

Enhanced Plasma Protein Purification Using Novel Polystyrene-Supported Chelating Resins: Experimental and Computational Insights into Buffer-Resin Interactions

Zina Benmamar

Université Ferhat Abbas

Salim Madani

`salim.madani@univ-setif.dz`

Université Ferhat Abbas

Kamel Mokhnache

Université Hassiba Benbouali

Souhib Bennaadja

Université Ferhat Abbas

Nouredine Charef

Université Ferhat Abbas

Lokmane Abdelouahed

Normandie Univ, INSA Rouen Normandie, UNIROUEN, LSPC EA

Marta F. Garcia

Normandie Univ, INSA Rouen Normandie, UNIROUEN, LSPC EA

Daniel L. Garcia

Normandie Univ, INSA Rouen Normandie, UNIROUEN, LSPC EA

Yacine Benguerba

Ferhat Abbas Sétif 1 University

Research Article

Keywords: Synthetic polystyrene, immobilized metal affinity chromatography, Protein, Human proteins, Buffer systems, COSMO-RS

Posted Date: October 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11075888/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

**Enhanced Plasma Protein Purification Using Novel Polystyrene-Supported Chelating Resins:
Experimental and Computational Insights into Buffer-Resin Interactions**

*Zina Benmamar ^a, Salim Madani ^a, Kamel Mokhnache ^b, Souhib Bennaadja ^e, Nouredine Charef ^a,
Lokmane Abdelouahed ^c, Marta F. Garcia ^d, Daniel L. Garcia ^d, Yacine Benguerba ^{f,*}*

*^aLaboratoire Biochimie Appliquée, Université Ferhat Abbas, Sétif 1, 19000, **Algeria***

*^b Département de génie des procédés, Université Hassiba Benbouali, Chlef, **Algeria***

*^c Normandie Univ, INSA Rouen Normandie, UNIROUEN, Laboratoire de Sécurité des Procédés
Chimiques, LSPC EA-4704, 76000 Rouen, **France***

*^d Instituto de Ciencia y Tecnologia de Polimeros (Ictp-Csic) C/Juan de la Cierva 3, 28006, Madrid-
Espana.*

^e Département de génie des procédés, Université ferhat abbas, setif, algeria

*^f Laboratoire de Biopharmacie Et Pharmacotechnie (LPBT), Ferhat Abbas Sétif 1 University, 19000,
Sétif, Algeria*

*** Corresponding author:** bennaadja souhib mail: Sohaib1170@gmail.com

Abstract

Plasma protein fractionation is important for biomedical and biotechnological applications. This study investigated poly(styrene-co-ethyl acrylate) (PSEAA) and Cu²⁺-functionalized PSEAA (PSEAA-Cu²⁺) under different buffer conditions. HEPES/imidazole and MOPS/imidazole systems with increasing imidazole concentrations were evaluated using chromatography and SDS-PAGE. PSEAA fractionated plasma proteins according to their interactions with the resin. With HEPES/imidazole, protein bands of approximately 31 and 33 kDa were detected at 50, 100, and 300 mM imidazole. With MOPS/imidazole, protein bands of approximately 63–65 kDa were observed at 100–700 mM imidazole, while bands of approximately 39 and 65 kDa were detected at 1 M imidazole. PSEAA-Cu²⁺ produced approximately 66 and 33 kDa protein bands with HEPES/imidazole and 59, 33, and 66 kDa protein bands with MOPS/imidazole. COSMO-RS calculations provided molecular-level insights into buffer–resin interactions and their potential effects on protein elution. Overall, both adsorbents demonstrated potential for plasma protein fractionation, although further optimization and protein-specific characterization are required for selective purification.

Keywords: Synthetic polystyrene, immobilized metal affinity chromatography, Protein, Human proteins, Buffer systems, COSMO-RS

1. Introduction

Synthetic chelating ion exchange resins are increasingly recognized for broad applications in diverse scientific and industrial fields. These resins are crucial in environmental remediation, mainly removing heavy metals from aquatic ecosystems. Additionally, they are extensively utilized in heterogeneous catalysis, solid electrolytes, the fabrication of ion-exchange membranes, the development of ion-selective electrodes, and the purification of industrial waste streams [1, 2]. Their unique ability to selectively bind and exchange ions makes them invaluable in analytical and preparative chromatography, driving advancements in biochemistry, chemical engineering, and materials science. Among chromatographic techniques, ion exchange chromatography (IEC) is widely regarded as one of the most effective methods for separating and purifying charged biomolecules, such as polypeptides, proteins, polynucleotides, and nucleic acids. Its high binding capacity, operational simplicity, broad applicability, and superior resolution contribute to its frequent use in biochemical and industrial settings [2, 3]. Ion exchangers can be naturally occurring or synthetically derived, with synthetic organic and inorganic resins produced through polymerization processes involving monomers such as styrene, acrylic compounds, phenols, and divinyl benzene. These polymerized materials provide a structurally robust and adaptable framework for ion exchange functionality, allowing precise control over resin properties such as pore size, ligand density, and selectivity [3-6].

Initially conceptualized and developed by Porath [8], immobilized metal affinity chromatography (IMAC) has evolved beyond its conventional biochemical applications. It extends into diverse fields, including environmental analysis, bioseparations, and materials science. IMAC exploits the specific affinity of biomolecules, particularly proteins, for immobilized transition metal ions such as Cu^{2+} , Ni^{2+} , Zn^{2+} , and Co^{2+} , facilitating selective and efficient separations [9]. Beyond biochemical applications, IMAC has demonstrated great potential in environmental studies. The ability to immobilize metal ions

such as Fe^{2+} , Co^{2+} , Cd^{2+} , and Ni^{2+} allows for the isolation and characterization of natural organic ligands and macromolecules in aquatic and terrestrial ecosystems. However, copper-binding ligands dominate scientific literature, primarily due to their environmental significance and analytical versatility [7]. The ability of IMAC to isolate specific biomolecules has made it indispensable in bioengineering, wastewater treatment, and nanomaterial functionalization. IMAC ligands, or metal chelates, present several notable advantages, including cost-effectiveness, chemical stability, high affinity and selectivity, ease of preparation, and versatility [10, 11]. A single chelating ligand system can effectively purify multiple proteins, while the chelating resin can be readily adapted to accommodate various metal ions, broadening its application scope significantly. However, several critical factors govern protein adsorption efficiency in IMAC, including the chemical nature of the chelating groups, the type and coordination chemistry of the immobilized metal ion, ligand density on the adsorbent surface, protein surface amino acid composition, and experimental conditions such as solution pH, ionic strength, buffer salt composition, and temperature [12].

Furthermore, optimal affinity interactions require that ligands be anchored to the adsorbent matrix without compromising their accessibility to target proteins. This is often accomplished by incorporating spacer arms, which distance the ligand from the resin matrix, enhancing ligand accessibility and improving binding efficiency [11, 13]. Building upon previous research on the sorption properties of polystyrene-ethylene amine-based adsorbents (PSEAA) for the capture of trace-level divalent metal ions such as Cu^{2+} , Zn^{2+} , Co^{2+} , and Ni^{2+} in natural aquatic environments [14], the present study explores a novel application of these materials in plasma protein fractionation and purification. Specifically, it investigates the catalytic potential of PSEAA and copper-functionalized PSEAA (PSEAA- Cu^{2+}) in facilitating differential protein adsorption and elution. It also examines the role of different buffer compositions in modulating protein binding and elution efficiency through metal chelate chromatography. To achieve this, both experimental techniques and computational modeling were employed. COSMOtherm computational methods [15, 16] were used to optimize

buffer conditions, elucidate the molecular interactions responsible for protein binding affinity to PSEAA and PSEAA-Cu²⁺, enhance elution efficiency, and mitigate undesirable side reactions during chromatographic processes. By integrating computational simulations with experimental chromatographic techniques, this study provides a predictive framework for rational adsorbent design, enabling the development of more efficient and selective metal-affinity resins. The rapidly expanding field of synthetic ion exchange resins and IMAC presents unparalleled biochemical purification, environmental remediation, and materials science opportunities. By leveraging the versatility of synthetic chelating resins and the precision of immobilized metal affinity interactions, researchers can develop innovative solutions for complex separation challenges. The current study demonstrates the potential of PSEAA and PSEAA-Cu²⁺ in plasma protein fractionation and purification, with computational modeling providing predictive insights for process optimization. These findings pave the way for advancements in chelate chromatography, biosensing technologies, and biomedical separation sciences.

2. Materials and Methods

2.1. Reagents

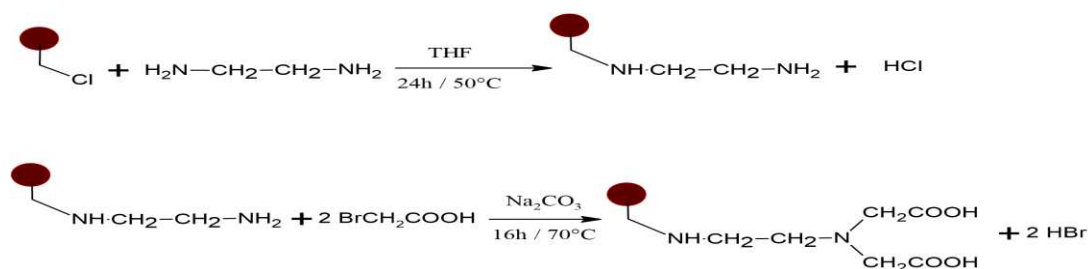
Chloromethylpolystyrene (Merrifield polymer), cross-linked with 5.5% divinylbenzene (DVB, 16-50 mesh, 5.5 mmol/g, Fluka), ethylenediamine (EDA), bromoacetic acid, dehydrated copper (II) acetate (98%), Tris(hydroxymethyl)aminomethane (Tris), Coomassie Brilliant Blue G-250 (CBB), hydrochloric acid (HCl), methanol, and polyacrylamide gel electrophoresis (PAGE) chemicals were procured from Merck. Sodium phosphate buffer components, including disodium hydrogen phosphate (Na₂HPO₄) and sodium dihydrogen phosphate (NaH₂PO₄), as well as sodium hydroxide (NaOH), polyethylene glycol (PEG), glycerol, acetic acid, HEPES, imidazole, 3-N-morpholinopropanesulfonic acid (MOPS), and ammonium persulfate, were obtained from Sigma.

