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The purification of natural coagulant extracted from common bean on IRA 958 Cl anion exchange resin

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Abstract: Natural coagulants are of organic nature and can increase organic load of treated water, thus they require purification in order to remove compounds that do not have coagulation activity. In this work natural coagulant was extracted from 50 g L\(^{-1}\) of ground common bean with 0.5 mol L\(^{-1}\) NaCl. Proteins from this crude extract were precipitated by adding ammonium-sulphate. After precipitation, separation and resolution of proteins, further purification was done by anion-exchange resin Amberlite IRA 958 Cl in batch process. Partially purified coagulant eluted by 2 mol L\(^{-1}\) NaCl solution achieved the highest coagulation activity of 53.3 % at dose of 1 mL L\(^{-1}\) although it contained the lowest amount of proteins, but just slightly lower coagulation activity of 49.8 % was achieved at more than 5 times lower dosage of the same fraction. Organic load in treated water when purified fraction was applied as coagulant was almost 4 times lower than in case of crude extract as coagulant.

Keywords: Proteins, coagulation activity, water clarification, organic load.

INTRODUCTION

In the era of evident and fast environmental degradation, usage of green and sustainable technologies has been in line with global efforts for nature and life preservation and water quality protection, which is one of the environmental aspects concerning not only scientists and environmentalists but all humans. The usage of natural coagulants extracted from different sources such as plants, animals and microorganisms, in water and wastewater treatment, though not a completely new idea, is a promising technique considering environmental and health protection.

Application of natural coagulants dates back centuries ago, but it was limited to household water treatment mainly in developing countries. In the past few decades, they have been attracting the attention of researchers around the world due to their advantages over conventional coagulants and flocculants (i.e. salts of

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alum and iron). Since the 60’s of the last century, many studies which drew attention to the potential harmful health influence (Alzheimer’s and Parkinson’s diseases, carcinogenic and neurotoxic effects) of residues of alum and synthetic organic flocculants in treated water were published\(^1\)\(^-\)\(^2\). Additionally, epidemiological and clinical investigations showed that increased content of iron in body is linked with increased risk of cancer, vascular diseases and neurological disorders\(^3\)\(^-\)\(^4\). Beside potential adverse health effects, remaining sludges of alum and iron are hazardous to the environment so that they cannot be disposed of into the surrounding while, on the other hand, high concentrations of metals complicate their further biological treatment. Also, the alum sludges are acidic, gelatinous, and difficult to dewater and to dispose of in the environment, and the lowering of pH of treated water and increase in conductivity are the additional disadvantages of alum coagulants\(^5\). Natural coagulants do not present a health threat in general; they are obtained from renewable sources and sludges remained after their application can be added to feed or fertilizers, or biologically treated.

The main drawback of natural coagulants application is an increase in organic matter content in treated water. Organic matter can cause color and unpleasant odor of water, may lead to increased microbial growth and will react with chlorine or other disinfectants giving toxic and carcinogenic byproducts during the disinfection process at the water treatment plant. This problem can be addressed by purification of the coagulant which can be accomplished by different techniques\(^6\)\(^-\)\(^9\). The importance of purification of natural coagulants is reflected through novel papers dealing with this\(^10\)\(^-\)\(^13\).

The most investigated plant in terms of natural coagulants preparation is tropical plant *Moringa oleifera*\(^5\)\(^,\)\(^7\),\(^14\)\(^-\)\(^17\). During our previous research we investigated the potential of common bean, as widely grown, cheap and easily available source of natural coagulants in the region of the Balkan, as well as Europe, and confirmed its crude seed extract as an effective coagulant\(^18\)\(^-\)\(^22\).

This paper deals with purification of common bean seed crude extract on IRA 958 Cl anion exchange resin the aim of which was to remove compounds which did not possess coagulation activity.

