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Original article

Fractionation of proteins from natural coagulant from common bean on ion-exchanger Amberlite™ IRA 900 Cl and comparison with Amberlite™ IRA 958 Cl

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Abstract

Turbidity reduction in water and wastewater treatment typically involves chemical coagulants like alum, iron salts, and acrylamide. While natural coagulants offer benefits, their main drawback is the organic load in treated water, which can be addressed through purification. In this work, proteins from a crude extract obtained with 0.5 mol L^{-1} NaCl from *Phaseolus vulgaris* seeds were first precipitated by ammonium sulfate and then redissolved and purified on the anion exchange resin Amberlite™ IRA 900 Cl in batch mode. Prior to their purification, optimization of adsorption conditions and elution was performed. The purification on Amberlite™ IRA 900 Cl was then compared with purification on Amberlite™ IRA 958 Cl. Amberlite™ IRA 900 Cl showed twice the adsorption capacity of Amberlite™ IRA 958 Cl. The organic load in treated water was over five times lower when the fraction purified on Amberlite™ IRA 900 Cl was used as a coagulant compared to when the crude extract was used.

Keywords natural coagulant, proteins, water clarification, coagulation activity, organic load, ion-exchange

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Introduction

Coagulation and flocculation, which are essential processes in the treatment of both surface waters and industrial wastewaters, involve the removal of turbidity and dissolved organic species from water. These processes are most commonly accomplished by the addition of conventional chemical-based coagulants/flocculants – alum and iron salts and synthetic organic polymers. Due to their potential to cause harmful health effects, including Alzheimer's and Parkinson's diseases, as well as carcinogenic and neurotoxic effects, vascular diseases and neurological disorders [1-5], and the difficulties associated with disposing of or treating large volumes of toxic sludges [6], the application of natural coagulants has been receiving increased attention not only from researchers around the world but also gradually from industrialists and stakeholders.

Although the advantages of natural coagulants over conventional ones are numerous such as having generally no detrimental effects on human health, originating from renewable sources and producing sludges that can be biologically treated or used as addition to fertilizers or feed depending on the composition of the treated water or wastewater, the primary disadvantage of their use is the organic load in the treated water. This can adversely affect water quality [7] by causing color, odor and taste problems and promoting microorganisms growth, might exert a chlorine demand and act as a precursor to health-hazardous disinfection by-products (DBPs) during chlorine disinfection and complicates its further processing [6]. However, this issue can be addressed by purifying the crude coagulant extracts, which can be accomplished by different techniques [6, 8-14].

As reported in the literature, natural coagulants are mostly purified by precipitating proteins from crude extracts with salt (e.g. ammonium sulfate) followed by ion-exchange chromatography (either batch or continuously operated column, single-step or two-step), or by using just one of these two methods. Ghebremichael et al. (2006) [9] investigated the purification of *Moringa oleifera* crude extract on CM Sepharose Fast Flow cation exchange resin in a batch mode. The authors demonstrated that the removal of organic and other compounds which did not exert coagulation activities from the crude extract could have been accomplished by this method. The obtained purified fractions had significantly lower nutrients content compared to the crude extract and did not increase organic matter content, expressed as COD, in the treated water even at high applied doses. *Moringa oleifera* crude extract was purified according to the same procedure in the paper of Ghebremichael et al. (2009) [15] as well. At the optimal dose, which was about eight times lower for the purified fraction compared to the crude extract, a turbidity reduction of 97% was achieved. Ndabigengesere and Narasiah (1998) [16] proposed a purification scheme for *Moringa oleifera* crude water extract that included protein precipitation by ammonium sulfate and adsorption on carboxymethyl cellulose in a column ion exchange chromatography. Their results revealed that the optimal dosage of the purified proteins was 50 to 100 times lower compared to the optimal dosage of alum or the crude extract. Unlike the crude extract, the purified proteins did not increase the organic matter and nutrient content of the treated water.

The tropical plant *Moringa oleifera* is the most extensively studied for the isolation and purification of natural coagulants [8-9, 11-13, 15-18]. Results from our earlier research in which we investigated the potential of common bean (*Phaseolus vulgaris*) as a readily available, cost-effective source of natural coagulants in the Balkan region and Europe, confirmed that its seed crude extract was an effective coagulant [19, 20]. Additionally, we explored various techniques for purifying common bean crude extract, including fractionation on the anion exchange resin Amberlite™ IRA 958 Cl [6].

This paper continues our previous work and focuses on purifying the crude extract of common bean seeds using Amberlite™ IRA 900 Cl anion exchange resin, with the aim of finding the most suitable purification method to remove compounds with no coagulation activity. The detailed optimization of the adsorption and elution conditions conducted in this work is significant, as it provides a solid foundation for future research and potential industrial applications. By comparing two different anion exchange resins, this research provides practical insights into optimizing the purification process of natural coagulants extracted from common bean seeds.

Materials and Methods

Extraction of the active component from common bean seeds

The natural coagulant was obtained from common bean (*P. vulgaris*) seeds by extraction with 0.5 mol L^{-1} NaCl according to established procedure [6]. Fig. S1 (Supplementary material) illustrates a flowchart of the experimental setup.

The active component precipitation

Proteins extracted from common bean seeds (Fig. S1, Supplementary material) underwent further processing through precipitation and dialysis according to procedure described in Prodanović et al. (2020) [6].

