Original Article

Avicenna Journal of Medical Biotechnology
Volume 18, Issue 1, 39-47
https://doi.org/10.18502/ajmb.v18i1.21044

Check for updates

Step-by-Step Preparation of Immobilized Recombinant *Staphylococcus aureus* Protein A (SpA): A Versatile Tool for Efficient Antibody Purification

Mahboobeh Nazari [1*], Zahra Salimzadeh [1], Amir-Hassan Zarnani [2,3], Roya Ghods [4] and Ramin Ghahremanzadeh [5]

1. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
2. Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
4. Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
5. Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

*** Corresponding author:**
Mahboobeh Nazari, Ph.D.,
Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
Tel: +98 21 22432020
Fax: +98 21 22432021
E-mail:
Ma.Nazari@avecinna.ac.ir,
nazari1980azar@yahoo.com
Received: 18 Aug 2025
Accepted: 1 Nov 2025

Abstract

Background: This study highlights the significance of using *Staphylococcus aureus* (*S. aureus*) Protein A (SpA) for antibody purification.

Methods: The gene encoding Protein A was isolated from *S. aureus* and cloned into the pET-28a vector. Following transformation into *Escherichia coli* (*E. coli)* BL21, recombinant Protein A was expressed and purified using a nickel affinity resin.

Results: The recombinant expression of protein A produced a yield of 50 *mg/L*, indicating a substantial production efficiency. The characterization of the recombinant protein through various ELISA tests confirmed its binding affinity to antibodies. Subsequently, the recombinant Protein A was immobilized on two different matrices: activated Sepharose 4B and amine-functionalized magnetic nanoparticles.

Conclusion: The immobilization on magnetic nanoparticles presents a versatile alternative, offering the advantages of rapid separation, high surface area, and ease of handling. Magnetic nanoparticles facilitate automation and reduce processing time, making them particularly attractive for clinical and industrial applications. These immobilized forms were used to efficiently purify serum IgG, demonstrating the potential of Protein A as an effective tool for antibody isolation in biotechnological applications.

Keywords: Enzyme-linked immuno sorbent assay (ELISA), Magnetite nanoparticles, Recombinant proteins, *Staphylococcus aureus protein A (SpA)*
To cite this article: Nazari M, Salimzadeh Z, Zarnani AH, Ghods R, Ghahremanzadeh R. Step-by-Step Preparation of Immobilized Recombinant *Staphylococcus aureus* Protein A (SpA): A Versatile Tool for Efficient Antibody Purification. Avicenna J Med Biotech 2026;18(1):39-47.

Introduction

Affinity adsorption is highly esteemed in bioseparation due to its selectivity for isolating native and recombinant proteins, enhancing yield when applied early in downstream processing [1,2]. Affinity chromatography exploits specific, reversible, non-covalent interactions between biomolecules and ligands, usually with association constants of 10^3–10^8 M^{-1}, enabling purification from dilute crude extracts and facilitating analysis of protein interactions [3,4]. Since its development in 1910, affinity chromatography has evolved to include a variety of ligands such as coenzymes, lectins, dyes, and biomimetic molecules, enabling the selective targeting of both natural "biospecific" and synthetic "pseudobiospecific" ligands [5].

Antibody production has significantly evolved *via* hybridoma technology, molecular engineering, and display platforms, resulting in widespread applications in diagnostics and immune-affinity chromatography [6-8]. Purification of antibodies is primarily achieved through Affinity chromatography, utilizing specific ligand–antibody interactions, with process optimization involving parameters like pH, ionic strength, and temperature [9-12].

Affinity chromatography relies on preparing an immobilized ligand on a suitable matrix—such as agarose, synthetic polymers, or inorganic supports—using coupling chemistries (*e.g.*, CNBr, carbodiimide) [13]. Magnetic beads and affinity membranes are modern

alternatives offering rapid, high-throughput, and gentle separation methods advantageous for proteomics and genomic workflows [13-15]. The choice of ligand, matrix, and conditions is critical for maximizing yield, purity, and reproducibility in antibody and protein purification [9].

Bacterial proteins with high affinity for antibodies, notably Staphylococcal protein A (SpA) and Streptococcal protein G (SpG), are widely utilized in antibody detection and purification [16-18]. These ligands, derived from bacterial cell walls, enable the capture of antibodies from multiple species, with affinity varying among subclasses. Protein A comprises five Ig-binding domains (designated E, D, A, B, and C), and structural analyses have demonstrated that it binds the Fc region of IgG at the interface between the CH2 and CH3 domains. Additionally, protein A can interact with the variable heavy chain region, specifically between CDR2 and CDR3 [19,20]. Protein G, derived from groups C and G Streptococcus, exhibits strong binding to the Fc portion of IgG and has been reported to have low-affinity interactions with the CH1 domain of the Fab region [18,21]. When selecting between these ligands, protein A is frequently preferred due to its minimal cross-reactivity with contaminants such as albumin, α_2-macroglobulin, and kinogen, which are known to bind protein G and potentially reduce purification efficiency [22].

In this study, the sequences of full-length of *Staphylococcus aureus* protein A (SpA) was isolated from the genomic DNA of *Staphylococcus aureus* (*S. aureus*). The corresponding gene was cloned into suitable expression vectors. The purified proteins were immobilized onto two platforms: Cyanogen bromide (CNBr)-activated Sepharose™ 4B and magnetic particle beads. The purification of immunoglobulin was then evaluated using various methods.