**EXPERIMENTAL**

*Extraction of active component*

Natural coagulant was obtained in the following way: common bean (*P. vulgaris*) seeds were ground and sieved through 0.4 mm sieve. An amount of a 50 g L\(^{-1}\) of the smaller fraction was suspended in 0.5 mol L\(^{-1}\) NaCl. The suspension was stirred 10 minutes using a magnetic stirrer in order to extract active coagulant, and then filtered through filter paper Macherey-Nagel MN 651/120 to obtain a crude extract.

*Precipitation of active component*

Proteins extracted from common bean seed were further processed by precipitation and dialysis. The crude extract was saturated to 80 % by adding (NH\(_4\))\(_2\)SO\(_4\) and centrifuged at
3000 rpm for 10 minutes. The precipitate was dissolved in 0.01 mol L$^{-1}$ phosphate buffer (pH 7) and dialysed overnight at 4 °C against Millipore water in dialysis bag with molecular cut-off 12-14 kDa.

**Optimization of resin binding conditions and elution of active component**

Prior to further purification of active component, the kinetic of proteins binding for anion exchange resin (the optimal binding time), the optimal resin/protein solution ratio, the influence of initial protein concentration, buffer ionic strength and pH on binding efficiency were examined. The optimization of binding conditions was conducted using dialysate extract, obtained according to the above explained procedure, in batch ion-exchange experiments with Amberlite™ IRA 958 Cl (Rohm and Haas) as matrix. Amberlite™ IRA 958 Cl is a macroporous strongly basic anion exchange resin having quaternary ammonium functionality in a crosslinked acrylic polymer matrix. Its shipping weight is 720 g L$^{-1}$ and total exchange capacity $\geq$ 0.80 eq L$^{-1}$ (Cl$^-$ form). The choice of the optimal binding conditions was made by measuring the amount of bound protein ($q$):

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

where $q$ is the amount of bound protein, mg of protein mL of resin$^{-1}$, $C_0$ is the initial protein concentration in the protein solution, mg mL$^{-1}$, $C$ is the protein concentration in solution in equilibrium, mg mL$^{-1}$ and $m / mL$ is the amount of the resin added in 1 mL of protein solution. Protein concentrations were measured according to Bradford with bovine serum albumin as standard.

Another parameter used for selection of optimal binding conditions was the binding efficiency ($E$):

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

After optimization of binding conditions, the kinetic of elution was examined when the optimal elution time was determined. 0.5 mol L$^{-1}$ NaCl was mixed with resin at the ratio 1:1. During 60 minutes samples of 0.1 mL were taken and analyzed for protein content.

**Purification of active component**

The binding of active component was done in a batch mode at previously determined optimal binding conditions. The dialysate extract was diluted to achieve certain initial protein concentration and mixed with Amberlite™ IRA 958 Cl. After that, the residual solution of protein was removed, and resin was washed for 15 minutes with 0.01 mol L$^{-1}$ phosphate buffer (pH 7) at resin/buffer ratio 1:1. The flushing buffer was removed. After binding, different concentrations (0.5 mol L$^{-1}$, 1 mol L$^{-1}$, 1.5 mol L$^{-1}$ and 2 mol L$^{-1}$) of NaCl solution were applied in consecutive order during 20 minutes for each solution and at resin/NaCl solution ratio 1:1 to accomplish elution of active components. Coagulation activity of obtained eluates was examined by jar tests in model water.

**Model water**

Coagulation activity of partially purified natural coagulant was assessed by jar test using synthetic turbid water. At the first place, kaolin was ground in a ceramic mortar and sieved through 0.4 mm sieve. Smaller fraction was used to prepare a 10 g L$^{-1}$ suspension in tap water. The suspension was stirred for 60 minutes on a magnetic stirrer to achieve uniform dispersion of kaolin particles, and left for 24 hours in order to achieve complete hydration of kaolin. Model water was prepared just before performing the coagulation tests, by adding this 1 % kaolin suspension to tap water to obtain the water with initial turbidity of 35 NTU (nephelometric turbidity units).
Coagulation test