N,N,N',N'-Tetramethyl ethylene diamine (TEMED) was purchased from Sigma-Aldrich. However, dehydrated copper (II) acetate (98%) was specifically obtained from Merck.

2.2. Methods

2.2.1. The stationary phase

The stationary phase employed in the chromatographic technique was a functional polymer synthesized previously by Charef and collaborators in the Biochemistry Laboratory, as depicted in Scheme 1 [14].



Scheme 1: Preparation of PSEAA [14].

2.2.2. preparation of human plasma

Blood samples from healthy donors were collected in glass tubes containing sodium citrate. Samples were centrifuged for 5 minutes at 3000 rpm using a Sigma 3K30 C centrifuge at 4°C, and the supernatants were stored at -20°C until further analysis.

2.2.3. Chromatographic experiments

All chromatographic procedures were performed using an automated chromatography system (Econo Liquid Chromatography System, Bio-Rad, USA) maintained at 25°C with a constant flow rate of 0.5 mL/min. Human plasma samples (0.6 mL), diluted five-fold in an appropriate loading buffer, were injected into a previously equilibrated column. The column was subsequently washed with the loading

buffer until the eluate absorbance approached zero. The buffer systems (pH 7.4) employed with the newly synthesized resin consisted of the following compositions: (1) Imidazole (20 mM–1 M)/HEPES (20 mM), (2) HEPES (20–100 mM)/EDTA (20 mM), and (3) Imidazole (15 mM–1 M)/MOPS (20 mM). When employing the PSEAA-Cu²⁺ resin, elution was performed using either imidazole (20–1000 mM)/HEPES (20 mM) or Imidazole (15–1000 mM)/MOPS (20 mM), with the latter buffer also utilized for resin regeneration.

2.2.3. Protein estimation and electrophoresis

Protein estimation was carried out according to Chong [17]. Bovine serum albumin (BSA) was used to construct the regression curve. The purity of proteins from different chromatographic peaks was checked using polyacrylamide gel electrophoresis (7.5%) in the presence of sodium dodecyl sulfate (SDS), as described by Zagst [18].

2.2.4. Computational approach:

This study employed the COSMO-RS (Conductor-like Screening Model for Real Solvents) theory to optimize molecular geometry and charge density through Density Functional Theory (DFT). The molecular optimization process was conducted using the COSMO-BP-TZVP template within the TmoleX software, which is integrated with the TURBOMOLE computational package, a widely used tool for advanced quantum chemical calculations [19,20].

The COSMO-BP-TZVP template utilizes the def-TZVP (Triple Zeta Valence Polarization) basis set in combination with the M06-2x functional level of DFT, providing high-precision results in modeling molecular structures and properties [21,22]. The COSMO solvation model was applied to accurately account for solvent effects, assuming infinite permittivity to simulate a realistic solution environment.

This model allows for predicting molecular interactions in various solvent conditions, offering deeper insights into solute-solvent dynamics.

The COSMO-RS calculations were performed using BIOVIA COSMOtherm version 18.0.0, a specialized computational tool designed explicitly for predicting the thermodynamic properties of liquid mixtures. In this study, the def-TZVP parametrization was incorporated into the COSMO-RS framework to enhance the accuracy and reliability of the expected results. This comprehensive approach provides a robust foundation for understanding complex molecular interactions and optimizing buffer systems for specific applications, ensuring precise control over protein binding affinity, elution efficiency, and chromatographic performance in experimental and industrial settings.

3. Results

3.1. Fractionation on the PSEAA resin

3.1.1. Imidazole-HEPES buffer

Figure 1 presents the results of human plasma fractionation on the polystyrene-supported ethyl amino acetic acid (PSEAA) adsorbent, demonstrating the sequential elution of plasma proteins under varying buffer conditions. Peak 1, observed following the washing step with 20 mM imidazole-HEPES buffer, represents the fraction of unbound proteins that did not interact with the PSEAA adsorbent, indicating their weak or negligible affinity for the resin. Subsequent elution steps at 50, 100, and 300 mM imidazole concentrations resulted in the release of two distinct proteins with molecular weights of 31 and 33 kDa, corresponding to Peaks 2, 3, and 4. The selective elution at these concentrations suggests differential binding affinities between the proteins and the PSEAA resin, with both proteins requiring moderately increased imidazole concentrations for displacement.

In contrast, no proteins were detected in the eluates corresponding to 500, 700, and 1000 mM imidazole concentrations (Peaks 5, 6, and 7), suggesting that all plasma proteins capable of binding to the resin had been efficiently eluted at lower imidazole concentrations. Finally, Peak 8, obtained after resin regeneration with 0.5 M NaOH, showed no detectable protein bands on SDS-PAGE, confirming the complete removal of bound biomolecules and the effective restoration of the adsorbent for subsequent fractionation cycles. This elution profile underscores the specificity and efficiency of the PSEAA adsorbent in fractionating human plasma proteins under controlled buffer conditions.

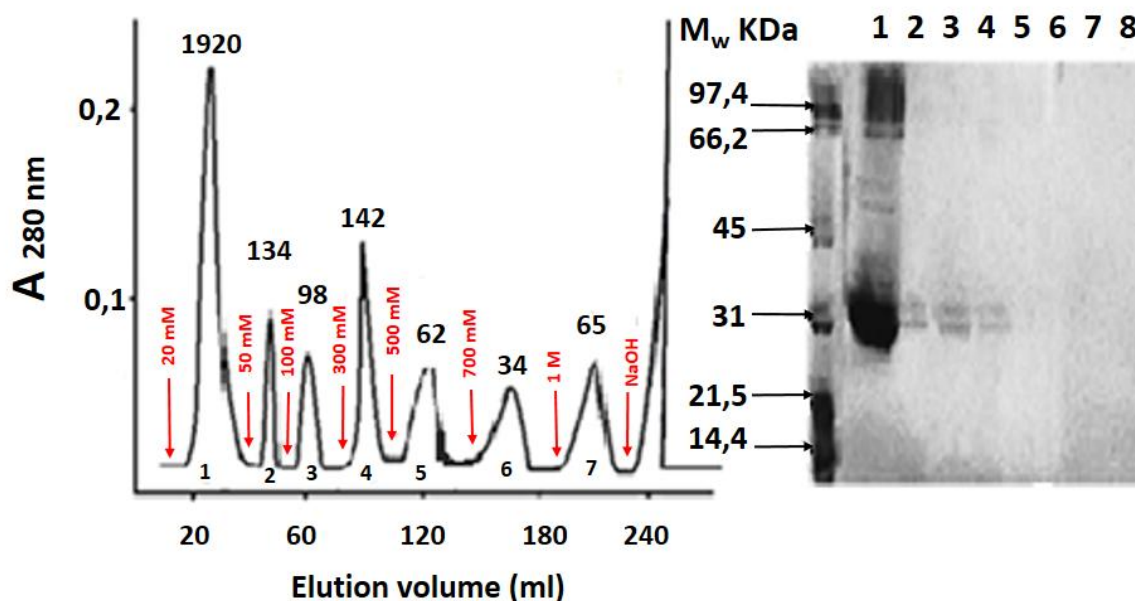


Figure 1. Chromatogram and SDS-PAGE (7.5%) pattern of human plasma fractions on PSEAA by imidazole-HEPES buffer (pH 7.4). Values upon the peaks correspond to protein concentrations (µg/ml), and those under each peak and upon the gel pattern indicate its number.

3.1.2. Imidazole-MOPS buffer

Figure 2 presents the results of human plasma fractionation using imidazole-MOPS buffer, highlighting the selective elution of plasma proteins under varying buffer concentrations. Peak 1, obtained after washing with 15 mM imidazole-MOPS, contains unadsorbed proteins that did not interact with the PSEAA resin, indicating a lack of affinity for the adsorbent under these conditions. No detectable protein bands were observed at 50 mM imidazole-MOPS (Peak 2), suggesting that proteins with weak binding affinity remained bound to the resin or required higher buffer concentrations for elution. However, at 100 mM and 300 mM imidazole-MOPS (Peaks 3 and 4), a pure protein of 63 kDa was successfully eluted, indicating its moderate binding strength to the resin. As the buffer concentration increased to 500 mM and 700 mM (Peaks 5 and 6), a pure protein of approximately 65 kDa was obtained, suggesting a stronger interaction with the adsorbent that necessitated higher imidazole concentrations for elution. At the highest elution strength, 1 M imidazole-MOPS and 0.5 M NaOH (Peaks 7 and 8), two proteins with molecular weights of 39 and 65 kDa were released. This final step facilitated the desorption of proteins with the highest binding affinity, demonstrating the effectiveness of imidazole-MOPS in selectively fractionating plasma proteins based on their binding interactions with the PSEAA resin. The observed elution pattern further emphasizes the role of buffer concentration in controlling protein recovery and purification efficiency in plasma fractionation studies.