Materials and Methods

Reagents

The following reagents and kits were used. Isopropyl-β-D-thiogalactopyranoside (IPTG), kanamycin (Roche); T4 DNA ligase, shrimp alkaline phosphatase, restriction enzymes, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS) linker (Thermo Fisher Scientific) (a heterobifunctional, water-soluble, zero-length carbodiimide crosslinker that is used to couple carboxyl groups to primary amines), Taq DNA polymerase, plasmid extraction kit, gel purification kit, PCR purification (QIAGEN Inc), Ni Sepharose™ High Performance histidine-tagged protein purification resin (Cytiva), CNBr-Activated Sepharose™ 4B (Cytiva) and pET-28a vector (Novagen), HRP-conjugated anti-histidine antibody (Roche). All experiments are repeated at least 3 times and spectroscopic results are typical experimental data.

Cloning of the staphylococcal protein A gene

The genomic DNA of *S. aureus* (PTCC1431) was extracted following the Sambrook protocol [23]. Primers designed based on the *S. aureus* protein A (SpA) gene sequence (accession no. M18264.1) were used to amplify the protein A gene.

Two primers were used: a forward primer with an *Nhe*I restriction site (5′-AACATAGCTAGCGCGCA-ACACGATGAAGCTCAAC-3′) and a reverse primer with a *Bam*HI restriction site (5′-AGGATCCTTATGT-CATTTACTGTATCACCAGGTTTAACGAC-3′), with restriction sites underlined. Genomic DNA served as the template in PCR amplification, performed with Taq DNA polymerase under the following conditions: initial denaturation at 94°*C* for 5 *min*, 30 cycles of 94°*C* for 1 *min*, 55°*C* for 30 *s*, 72°*C* for 1 *min*, and a final extension at 72°*C* for 5 *min*.

The PCR product was purified to remove excess primers. Digestion with *Nhe*I/ *Bam*HI enzymes generated compatible ends, allowing ligation into similarly digested and dephosphorylated pET28a (+) vector resulting in recombinant protein A tagged with a His_6 at the N-terminus. The gene's sequence was confirmed via sequencing. The ligation mixture was transformed into *Escherichia coli* BL21 (DE3) competent cells *via* electroporation. All procedures—plasmid preparation, PCR, restriction digestion, gel electrophoresis, ligation, and transformation—were conducted following the protocols described by Sambrook and Russell [23].

Sequencing pET28a (+) vectors containing *Staphylococcus aureus* protein-A sequenced using an automatic sequencer (MWG) by the T7 promoter and T7 terminator universal primers.

Protein expression and purification and western blot analysis

Five *ml* of Terrific Broth (TB) Medium containing 50 *mg/ml* kanamycin with a fresh bacterial colony harboring the expression plasmid was inoculated and grown at 37°*C* overnight. Then 200 *ml* of medium with 500 *ml* overnight cultures was inoculated and grown at 37°*C* with vigorous shaking until the OD_{600} reached 0.9. Then, IPTG was added to the solution to a final concentration of 0.5 *mM*, and the mixture incubated at 22°*C* overnight with vigorous shaking. The cells were harvested by centrifugation at 5000 *g* for 15 *min* [24]. The cell pellet was resuspended in lysis buffer [50 *mM* Tris-HCl, 300 *mM* NaCl, 10 *mM* imidazole, and 1 *mM* PMSF (add fresh) (pH=7.80)]. Purification of His_6-tagged fusion protein was performed with the Ni-NTA Sepharose™ High Performance histidine-tagged protein purification resin as described by the manufacturer (cytiva). Protein was eluted in buffer containing (50 *mM* Tris-HCl, 300 *mM* NaCl, and 300 *mM* imidazole, pH=7.80). Protein concentrations were measured using the Bradford assay [25], and purity was evaluated by SDS-PAGE on a 12% gel. Proteins were transferred to nitrocellulose membranes, blocked with 5% skimmed milk in Tris-buffered saline with Tween-20 (TBST), and probed with HRP-conjugated anti-histidine antibody (1:50,000 dilution). Detection was performed

using ECL reagents followed by exposure to autoradiography film [26].

Characterization of purified pr A with Enzyme-Linked ImmunoSorbent Assay (ELISA)

Direct ELISA: Recombinant SpA was coated with 5 *µg/ml* on ELISA strip and HRP- Sheep Anti mouse Ig and F(ab)2 HRP Sheep Anti mouse Ig used as immune conjugate.

Indirect ELISA: Recombinant SpA was coated with 5 *µg/ml* on ELISA strip, then human IgM with affinity to protein A used as second layer and F(ab)2 HRP Sheep Anti mouse Ig used as immune conjugate.

Titration of conjugates on commercial pr A and recombinant pr A produced in this study: In the ELISA assays, two form of Protein A (commercial pr A, sigma P6031, and recombinant SpA produced in this study) were immobilized onto the wells of the ELISA strip at a concentration of 5 *µg/ml*. Immunoconjugates, specifically HRP-conjugated sheep anti-mouse IgG and F(ab')2 HRP sheep anti-mouse IgG, were titrated over a dilution range from 1/250 to 1/32,000.

In a separate assay, recombinant SpA (5 *µg/ml*) was again coated onto the strip, with mouse IgG introduced as the second-layer at concentrations ranging from 2 to 15 *µg/ml*. The detection was carried out using F(ab')2 HRP sheep anti-mouse IgG as the conjugate.