Optimization of adsorption conditions and elution of bound proteins

Before proceeding with the purification of the active component, we investigated the kinetics of protein binding to anion exchange resin (optimal binding time), the best resin-to-protein solution ratio, and the effects of initial protein concentration, buffer ionic strength, and pH on binding efficiency (Fig. S1, Supplementary material). Optimization of these binding conditions was carried out using the dialysate extract obtained as described above, in batch ion-exchange experiments with Amberlite™ IRA 900 Cl (Rohm and Haas) as the matrix. Amberlite™ IRA 900 Cl is a macroporous polystyrene type 1 strongly basic anion exchange resin having trimethylammonium as functional groups. Its shipping weight is 700 g L^{-1} and total exchange capacity ≥ 1.00 eq L^{-1} (Cl^- form). The optimal adsorption conditions were selected based on measurements of the amount of bound protein (q) derived from a mass balance relationship:

$$q = (C_0 - C) / m \quad (1)$$

where q represents the quantity of adsorbed protein, mg of protein mL^{-1} of resin, C_0 denotes the initial protein concentration in the protein solution, mg mL^{-1}, C signifies the protein concentration in equilibrium in the solution, mg mL^{-1} and m / mL denotes the volume of resin added per mL of protein solution. Protein concentrations were determined according to Bradford method using bovine serum albumin as the standard [21].

Another parameter considered for determining the optimal conditions for adsorption was the binding efficiency (E):

$$E\ (\%) = (C_0 - C) \cdot 100 / C_0 \quad (2)$$

Following the optimization of adsorption conditions, the binding of proteins in a column was done in the next experiment as the first step, followed by elution in a linear concentration gradient of NaCl solution in order to precisely determine the NaCl solutions' concentrations for elution of proteins in batch mode (Fig. S1, Supplementary material). This was done in the following way. A certain amount of the dialysate extract was loaded onto a laboratory column (10 mm diameter glass column) packed with 10 mL of Amberlite™ IRA 900 Cl previously equilibrated with 2 BVs (BV – bed volume) of 0.01 mol L^{-1} phosphate buffer (pH 7). The 0.01 mol L^{-1} phosphate buffer (pH 7) was then flushed through the column at a flow rate of 1 mL min^{-1}, carrying proteins through the matrix. Those proteins which were not bound left the column with the phosphate buffer. Flushing continued until no more proteins were detected in the effluent. Active components were then eluted from the resin using a linear gradient of concentration of NaCl solution (in 0.01 mol L^{-1} phosphate buffer, pH 7) from 0 to 1 mol L^{-1} at a flow rate of 1 mL min^{-1}. Protein content according to Bradford [21], with bovine serum albumin as the standard, as well as coagulation activities of the fractions (2 mL) were determined. The resulting data provided the elution diagram used to determine the precise concentrations of NaCl solutions for the step elution of proteins in the subsequent batch mode purification experiment.

The active component purification

The active component was bound in batch mode under the previously determined optimal conditions (Fig. S1, Supplementary material). The dialysate extract was diluted to achieve a specific initial protein concentration and mixed with Amberlite™ IRA 900 Cl. Subsequently, the residual protein solution was removed and the resin was washed for 15 minutes with 0.01 mol L^{-1} phosphate buffer (pH 7) at a resin-to-buffer ratio of 1:1. The buffer was then drained. Following adsorption, solutions of NaCl at various concentrations (0.25 mol L^{-1}, 0.38 mol L^{-1}, 0.5 mol L^{-1} and 1 mol L^{-1}) in 0.01 mol L^{-1} phosphate buffer (pH 7) were sequentially applied for 15 minutes each, at a resin-to-NaCl solution ratio of 1:1, to elute the active components. Protein concentrations, determined using the Bradford method [21], and coagulation activities were assessed in the collected eluates.

Model water

The coagulation effectiveness of natural coagulant was evaluated using jar tests with synthetic turbid water with an initial turbidity of 35 NTU (nephelometric turbidity units), prepared according to procedure described in Prodanović et al. (2020) [6]. The isoelectric point (pI) of the kaolin suspension was 2.56, indicating that the colloidal kaolin particles were negatively charged at the pH of the model water used in the coagulation tests.

Coagulation test

Coagulation effectiveness was evaluated using jar tests conducted in a VELP FC6S jar tester, using model water with an initial turbidity of 35 NTU. The pH of the model water was adjusted to 9 by adding 33% NaOH, following established protocols [22]. Jar tests involved adding 0.5 mL L^{-1} or 1 mL L^{-1} of eluates to the model water according to procedure described in Prodanović et al. (2020) [6]. Having them completed, coagulation activity was calculated:

$$CA\ (\%) = (T_b - T_s) \cdot 100 / T_b \quad (3)$$

where T_b and T_s are the turbidities of the blank and the sample, respectively.

Analytical methods

Turbidity was assessed with a nephelometric turbidimeter (WTW TURB 550/550 IR) and reported in nephelometric turbidity units (NTU). Permanganate demand was determined in an acidic environment following the Kübel-

Tiemann method [23]. Protein concentrations were measured using the Bradford method [21], with bovine serum albumin as the standard.

Statistical analysis

All analyses were conducted in triplicate, and the results were presented as means ± standard deviation (SD). Differences between mean values were considered significant at a confidence level of $p < 0.05$, determined through one-way ANOVA followed by Tukey's test.

Results

Results presented in various papers show that proteins are among the most important compounds from plant material which possess coagulation ability [8-9, 24]. Considering these findings and the fact that the common bean is rich in proteins, they were precipitated from the common bean crude extract using $(NH_4)_2SO_4$ and dialyzed, and the dialysate extract was employed in the subsequent purification.