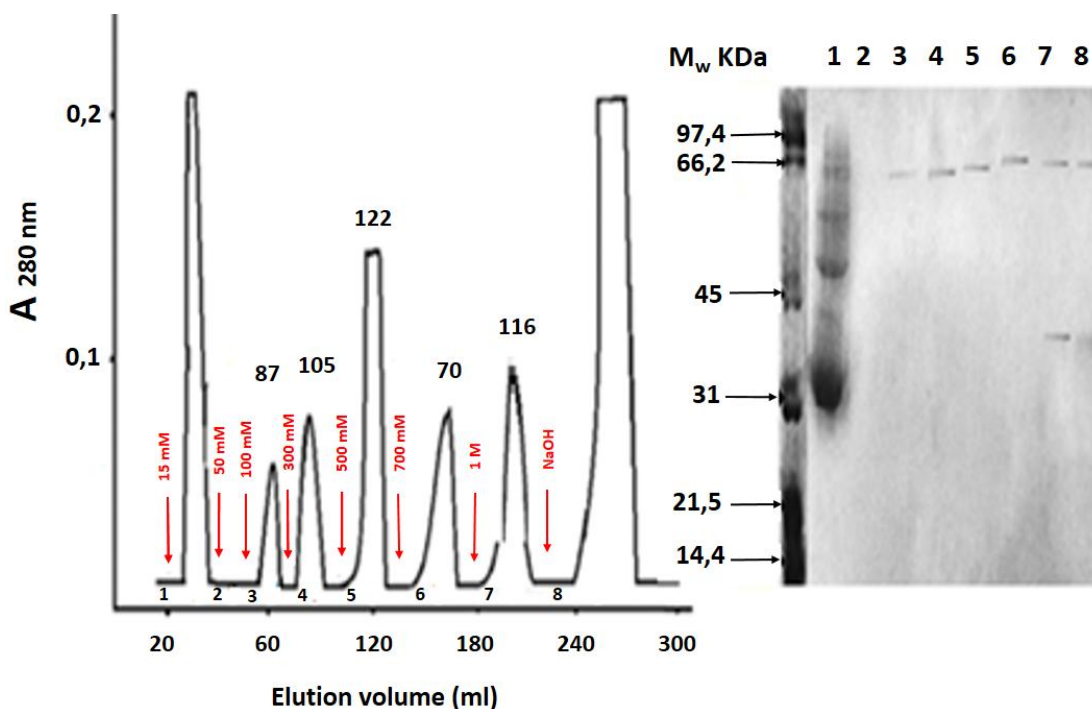


Figure 2. Chromatogram and SDS-PAGE (7.5%) pattern of human plasma fractions on PSEAA by Imidazole-MOPS buffer (pH 7.4). Values upon the peaks correspond to protein concentrations ($\mu\text{g/ml}$), and those under each peak and upon the gel pattern indicate its number

3.2. Fractionation on the resin PSEAA- Cu^{+2}

3.2.1. Imidazole-HEPES buffer

Figure 3 presents the results of serum fractionation using PSEAA $\text{Cu}(\text{II})$, demonstrating the selective elution of proteins under varying buffer concentrations. The first peak, obtained after washing with a 20 mM adsorption buffer, contains proteins not adsorbed by the resin, indicating their weak interaction or lack of affinity with the PSEAA $\text{Cu}(\text{II})$ complex. Elution at 300 mM (Peak 2) released a mixture of two proteins with molecular weights of 66 kDa and 33 kDa, suggesting that these proteins exhibit moderate binding affinity to the $\text{Cu}(\text{II})$ -functionalized resin. The presence of both proteins in a single peak indicates co-elution, possibly due to similar binding strengths at this concentration. At

higher imidazole concentrations of 500 mM, 700 mM, and 1 M (Peaks 3, 4, and 5, respectively), the same 33 kDa and 66 kDa proteins were eluted, indicating that both proteins continued to be released across these concentrations. This suggests their interaction with the PSEAA Cu(II) resin was relatively strong, requiring higher buffer concentrations for complete desorption.

The final peak (Peak 6) was obtained after resin regeneration with 0.5 M NaOH. Since no protein bands were detected in this fraction, it can be concluded that the elution process was efficient, with all proteins successfully desorbed using the previous elution steps. These results confirm the effectiveness of PSEAA Cu(II) in selectively binding and fractionating serum proteins, with buffer concentration playing a key role in modulating protein elution.

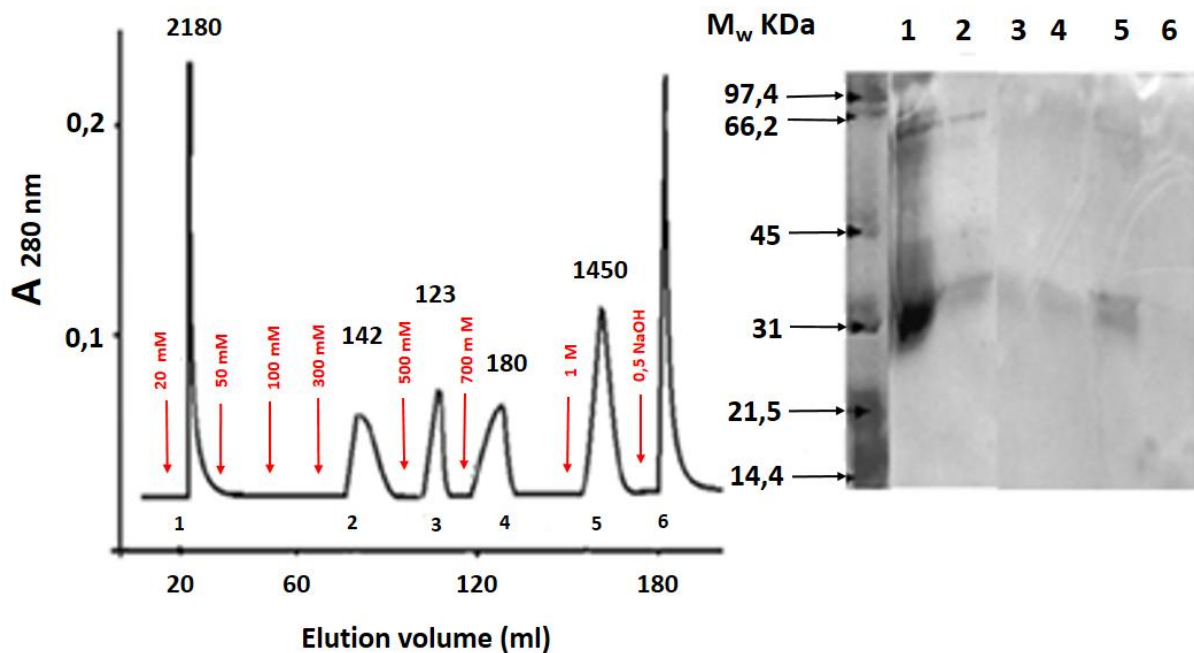


Figure 3. Chromatogram and SDS-PAGE (7.5%) pattern of human plasma fractions on PSEAA-Cu(II) by HEPES /imidazole buffer (pH 7.4). Values upon the peaks correspond to protein concentrations ($\mu\text{g/ml}$), and those under each peak and upon the gel pattern indicate its number.

3.2.2. Imidazole-MOPS buffer

Figure 4 presents the fractionation results of **human plasma** using PSEAA-Cu²⁺, illustrating proteins' selective binding and elution under different buffer conditions. The first peak, obtained after washing with 15 mM adsorption buffer, contains proteins not retained by the resin, indicating their weak or negligible interaction with the PSEAA-Cu²⁺ complex. These unadsorbed proteins either lack sufficient metal-binding sites or exhibit low affinity under these conditions. Subsequent elution steps using increasing imidazole concentrations in MOPS buffer resulted in the release of a mixture of three proteins with molecular weights of approximately 59, 33, and 66 kDa. The presence of multiple proteins in each fraction suggests overlapping elution profiles, likely due to varying degrees of interaction between these proteins and the Cu²⁺-functionalized resin. The progressive release of proteins across increasing buffer concentrations implies a differential affinity-based elution mechanism, where proteins with weaker interactions desorb first. At the same time, those with stronger binding require higher buffer concentrations for displacement.

These results confirm the efficacy of PSEAA-Cu²⁺ in fractionating human plasma proteins and demonstrate the role of buffer concentration and composition in controlling protein elution. Identifying distinct molecular weight fractions further highlights the potential of metal-affinity chromatography in bioseparations, particularly for applications requiring selective protein purification.

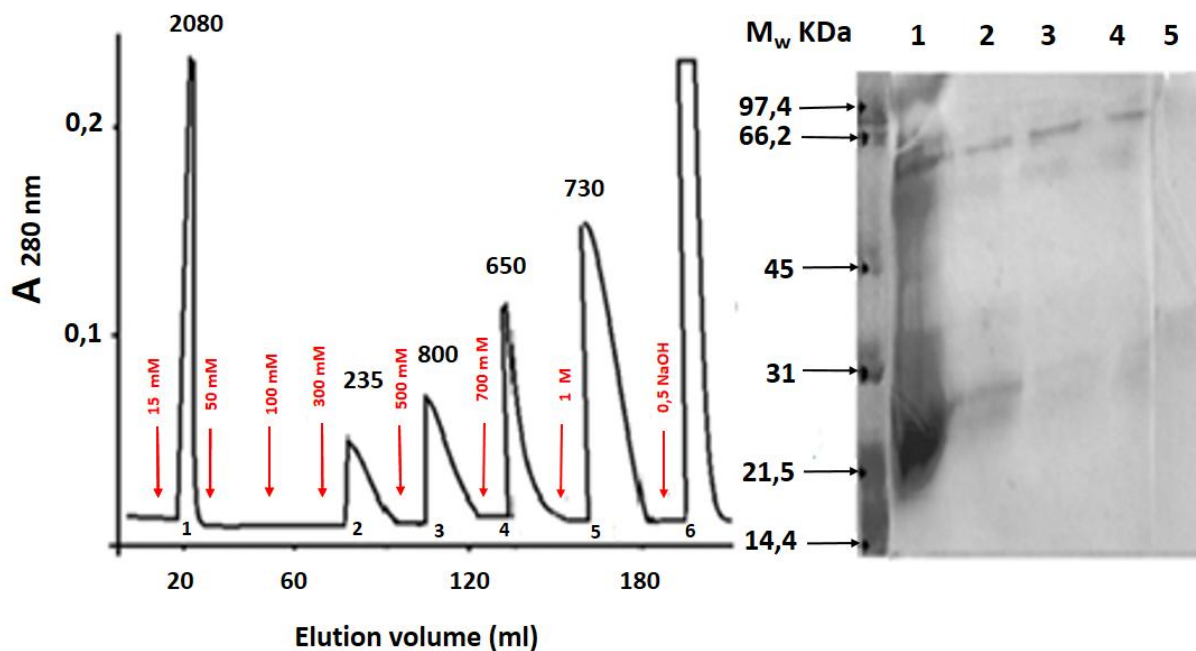


Figure 4: Chromatogram and SDS-PAGE (7.5%) pattern of human plasma fractions on PSEAA-Cu⁺² by MOPS/imidazole buffer (pH 7.4). Values upon the peaks correspond to protein concentrations (µg/ml), and those under each peak and upon the gel pattern indicate its number.