Investigation of recombinant SpA folding with ELISA: In order to assess the correct folding of recombinant SpA the binding ability of protein a to Fab domain of of IgM was evaluated by ELISA. In this assay recombinant SpA (5 *µg/ml*) was coated onto the strip, with human IgM introduced as the second. The detection was carried out using F(ab')2 HRP sheep anti-mouse IgG as the conjugate.

During all assays, non-specific bindings were minimized by washing with phosphate-buffered saline containing 0.05% Tween-20. To prevent non-specific adsorption, blocking was performed using 5% skim milk in Phosphate-Buffered Saline (PBS) buffer. The enzymatic reaction was developed with TMB (3,3',5,5'-Tetramethylbenzidine) substrate, and the reaction was terminated with 20% sulfuric acid. All experiments were conducted in triplicate. The optical density of each well was measured at 450 *nm* using a Biotek microplate reader.

Preparation of Affinity chromatography platforms

Preparation of recombinant SpA immobilized on CNBr-activated sepharose 4B: The selection of the immobilization medium is critical for the efficiency of the purification process. The media are prepared by covalently attaching the chosen ligand to the affinity support. In this study, two types of matrices were employed: a commercial cyanogen bromide-activated support and magnetite nanoparticle ($MnFe_2O_4@SiO_2@NH_2$) that was previously synthesized in our laboratory [27]. The purified proteins were subjected to dialysis against coupling buffer (0.1 *M* $NaHCO_3$, pH=8.3, containing 0.5 *M* NaCl) using a 12 *kDa* molecular weight cutoff dialysis membrane (Sigma).

One *g* of CNBr-activated Sepharose 4B matrix powder was dissolved in 200 *ml* of 1 *mM* HCl, with supernatant removed by mild centrifugation between successive additions. The activated Sepharose was then transferred into coupling buffer (0.1 *M* $NaHCO_3$, pH=8.3, containing 0.5 *M* NaCl). Recombinant SpA (5 to 20 *mg*) was added to the support, and the mixture was thoroughly mixed and stirred at room temperature for 2-4 *hr* to facilitate covalent immobilization. Subsequently, the mixture was transferred to an empty column and washed with 50 *ml* of 0.1 *M* Tris-HCl buffer. The support was further washed through three alternating pH cycles: each cycle included a wash with 0.1 *M* acetic acid/sodium acetate buffer, pH=4.0, containing 0.5 *M* NaCl, followed by a wash with 0.1 *M* Tris-HCl buffer, pH=8.0, with 0.5 *M* NaCl. Finally, the resin containing the immobilized ligand was equilibrated with 0.15 *M* sodium phosphate buffer, pH=7.2, at room temperature for use in subsequent purification procedures.

Preparation of recombinant SpA immobilized on magnetic beads: The functionalized amino nanoparticle from previous study $MnFe_2O_4@SiO_2@NH_2$ was dispersed in 10 *ml* of ethanol using ultrasonic treatment to achieve a uniform suspension [27]. The resulting homogeneous dispersion was transferred to a round-bottom flask. Subsequently, 26 *mM* of EDC and 10 *mM* of NHS, were separately dissolved in 0.1 *M* MES buffer adjusted to pH=6.3. These solutions were combined with 1 *ml* of magnetic beads at a concentration of 5 *mg/ml* and incubated for 2 *hr* at room temperature under continuous agitation. Following incubation, the particles were isolated using a magnetic field, washed twice with phosphate-buffered saline (PBS, pH=7.2), and then incubated with 5 *mg/ml* of recombinant SpA. The mixture was maintained at room temperature with constant mixing for 3 *hr*. The functionalized nanoparticles with immobilized SpA were then collected magnetically. The immobilization process was terminated by adding glycine and allowing the reaction to proceed for an additional 30 *min*. The concentrations of protein A in the supernatant before and after the coupling reaction were measured to determine the efficiency of labeling, which was expressed as the ratio of unbound (free) to bound SpA [26].

Purification of IgG from human plasma with immobilized recombinant SpA

Human plasma obtained from anticoagulated blood was subjected to centrifugation at 200 *g* for 10 *min*. A 2.5 *ml* aliquot was then diluted fivefold with 0.15 *M* sodium phosphate buffer at pH=7.2. This diluted sample was applied to affinity beads, and subsequent washing with the same phosphate buffer continued until the absorbance at 280 *nm* was reduced to below 0.002, indicating minimal non-specific protein binding. Proteins bound to the beads were eluted using specific

elution buffers. The purity of the eluted proteins was assessed using SDS-PAGE with a 12.5% (w/v) polyacrylamide gel stained to visualize protein bands. The separation was carried out using an automated FPLC system equipped with a fraction collector (Pharmacia, Sweden). Collected fractions were dialyzed against 0.01 M Tris-HCl buffer at pH=7.0 and stored at 0°*C* until further analysis.

Determination of the apparent capacity of immobilized particles for IgG and stability of ligand in 1 M NaOH

A resin conjugated with recombinant SpA was equilibrated using PBS at pH=7.2. A solution of pure human IgG (4 *mg/ml*; 1.5 *ml*) was then introduced to the resin. The flow-through was collected and continuously recirculated through the resin until the absorbance at 280 *nm* reached a stable level. Subsequently, the IgG bound to the resin was eluted using a 0.025 *M* citric acid buffer at pH=2.4 (10 *ml*). To evaluate the stability and reusability of the SpA-immobilized resin post-IgG purification, the resin was treated with 1 *M* NaOH and incubated for 18 *hr* at 22°*C*. Subsequently, the resin was re-equilibrated with 0.15 *M* sodium phosphate buffer at pH=7.2. The IgG binding capacity was then reassessed to determine any changes resulting from the alkaline treatment.