Optimization of adsorption conditions

To initiate the optimization of binding conditions, an adsorption kinetic study was conducted at room temperature. The dialysate extract was mixed with 0.01 mol L^{-1} phosphate buffer (pH 7) and diluted to achieve initial protein concentrations of 0.3919 mg mL^{-1} and 0.2770 mg mL^{-1}. The resin was equilibrated with 0.01 mol L^{-1} phosphate buffer (pH 7) during 15 minutes at a resin-to-buffer ratio 1:1. The diluted dialysate solutions were mixed with the resin at a ratio of 1 part resin to 1 part solution, and the mixtures were stirred at 80 rpm. Samples of 0.1 mL were taken at specific time intervals over 120 minutes and analyzed for protein content. The quantities of adsorbed protein (q) were calculated and the results are shown on Fig. S2 (Supplementary material). A binding efficiency of 91.84% was achieved in the solution with an initial protein concentration of 0.2770 mg mL^{-1} after 2 hours. However, considering that this is a lengthy period and that the majority of proteins were adsorbed within the first 30 minutes, this contact time was chosen for the anion exchange resin Amberlite™ IRA 900 Cl and the dialysate extract solution for the following experiments.

Results from an experiment assessing the amount of proteins bound by Amberlite™ IRA 900 Cl at different pH values illustrate the influence of buffer pH on the binding process. A phosphate buffer was used to dilute the dialysate extract to achieve specific initial protein concentration and the effect of its pH on adsorption was examined at three values: 5, 7 and 9. As shown in Fig. S3 (Supplementary material), pH 5 does not support binding, which was expected since it is close to the isoelectric point (pI) of the dialysate extract obtained from common bean (between pH 4 and 5.5) [10]. Adsorption at pH 7 and 9 exhibited similar patterns, with a slightly higher amount of bound proteins at pH 7. Given this and considering that working at a neutral pH is more advantageous from both economic and environmental perspectives compared to a basic pH, pH 7 was selected for further experiments. Similar observations were noted in the adsorption of proteins extracted from common bean on the Amberlite™ IRA 958 Cl anion exchange resin [6].

Beside determining the optimal pH for protein adsorption, it is also necessary to establish the optimal ionic strength of the buffer. The impact of the phosphate buffer's ionic strength on adsorption was studied by assessing the binding efficiency of proteins to the matrix across a concentration range of 10 – 100 mmol L^{-1} at pH 7. The initial concentration of protein in the solution was set to 0.390 mg mL^{-1}. Protein solutions were mixed with the matrix for 30 minutes at 80 rpm and matrix-to-protein solution ratio 1:1. The results are presented at Fig. S4 (Supplementary material). The highest binding efficiency was observed with a 0.01 mol L^{-1} buffer, and thus this concentration was chosen for further work.

The determination of the optimal resin volume needed for the adsorption and purification of protein extracted from common bean was carried out through experiments where a consistent amount of resin was used, but the volumes of dialysate extract varied. The dialysate extract was diluted with 0.01 mol L^{-1} phosphate buffer (pH 7) to achieve an initial protein concentration of approximately 0.4 mg mL^{-1}. Subsequently, it was mixed with the resin for 30 minutes at 80 rpm, followed by an analysis of the protein content. Binding efficiencies were then calculated based on the collected data. The results of these experiments are presented in Fig. S5 (Supplementary material). Since the resin-to-protein solution ratio of 1:1 yielded the highest binding efficiency, it was used in subsequent experiments.

The influence of initial protein concentration in the protein solution on adsorption efficiency and the quantity of bound protein was also examined. The dialysate extract was diluted with 0.01 mol L^{-1} phosphate buffer (pH 7) to prepare protein solutions with varying initial concentrations. These solutions were mixed with the resin for 30 minutes at 80 rpm, maintaining a ratio of 1 part resin to 1 part solution. After separation, the protein content in the liquid phase was measured, and binding efficiency as well as the amount of bound protein was calculated. Fig. S6 (Supplementary material) illustrates the results obtained.

Optimization of elution

After optimizing the adsorption conditions and before purifying the active component, the elution process was

optimized using a column. 1 mL of the dialysate extract, which contained 6.8535 mg mL^{-1} of proteins, was added to Amberlite™ IRA 900 Cl anionite previously equilibrated with 10 mmol L^{-1} phosphate buffer (pH 7). During this step, 70.1% of loaded proteins were adsorbed which corresponded to the previously determined resin capacity. After completing the adsorption, the resin was rinsed with 10 mmol L^{-1} phosphate buffer (pH 7). The following elution was done by the linear gradient of concentration of NaCl solution from 0 to 1 mol L^{-1} at a flow rate of 1 mL min^{-1}. The protein concentrations (C_p) of the obtained fractions are presented on the elution diagram (Fig. S7, Supplementary material). Three protein peaks are observed on Fig. S7 whose maximums were in fractions 6, 8 and 14. As can be seen from Fig. S7, these protein peaks were utilized to precisely determine the NaCl solution concentrations for further purification experiments and those were: 0.25 mol L^{-1}, 0.38 mol L^{-1}, 0.5 mol L^{-1} and 1 mol L^{-1}.