Coagulation activities were assessed by jar tests in jar tester VELP FC6S using model water of initial turbidity 35 NTU. pH value of model water was adjusted to 9 by adding 33 % NaOH, in accordance with previous investigations\(^\text{18,24}\). Jar tests were carried out by adding different amounts of eluates to 200 mL of model water. After fast stirring at 200 rpm for 1 minute in order to disperse the coagulant, it was continued with slower stirring at 60 rpm for 30 minutes in order to promote the flocculation of the kaolin particles present in the model water, and after that systems were left for 1 h for sedimentation. The same coagulation test was conducted with no coagulant as blank. After sedimentation for 1 h, residual turbidity was determined in upper clarified liquid using turbidimeter WTW Turb 550/550IR and coagulation activity was calculated.

\[
CA, \% = \frac{(T_b - T_s)}{T_b} \times 100
\]

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

Analytical methods

Turbidity was measured using a turbidimeter (WTW TURB 550/550 IR) and it was expressed in nephelometric turbidity units. Permanganate demand was determined in an acid medium according to Kübel-Tiemann method\(^\text{25}\).

Statistical analysis

All analyses were run in triplicate and the results were expressed as means ± standard deviation (SD). Mean values were considered significantly different at \(p < 0.05\) confidence level, after the performance of the one-way ANOVA statistical analysis followed by Tukey’s test.

RESULTS AND DISCUSSION

According to literature data, proteins are one of compounds from plant material which possess coagulation ability\(^\text{6-7,26}\). Thus, the coagulation active components in the current study were precipitated by ammonium sulphate and dialysed, and dialysate extract was used for further purification.

Optimization of binding conditions

As a first step of binding optimization, the kinetic of proteins binding for anion exchange resin at room temperature was studied. The dialysate extract was diluted with 0.01 mol L\(^{-1}\) phosphate buffer (pH 7) to achieve the initial protein concentration of 0.401 mg mL\(^{-1}\) and 0.239 mg mL\(^{-1}\). The resin was equilibrated with 0.01 mol L\(^{-1}\) phosphate buffer (pH 7) for 15 minutes. The diluted dialysate solutions were added to resin at resin/solutions ratio 1:1 and the mixtures were stirred at 100 rpm. Solution samples of 0.1 mL were collected in certain time intervals for 120 minutes and analyzed for protein content. The amounts of bound protein (\(q\)) were calculated and results are presented on Figure 1.

As can be seen from Figure 1, the rate of protein adsorption was high at the beginning of the adsorption. The highest binding efficiency was achieved in the first fifteen minutes of the binding process and after that period of time the process became unstable. Hence, 15 minutes was chosen as binding time for the following experiments. Results of performed statistical analysis suggested that
there was a significant difference in the binding efficiencies in protein solutions of different initial protein concentrations. For binding time of 15 minutes, in protein solution of higher initial protein concentration binding efficiency of 52.82 % was attained, while in protein solution of lower initial protein concentration higher binding efficiency, 75.12 %, was achieved.

![Graph showing adsorption kinetic of dialysate extract from common bean seed on anion exchange resin Amberlite™ IRA 958 Cl at 10 mmol L⁻¹ phosphate buffer, pH 7](image)

The estimate of optimum volume of resin required for adsorption and purification of protein extracted from common bean was based on the experiments conducted with constant amount of resin but varying volumes of dialysate extract. The dialysate extract was diluted with 0.01 mol L⁻¹ phosphate buffer (pH 7) to obtain the protein solution with initial protein concentration of 0.260 mg mL⁻¹. It was afterwards mixed with resin for 15 minutes at 100 rpm and after that analyzed for protein content. Based on obtained data, binding efficiencies were calculated. Results of experiments are shown on Figure 2.

Results revealed that increase in protein solution volume led to a decrease in percentage of adsorbed protein, and the resin/protein solution ratio 1:0.5 gave the highest binding efficiency, when 88.46 % of protein was adsorbed. This ratio was applied in succeeding experiments.