Results

Construction of expression plasmid pET28a-Staphylococcus aureus Protein A (SpA)

A gene fragment encoding *S. aureus* Protein A (SpA) was amplified *via* Polymerase Chain Reaction (PCR) utilizing specific primers and genomic DNA from *S. aureus* as the template. The resulting DNA segment, encompassing the targeted gene, was subsequently inserted into the pET28a (+) plasmid vector for cloning purposes. The *SpA* gene spans 1146 base pairs, commencing with an ATG start codon and ending with a TAA stop codon (Figure 1).

Staphylococcus aureus protein A (SpA) expression, purification and characterization with WB and ELISAs

The *SpA* gene fragment was highly expressed from the pET28a-SpA plasmid construct. The expressed protein was fused with an MGSSHHHHHHSSGLVP RGSHM affinity tag, enabling straightforward purification via a single-step His-tag affinity chromatography process. Molecular weight estimation through SDS-PAGE analysis indicated a size of approximately 42 *kDa* (Figure 2A). The SDS-PAGE profile displayed a single band at roughly 42 *kDa*, which aligned with the theoretical molecular weight derived from the amino acid sequence. The western blot analysis with anti-his

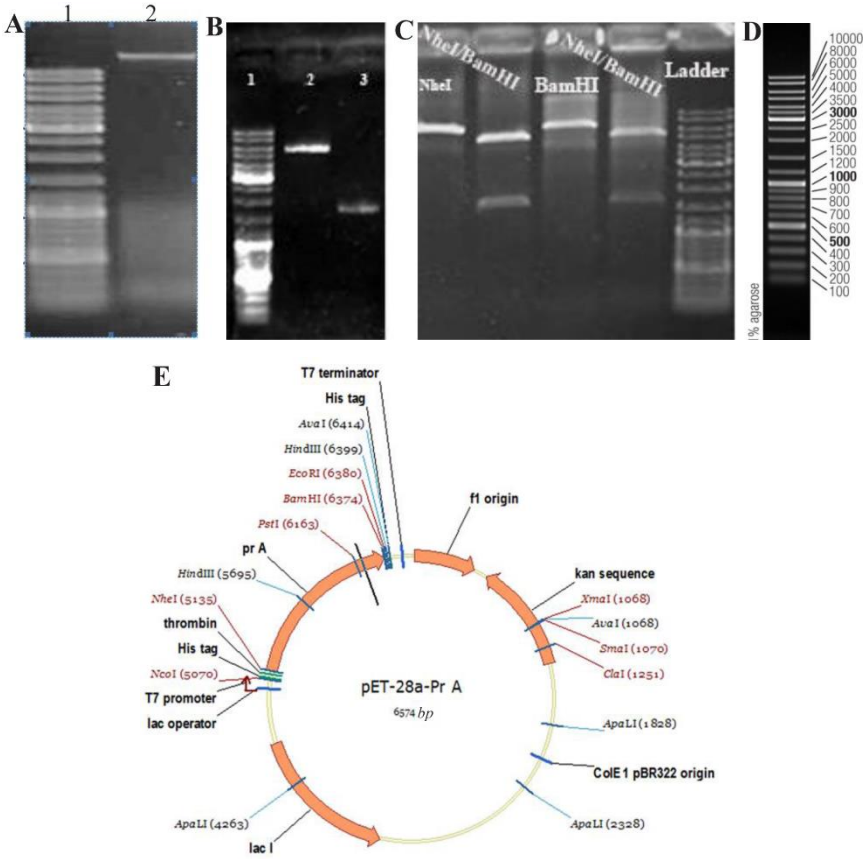

Figure 1. A) Gel electrophoresis image displaying genomic DNA extracted from *S. aureus* culture. Lane 1 contains the Gene Ruler DNA Ladder Mix, serving as a size reference, while Lane 2 shows the extracted genomic DNA. B) Gel image illustrating the digestion products: Lane 1 with the DNA ladder; Lane 2 exhibiting the *SpA* gene after digestion with *Nhe*I and *Bam*HI restriction enzymes; Lane 3 showing the pET-28a vector digested with the same enzymes. C) Confirmation of plasmids harboring the Protein A (SpA) gene *via* double digestion with *Nhe*I and *Bam*HI. D) The Gene-Ruler DNA Ladder Mix used in all DNA gel electrophoresis. E) Snap gene result of SpA cloning in pET-28a plasmid.

