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Original scientific paper

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 the organic load of treated water and 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 addition of ammonium sulphate. After the precipitation, separation and resolution of proteins, further purification was performed using the anion-exchange resin Amberlite IRA 958 Cl in a batch process. Partially purified coagulant eluted with 2 mol L^{-1} NaCl solution achieved the highest coagulation activity of 53.3 % at a dose of 1 mL L^{-1} although it contained the lowest amount of proteins, but a slightly lower coagulation activity of 49.8 % was achieved at more than 5 times lower dosage of the same fraction. The organic load in treated water when the purified fraction was applied as coagulant was almost 4 times lower than in case of the crude extract as coagulant.

Keywords: proteins; coagulation activity; water clarification; organic load.

INTRODUCTION

In the era of evident and fast environmental degradation, the usage of green and sustainable technologies are 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.

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Application of natural coagulants dates back centuries, but it was limited to household water treatment mainly in developing countries. In the past few decades, they have attracted the attention of researchers around the world due to their advantages over conventional coagulants and flocculants (*i.e.* salts of alum and iron). Since the 60's of the last century, many studies that 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 an increased content of iron in the body is linked with increased risk of cancer, vascular diseases and neurological disorders.[3,4] Beside the potential adverse health effects, the remaining alum and iron sludges are hazardous to the environment and so cannot be disposed of into the surroundings while, on the other hand, high concentrations of metals complicate their further biological treatment. In addition, 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 additional disadvantages of alum coagulants.[5] Natural coagulants do not present a health threat in general. They are obtained from renewable sources and the sludges remaining after their application can be added to feed or fertilizers, or biologically treated.

The main drawback of the application natural coagulants is an increase in organic matter content in treated water. Organic matter can cause colour and an unpleasant odour of water, may lead to increased microbial growth and will react with chlorine or other disinfectants giving toxic and carcinogenic by-products 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 subject.[10–13]

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

The present paper deals with the purification of the crude extract of common bean seed on IRA 958 Cl anion exchange resin the aim of removing compounds that did not possess coagulation activity.

EXPERIMENTAL

Extraction of active component

To obtain the natural coagulant, common bean (*P. vulgaris*) seeds were ground and sieved through 0.4 mm sieve. The smaller fraction, 50 g L^{-1}, was suspended in 0.5 mol L^{-1} NaCl. The suspension was stirred 10 min using a magnetic stirrer in order to extract the 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 $(NH_4)_2SO_4$ and centrifuged at 3000 rpm for 10 min. The precipitate was dissolved in 0.01 mol L^{-1} phosphate buffer (pH 7) and dialysed overnight at 4 °C against Millipore water in a dialysis bag with a molecular cut-off of 12–14 kDa.

Optimization of resin binding conditions and elution of active component

Prior to further purification of the active component, the kinetics of protein binding to the 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 the binding efficiency were examined. The optimization of binding conditions was conducted using a dialysed extract, obtained according to the above-explained procedure, in batch ion-exchange experiments with Amberlite^{TM} IRA 958 Cl (Rohm and Haas) as the matrix. Amberlite^{TM} IRA 958 Cl is a macroreticular strong base anion exchange resin having quaternary ammonium functionality in a crosslinked acrylic polymer matrix. Its shipping weight is 720 g L^{-1} and its total exchange capacity is ≥ 0.80 eq L^{-1} (Cl^- form). The choice of the optimal binding conditions was made by measuring the amount of bound protein (q):

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

where q is the amount of bound protein, mg protein mL^{-1} of resin, 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. The protein concentrations were measured according to Bradford[23] with bovine serum albumin as the standard.

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

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

After optimization of the binding conditions, the kinetics of elution was examined when the optimal elution time was determined. NaCl (0.5 mol L^{-1}) was mixed with resin at a 1:1 ratio. Samples of 0.1 mL during 60 min were taken and analyzed for their protein content.

Purification of the active component

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

Model water

The coagulation activity of partially purified natural coagulant was assessed by the jar test using synthetic turbid water. First, kaolin was ground in a ceramic mortar and sieved

through 0.4 mm sieve. The smaller fraction was used to prepare a 10 g L^{-1} suspension in tap water. The suspension was stirred for 60 min on a magnetic stirrer to achieve uniform dispersion of the kaolin particles, and left for 24 h in order to achieve complete hydration of the kaolin. Model water was prepared just before performing the coagulation tests, by adding this 1 % kaolin suspension to tap water to obtain water with an initial turbidity of 35 NTU (nephelometric turbidity units).