The effect of initial protein concentration in protein solution on binding efficiency and amount of bound protein was investigated too. The dialysate extract was diluted with 0.01 mol L⁻¹ phosphate buffer (pH 7) to obtain protein solutions with different initial protein concentrations. These solutions were mixed with matrix for 15 minutes at 100 rpm and matrix/solution ratio 1:0.5. After separation, protein content was measured in liquid phase and binding efficiency and amount of bound protein were calculated. Figure 3 shows obtained results.
Fig. 2. The influence of resin/protein solution ratio ($V_r : V_{ps}$) on binding of proteins on anion exchange resin Amberlite™ IRA 958 Cl at 10 mmol L$^{-1}$ phosphate buffer, pH 7; different letters indicate significant differences between samples ($p < 0.05$).

Fig. 3. The effect of initial protein concentration on binding efficiency and amount of bound proteins on anion exchange resin Amberlite™ IRA 958 Cl at 10 mmol L$^{-1}$ phosphate buffer, pH 7.

According to results presented in Figure 3, the highest binding efficiency was attained when initial protein concentration was 0.248 mg mL$^{-1}$. At higher initial protein concentrations the amount of bound protein increased and achieved the highest value at initial protein concentration 0.583 mg mL$^{-1}$. However, the binding efficiency was significantly lower at higher initial protein concentrations, i.e. more unbound proteins remained in solution.

The effect of ionic strength of buffer on the adsorption was investigated by measuring the binding efficiency of proteins to matrix in phosphate buffer within...
concentration range 10 – 100 mmol L\(^{-1}\) at pH 7. The initial protein concentration in solution was adjusted to 0.260 mg mL\(^{-1}\). Obtained protein solutions were mixed with matrix for 15 minutes at 100 rpm and matrix/protein solution ratio 1:0.5. Results are presented at Figure 4. The increase of buffer ionic strength from 0.01 mol L\(^{-1}\) to 0.05 mol L\(^{-1}\) did not significantly affect adsorption of proteins. However, at buffer ionic strength 0.1 mol L\(^{-1}\), amount of adsorbed protein decreased. This can be explained by the competition for adsorption sites at matrix between protein ions and buffer ions which increases when buffer ions are present in higher concentrations\(^{27}\). The highest binding efficiency was observed with 0.05 mol L\(^{-1}\) buffer, but it was just slightly higher than the one obtained with 0.01 mol L\(^{-1}\) buffer, which was better from economic point of view. Hence, 0.01 mol L\(^{-1}\) phosphate buffer was chosen for further work.

![Figure 4](image-url)

**Fig. 4.** The effect of concentration of phosphate buffer (\(C_b\)) on the adsorption of proteins extracted from common bean on anion exchange resin Amberlite\textsuperscript{TM} IRA 958 Cl at pH 7; different letters indicate significant differences between samples (\(p < 0.05\))

According to our previous research (data not shown)\(^8\), isoelectric point (pI) of dialysate extract obtained from common bean is between pH 4 and 5.5. Thus, the effect of pH of buffer on the amount of bound proteins from dialysate extract on anion exchange resin was studied within pH range 7 – 9. In order to find the optimal pH of the buffer for the adsorption, dialysate extract was diluted in universal McIlvaine buffer having pH from 7 to 9 with an increment of increase of pH 0.5. The initial protein concentration was around 0.260 mg mL\(^{-1}\). The matrix and the protein solution were mixed at ratio 1:0.5 for 15 minutes and 100 rpm. Results (Figure 5) revealed that the maximum of protein adsorption was achieved at pH 7 and 7.5. Considering this, further experiments were conducted at pH 7. Statistical analysis showed significant difference between the protein adsorptions at pH 8 and 8.5 in comparison to that achieved at other pH values.
Optimization of elution