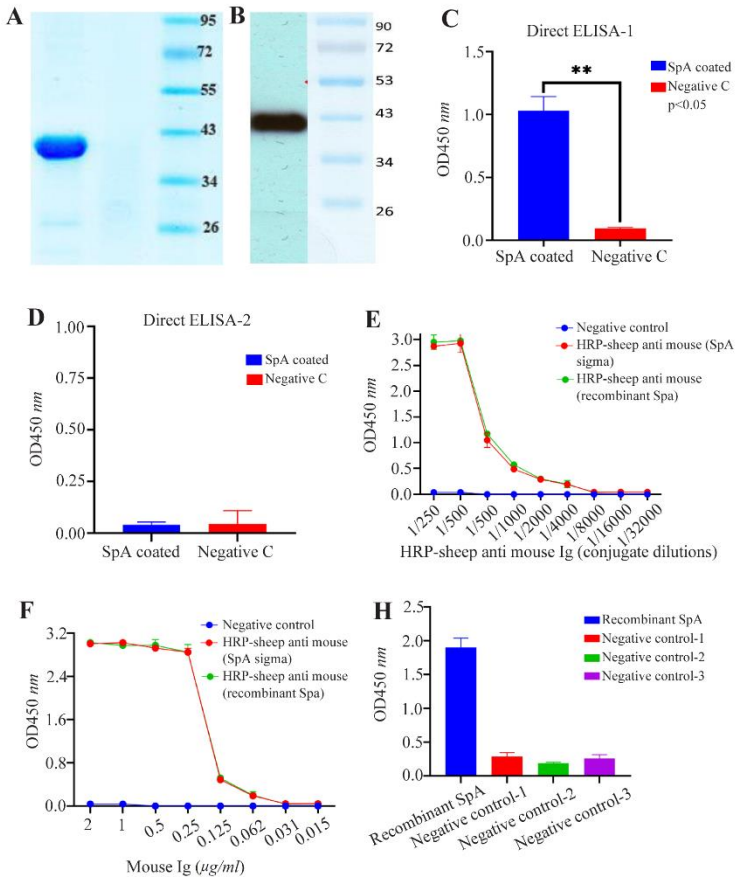

Figure 2. Summarizes the characterization and binding assessment of recombinant SpA: A) SDS-PAGE analysis of purified recombinant SpA was performed using a 12% gel, with samples alongside molecular weight markers stained with Coomassie Blue R-250. The estimated molecular weight of the purified protein was determined based on the marker proteins. B) Western blotting of recombinant SpA was conducted using Horseradish Peroxidase (HRP)-conjugated anti-His tag antibody to confirm the presence of the His-tagged recombinant protein. C and D) Enzyme-linked immunosorbent assay (ELISA) experiments involved coating ELISA strips with 5 *µg/ml* of recombinant SpA, followed by incubation with HRP-conjugated sheep anti-mouse Ig and F(ab')2 HRP-conjugated sheep anti-mouse Ig, respectively, to evaluate binding specificity. E) Titration curves from direct ELISA showed the binding of HRP-conjugated sheep anti-mouse Ig to plates coated with commercial SpA (Sigma, red line) and recombinant SpA (green line), indicating comparable reactivity. F) Indirect ELISA titration assessed the binding of mouse immunoglobulin (ranging from 2 to 0.015 *µg/ml*) to plates coated with protein A, using F(ab')2 HRP sheep anti-mouse IgG as the detection conjugate. H) The interaction of coated SpA with VH3 of IgM was examined, along with detection using F(ab')2 HRP sheep anti-mouse IgG; controls included NC-1 (no coating), NC-2 (absence of IgM), and NC-3 (absence of conjugate), to verify specific binding.

antibody confirms the single correct band (Figure 2B). The production yield of recombinant Staphylococcal Protein A (SPa) in *E. coli* varies significantly based on the expression system, cultivation conditions, and optimization strategies employed. One study optimized the expression and secretion of a truncated form of SPa using its native signal sequence. The optimization included factors such as lactose concentration, glycine concentration, induction time, optical density, and temperature. This resulted in a fivefold increase in the secretion of SPa [28]. The use of the *E. coli* X-press strain facilitated extracellular recombinant protein production, achieving extracellular SPa titers of up to 349 *mg/g*, comprising up to 90% of the total soluble product [29]. A protocol developed for extracellular production of SPa in a stirred tank bioreactor yielded 5.5 *g/L* of the secreted target protein. The process included cell removal by centrifugation, concentration, and purification by anion exchange chromatography, achieving a total process yield of 90% and final purity of ≥95% [30]. These findings highlight the potential for achieving high yields of recombinant SPa in *E. coli* through careful optimization of expression systems and cultivation conditions. In this study, the recombinant expression of protein A produced a yield of 50 *mg/L*, indicating a substantial production efficiency without any optimization. The protein yield reported in this study is compared with that of another study. The yield was found to be significant (Table 1).

In two direct Enzyme-Linked Immunosorbent Assays (ELISAs), recombinant SpA demonstrated specific binding affinity for the Fc region of human IgG, with no reactivity observed towards the F(ab')2 fragment, consistent with its established binding profile.

Table 1. Comparison of different protein yield

Expression system	Yield	Purity	Notes	Ref
E. coli (native signal sequence)	Increased 5-fold	Not specified	Optimization of multiple factors	[28]
E. coli X-press strain	349 *mg/g*	Not specified	Extracellular production	[29]
Stirred tank bioreactor	5.5 *g/L*	≥95%	Fed-batch cultivation, anion exchange chromatography	[30]

Positive control experiments yielded high Optical Density (OD) readings at 450 *nm*, confirming the functional integrity of the Horseradish Peroxidase (HRP)-conjugated secondary antibody used in the assays (Figures 2C and D). Additionally, indirect ELISA tests confirmed that the recombinant SpA retained its ability to interact with anti-SpA antibodies (Figure 2E).

A titration experiment comparing commercial (Sigma) and recombinant SpA, each coated at a concentration of 0.5 *µg/ml*, involved titrating HRP-conjugated sheep anti-mouse Ig from 1/250 to 1/32,000. The resulting curves demonstrated similar reactivity patterns between the two protein sources and indicated that both could effectively detect antibodies at lower conjugate concentrations. The optimal conjugate dilution for specific antigen detection was determined to be 1/1,000 (Figure 2E).