4. Discussion

Experimental results demonstrate the feasibility of plasma protein separation using different buffer solutions with PSEAA, confirming its efficacy in selective protein purification. The polyacrylamide gel electrophoresis (PAGE, 7.5%) analysis of fractionated human plasma revealed protein bands of approximately 31 and 33 kDa using HEPES/imidazole buffer at 50, 100, and 300 mM imidazole. The absence of visible protein bands in other peaks can be attributed to the low concentration of desorbed proteins, indicating that only specific proteins with strong interactions with the resin were eluted under these conditions. With MOPS/imidazole buffer, protein bands of approximately 63–65 kDa were detected at 100, 300, 500, and 700 mM imidazole, while bands of approximately 39 and 65 kDa were detected at 1 M imidazole, suggesting the effectiveness of this buffer system in selectively eluting

high-affinity plasma proteins. Similarly, the same buffer systems (MOPS/imidazole and HEPES/imidazole) were applied to PSEAA-Cu²⁺. Similarly, the HEPES/imidazole and MOPS/imidazole buffer systems were applied to PSEAA-Cu²⁺. HEPES/imidazole resulted in approximately 66 and 33 kDa protein bands, whereas MOPS/imidazole resulted in approximately 59, 33, and 66 kDa protein bands.

. These findings are consistent with previous research conducted by Nascimento [23-25], which demonstrated that sepabeads-IDA-Cu²⁺ and sepabeads-IDA-Ni²⁺ chelate supports selectively adsorbed only those proteins capable of forming multiple support-protein bonds in the presence of 50 mM imidazole. This result confirms that only naturally occurring proteins capable of establishing strong coordination interactions are retained on immobilized metal affinity chromatography (IMAC) resins under competitive conditions. Imidazole is widely recognized as an effective elution agent in IMAC, as it competes with protein residues for binding to immobilized metal ions. The results reinforce that imidazole is one of the best eluents, effectively displacing bound proteins at specific concentrations. Low imidazole concentrations are typically used to minimize host cell protein binding, while slightly higher concentrations are necessary to selectively displace target proteins, ensuring optimal purification efficiency [23, 26]. Protein adsorption on IMAC matrices is governed by multi-point interactions between immobilized metal ions and specific amino acid residues, particularly histidine, cysteine, and tyrosine, on the protein surface [27, 28]. These interactions define binding affinity, selectivity, and elution efficiency, making IMAC a powerful tool for selective protein fractionation. The present findings contribute to a deeper understanding of protein-metal interactions in chromatography, paving the way for optimized buffer conditions tailored for specific protein purification applications.

5. Interaction Modeling Using COSMO-RS

5.1. Preparation of buffers

The buffer formulations in this study were prepared using varying ratios of HEPES, IMIDAZOLE, and MOPS, ranging from simple 1:1 mixtures to more complex formulations with increased IMIDAZOLE concentrations (e.g., 1:50 ratios). The objective of these formulations is to systematically investigate how buffer composition and concentration ratios influence their chemical behavior and their suitability for specific experimental conditions [29]. By analyzing these interactions, researchers can optimize buffer formulations for enhancing protein stability, solubility, and functional integrity in various biochemical and pharmaceutical applications.

Optimizing buffer systems is critical for ensuring efficient protein purification and stabilization, particularly in chromatographic and biochemical assays. COSMOtherm, a computational modeling tool based on the COSMO-RS (Conductor-like Screening Model for Real Solvents) theory, allows for the prediction of thermodynamic properties of liquid mixtures [40,41]. This capability makes it highly valuable for evaluating buffer compatibility and performance under different conditions [30,31]. Computational approaches such as COSMOtherm simulations provide insights into buffer-protein and -resin interactions, leading to better experimental design and reproducibility.

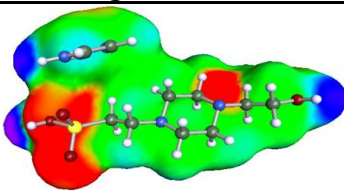
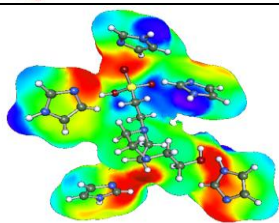
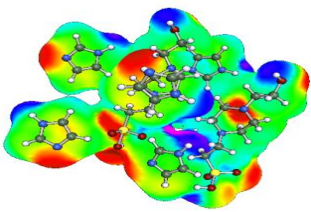
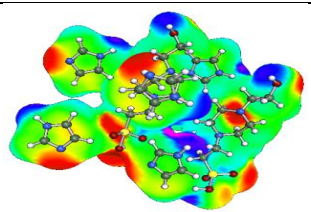
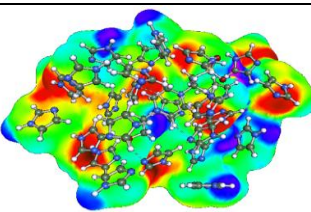
A table summarizing various buffer preparations lists different component combinations and their respective concentrations. Examples include:

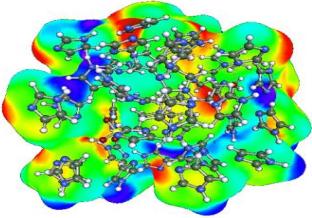
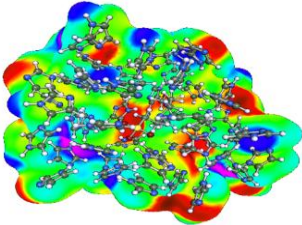
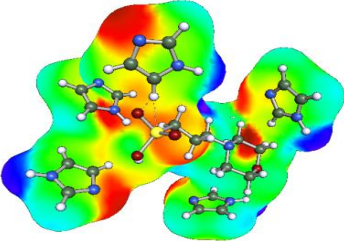
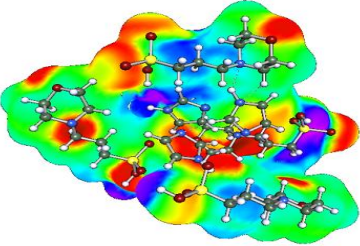
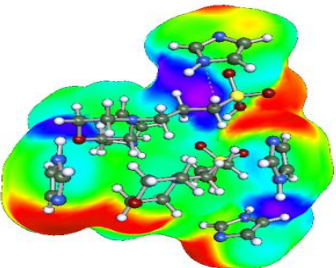
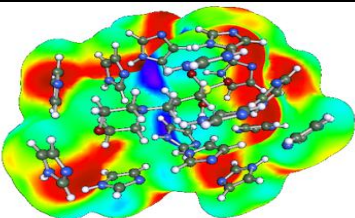
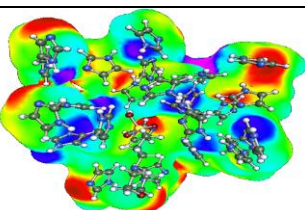
- 1HEPES_1IMIDAZOLE represents a buffer solution containing 20 mM HEPES and 20 mM IMIDAZOLE.
- 2MOPS_5IMIDAZOLE, denoting a solution with 20 mM MOPS and 50 mM IMIDAZOLE.

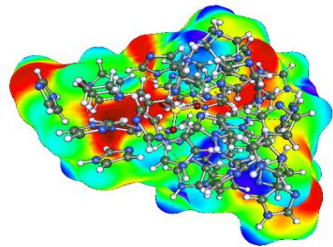
In this study, a key focus is determining the interaction energy between these buffer solutions and polystyrene-supported (carboxy, 2-(ethyl amino)ethyl amino) acetic acid (PSEAA) and PSEAA-Cu²⁺. By analyzing these interactions, researchers can gain deeper insights into binding affinities, elution efficiencies, and buffer compatibility with adsorbents, ultimately optimizing protein fractionation and

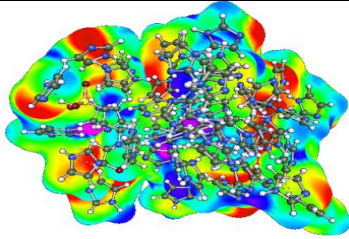
purification conditions. The accompanying table presents the computed interaction values, which serve as a critical reference for selecting the most effective buffer formulations for chromatography and protein separation.

Table 1: the composition and ratio of various buffer formulations, specifically designed with combinations of HEPES, IMIDAZOLE, and MOPS.

Buffers N°	Ratio	Compositions	Sigma surface
Buff01	1:1	1HEPES1IMIDAZOLE	
Buff02	1:5	1HEPES5IMIDAZOLE	
Buff03	2:5	2HEPES5IMIDAZOLE	
Buff04	1:15	1HEPES15IMIDAZOLE	
Buff05	1:25	1HEPES25IMIDAZOLE	

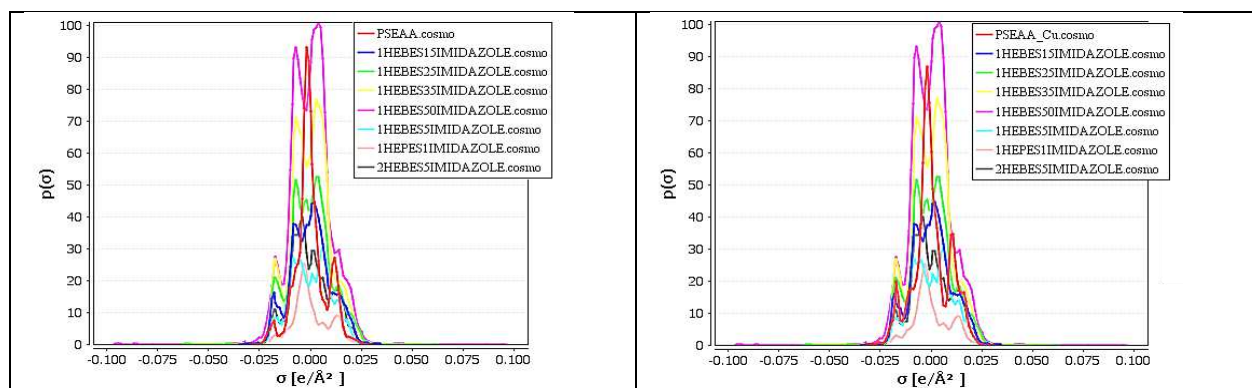
Buff06	1:35	1HEPES35IMIDAZOLE	
Buff07	1:50	1HEPES50IMIDAZOLE	
Buff08	1:5	1MOPS5IMIDAZOLE	
Buff09	3:4	3MOPS4IMIDAZOLE	
Buff10	2:5	2MOPS5IMIDAZOLE	
Buff11	1:15	1MOPS15IMIDAZOLE	
Buff12	1:25	1MOPS25IMIDAZOLE	