The active component purification and coagulation study

The dialysate extract was mixed with 10 mmol L^{-1} phosphate buffer (pH 7) to achieve an initial protein concentration of 0.2753 mg mL^{-1}. This protein solution was added to the anion exchange resin Amberlite™ IRA 900 Cl, which had been equilibrated with 10 mmol L^{-1} phosphate buffer (pH 7) before adsorption. The binding process was conducted under previously optimized conditions. The amount of bound proteins was 0.2556 mg mL^{-1}, resulting in a binding efficiency of 92.86%. Following this, the resin was washed with 10 mmol L^{-1} phosphate buffer (pH 7) at 80 rpm and a resin-to-buffer ratio of 1:1 for 15 minutes. Elution was carried out using a step gradient of NaCl solutions (0.25 mol L^{-1}, 0.38 mol L^{-1}, 0.5 mol L^{-1} and 1 mol L^{-1}) for 15 minutes each step, at a resin-to-NaCl solution ratio of 1:1. This process yielded four fractions, and the protein contents of each fraction were determined (Table 1).

Table 1. Protein concentrations in fractions obtained by eluting bound proteins from common bean using Amberlite™ IRA 900 Cl anion exchange resin with a step gradient of NaCl solution

Fraction:	CNaCl (mol L-1):	Cp (mg mL-1) in fractions:
I	**0.25**	0.0067
II	**0.38**	0.0208
III	**0.50**	0.0350
IV	**1.00**	0.0514

With an increase of concentration of NaCl solution, the protein content in the fractions increased too. An elution efficiency of 44.56% was achieved, which is similar to that achieved during column elution.

To investigate the relationship between protein concentrations in specific fractions and their ability to coagulate, fractions number 6, 8 and 14 from column chromatography and II, III and IV from batch chromatography were tested as coagulants in synthetic turbid water with an initial turbidity of 35 NTU at pH 9. The outcomes of coagulation tests for these fractions, concerning the dosage of the applied coagulant, are illustrated in Fig. 1 and 2, respectively.

As can be seen from Fig. 1, fractions 6 and 14 show better coagulation activities at higher applied doses, whereas fraction 8 performs better at a lower dose. Fraction 8 demonstrates nearly the same coagulation activity at half the dose compared to fraction 6 at a dose of 1 mL L^{-1}, despite having approximately the same protein content. The best coagulation activity (42.9%) is achieved by fraction 14 whose protein content is also the highest. However, the protein content in the 14th fraction is about 4 times higher than those in fractions 6 and 8, while its coagulation activity is only 4% higher and achieved at twice the dose compared to fraction 8th.

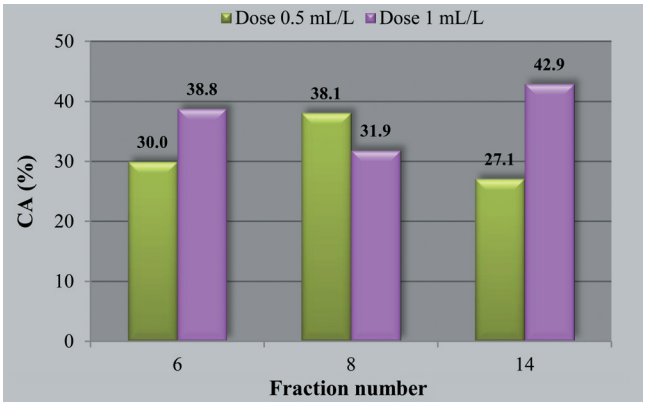

Fig. 1. Coagulation activities of fractions obtained in a continuous (column) mode of operation with anion exchange resin Amberlite™ IRA 900 Cl using a linear gradient of NaCl solution concentrations from 0 to 1 mol L^{-1}

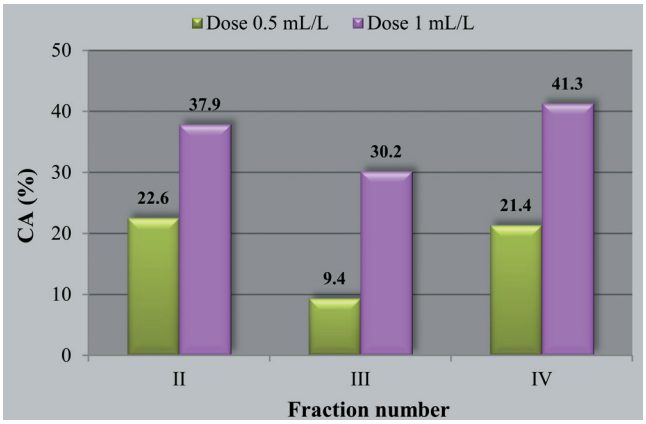

Fig. 2. Coagulation activities of fractions obtained through batch operation with Amberlite™ IRA 900 Cl anion exchange resin using a step gradient of NaCl solution

Fractions obtained in batch mode (Fig. 2) show significantly better coagulation activities at higher applied doses. As observed in the previous experiment (Fig. 1), the fraction with the highest protein concentration (IV fraction) also exibits the best coagulation ability. The protein content in this fraction is about 2.5 times higher than that in the II fraction whose coagulation activity is only about 4% lower. Based on protein concentration, the optimal coagulation doses were 0.0514 mg L^{-1} for fraction IV and 0.0208 mg L^{-1} for fraction II.

Discussion

Optimization of adsorption conditions

Before proceeding with the purification of the active component, we investigated the kinetics of protein binding to anion exchange resin (optimal binding time), the best resin-to-protein solution ratio, and the effects of initial protein concentration, buffer ionic strength, and pH on binding efficiency.

