorption of biotin at 200 nm, but this is compensated by the fact that the quantitative assessment is based on the decrease in the peak area of a standard biotin solution added in excess during incubation with SAV particles. It should be noted that chromatographic-mass spectrometry is becoming a routine method of analysis, and its application significantly increases the sensitivity of the proposed method. For example, in (Weimann et al., 2021), the authors quantified

biotin in serum or plasma down to 3 nmol/L. In our opinion, the proposed method, in principle, can be extended to assess the interaction of biotin not only with streptavidin bound to the solid phase but also with free streptavidin in a homogeneous medium, if appropriate chromatographic separation conditions are selected. Separating low-molecular-mass biotin from the streptavidin-biotin complex, given the capabilities of HPLC, seems to be a simple task.

The biotin-binding activity of synthesized particles depends on numerous factors, including particle size, density, and conjugation efficiency. Our HPLC method is a valuable tool for assessing streptavidin activity and optimizing experimental conditions. Testing commercial streptavidin MPs (NEBio, Table 2) revealed high binding capacity values per mg, highlighting the importance of considering specific mass and other particle characteristics for accurate comparisons. For example, light particles with the same mass and diameter have a larger total surface area and, accordingly, a higher activity than heavier magnetic particles. The HPLC method we have developed allows for studying the patterns of affinity sorption of biotin.

Conclusions. This research underscores the potential of SAMP for biomedical applications, including early diagnostics and personalized therapeutic approaches. The developed HPLC method offers a robust tool for assessing streptavidin-functionalized magnetic particle activity, advancing biotechnology and nanomedicine.

Conflicts of Interest. The authors declare that there are no conflicts of interest.

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ОЦІНКА ЗВ'ЯЗУЮЧОЇ ЗДАТНОСТІ СТРЕПТАВІДИНОВИХ МАГНІТНИХ ЧАСТИНОК ЗА ДОПОМОГОЮ HPLC: ЗНАЧЕННЯ ДЛЯ БІОТЕХНОЛОГІЇ ТА НАНОМЕДИЦИНИ

Функціоналізовані стрептавідином магнітні частинки (SAMP) є незамінним інструментом у біотехнології та біомедичних дослідженнях завдяки їхнім унікальним фізико-хімічним властивостям та високо специфічним біохімічним взаємодіям. Ці частинки покращують аналіз біомолекул, імуноаналізи, діагностику та процеси очищення. Стрептавідин, відомий своєю високою афінністю до біотину, діє як ідеальний біосумісний ланцюг, що сприяє точним біохімічним маніпуляціям. **Метою** цього дослідження було синтезувати та охарактеризувати SAMP і оцінити їхню зв'язуючу активність до біотину за допомогою вискоефективної рідинної хроматографії (HPLC). **Методи.** Рекombінантний стрептавідин був отриманий в *E. coli*, а магнітні частинки з ядром оксиду заліза та силікагелевою оболонкою були функціоналізовані аміно- та карбоксильними групами для кон'югації стрептавідину. Синтез включав активацію карбоксильних груп N-гідроксисукцинімідом (NHS) та N,N'-діциклогексилкарбодіімідом (DCC), яка забезпечувала ковалентне зв'язування зі стрептавідином. **Результати.** Отримані SAMP мали гідродинамічний діаметр близько 1 мкм та індекс полідисперсності близько 26%, що свідчить про однорідний розподіл за розміром. Для визначення активності SAMP був застосований неописаний раніше прямий метод оцінки взаємодії стрептавідин-біотин за допомогою HPLC, який виявився надійним і дозволив усунути обмеження непрямих методів, таких як спектрофотометричний тест з використанням барвника 4-гідроксіязобензен-2'-карбонової кислоти (НАВА). Зв'язуюча здатність була оцінена на рівні близько 0,21 μg вільного біотину на 1 мг частинок. Порівняльний аналіз показав, що SAMP, виготовлені з власним стрептавідином, мали більшу активність (852 пМоль/мг) порівняно з комерційним стрептавідином (683 пМоль/мг). **Висновки.** Це дослідження підкреслює потенціал SAMP для біомедичних застосувань, включаючи ранню діагностику та персоналізовані терапевтичні підходи. Розроблений авторами метод HPLC пропонує надійний інструмент для оцінки активності магнітних частинок, функціоналізованих стрептавідином, сприяючи розвитку біотехнології та наномедицини.

Ключові слова: функціоналізовані стрептавідином магнітні частинки, зв'язуюча активність біотину, HPLC, наномедицина, біотехнологія.