Buff13	1:35	1MOPS35IMIDAZOLE	
--------	------	------------------	--

Buff14	1:50	1MOPS50IMIDAZOLE	
--------	------	------------------	--

5.2. Sigma profile of the hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD)

The sigma profiles (Figure 5) provide valuable insights into the role of imidazole in buffer formulations and its interactions with polystyrene-supported (carboxy, 2-(ethyl amino)ethyl amino) acetic acid (PSEAA). Sharp peaks in the negative σ range suggest that imidazole enhances molecular interactions, making it a crucial component in optimizing buffer performance. This effect can be attributed to the nitrogen-containing heterocyclic structure of imidazole, which possesses strong hydrogen-bond donor and acceptor properties. As the imidazole concentration increases, these interactions intensify, leading to greater binding efficiency with PSEAA.



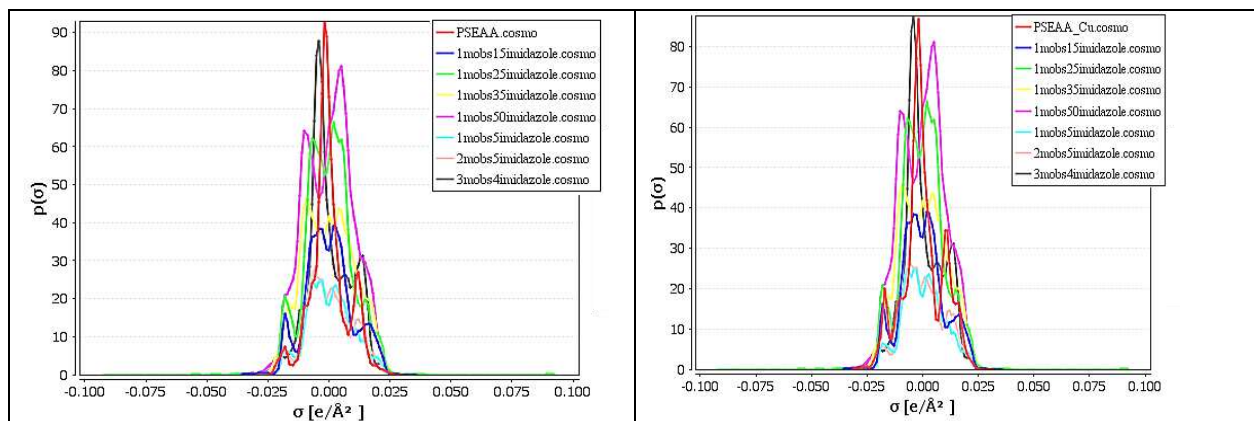


Figure 5. Sigma Profiles of PSEAA with and without Copper-Enhanced Buffer

Buffers with higher imidazole concentrations, such as 1HEPES50IMIDAZOLE, consistently exhibit stronger interactions, primarily due to the increased availability of donor and acceptor sites. This is evident in the enhanced polar interactions observed in the system, which result in increasingly negative ΔG values. The more negative Gibbs free energy values indicate higher thermodynamic stability of the interaction system, reinforcing that imidazole significantly stabilizes the molecular environment in buffer formulations. This stabilization effect is crucial in optimizing protein adsorption, elution efficiency, and chromatographic performance, making imidazole essential for practical biochemical and biotechnological applications [32].

This study underscores the unique interactions between different buffer systems, including HEPES, Imidazole, and MOPS, with polystyrene-supported PSEAA, highlighting their distinct roles in molecular binding and stability. HEPES is fundamental in maintaining a stable pH, but its direct interactions with PSEAA are weaker than imidazole. However, in dual-buffer systems where HEPES and imidazole are combined, HEPES contributes to system stability, while imidazole provides highly interactive sites that enhance binding strength. Sigma profiles for these combined buffer systems display sharper and more intense peaks in the negative σ range, confirming the synergistic effect of these buffers for optimal interactions [33]. Including copper ions (Cu^{2+}) significantly enhances these interactions by coordinating with PSEAA and imidazole functional groups. Copper ions interact with

carboxy and amino groups of PSEAA and the nitrogen atoms in imidazole, leading to increased molecular stability. This metal coordination effect strengthens protein binding, facilitates catalytic pathways, and enhances the functional properties of these buffer systems, making them valuable in catalysis and advanced material applications.

Dynamic simulations of imidazole mobility reveal its flexibility, allowing it to explore diverse interaction pathways with PSEAA. The broader sigma profiles in these simulations reflect enhanced interaction strength and adaptability, making imidazole-based buffer systems particularly effective in dynamic environments. This adaptability is particularly relevant in fluctuating experimental conditions, such as temperature variations or continuous flow systems. These findings have broad implications for both material and biological applications. In material science, the strong interactions observed at higher imidazole concentrations can be exploited for surface functionalization or stabilizing polymer matrices like PSEAA, with potential applications in selective adsorption, catalysis, and drug delivery. In biological applications, imidazole-based buffers can enhance biomolecular interactions, mimicking histidine residues in proteins, making them suitable for enzyme stabilization, affinity chromatography, and biocompatible coatings.

In contrast, MOPS exhibits weaker interactions with PSEAA than imidazole, as indicated by less pronounced sigma profile peaks in the negative σ range. The interactions of MOPS with PSEAA are primarily driven by its sulfonic acid group, which forms ionic bonds with the resin. However, MOPS does contribute to stabilizing the buffer environment by enhancing hydrogen bonding and van der Waals interactions, particularly when combined with imidazole in dual-buffer systems. The synergy between MOPS and imidazole leads to broader and more intense interaction profiles, suggesting that MOPS can enhance the overall stability of imidazole-based systems.

Dynamic simulations involving MOPS reveal moderate peak broadening, indicating limited flexibility and weaker interactions than imidazole. The morpholine ring in MOPS restricts its binding potential,

favoring ionic interactions over extensive hydrogen bonding. When copper ions are introduced into MOPS-containing systems, additional coordination interactions are observed, altering charge distribution and enhancing electrostatic interactions with PSEAA. However, these interactions remain weaker than those observed in imidazole-Cu²⁺ systems, suggesting that MOPS is best utilized as a secondary buffer in dual-buffer formulations.

The advantages of MOPS lie in its ability to provide stability and moderate interactions, making it particularly useful in applications requiring controlled surface properties or stabilizing colloidal suspensions under mild conditions. When combined with imidazole, the synergistic effects contribute to enhanced binding and surface functionalization, making such systems highly effective for applications in material science and biological research [34].

5.3. PSEAA with and without Copper in Buffers combination

Tables 2 and 3 comprehensively evaluate the interactions between different buffer formulations and polystyrene-supported materials, PSEAA and PSEAA-Cu²⁺. The parameters analyzed include Chemical Potential (CP), Log10 Partial Pressure (PP), Free Energy (ΔG), Total Mean Interaction Energy (TMIE), Hydrogen-Bond Interaction Energy (H-BIE), and van der Waals (vdW) Energy. These parameters offer critical insights into the relative binding strength, stability, and energetic favorability of different buffer compositions when interacting with PSEAA and PSEAA-Cu²⁺.

Table 2 provides detailed insights into the interaction dynamics between PSEAA and various buffer formulations, highlighting the superior performance of HEPES-imidazole buffers over MOPS-imidazole buffers regarding interaction strength and stability. The free energy (ΔG) values demonstrate that 1HEPES50IMIDAZOLE exhibits a significantly stronger interaction (-187.54 kcal/mol) compared to 1MOPS50IMIDAZOLE (-147.90 kcal/mol), suggesting that HEPES contributes more effectively to stabilizing PSEAA interactions. This can be attributed to HEPES' sulfonic acid

functional group, which enhances hydrogen bonding and electrostatic stabilization. Similarly, the total mean interaction energy (TMIE) values follow the same trend, with 1HEPES50IMIDAZOLE reaching -187.54 kcal/mol, whereas 1MOPS50IMIDAZOLE achieves only -147.90 kcal/mol, reinforcing the superior stabilizing effect of HEPES buffers due to their extensive hydrogen bonding networks and effective electrostatic interactions [33-35]. The role of imidazole in enhancing these interactions is evident across all buffer systems, as increasing the imidazole concentration significantly strengthens interactions with PSEAA, regardless of whether the base buffer is HEPES or MOPS. For instance, 1HEPES5IMIDAZOLE exhibits a ΔG of -60.99 kcal/mol, while 1HEPES50IMIDAZOLE shows a much stronger interaction of -187.54 kcal/mol. Similarly, in MOPS buffers, 1MOPS5IMIDAZOLE has a ΔG of -48.53 kcal/mol, which intensifies to -147.90 kcal/mol in 1MOPS50IMIDAZOLE. These findings confirm that higher imidazole concentrations amplify interaction strength, primarily due to imidazole's nitrogen-containing heterocyclic structure, which provides additional donor and acceptor sites for hydrogen bonding and van der Waals interactions [33-35].