Following optimization of binding conditions and prior to purification of active component, optimization of elution was done. Dialysate extract was diluted with 10 mmol L\(^{-1}\) phosphate buffer (pH 7) to obtain protein solution with initial protein concentration of 0.262 mg mL\(^{-1}\). The protein solution was added to Amberlite™ IRA 958 Cl anionite which was previously equilibrated with 10 mmol L\(^{-1}\) phosphate buffer (pH 7). The resin/protein solution ratio was 1:0.5 and the system was mixed for 15 minutes at 100 rpm. After completion of adsorption, the remained protein solution was drained and matrix was washed with 10 mmol L\(^{-1}\) phosphate buffer (pH 7). The elution was done with 0.5 mol L\(^{-1}\) NaCl solution at resin/solution ratio 1:1. The kinetic of elution was monitored during 60 minutes. Solution samples of 0.1 mL were collected in certain time intervals and analysed for protein content. Optimal elution time of 20 min was determined from obtained results, which are presented on Figure 6.
According to ANOVA, there were significant (p<0.05 confidence level) differences in protein contents in fractions obtained during first 40 minute of elution of proteins from common bean seed from anion exchange resin Amberlite TM IRA 958 Cl. However, performed statistical analysis suggested that there were insignificant differences between protein contents of samples collected after 40 min.

**Purification of active component and coagulation study**

Dialysate extract was diluted with 10 mmol L⁻¹ phosphate buffer (pH 7) to obtain protein solution with initial protein concentration of 0.261 mg mL⁻¹. The protein solution was added to Amberlite™ IRA 958 Cl anion exchange resin, equilibrated with 10 mmol L⁻¹ phosphate buffer (pH 7) prior to adsorption, and binding was done at previously determined optimal conditions. The amount of adsorbed proteins was 0.230 mg mL⁻¹, in other words binding efficiency of 88.35 % was achieved. After washing with 10 mmol L⁻¹ phosphate buffer (pH 7) at 100 rpm and resin/buffer ratio 1:1 during 15 minutes, elution was performed by step gradient of NaCl solution during 20 minutes for each step and at resin/NaCl solution ratio 1:1. In this way four fractions were obtained in which protein contents were determined (Figure 7).

With increase of concentration of NaCl solution, protein content in fractions decreased. Elution efficiency of 87.37 % was achieved, i.e. 12.63 % of bound proteins remained at resin.

In order to determine whether there was correlation between proteins’ concentrations in fractions and their coagulation ability, fractions were examined as coagulants in coagulation tests. The main aim of coagulation/flocculation process is turbidity removal, thus coagulation activities of fractions were estimated in synthetic turbid water of initial turbidity 35 NTU at pH 9. Results of coagulation tests of fractions in relation to applied coagulant dose are shown in Figure 8.

Significant differences in coagulation’s behaviour of fractions obtained by elution with different NaCl concentrations were confirmed by ANOVA statistical analysis.
Although it contained the lowest amount of proteins, the fraction obtained by elution with 2 mol L\(^{-1}\) NaCl showed the best coagulation ability. Fraction obtained with 1.5 mol L\(^{-1}\) NaCl showed similar behaviour to fraction obtained with 2 mol L\(^{-1}\) NaCl, which imposes that they contained proteins of similar characteristics considering coagulation, but with lower coagulation activities. The highest coagulation activity of fraction obtained with 2 mol L\(^{-1}\) NaCl was 53.3 % at applied dose 1 mL L\(^{-1}\), but just slightly lower coagulation activity of 49.8 % was achieved at more than 5 times lower dosage (0.17 mL L\(^{-1}\)) of this fraction, thus it can be considered as optimal one. Calculated on the basis of proteins concentration, the optimal coagulation dose was 0.0041 mg L\(^{-1}\).

In our previous investigation when Amberlite\textsuperscript{TM} IRA 900 Cl was used for purification of coagulant from common bean in continual mode, the highest coagulation activity of 72.3 % was obtained at dose of purified fraction 0.081 mg L\(^{-1}\). When compared to results from that study, optimal dose of fraction obtained by purification on Amberlite\textsuperscript{TM} IRA 958 Cl was almost 20 times lower than dose of fraction obtained by purification on Amberlite\textsuperscript{TM} IRA 900 Cl, and when calculated on the basis of proteins which were added in model water, coagulation activity obtained with optimal dose in this research was almost 14 times higher than that of purified fraction obtained on Amberlite\textsuperscript{TM} IRA 900 Cl.