In a separate titration assay, coating SpA at 5 *µg/ml* followed by titration with mouse Ig revealed that the recombinant protein exhibited comparable reactivity to the commercial standard (Figure 2F). Furthermore, Figure 2H depicts the interaction between recombinant SpA and the Fab region of IgM (VH3), highlighting alternative binding sites on SpA distinct from its classical IgG Fc-binding region. Prior research has shown that IgG F(ab')2 fragments can also bind to SpA, with this interaction serving as a marker for Ig light chains encoded by the V.3 gene family in humans. This binding occurs across multiple immunoglobulin classes (IgM, IgA, IgG) and their F(ab')2 fragments, suggesting that SpA's specificity is largely influenced by germline *V.3* gene sequences external to the conventional antigen-binding site [31].

Assessment of SpA conjugation on CNBr-activated sepharose 4B and magnetic nanoparticles

Figure 3A depicts the schematic process for preparing recombinant SpA immobilized on CNBr-activated Sepharose 4B. SpA was conjugated at varying concentrations, and the amount of protein bound to the matrix was measured. The non-bound fractions were collected and analyzed via 12% SDS-PAGE, as shown in Figure 3B. Based on data summarized in table 2 and illustrated in figure 3C, the yields of purified human IgG obtained using the immobilized recombinant SpA were (12.4, 13.1, 14.1, and 15.7 *mg*) corresponding to 5, 10, 15, and 20 *mg* of SpA per/*ml* of CNBr-activated Sepharose 4B, respectively. These results suggest that immobilizing 5 *mg* of recombinant SpA per *ml* of resin

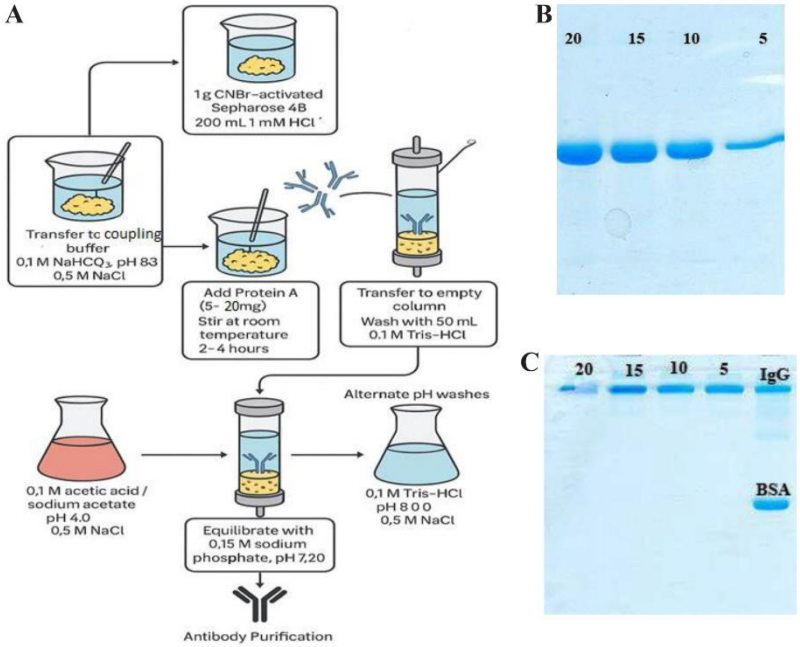

Figure 3. A) Schematic presentation of immobilized recombinant SpA on CNBr-Activated Sepharose 4B and then human IgG purification. B) SpA protein which didn't bound to resin. C) the purified human IgG after immobilization of SpA on to the resins (20,15, 10 and 5) show the initial amount of recombinant SpA used in immobilization process.

Table 2. The binding capacity of CNBr-activated Sepharose 4B conjugated with recombinant SpA for purifying human serum IgG

Recombinant SpA immobilized per 1 *ml* of CNBr-activated Sepharose 4B (*mg/ml*)	5	10	15	20
Amount of IgG purified (*mg*)	12.4	13.1	14.1	15.7

It details the amount of IgG captured per ml of resin, indicating the efficiency of this affinity matrix in immunoglobulin purification.