Table 2 Interaction between PSEAA and Buffer Combinations

Mixture	CP kcal/mol	PP [mbar]	ΔG kcal/mol	TMIE kcal/mol	H-BIE Kcal/mol	VdW Kcal/mol
PSEAA	-16.42548	-21.20363	-1904314.83	-71.38129	-44.43065	-40.53679
1HEPES1IMIDAZOLE	-6.45423	-12.88936	-847660.991	-28.10267	-19.20269	-13.69298
PSEAA	-14.38125	-19.70516	-1904312.79	-70.17706	-44.79254	-40.81190
1HEPES5IMIDAZOLE	-8.75976	-10.84266	-1415361.92	-60.99910	-42.29555	-29.65739
PSEAA	-16.33606	-21.13807	-1904314.74	-73.23076	-47.01945	-41.06234
2HEPES5IMIDAZOLE	-11.11199	-4.70794	-2121256.53	-72.53869	-51.31277	-33.48597
PSEAA	-17.11611	-21.70987	-1904315.52	-76.62815	-49.45708	-41.36063
1HEPES15IMIDAZOLE	-13.78072	26.19919	-2835266.56	-86.38224	-56.74552	-43.96899
PSEAA	-19.33778	-23.33840	-1904317.75	-82.66435	-55.64037	-41.37708
1HEPES25IMIDAZOLE	-15.77916	68.91053	-4255425.42	-107.41675	-71.13790	-54.38124
PSEAA	-26.25528	-28.40907	-1904324.66	-135.98402	-102.15106	-41.50660
1HEPES35IMIDAZOLE	-18.39275	133.39246	-5674952.54	-127.84317	-75.94495	-76.95115
PSEAA	-21.49249	-24.91784	-1904319.90	-105.90952	-73.86919	-41.50693
1HEPES50IMIDAZOLE	-30.94199	301.53424	-7804962.87	-187.54118	-117.69838	-101.72578
PSEAA	-16.56874	-21.30864	-1904314.98	-72.52787	-46.32892	-40.87837
1MOPS5IMIDAZOLE	-8.28423	-18.21162	-1356241.29	-48.53221	-32.97807	-24.30642
PSEAA	-14.91825	-20.09879	-1904313.33	-69.45945	-43.95678	-40.17894

2MOPS5IMIDAZOLE	-6.99138	-11.29764	-2002474.77	-49.97349	-34.01884	-25.11308
PSEAA	-15.31217	-20.38755	-1904313.72	-68.99846	-43.35157	-40.53905
3MOPS4IMIDAZOLE	-15.56910	31.87597	-3010917.01	-97.84357	-68.42228	-48.17235
PSEAA	-17.31088	-21.85264	-1904315.72	-73.19140	-45.75182	-41.45942
1MOPS15IMIDAZOLE	-11.33939	17.27263	-2776358.28	-73.85913	-47.45160	-39.30468
PSEAA	-17.53396	78.84170	-4992501.88	-112.18339	-84.32964	-49.07014
1MOPS25IMIDAZOLE	-18.00570	80.83643	-4196464.39	-115.52280	-70.28250	-67.11192
PSEAA	-16.23521	-21.06415	-1904314.64	-73.97988	-47.67639	-41.29153
1MOPS35IMIDAZOLE	-13.69578	88.13854	-5613555.60	-103.13815	-66.62014	-55.39275
PSEAA	-15.04041	-20.18834	-1904313.45	-73.52486	-47.10389	-40.76579
1MOPS50IMIDAZOLE	-21.50505	216.13716	-7742529.79	-147.90381	-89.91290	-84.90355

The influence of imidazole concentration on hydrogen-bond interaction energy (H-BIE) and van der Waals (vdW) energy further supports its role in strengthening buffer-PSEAA interactions. In HEPES buffers, increasing imidazole content from 100 mM to 1000 mM leads to a substantial increase in H-BIE from -42.30 kcal/mol to -117.69 kcal/mol, while vdW energy improves from -29.65 kcal/mol to -101.72 kcal/mol. A similar but less pronounced trend is observed in MOPS buffers, where 1MOPS50IMIDAZOLE has an H-BIE of -89.91 kcal/mol, which is still significantly stronger than 1MOPS5IMIDAZOLE (-32.97 kcal/mol). These results indicate that higher imidazole ratios significantly enhance interaction strength by increasing hydrogen bonding and van der Waals forces, particularly in HEPES-based buffer systems [33-35]. The partial pressure (PP) values provide further insight into the stability of buffer-PSEAA interactions, with higher PP values indicating more excellent stability. HEPES-imidazole buffers consistently exhibit higher PP values than MOPS-imidazole buffers, confirming their superior interaction stability. For example, the PP for 1HEPES50IMIDAZOLE is 301.53 mbar, while 1MOPS50IMIDAZOLE has a lower PP of 216.14 mbar. This suggests that when combined with higher imidazole concentrations, HEPES buffers provide the most stable interaction systems with PSEAA [33-35].

Overall, the results from Table 2 underscore the superior interaction capabilities of HEPES-imidazole buffer systems compared to MOPS-imidazole buffers. HEPES buffers exhibit stronger free energy interactions, higher total mean interaction energy, and improved hydrogen bonding and van der Waals interactions, particularly at higher imidazole concentrations. These findings suggest that

HEPES-imidazole systems are better suited for applications requiring enhanced stability and strong molecular interactions, such as protein purification, chromatography, and biomolecular stabilization [33-35].

Table 3 explores the interaction of various buffer formulations with PSEAA-Cu²⁺, revealing significant alterations in interaction dynamics and a general reduction in interaction strength across all buffers. However, Cu²⁺ plays a crucial role in stabilizing the system through specific coordination interactions despite this reduction. The free energy (ΔG) values demonstrate that 1HEPES50IMIDAZOLE experiences a decrease from -187.54 kcal/mol with PSEAA to -114.80 kcal/mol with PSEAA-Cu²⁺, while 1MOPS50IMIDAZOLE decreases from -147.90 kcal/mol to -83.98 kcal/mol. This reduction can be attributed to Cu²⁺'s competitive binding at hydrogen-bonding sites, which disrupts hydrogen bonding and van der Waals interactions.

Table 3: Interaction between PSEAA-Cu²⁺ and Buffer Combinations

MIXTURE	CP kcal/mol	PP [mbar]]	ΔG kcal/mol	TMIE kcal/mol	H-BIE Kcal/mol	VdW Kcal/mol
PSEAA-Cu ²⁺	-11.93570	82.94533	-4992496.28	-88.87676	-57.91761	-47.73762
1HEPES1IMIDAZOLE	-6.45423	-12.88936	-847660.991	-28.10267	-19.20269	-13.69298
PSEAA-Cu ²⁺	-11.11189	83.54921	-4992495.46	-82.68023	-52.76755	-48.19753
1HEPES5IMIDAZOLE	-8.75976	-10.84266	-1415361.92	-60.99910	-42.29555	-29.65739
PSEAA-Cu ²⁺	-11.95897	82.92828	-4992496.31	-85.13894	-54.53599	-48.33636
2HEPES5IMIDAZOLE	-11.11199	-4.70794	-2121256.53	-72.53869	-51.31277	-33.48597
PSEAA-Cu ²⁺	-12.77527	82.32991	-4992497.12	-87.11274	-55.49184	-48.79204
1HEPES15IMIDAZOLE	-13.78072	26.19919	-2835266.56	-86.38224	-56.74552	-43.96899
PSEAA-Cu ²⁺	-14.78023	80.86024	-4992499.13	-91.23091	-59.69739	-48.91407
1HEPES25IMIDAZOLE	-15.77916	68.91053	-4255425.42	-107.41675	-71.13790	-54.38124
PSEAA-Cu ²⁺	-23.01723	74.82234	-4992507.36	-136.80998	-100.32066	-49.10300
1HEPES35IMIDAZOLE	-18.39275	133.39246	-5674952.54	-127.84317	-75.94495	-76.95115
PSEAA-Cu ²⁺	-17.09360	79.16449	-4992501.44	-114.79746	-79.06370	-49.04849
1HEPES50IMIDAZOLE	-30.94199	301.53424	-7804962.87	-187.54118	-117.69838	-101.72578
PSEAA-Cu ²⁺	-11.68109	83.13197	-4992496.03	-81.45932	-51.01280	-48.23498
1MOPS5IMIDAZOLE	-8.28423	-18.21162	-1356241.29	-48.53221	-32.97807	-24.30642
PSEAA-Cu ²⁺	-10.96197	83.65910	-4992495.31	-80.63367	-50.73497	-47.47013
2MOPS5IMIDAZOLE	-6.99138	-11.29764	-2002474.77	-49.97349	-34.01884	-25.11308
PSEAA-Cu ²⁺	-11.64448	83.15880	-4992495.99	-86.70813	-56.77306	-47.81839
3MOPS4IMIDAZOLE	-15.56910	31.87597	-3010917.01	-97.84357	-68.42228	-48.17235
PSEAA-Cu ²⁺	-12.01902	82.88426	-4992496.37	-82.33421	-50.5954	-48.81376

1MOPS15IMIDAZOLE	-11.33939	17.27263	-2776358.28	-73.85913	-47.45160	-39.30468
PSEAA-Cu²⁺	-17.53396	78.84170	-4992501.88	-112.18339	-84.32964	-49.07014
1MOPS25IMIDAZOLE	-18.00570	80.83643	-4196464.39	-115.52280	-70.28250	-67.11192
PSEAA-Cu²⁺	-12.52618	82.51250	-4992496.87	-83.21386	-52.09503	-48.78521
1MOPS35IMIDAZOLE	-13.69578	88.13854	-5613555.60	-103.13815	-66.62014	-55.39275
PSEAA-Cu²⁺	-11.44058	83.30827	-4992495.79	-83.98859	-52.94293	-48.22689
1MOPS50IMIDAZOLE	-21.50505	216.13716	-7742529.79	-147.90381	-89.91290	-84.90355