Coagulation test

Coagulation activities were assessed by jar tests in a jar tester VELP FC6S using model water of initial turbidity 35 NTU. The pH value of the model water was adjusted to 9 by addition of 33 % NaOH, in accordance with previous investigations.[18,24] The jar tests were performed by addition of different amounts of eluates to 200 mL of model water. After fast stirring at 200 rpm for 1 min in order to disperse the coagulant, it was continued with slower stirring at 60 rpm for 30 min in order to promote the flocculation of the kaolin particles present in the model water, and then the systems were left for 1 h for sedimentation. As the blank, the same coagulation test was conducted but with no coagulant. After sedimentation for 1 h, the residual turbidity was determined in the upper clarified liquid using a WTW Turb 550/550IR turbidimeter and the coagulation activity was calculated:

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

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

Analytical methods

The turbidity was measured using a turbidimeter (WTW TURB 550/550 IR) and it was expressed in nephelometric turbidity units. The permanganate demand was determined in an acid medium according to the Kübel–Tiemann method.[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 performance of the one-way ANOVA statistical analysis followed by Tukey's test.

RESULTS AND DISCUSSION

According to literature data, proteins are compounds from plant material that possess coagulation ability.[6–7,26] Thus, the coagulation active components in the current study were precipitated by ammonium sulphate, dialysed, and dialysed extract was used for further purification.

Optimization of the binding conditions

As the first step of binding optimization, the kinetics of proteins binding to the anion exchange resin was studied at room temperature. The dialysed extract was diluted with 0.01 mol L^{-1} phosphate buffer (pH 7) to achieve an initial protein concentration of 0.401 and 0.239 mg mL^{-1}. The resin was equilibrated with 0.01 mol L^{-1} phosphate buffer (pH 7) for 15 min. The diluted dialysed solutions were added to the resin at a resin/solution ratio of 1:1 and the mixtures were stirred at 100 rpm. Samples (0.1 mL) of the solution were collected in certain inter-

vals for 120 min and analyzed for their protein content. The amounts of bound protein (q) were calculated and results are presented in Fig. 1.

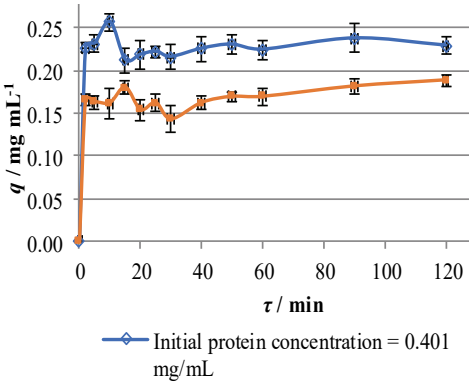

Fig. 1. Adsorption kinetics of the dialysed extract from common bean seed by the anion exchange resin Amberlite^{TM} IRA 958 Cl at 10 mmol L^{-1} phosphate buffer, pH 7.

As could be seen from Fig. 1, the rate of protein adsorption was high at the beginning of the adsorption. The highest binding efficiency was achieved in the first 25 min of the binding process and thereafter, the process became unstable. Hence, 15 min was chosen as the binding time for the following experiments. The results of the performed statistical analysis suggested that there was significant difference in the binding efficiencies in protein solutions of different initial protein concentrations. For a binding time of 15 min, in the protein solution of higher initial protein concentration, a binding efficiency of 52.82 % was attained, while in protein solution of lower initial protein concentration, a higher binding efficiency, 75.12 %, was achieved.

The estimate of the 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 the volumes of dialysed extract. The dialysed extract was diluted with 0.01 mol L^{-1} phosphate buffer (pH 7) to obtain a protein solution with initial protein concentration of 0.260 mg mL^{-1}. It was afterwards mixed with resin for 15 min at 100 rpm and after that analyzed for protein content. Based on obtained data, binding efficiencies were calculated. The results of these experiments are shown in Fig. 2.

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

The effect of initial protein concentration in the protein solution on the binding efficiency and the amount of bound protein was also investigated. The dialysed 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 in liquid phase was measured and the binding efficiency and amount of bound protein were calculated. The obtained results are shown in Fig. 3.