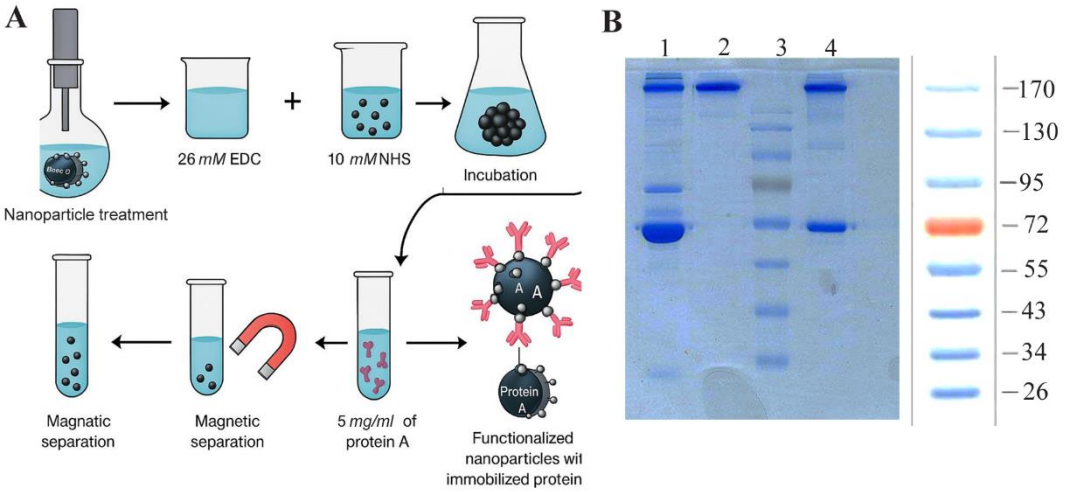

Figure 4. Illustrates the process and results of IgG purification using recombinant SpA immobilized on magnetic nanoparticles: A) A schematic diagram depicting the immobilization of recombinant SpA on $MnFe_2O_4$@SiO_2@NH_2) magnetic nanoparticles. B) SDS-PAGE analysis showing the purification of human IgG. Lane 1; displays the IgG in its reduced and Lane 2; IgG in non-reduced forms purified using 5 *mg* of immobilized SpA, Lane 3, prestain protein markers included for size reference. Lane 4 confirms the purified IgG in its reduced form, obtained using 10 *mg* of SpA immobilized on the nanoparticle matrix.

Table 3. The binding capacity of $MnFe_2O_4$@SiO_2@NH_2) magnetic nanoparticles functionalized with recombinant SpA for isolating human serum IgG

Recombinant SpA immobilized per 5 *mg* of magnetic nanoparticle	5	10	15
Amount of IgG purified (*mg*)	1.4	4.33	7.29

The table provides quantitative measurements of IgG binding efficiency, reflecting the potential of these magnetic nanomaterials as an alternative affinity purification platform

offers a cost-effective approach for consistent IgG purification, with the resin efficiency and binding capacity reaching approximately 65%.

In another assay the 5 *mg/ml* nanoparticles were coupled *via* their surface amino group to the carboxyl groups on the (5, 10 and 15 *mg*) of SpA using the EDC/NHS conjugation method (Figure 4A). The amount of IgG captured by above mentioned of SpA was 1.4, 4.3 and 7.2 *mg* respectively (Figure 4B and Table 3).

Discussion

The present results showed that protein A could capture human plasma IgG successfully, which indicated the bioactivity of recombinant SpA was retained after its attachment to $MnFe_2O_4$@SiO_2@NH_2.

In comparison to the long operation time, solvent consumption, and problems with protein solubility associated with affinity chromatography, the conventional method for antibody purification, we have demonstrated that use of nonmagnetic beads is a much simpler and more versatile system for antibody purification. Magnetic separation can significantly shorten the purification process by quick retrieval of affinity beads at each step (*e.g.*, binding, wash, and elution), and reduce sample dilution usually associated with traditional column-based elution.

Conclusion

The production of recombinant Protein A (SpA) and its stabilization on CNBr-activated Sepharose 4B and magnetic nanoparticles advance IgG purification. Recombinant SpA, derived from *S. aureus* and expressed in *E. coli*, provides a consistent, high-affinity IgG ligand, reducing variability from native sources and enabling large-scale, cost-effective production with high purity and activity. Immobilizing recombinant SpA on CNBr-activated Sepharose 4B provides a stable matrix suitable for repeated use, maintaining its binding capacity through multiple purification cycles. This meth-

od benefits from the strong covalent linkage, which enhances the durability and reusability of the affinity matrix. Immobilization on magnetic nanoparticles offers rapid separation, high surface area, and ease of handling, facilitating automation and reducing processing time—beneficial for clinical and industrial applications.

Both platforms maintain SpA activity and IgG affinity, enabling efficient purification. The choice between Sepharose and magnetic nanoparticles depends on specificity, process scale, and operational convenience. Overall, stabilizing recombinant SpA on these platforms enhances efficiency, reusability, and scalability in IgG purification, supporting biomedical research, diagnostics, and therapeutic antibody production.

Acknowledgement

This research was funded by the Avicenna Research Institute (Grant number 910309-031) and conducted in accordance with the approval of the ethics committee IR.ACECR.Avicenna.REC.92.927. We extend our gratitude to Mrs. Somayeh Najafzadeh and Leila Balay-Goli for their valuable assistance and collaboration.

Conflict of Interest

The authors confirm that the content of this article has no conflicts of interest. Using artificial intelligence bots: We utilized AI-powered tools to check grammar and enhance the academic quality of the text, which was primarily written by the authors.