The role of the imidazole ratio remains critical in the presence of Cu²⁺. Higher imidazole concentrations partially compensate for the reduction in interaction strength caused by Cu²⁺, maintaining substantial interaction energy. For example, 1HEPES5IMIDAZOLE exhibits a ΔG of -85.14 kcal/mol with PSEAA-Cu²⁺, compared with -61.00 kcal/mol for PSEAA. For the HEPES/imidazole system, increasing the imidazole proportion was generally associated with more negative ΔG values, although the magnitude of this effect depended on the PSEAA/PSEAA-Cu²⁺ system and buffer composition. A similar trend is observed in MOPS buffers, where a similar trend was observed for MOPS/imidazole systems. The ΔG value changed from -48.53 kcal/mol for PSEAA to -80.63 kcal/mol for PSEAA-Cu²⁺ at the 1MOPS5IMIDAZOLE composition when imidazole concentration. These findings suggest that imidazole continues to be a dominant factor in enhancing interaction strength, even in the presence of Cu²⁺, likely due to its nitrogen-containing heterocyclic structure, which facilitates multi-point interactions with the metal ion. The hydrogen-bond interaction energy (H-BIE) and van der Waals (vdW) energy values further highlight the impact of Cu²⁺ coordination. Both interaction types decline with Cu²⁺, but their strength remains relatively higher in systems with increased imidazole concentrations. For example, 1HEPES50IMIDAZOLE sees its H-BIE drop from -117.69 kcal/mol (PSEAA) to -79.06 kcal/mol (PSEAA-Cu²⁺), while vdW energy decreases from -101.72 kcal/mol to -49.05 kcal/mol. In MOPS buffers, a similar trend is observed: H-BIE for 1MOPS50IMIDAZOLE declines from -89.91 kcal/mol to -52.94 kcal/mol, while vdW energy decreases from -84.90 kcal/mol to -48.23 kcal/mol. These reductions confirm that Cu²⁺ weakens interaction strength overall. The comparison between HEPES/imidazole and MOPS/imidazole systems indicates that their relative interaction strengths depend on both the resin composition and buffer formulation. For PSEAA, the HEPES/imidazole system generally exhibited more negative ΔG

values at comparable high imidazole ratios, whereas for PSEAA-Cu²⁺ the differences between the two buffer systems were less pronounced and depended on the specific composition [36]. Interestingly, partial pressure (PP) values increase in the presence of Cu²⁺, suggesting that while interaction strength is reduced, overall system stability is enhanced. For instance, The partial pressure values also differed between the non-functionalized and Cu²⁺-functionalized systems. For example, at the 1HEPES50IMIDAZOLE composition, the PP was 301.53 mbar for PSEAA and 83.13 mbar for PSEAA-Cu²⁺. These results indicate that Cu²⁺ functionalization substantially alters the thermodynamic behavior of the buffer–resin system.

Still, HEPES-imidazole systems mitigate this effect more effectively than MOPS-imidazole systems. The presence of Cu²⁺ stabilizes the buffer-PSEAA system but simultaneously reduces interaction strength, making it ideal for applications requiring controlled interaction dynamics rather than maximum binding strength. HEPES-imidazole systems are well-suited for applications demanding strong binding and stability, such as catalysis, surface functionalization, and biomolecular adsorption. In contrast, MOPS-imidazole systems may be preferable for applications requiring moderate binding, particularly in environments where Cu²⁺ coordination enhances specific interactions, such as plasma protein cleavage, chromatography, and mild catalytic systems [37-39].

6. Conclusions

This study successfully demonstrated that chromatographic purification of human plasma proteins is feasible using novel polystyrene-supported (PSEAA) resin and its Cu²⁺-functionalized counterpart (PSEAA-Cu²⁺). The nature of the buffer system strongly influenced the adsorption and purification process, as it dictated the extent of mixed-mode interactions, including ion exchange and immobilized metal affinity chromatography (IMAC). The chromatographic experiments demonstrated the potential of PSEAA and PSEAA-Cu²⁺ to fractionate plasma proteins, as evidenced by the detection of distinct protein bands at different molecular weights under different buffer and imidazole conditions.

However, protein-specific identification requires additional analytical characterization beyond SDS-PAGE. The methodologies employed in this study were simple, cost-effective, and simple and potentially cost-effective, offering significant advantages over conventional chromatography techniques that rely on expensive and chemically unstable ligands. The observed chromatographic performance and compatibility of PSEAA and PSEAA-Cu²⁺ with the tested buffer conditions make them attractive alternatives to widely used adsorbents in protein purification. Additionally, the computational analysis provided valuable insights into the thermodynamic properties of different buffer systems, revealing the critical role of imidazole in enhancing the binding strength of both PSEAA and PSEAA-Cu²⁺.

The COSMO-RS analysis demonstrated that buffer composition and imidazole proportion substantially influence the thermodynamic interactions of PSEAA and PSEAA-Cu²⁺. The relative interaction strength of HEPES/imidazole and MOPS/imidazole systems depended on the resin composition and buffer formulation. These computational findings provide molecular-level insights into the experimentally observed differences in protein elution and support further optimization of buffer conditions for plasma protein fractionation. Imidazole facilitated stronger hydrogen bonding and van der Waals interactions, while Cu²⁺ introduced stabilizing coordination effects despite reducing overall interaction strength. HEPES-imidazole buffers were ideal for applications requiring robust protein cleavage owing to their higher interaction energy, stability, and superior hydrogen bonding capacity. In contrast, MOPS-imidazole buffers were more suitable for milder and more controlled protein purification processes, making them advantageous in scenarios where selectivity and fine-tuned protein retention are required.

This research underscores the potential of PSEAA and PSEAA-Cu²⁺ as efficient, stable, and low-cost adsorbents for plasma protein purification, offering an alternative to traditional affinity chromatography resins. The combined experimental and computational approach provides a deeper

understanding of how buffer composition influences protein adsorption, which can guide future applications in biotechnology, clinical diagnostics, and therapeutic protein production. Moving forward, further optimization of resin formulations and buffer compositions, along with expanding this approach to other biologically significant proteins, will enhance the applicability of this purification strategy in both academic research and industrial-scale protein isolation.

Author Contributions

Zina Benammar, Salim Madani, and Kamel Mokhnache conducted the protein purification experiments. **Zina Benammar, Salim Madani, Souhib Bennaadja, and Noureddine Charef** were responsible for study design and manuscript drafting. **Lekhmici Arrar, Lokmane Abdelouahed, Marta F. Garcia, Daniel L. Garcia, Manawwer Alam, and Yacine Benguerba** critically reviewed and revised the final manuscript. All authors contributed to the research process reviewed, and approved the final version of the manuscript for publication.

Declaration of Competing Interests

The authors declare that they have no known financial or personal conflicts of interest that could have influenced the research findings presented in this manuscript.

Availability of Data

No external datasets were used in the research described in this article.

References

- [1] N. C. Das, Y. U. Rathod, V. U. Pandit, W. B. Gurnule, Studies of chelation ion-exchange properties of copolymer resin derived from 1,5-diaminonaphthalene, 2,4-dihydroxy- propiophenone and formaldehyde, Mater. Today: Proc. 53 (2022) 80-85.
<https://doi.org/10.1016/j.matpr.2021.12.370>

- [2] S. Doria, J. Yost, Z. Gagnon, Free-flow biomolecular concentration and separation of proteins and nucleic acids using téichophoresis, *Talanta*, 255 (2023)124-198.<https://doi.org/10.1016/j.talanta.2022.12419>
- [3] C. Wang, D. Zhu, J. Zhang, Homochiral iron-based γ -cyclodextrin metal-organic framework for stereoisomer separation in the open tubular capillary electrochromatography, *J. Pharm. Biomed.* 215 (2022) 114777. <https://doi.org/10.1016/j.jpba.2022.114777>
- [4] N. Babanejad, K. Mfoafo, E. Zhang, Y. Omid, R. Razeghifard, H. Omidian, Applications of cryostructures in the chromatographic separation of biomacromolecules, *J. Chromatogr. A.* 1683 (2022) 463-546. <https://doi.org/10.1016/j.chroma.2022.463546>
- [5] S. Madani, N. Charef, A. Hellal, D. L Garcia, M. F Garcia, L. Arrar, M.S. Mubarak, Synthesis, density functional theory studies, and sorption properties toward some divalent heavy metal ions of a new polystyrene-supported 4-(5-mercapto-1,3,4-thiadiazol-2-ylimino) pentan-2-one polymer, *J. Appl. Polym. Sci.* 137, (2019), 48289.<https://doi.org/10.1002/app.48289>
- [6] N. Guemmaz, S. Madani, K. Mokhnache, A. Karbab, D. L. Garcia, M. F. Garcia, L. Arrar, N. Charef, M. S. Mubarak, Synthesis, characterization, sorption, and antibacterial properties of a new polystyrene-supported o-hydroxy-acetophenone diethylenetriamine polymer, *Int. J. Polym. Mater.* 37, (2023), 2288096. <https://doi.org/10.1080/00914037.2023.2288096>
- [7] R. Irankunda, J. A. C. Echavarría, C. Paris, L. Stefan, S. Desobry, K. Selmeczi, L. Muhr, L. Canabady-Rochelle, Metal-Chelating Peptides Separation Using Immobilized Metal Ion Affinity Chromatography: Experimental Methodology and Simulation, *Separations*, 9 (2022) 370-387, <https://doi.org/10.3390/separations9110370>
- [8] O. Kokhan, D. R. Marzolf, Detection and quantification of transition metal leaching in metal affinity chromatography with hydroxy naphthol blue, *Anal. Biochem.* 582 (2019) 113347, <https://doi.org/10.1016/j.ab.2019.113347>. K
- [9] T. S. Choi, F. A. Tezcan, Overcoming universal restrictions on metal selectivity by protein design, *Nature* volume 603 (2022), 522–527. <https://doi.org/10.1038/s41586-022-04469-8>