Results from the kinetic study presented in Fig. S2 show that there are no significant changes in the quantity of adsorbed proteins at higher initial protein concentration after the 30th minute. In the case of the lower initial protein concentration, the amount of bound proteins increases quite fast during the first 30 minutes and continues to increase afterwards, but at a slower rate. When comparing the adsorption kinetics of the dialysate extract from common bean seeds on the anion exchange resin Amberlite™ IRA 900 Cl with those on Amberlite™ IRA 958 Cl [6], it is evident that binding on Amberlite™ IRA 900 Cl is much more stable. The binding efficiency is similar for both resins at higher initial protein concentrations and it was around 50%, while at lower initial protein concentrations Amberlite™ IRA 900 Cl performed significantly better, though the contact time required for that binding efficiency was also longer.

The impact of the phosphate buffer's ionic strength on adsorption was studied by assessing the binding efficiency of proteins to the matrix across a concentration range of 10 – 100 mmol L^{-1} at pH 7 and the results are presented at Fig. S4 (Supplementary material). Having the same initial protein concentration in all four experiments, with the decrease in the amount of bound proteins the binding efficiency also decreased. This behavior differs from that observed with Amberlite™ IRA 958 Cl [6], where the ionic strength of the buffer did not significantly affect binding. The decrease in binding efficiency with increased buffer ionic strength can be attributed to competition between other ions at higher concentrations and the proteins for adsorption sites [25]. However, even when the buffer ionic strength was increased tenfold, the adsorption capacity of Amberlite™ IRA 900 Cl decreased by only 30% approximately.

The results from the experiments for the determination of the optimal resin volume needed for the adsorption and purification of protein extracted from common bean (Fig. S5, Supplementary material) indicated that an increase in the volume of the protein solution resulted in a reduction in the percentage of bound protein, which can be attributed to indirect decrease in the number of active adsorption sites in relation to their concentration. The resin-to-protein solution ratio of 1:1 yielded the highest binding efficiency, with 49.65% of the protein being adsorbed.

According to the results presented in Fig. S6, the maximum amount of proteins from common bean that can be adsorbed on Amberlite™ IRA 900 Cl under the optimal conditions is 0.5245 mg mL^{-1} and in the same experiment almost the highest binding efficiency is attained, 75.9%. Upon further increase of the initial protein concentration above 0.691 mg mL^{-1} the amount of bound proteins decreases as well as binding efficiency. Comparing these results with those of Prodanović et al. (2020) [6], it can be concluded that Amberlite™ IRA 900 Cl can bind approximately twice the amount of proteins compared to Amberlite™ IRA 958 Cl from a protein solution with an initial concentration of around 0.6 mg mL^{-1} (when the maximum amounts of proteins are bound on both resins).

Data from Fig. S6 also show that not all proteins are adsorbed even at the lowest initial protein concentrations despite resin's capacity to do so, which is the result of the equilibrium established between the amounts of adsorbed proteins and those remaining in the solution. In examining the kinetics of adsorption of dylisate proteins obtained in the same way on Amberlite™ IRA 900 Cl, Antov et al. (2010) [10] came to the same value for resin capacity by applying Langmuir adsorption model, i.e. it was 0.51 mg mL^{-1} according to their findings. Authors [10] also stated that this capacity was significantly lower than that for proteins from *Moringa oleifera* [9], attributing the diffrence to the molecular weight of the proteins. Namely, the estimated molecular weight of the dimeric *M. oleifera* protein was 13 kDa, whereas, according to literature data [26], the subunit of faseolin, the main storage protein of common bean was trimer with a molecular weight of 50 kDa, limiting its binding to the surface of ion-exchange beads.

The active component purification and coagulation study

In our previous investigation [10], when Amberlite™ IRA 900 Cl was utilised to purify natural coagulant from common bean in continuous mode, we achieved the highest

coagulation activity of 72.3% at a purified fraction dose of 0.081 mg L^{-1}. In contrast, the optimal doses of the purified fractions obtained using Amberlite™ IRA 900 Cl in batch mode, in the present work, were significantly lower: nearly 2 times lower for fraction IV and 4 times lower for fraction II. Furthermore, when normalized based on the proteins added to the model water, the coagulation activity achieved with the optimal dose of fraction II in this study was twice as high as that obtained with the purified fraction from the continuous mode process.

Okuda et al. (2001) [22] purified *Moringa oleifera* coagulant on Amberlite IRA-900 anion exchange resin, the same ion-exchanger that was applied in this research. They obtained a fraction with high coagulation activity of ~90%, but their optimal dosage was 20 times higher than the one achieved in this investigation. Baptista et al. (2017) [11] fractionated protein coagulants from *Moringa oleifera* seeds based on their solubility in different extraction systems according to Osborne. One fraction obtained through extraction with 0.5 mol L^{-1} NaCl, the same solvent used in our study, was identified as globulin (II) [11]. It exhibited a turbidity removal efficiency of approximately 30% in surface water with an initial turbidity of 102.42 NTU. However, the authors noted that this result was comparable to the control analysis (without *Moringa oleifera* addition), suggesting that the turbidity reduction was primarily due to gravity settling of particles rather than the coagulant's action. In contrast, fractions obtained through water extraction achieved significantly higher turbidity removal efficiencies, ranging from 79% to 89%. The enhanced coagulation efficiencies of these fractions compared to those observed in our study may be due to the initial turbidity of the water treated in Baptista et al.'s study [11], which was approximately 1.5 times higher than that of our experimental model water. Literature indicates that natural coagulants tend to exhibit greater effectiveness in removing particles from more turbid waters [7, 15]. Furthermore, the dosage applied in Baptista et al.'s study [11] was over six hundred times higher than the optimal dosage achieved in our present work. This discrepancy underscores the practical importance of achieving effective coagulation at lower dosages, especially in large-scale applications. Similarly, Sánchez-Martín et al. (2010) [27] and Ghebremichael et al. (2009) [15] achieved high coagulation activity of ~95% in surface waters of high initial turbidity (170 NTU and 120 NTU, respectively) with *M. oleifera* coagulant purified on CM-Sepharose, but their optimal dosages were about 25 and 330 times higher than the optimal dosage achieved in this work. These findings suggest that additional examination of the purified proteins from common bean seeds should involve testing a wider range of dosages and waters with higher initial turbidities.