Baptista et al.\textsuperscript{10} fractionated protein coagulants from Moringa oleifera seed based on their solubility in different extraction systems. According to their results, fraction obtained by extraction with 0.5 mol L\(^{-1}\) NaCl which corresponded to globulin (II)\textsuperscript{10} showed turbidity removal of about 30 % in surface water whose initial turbidity was 102.42 NTU. As authors claimed, presented removal value was similar to the analysis control (without addition of Moringa oleifera), meaning that it was inherent to the decanting process through gravity of particles and not due to an effective action of coagulant tested. However, fractions obtained by water extraction showed high turbidity removal ranging from 79 – 89 %. Higher coagulation activities of these fractions compared to
those of fractions obtained in this work can be attributed to the fact that the initial turbidity of treated water in the paper of Baptista et al.\textsuperscript{10} was almost 1.5 times greater than that of model water used in experiments presented in this paper. Literature data show that natural coagulants achieve higher efficiency of particles removal in more turbid waters\textsuperscript{28-31}. Moreover, the applied dosage of fractions in the paper of Baptista et al.\textsuperscript{10} was more than three thousand time higher than optimal dosage achieved in present work.

Organic matter in water before and after coagulation tests with common bean crude extract and fraction obtained with 2 mol L\textsuperscript{-1} NaCl at optimal doses was assessed to determine the increase in organic load. Results revealed that crude extract and purified fraction increased organic matter content in treated water by 68 and 19 \%, respectively. This was in accordance with the low protein content in tested fraction, but it could be explained by absence of other organic compounds in purified fraction as well.

**CONCLUSION**

Evaluation of fractions obtained by purification of common bean seed crude extract on Amberlite™ IRA 958 Cl anion exchange resin for their suitability for model water clarification revealed that they were efficient. The highest coagulation activity of 53.3 \% was achieved by fraction obtained with 2 mol L\textsuperscript{-1} NaCl at dose 1 mL L\textsuperscript{-1}. Optimal dose of fraction obtained by purification on Amberlite™ IRA 958 Cl was almost 20 times lower than optimal dose of fraction obtained by purification on Amberlite™ IRA 900 Cl, while its coagulation activity was almost 14 times higher, when calculated on the basis of proteins which were added in model water. Organic load in treated water when purified fraction was applied as coagulant was almost 4 times lower than in case of crude extract as coagulant which proved Amberlite™ IRA 958 Cl anion exchange resin as appropriate for purification of natural coagulant from common bean seed.

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**ИЗВОД**

ПРЕЧИЩАЊЕ ПРИРОДНОГ КОАГУЛАНТА ИЗ СЕМЕНА ПАСУЉА НА АНЈОНСКОМ МЕЊАЧУ ЈОНА IRA 958 CL

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Природни коагуланти представљају органска јединења и могу повећати садржај органских материја у третиранијој води, те их је стога потребно пречистити како би се уклониле материје које немају коагулациону способност. У овом раду је природни
коагулат екстракован са 0.5 мол L⁻¹ раствором NaCl из 50 г L⁻¹ самлевеног семена пасуља. Протеини из сировог екстракта су исталожени додавањем амонијум-сулфата. Након таложења, одвајања талога и поновог растварања протеина, даље пречишћавање је изведено на анјонској јоноизменичкој смоли Amberlite IRA 958 Cl у водном поступку. Делимично пречишћени коагулат који је добијен елуирањем са изведено на анјонској јоноизменичкој смоли Amberlite IRA 958 Cl у шаржном поступку. Пречишћене фракције као коагуланта у поре органских материја у обрађеној води је било око 4 пута ниже у случају примене ове активност од 49,8 %, фракција садржала најмању концентрацију протеина, док активност од 53,3 % је острвала највећу коагулациону активност од 53,3 % иако је та фракција садржала најмању концентрацију протеина. Незнатно нису коагулациону активност од 49,8 % је подсетила фракција постигла при 5 пута ниже дози. Повећање садржаја сировог екстракта на 0.5 ml L⁻¹ је стиже при 5 пута ниже дози.

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