- [10] L. Cristina d. Góes, E. A. Miranda, S.M.A. Bueno, Interaction of histidine-tagged human proinsulin with immobilized nickel ion: Effect of chelating ligand and thermodynamics analysis. *Colloids Surf. A: Physicochem*, 369 (2010) 176–185. <https://doi.org/10.1016/j.colsurfa.2010.08.019>
- [11] H. Zheng, F. Wei, J. Tian, C. Wang, C. Xue, Preparation of nickel-chelated iminodiacetate-functionalized macroporous agarose monolith using modular and clickable building blocks for affinity separation of histidine-tagged recombinant proteins, *J. Chromatogr. A*, 1682 (2022), 463509. <https://doi.org/10.1016/j.chroma.2022.463509>
- [12] J. J Lee, D. F Bruley, K. A Kang, Effect of pH and Imidazole on protein C purification from Cohn fraction IV-1 by IMAC, *Adv. Exp. Med. Biol*, 61 (2007) 599. https://doi.org/10.1007/978-0-387-71764-7_9
- [13] T.N. Lin, S.C. Lin, Metal chelate-epoxy bifunctional membranes for selective adsorption and covalent immobilization of a His-tagged protein, *J. Biosci. Bioeng*, 133 (2022) 258-264. <https://doi.org/10.1016/j.jbiosc.2021.11.007>
- [14] N. Charef, Z. Benmamar, A. Baghiani, M. S. Mubarak, L. Arrar Preparation of a new polystyrene supported-ethylenediamine dicarboxylic acid resin and its sorption behavior toward divalent metal ions, *Solvent Extr. Ion Exch*, 30, (2012), 101-112. <https://doi.org/10.1080/07366299.2011.581070>
- [15] N. Hyttinen, N. L. Prisle. Improving Solubility and Activity Estimates of Multifunctional Atmospheric Organics by Selecting Conformers in COSMOtherm. *The Journal of Physical Chemistry A*, 2020, 124 (23), 4801–4812, <https://doi.org/10.1021/acs.jpca.0c04285>
- [16] J. Iqbal, N. Muhammad, A. Rahim, A. S. Khan, Z. Ullah, G. Gonfa, P. Ahmad, COSMO-RS predictions, hydrogen bond basicity values and experimental evaluation of amino acid-based ionic liquids for lignocellulosic biomass dissolution, *Journal of Molecular Liquids*, 2019, 273, 215-221, <https://doi.org/10.1016/j.molliq.2018.10.044>.
- [17] N. F. M. Chong, H. Hussain, N. E. Hamdin, D. H. S. Wee, M. Nisar, W. J. Yan, B. Y. G. Lau, N. Rahmad, Higher resolution protein band visualization via improvement of colloidal CBB-G staining by gel fixation, *Chem. Biol. Technol. Agric*. 9 (2022) 32-41 <https://doi.org/10.1186/s40538-022-00297-0>

- [18] H. Zagst, C. Elgert, S. Behrends et al. Combination of strong anion exchange liquid chromatography with microchip capillary electrophoresis sodium dodecyl sulfate for rapid two-dimensional separations of complex protein mixtures. *Anal Bioanal Chem* 414, (2022).1699–1712. <https://doi.org/10.1007/s00216-021-03797-4>
- [19] TURBOMOLE V7.3 2018. a Development of University of Karlsruhe and Forschungszentrum Karlsruhe GmbH. 1989–2007. TURBOMOLE GmbH. since 2007. Available online: <http://www.turbomole.com> (accessed on 9 July 2018).
- [20] A. Klamt. The COSMO and COSMO-RS solvation models. *Wiley Interdisciplinary Reviews: Computational Molecular Science*. (2011), 1(5), 699–709. <https://doi.org/10.1002/wcms.56>
- [21] Y. Zhao, D. G. Truhlar. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: Two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theoretical Chemistry Accounts*, (2008). 120(1-3), 215-241. <https://doi.org/10.1007/s00214-007-0310-x>
- [22] F. Weigend, R. Ahlrichs. Balanced basis sets of split valence, triple zeta valence, and quadruple zeta valence quality for H to Rn: Design and assessment of accuracy. *Physical Chemistry Chemical Physics*, (2005), 7(18), 3297. <https://doi.org/10.1039/b508541a>
- [23] R.G. Nascimento, M.C.P. Porfírio, A.N. Alves et al. Characterization and Thermodynamic Behavior of Protein Adsorption on a Macroporous Monolithic Polymeric IMAC Matrix. *J Polym Environ* 31(2023), 2641–2652. <https://doi.org/10.1007/s10924-022-02751-4>
- [24] C. Sanchez-Cano, M. Carril, Recent Developments in the Design of Non-Biofouling Coatings for Nanoparticles and Surfaces, *Int. J. Mol. Sci*, 21. (2020), 1007. <https://doi.org/10.3390/ijms21031007>
- [25] C. L. Kielkopf, W. Bauer, I. L. Urbatsch, Purification of Polyhistidine-Tagged Proteins by Immobilized Metal Affinity Chromatography, *Cold Spring Harb Protoc*, 2020(6), [pdb.prot102194](https://doi.org/10.1101/pdb.prot102194). doi:10.1101/pdb.prot102194.
- [26] N. Lingg, C. Öhlknecht, A. Fischer, M. Mozgovicz, T. Scharl, C. Oostenbrink, A. Jungbauer, Proteomics analysis of host cell proteins after immobilized metal affinity chromatography:

Influence of ligand and metal ions, *Journal of Chromatography A*, (2020), 1633, 461649.
<https://doi.org/10.1016/j.chroma.2020.461649>

- [27] K. Kannan, J. Mukherjee, P. Mishra, M. N. Gupta, Nickel Ferrite Nanoparticles as an Adsorbent for Immobilized Metal Affinity Chromatography of Proteins, *Journal of chromatographic Science*, 59,3, 2021, Pages 262–268. <https://doi.org/10.1093/chromsci/bmaa102>
- [28] H. S. D. R. Hamacek, I. T. L. Bresolin, I. F. Fioravante, S. M. A. Bueno, Synthesis and characterization of chitosan-polyacrylamide cryogels for the purification of human IgG by IMAC, *Process Biochemistry*, 131, 2023, 199-209. <https://doi.org/10.1016/j.procbio.2023.06.021>
- [29] J. A. Townsend, J. E. Keener, Z. M. Miller, J. S. Prell, M. T. Marty. Imidazole derivatives improve charge reduction and stabilization for native mass spectrometry. *Analytical Chemistry*. 2019, 91, 14765–14772. <https://doi.org/10.1021/acs.analchem.9b04263>
- [30] T. Yin, P. Gao, A. Xu, E.G.Saldanha. Enhanced Prediction of Molecular Properties Using Transfer Learning on Sigma Profiles, 2024, available at Research Square. <https://doi.org/10.21203/rs.3.rs-5278418/v1>
- [31] W. Witek, J. Sliwiak, M. Rawski, M. Ruszkowski, Targeting imidazole-glycerol phosphate dehydratase in plants: novel approach for structural and functional studies, and inhibitor blueprinting. *Frontiers in Plant Science*. 2024, 15:1343980. <https://doi.org/10.3389/fpls.2024.1343980>
- [32] D. Su, F. Xu, H. Wang, J. Xie, S. Wang, M. Jiang, M. Feng, , Z. Zhang , Z. Song, N. Liu. Polystyrene-supported imidazolium acidic ionic liquids: Highly efficient catalysts for the synthesis of bisphenols. *New Journal of Chemistry*, 2023,47, 9894-9904, <https://doi.org/10.1039/D3NJ01453C>.
- [33] S. Chen, Z. Li, Y. Wu, N. Mahmood, F. Lortie, J. Bernard, W.H Binder, J. Zhu. Hydrogen-Bonded Supramolecular Polymer Adhesives: Straightforward Synthesis and Strong Substrate Interaction. *Angewandte Chemie International Edition*. 2022. 61, 27. 134 <https://doi.org/10.1002/anie.202203876>
- [34] W. Inam, R. Bhadane, R. N. Akpolat, R. A. Taiseer, S. K. Filippov, O. M. H. Salo-Ahen, J. M. Rosenholm, H. Zhang, Interactions between polymeric nanoparticles and different buffers as investigated

by zeta potential measurements and molecular dynamics simulations. VIEW. 2022, 3, 20210009. <https://doi.org/10.1002/VIW.20210009>

- [35] M. Taha, J.A.P. Coutinho. Organic-phase biological buffers for biochemical and biological research in organic media. *Journal of Molecular Liquids*, 2016. 221, 197–205. <https://doi.org/10.1016/j.molliq.2016.05.052>
- [36] S. Brudar, B. Hribar-Lee. Effect of Buffer on Protein Stability in Aqueous Solutions: A Simple Protein Aggregation Model. *The Journal of Physical Chemistry B*, 2021. 125(10), 2504–2512. <https://doi.org/10.1021/acs.jpcb.0c10339>
- [37] S. Gong, S. Kirmizialtin, A. Chang, J.E. Mayfield. Kinetic and thermodynamic analysis defines roles for two metal ions in DNA polymerase specificity and catalysis. *Journal of Biological Chemistry*, 2020. 296, 100184. <https://doi.org/10.1074/jbc.ra120.016489>
- [38] A.R. Ferré-D'Amaré, W.C. Winkler. The Roles of Metal Ions in Regulation by Riboswitches. *Metallomics in Life Sciences*, 2011. 9, 141–173. <https://doi.org/10.1039/9781849732512-00141>
- [39] L. Aroui, S. Madani, I. Bousnoubra, A. Boublia, I. Lakikza S. I. Aouni, L. Abdelouahed, B. Ernst, M. Alam, Y. Benguerba. Enhanced degradation of crystal violet using PANI-ZnO nanocomposites: Electro-oxidation and photocatalysis studies. *Journal of Molecular Liquids*. 2024, 412, 125818. <https://doi.org/10.1016/j.molliq.2024.125818>
- [40] Bennaadja S, Soltani EK, Ghernaout D, Albrahim M, Benguerba Y (2025) Sustainable drug delivery innovations: enhancing ferulic acid release using deep eutectic solvents in dimyristoylphosphatidylcholine (DMPC) systems. *J Mol Liq* 429:127572. <https://doi.org/10.1016/j.molliq.2025.127572>
- [41] Bennaadja, S., Soltani, E. K., Sobhi, W., & Benguerba, Y. (2026). Computational screening of natural deep eutectic solvents for improving naringenin solubilization and membrane behavior in DMPC liposomes using COSMO-RS and COSMOmic. *Journal of Molecular Modeling*, 32(1), 333. <https://doi.org/10.1007/s00894-026-06872-8>

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [graphicalabstract.jpg](#)