References

1. Cramer SM, Holstein MA. Downstream bioprocessing: recent advances and future promise. Current Opinion in Chemical Engineering 2011 Oct 1;1(1):27-37.
2. Shanu K, Choudhary S, Kumari S, Anu K, Devi S. Downstream Processing for Bio-product Recovery and Purification. InRecent Advances in Bioprocess Engineering and Bioreactor Design 2024 May 14 (pp. 139-169). Singapore: Springer Nature Singapore.
3. Roque AC, Silva CS, Taipa MA. Affinity-based methodologies and ligands for antibody purification: advances and perspectives. J Chromatogr A. 2007 Aug 10; 1160(1-2):44-55.
4. Hunt B, Goddard C, Middelberg AP, O'Neill BK. Economic analysis of immunoadsorption systems. Biochemical Engineering Journal 2001 Dec 1;9(2):135-45.
5. Roque AC, Lowe CR. Advances and applications of de novo designed affinity ligands in proteomics. Biotechnol Adv 2006 Jan-Feb;24(1):17-26.
6. Baral TN, MacKenzie R, Arbabi Ghahroudi M. Single-domain antibodies and their utility. Curr Protoc Immunol 2013 Nov 18;103:2.17.1-2.17.57.
7. Mitra S, Tomar PC. Hybridoma technology; advancements, clinical significance, and future aspects. J Genet Eng Biotechnol 2021 Oct 18;19(1):159.
8. Roque AC, Lowe CR, Taipa MA. Antibodies and genetically engineered related molecules: production and purification. Biotechnol Prog 2004 May-Jun;20(3):639-54.
9. Arora S, Saxena V, Ayyar BV. Affinity chromatography: A versatile technique for antibody purification. Methods 2017 Mar 1;116:84-94.
10. Gagnon P. Technology trends in antibody purification. J Chromatogr A 2012 Jan 20;1221:57-70.
11. Fassina G, Ruvo M, Palombo G, Verdoliva A, Marino M. Novel ligands for the affinity-chromatographic purification of antibodies. J Biochem Biophys Methods 2001 Oct 30;49(1-3):481-90.
12. Denizli A. Purification of antibodies by affinity chromatography. Hacettepe Journal of Biology and Chemistry 2011;39(1):1-8.
13. Kim HS, Hage DS. Immobilization methods for affinity chromatography. Handbook of affinity chromatography 2006;2:35-78.
14. Bacon K, Lavoie A, Rao BM, Daniele M, Menegatti S. Past, Present, and Future of Affinity-based Cell Separation Technologies. Acta Biomater 2020 Aug;112:29-51.
15. Alvikas J, Neal MD. Modern Techniques for DNA, RNA, and Protein Assessment. Success in Academic Surgery: Basic Science 2019 Apr 30:65-104.
16. Rigi G, Ghaedmohammadi S, Ahmadian G. A comprehensive review on staphylococcal protein A (SpA): Its production and applications. Biotechnol Appl Biochem 2019 May;66(3):454-464.
17. Lee HG, Kang S, Lee JS. Binding characteristics of staphylococcal protein A and streptococcal protein G for fragment crystallizable portion of human immunoglobulin G. Comput Struct Biotechnol J 2021 Jun 4;19:3372-3383.
18. Zarrineh M, Mashhadi IS, Farhadpour M, Ghassempour A. Mechanism of antibodies purification by protein A. Anal Biochem 2020 Nov 15;609:113909.
19. Wines BD, Trist HM, Farrugia W, Ngo C, Trowsdale J, Areschoug T, et al. A conserved host and pathogen recognition site on immunoglobulins: structural and functional aspects. Adv Exp Med Biol 2012;946:87-112.
20. Choe W, Durgannavar TA, Chung SJ. Fc-Binding Ligands of Immunoglobulin G: An Overview of High Affinity Proteins and Peptides. Materials (Basel) 2016 Dec 8;9(12):994.
21. Ayyar BV, Arora S, Murphy C, O'Kennedy R. Affinity chromatography as a tool for antibody purification. Methods 2012 Feb;56(2):116-29.
22. J. Sambrook, E. Fritsch, T. Maniatis, Molecular cloning: a laboratory manual 2012 Edition (Final), Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 4th, 2012. 448.
23. Nazari M, Zarnani AH, Ghods R, Emamzadeh R, Najafzadeh S, Minai-Tehrani A, et al. Optimized protocol for soluble prokaryotic expression, purification and structural analysis of human placenta specific-1(PLAC1). Protein Expr Purif 2017 May;133:139-151.

24. He F. Bradford protein assay. Bio-protocol. 2011 Mar 20:e45.

25. Nazari M, Emamzadeh R, Masoudi-Khoram N, Nazari M. Immobilization of albumin binding domain (ABD) on Sepharose 4B and magnetic particle for efficient single-step purification of human serum albumin. J Chromatogr B Analyt Technol Biomed Life Sci 2025 Jul 1;1261: 124655.

26. Rashid Z, Naeimi H, Zarnani AH, Nazari M, Nejad-moghaddam MR, Ghahremanzadeh R. Fast and highly efficient purification of 6× histidine-tagged recombinant proteins by Ni-decorated MnFe2O4@ SiO2@ NH2@ 2AB as novel and efficient affinity adsorbent magnetic nanoparticles. RSC Advances 2016 Apr 12;6(43):36840-8.

27. Rigi G, Mohammadi SG, Arjomand MR, Ahmadian G, Noghabi KA. Optimization of extracellular truncated staphylococcal protein A expression in Escherichia coli BL21 (DE3). Biotechnol Appl Biochem 2014 Mar-Apr; 61(2):217-25.

28. Kastenhofer J, Rettenbacher L, Feuchtenhofer L, Mairhofer J, Spadiut O. Inhibition of E. coli Host RNA Polymerase Allows Efficient Extracellular Recombinant Protein Production by Enhancing Outer Membrane Leakiness. Biotechnol J 2021 Mar;16(3):e2000274.

29. Freiherr von Roman M, Koller A, von Rüden D, Berensmeier S. Improved extracellular expression and purification of recombinant Staphylococcus aureus protein A. Protein Expr Purif 2014 Jan;93:87-92.

30. Hillson JL, Karr NS, Oppliger IR, Mannik M, Sasso EH. The structural basis of germline-encoded VH3 immunoglobulin binding to staphylococcal protein A. J Exp Med 1993 Jul 1;178(1):331-6.