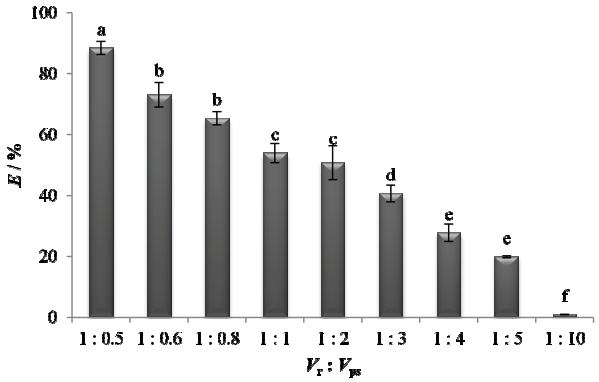

Fig. 2. The influence of resin/protein solution ratio (V_r:V_{ps}) on the binding of proteins on the anion exchange resin Amberlite^{TM} IRA 958 Cl at 10 mmol L^{-1} phosphate buffer, pH 7; the different letters indicate significant differences between the samples ($p < 0.05$).

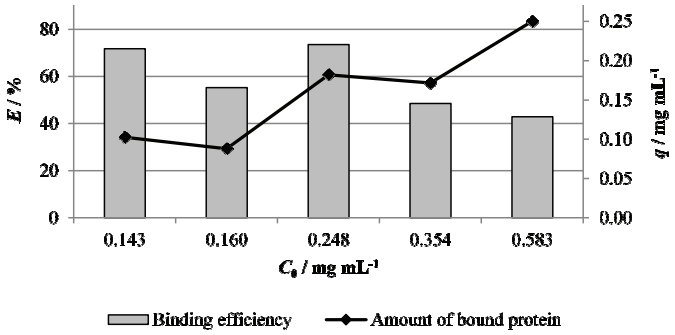

Fig. 3. The effect of initial protein concentration on binding efficiency and amount of bound proteins on the Amberlite^{TM} IRA 958 Cl anion exchange resin at 10 mmol L^{-1} phosphate buffer, pH 7.

According to results presented in Fig. 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 an initial protein concentration of 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 the buffer on the adsorption was investigated by measuring the binding efficiency of proteins to the matrix in phosphate buffer within the concentration range 10–100 mmol L^{-1} at pH 7. The initial protein con-

centration in solution was adjusted to 0.260 mg mL^{-1}. The obtained protein solutions were mixed with the matrix for 15 min at 100 rpm and a matrix/protein solution ratio of 1:0.5. The results are presented at Fig. 4. An increase in the ionic strength of the buffer from 0.01 to 0.05 mol L^{-1} did not significantly affect the adsorption of proteins. However, at buffer ionic strength 0.1 mol L^{-1}, the amount of adsorbed protein decreased. This could be explained by the competition for adsorption sites at the 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 is economically better. Hence, 0.01 mol L^{-1} phosphate buffer was chosen for further work.

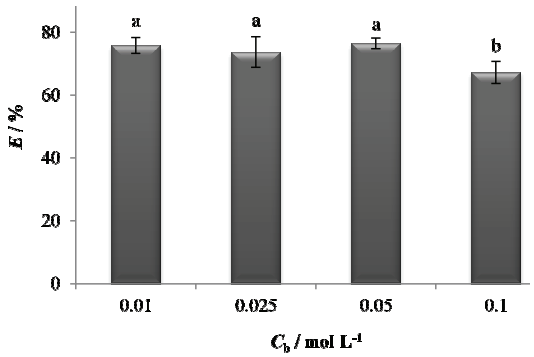

Fig. 4. The effect of phosphate buffer concentration (C_b) on the adsorption of proteins extracted from common bean on the anion exchange resin Amberlite™ IRA 958 Cl at pH 7; different letters indicate significant differences between the samples ($p < 0.05$).