In another study by Baptista et al. (2015) [12], ultrafiltration using a hollow fiber polyethersulfone membrane with a MWCO 50 kDa was employed to purify water- and NaCl- extracted coagulants from *M. oleifera*. The obtained fractions were examined in surface water of initial turbidity 75 NTU. Fractions originating from the water extract showed no coagulation activity. On the other hand, fractions obtained from the saline extract exhibited ~90% turbidity removal at a dose of 13.78 mg L^{-1}, while the control (without coagulant) had a turbidity removal of ~45%. This turbidity removal was similar to the result achieved with the IV fraction in our study, but the applied dose was more than 250 times higher suggesting that coagulating compounds extracted by NaCl solution from common bean seed are more active than those from *M. oleifera*.

The organic content in model waters was assessed before and after conducting coagulation tests using the crude extract from common bean, as well as fractions II and IV at their optimal doses (1 mL L^{-1}). This evaluation aimed to quantify the increase in organic load and assess the impact of natural coagulant purification. The findings showed that the crude extract and purified fractions raised the organic matter content in treated water by 68%, 13% and 20%, respectively. These results indicate a positive effect of purification which removed organic compounds with low or no coagulation abilities from the crude extract. The organic load of the II fraction was lower than that of the IV fraction which is in accordance with its lower protein content. Additionally, the II fraction showed slightly better results regarding organic load than the purified fraction obtained on Amberlite™ IRA 958 Cl [6], while the IV fraction demonstrated similar performance. The II fraction also performed similarly to the fraction purified on Amberlite™ IRA 900 Cl in continuous mode [10].

Conclusions

Assessing the fractions obtained through the purification of common bean seed crude extract using Amberlite™ IRA 900 Cl anion exchange resin in batch mode for their applicability in clarifying model water led to the following conclusions:

Amberlite™ IRA 900 Cl can bind approximately twice the amount of proteins compared to Amberlite™ IRA 958 Cl and the binding is significantly more stable;

The highest coagulation activity of 41.3% was achieved by fraction obtained with 1 mol L^{-1} NaCl at dose of 1 mL L^{-1}, while slightly lower coagulation activity (38.8%) was achieved by fraction obtained with 0.38 mol L^{-1} NaCl at the

same dose, despite its protein content being 2.5 times lower. For a more comprehensive evaluation, additional testing of the fractions obtained in this study at different doses is required, as they were assessed only at two doses;

The optimal dose of the fraction obtained by purification on Amberlite™ IRA 900 Cl in batch mode was 4 times lower than the optimal dose of the fraction obtained by purification on Amberlite™ IRA 900 Cl in continuous mode, while its coagulation activity was 2 times higher when calculated based on the proteins added to the model water;

The elution diagram of the dialysate extract from common bean seed using anion exchange resin Amberlite™ IRA 900 Cl, with a linear gradient of NaCl solution concentrations ranging from 0 to 1 mol L^{-1} revealed two smaller protein peaks and a larger protein peak corresponding to NaCl solution concentrations between 0.5 and 1 mol L^{-1}. The shape of this large peak suggests that additional proteins may not have been completely separated. Therefore, further fractionation should be attempted using an exponential gradient of NaCl solution concentrations within the range corresponding to this large peak;

The organic load in treated water when using the purified fraction (fraction II) as coagulant was more than 5 times lower than when using the crude extract as coagulant which proved Amberlite™ IRA 900 Cl anion exchange resin as effective for purifying natural coagulant from common bean seed.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Supplementary material

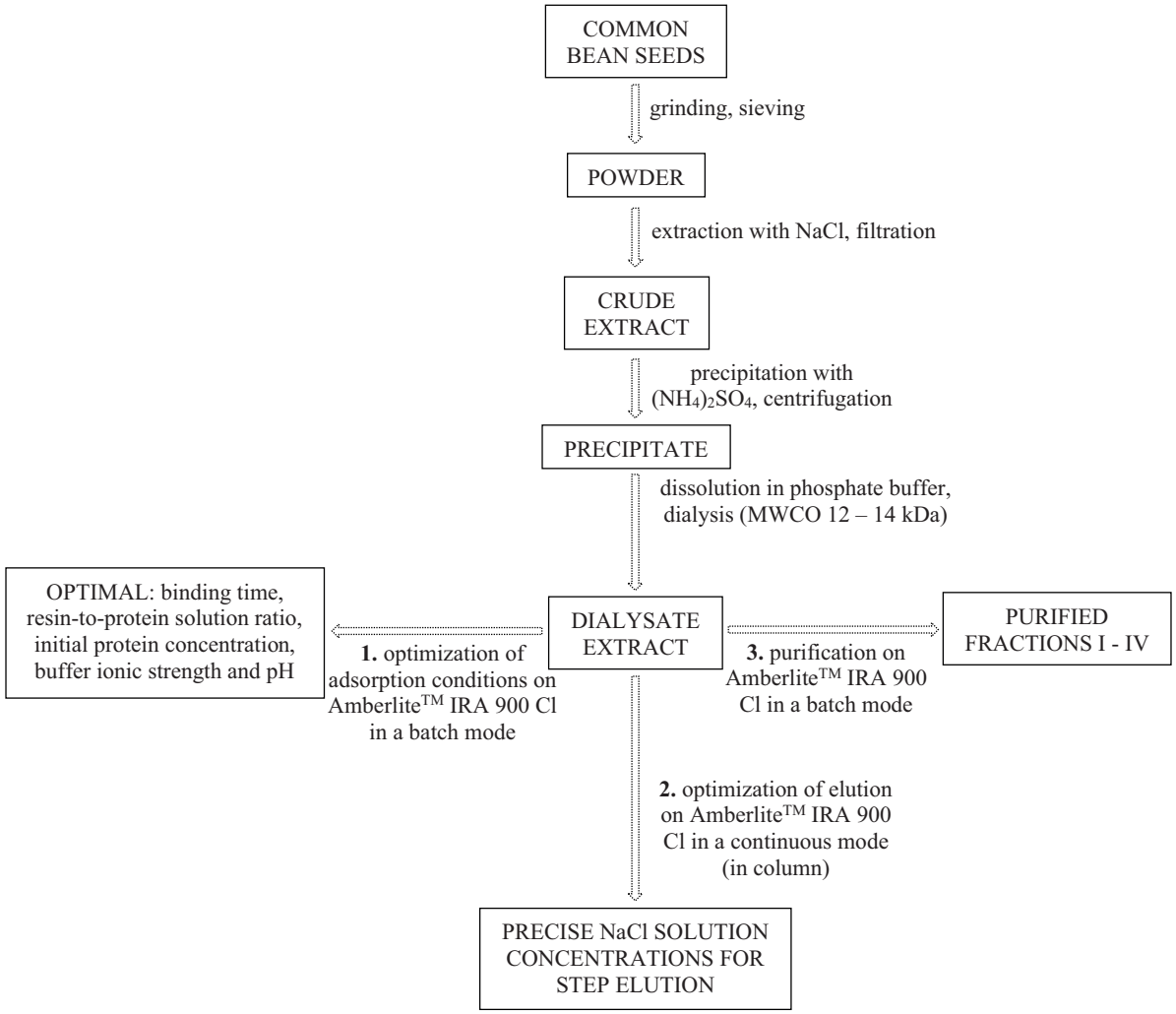

Fig. S1. Flow diagram showing the processing protocol of *Phaseolus vulgaris* seeds

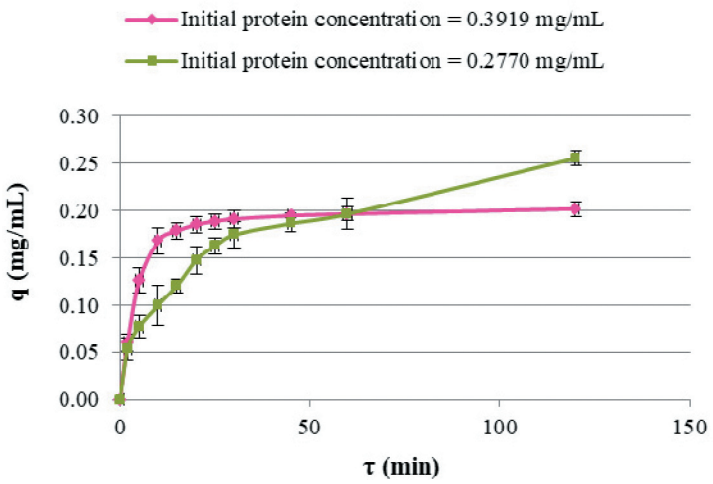

Fig. S2. Adsorption kinetics of dialysate extract from common bean seed on anion exchange resin Amberlite™ IRA 900 Cl in 10 mmol L^{-1} phosphate buffer, pH 7

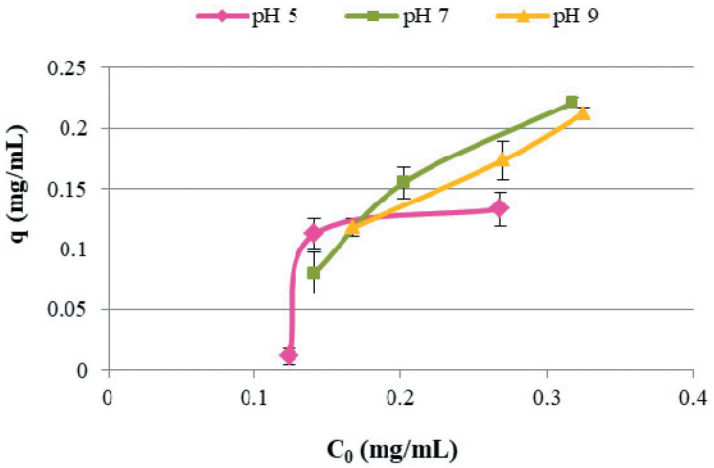

Fig. S3. The amount of proteins extracted from common bean bound on anion exchange resin Amberlite™ IRA 900 Cl at different pH values and initial protein concentrations