According to previous research (data not shown),[8] the isoelectric point (p*I*) of the dialysed extract obtained from common bean is between pH 4 and 5.5. Thus, the effect of the pH of the buffer on the amount of bound proteins from dialysed extract on the anion exchange resin was studied within the pH range 7––9. In order to find the optimal pH of the buffer for the adsorption, the dialysed extract was diluted in universal McIlvaine buffer having pH from 7 to 9 with an increment pH increase of 0.5. The initial protein concentration was around 0.260 mg mL^{-1}. The matrix and the protein solution were mixed in a ratio 1:0.5 for 15 min at 100 rpm. The results (Fig. 5) revealed that the maximum of protein adsorption was achieved at pH 7 and 7.5. Considering this, further experiments were

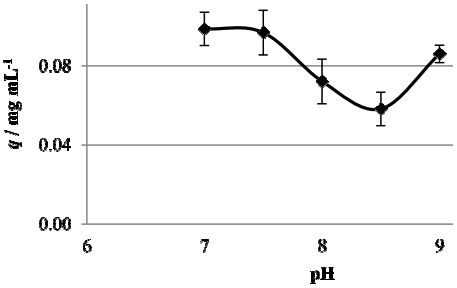

Fig. 5. The effect of the buffer pH on the adsorption of proteins extracted from common bean on the anion exchange resin Amberlite™ IRA 958 Cl.

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 the elution

Following optimization of binding conditions and prior to purification of active component, optimization of elution was performed. The dialysed extract was diluted with 10 mmol L^{-1} phosphate buffer (pH 7) to obtain a protein solution with an initial protein concentration of 0.262 mg mL^{-1}. The protein solution was added to Amberlite^{TM} IRA 958 Cl resin, which had previously been equilibrated in 10 mmol L^{-1} phosphate buffer (pH 7). The resin/protein solution ratio was 1:0.5 and the system was mixed for 15 min at 100 rpm. After completion of the adsorption, the remaining protein solution was drained and the matrix was washed with 10 mmol L^{-1} phosphate buffer (pH 7). The elution was performed with 0.5 mol L^{-1} NaCl solution at a 1:1 resin/solution ratio. The kinetics of elution were monitored during 60 min. Solution samples of 0.1 mL were collected in certain time intervals and analysed for their protein content. From the obtained results, presented in Fig. 6, an optimal elution time of 20 min was determined.

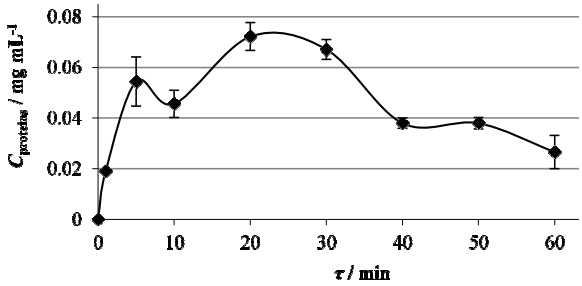

Fig. 6. Kinetics of the elution of proteins from common bean seed on anion exchange resin Amberlite^{TM} IRA 958 Cl with 10 mmol L^{-1} phosphate buffer, pH 7.

According to ANOVA, there were significant ($p < 0.05$ confidence level) differences in protein contents in fractions obtained during the first 40 min of elution of proteins from common bean seed from anion exchange resin Amberlite TM IRA 958 Cl. However, the performed statistical analysis suggested that there were insignificant differences between proteins contents of samples collected after 40 min.

Purification of active component and coagulation study

Dialysed extract was diluted with 10 mmol L^{-1} phosphate buffer (pH 7) to obtain a protein solution with an initial protein concentration of 0.261 mg mL^{-1}. The protein solution was added to Amberlite^{TM} IRA 958 Cl anion exchange resin, which had previously been equilibrated with 10 mmol L^{-1} phosphate buf-

fer (pH 7), and the binding was performed under the previously determined optimal conditions. The amount of adsorbed proteins was 0.230 mg mL^{-1}, *i.e.*, a 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 of 1:1 during 15 min, elution was performed by step gradient of NaCl during 20 min for each step and at a resin/NaCl solution ratio 1:1. In this way, four fractions were obtained, the protein contents of which were determined (Fig. 7).

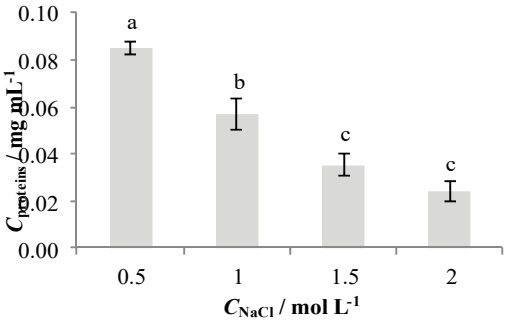

Fig. 7. Protein contents in fractions obtained by elution of bound common bean proteins from the anion exchange resin Amberlite^{TM} IRA 958 Cl in step gradient of NaCl solutions; different letters indicate significant differences between samples ($p < 0.05$).