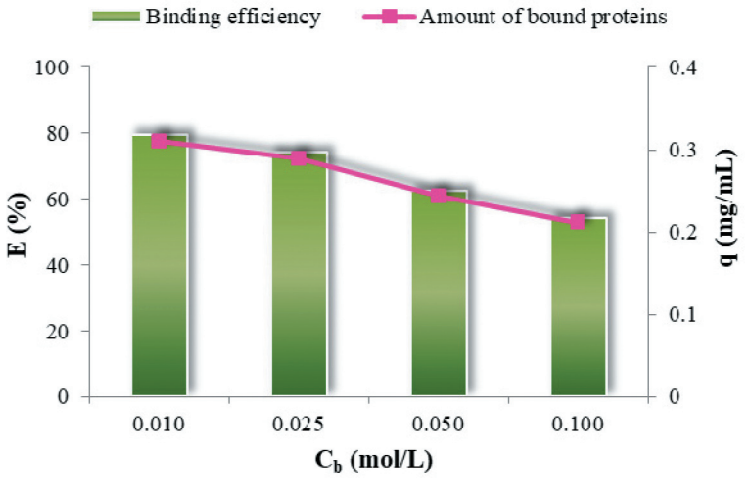

Fig. S4. The effect of concentration of phosphate buffer (C_b) on the adsorption of proteins extracted from common bean onto Amberlite™ IRA 900 Cl anion exchange resin at pH 7

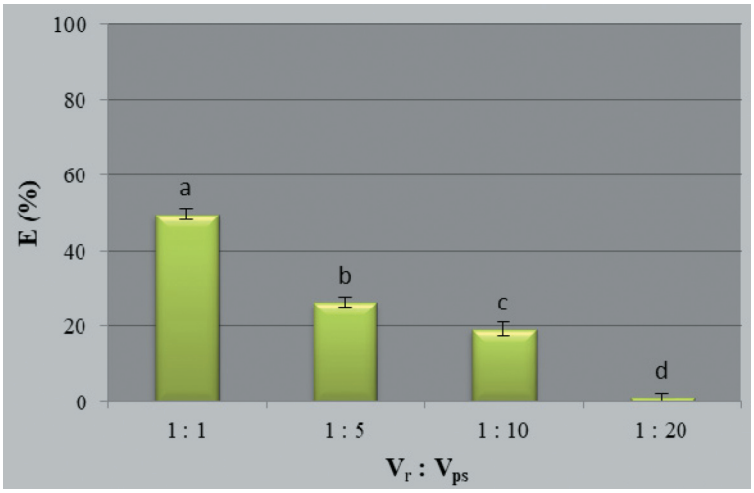

Fig. S5. The impact of the resin-to-protein solution ratio ($V_r : V_{ps}$) on the binding of proteins to Amberlite™ IRA 900 Cl anion exchange resin in 10 mmol L^{-1} phosphate buffer, pH 7; different letters denote significant differences between samples ($p < 0.05$)

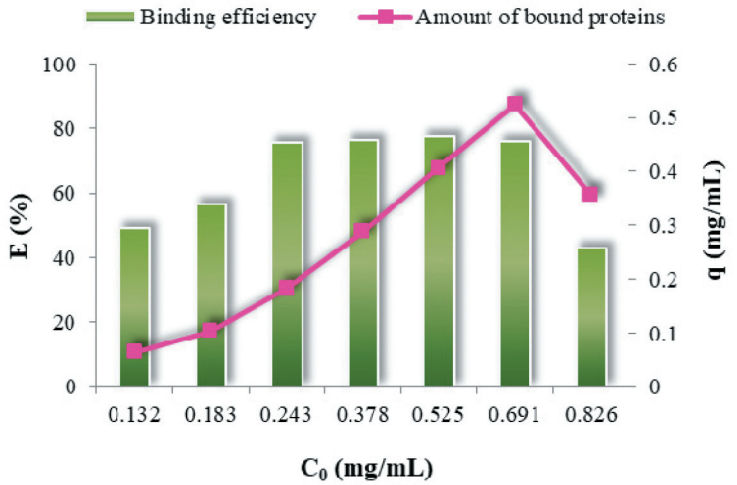

Fig. S6. The influence of initial protein concentration on the binding efficiency and the quantity of proteins bound to Amberlite™ IRA 900 Cl anion exchange resin in 10 mmol L^{-1} phosphate buffer, pH 7

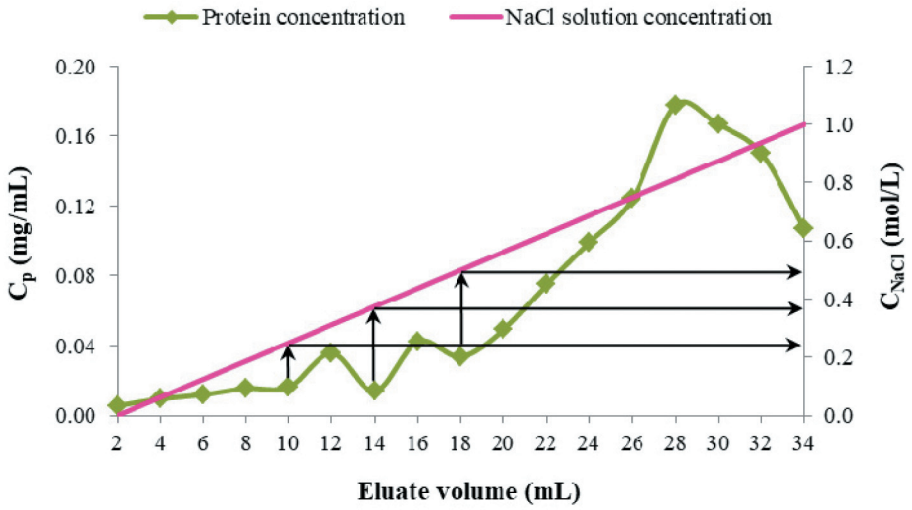

Fig. S7. Elution diagram of dialysate extract from common bean seed using anion exchange resin Amberlite™ IRA 900 Cl