With increasing concentration of the NaCl solution, the protein content of the fractions decreased. An elution efficiency of 87.37 % was achieved, *i.e.*, 12.63 % of the proteins remained bound to the resin.

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

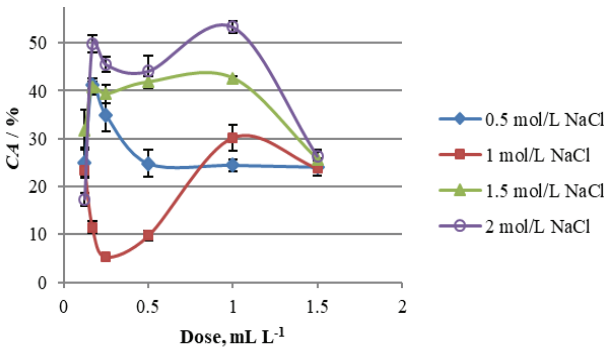

Fig. 8. Coagulation activity of the fractions purified on anion exchange resin Amberlite TM IRA 958 Cl at different doses.

Significant differences in coagulation behaviour of the 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. The fraction obtained with 1.5 mol L^{-1} NaCl showed similar behaviour to the 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 the fraction obtained with 2 mol L^{-1} NaCl was 53.3 % at an applied dose 1 mL L^{-1}, but only a 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 could be considered as optimal one. Calculated based on proteins concentration, the optimal coagulation dose was 0.0041 mg L^{-1}.

In a previous investigation[8] when Amberlite^{TM} IRA 900 Cl was used for purification of coagulant from common bean in the continual mode, the highest coagulation activity of 72.3 % was obtained at dose of purified fraction 0.081 mg L^{-1}. When compared to the results from that study, the optimal dose of the fraction obtained by purification on Amberlite^{TM} IRA 958 Cl was almost 20 times lower than the dose of fraction obtained by purification on Amberlite^{TM} IRA 900 Cl, and when calculated on the basis of proteins that were added in the model water, the coagulation activity obtained with optimal dose in the present research was almost 14 times higher than that of the purified fraction obtained on Amberlite^{TM} IRA 900 Cl.

Baptista *et al.*[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 that corresponded to globulin (II)[10] showed turbidity removal of about 30 % in surface water the initial turbidity of which was 102.42 NTU. As the authors claimed, the 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 the coagulant tested. However, fractions obtained by water extraction showed high turbidity removal ranging from 79–89 %. The higher coagulation activities of these fractions compared to those of the fractions obtained in this work could be attributed to the fact that the initial turbidity of the treated water in the paper of Baptista *et al.*[10] was almost 1.5 times greater than that of the model water used in experiments presented in the present paper. Literature data show that natural coagulants achieve higher efficiency of particles removal in more turbid waters[28–31]. Moreover, the applied dosage of the fractions in the paper of Baptista *et al.*[10] was more than three thousand time higher than optimal dosage achieved in the present work.

Organic matter in the water before and after coagulation tests with common bean crude extract and the fraction obtained with 2 mol L^{-1} NaCl at the optimal

doses was assessed to determine the increase in organic load. The results revealed that the crude extract and purified fraction had increased organic matter content in treated water by 68 and 19 %, respectively. This was in accordance with the low protein content in the tested fraction, but it could also be explained by the absence of other organic compounds in purified fraction.

CONCLUSIONS

Evaluation of fractions obtained by purification of common bean seed crude extract on Amberlite^{TM} 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 the fraction obtained with 2 mol L^{-1} NaCl at a dose of 1 mL L^{-1}. The optimal dose of the fraction obtained by purification on Amberlite^{TM} IRA 958 Cl was almost 20 times lower than the optimal dose of the fraction obtained by purification on Amberlite^{TM} IRA 900 Cl, while its coagulation activity was almost 14 times higher.

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ИЗВОД

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

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Природни коагуланти представљају органска једињења и могу повећати садржај органских материја у третираној води, те их је стога потребно пречистити како би се уклониле материје које немају коагулациону способност. У овом раду је природни коагулант екстрахован са 0,5 mol L^{-1} раствором NaCl из 50 g L^{-1} самлевеног семена пасуља. Протеини из сировог екстракта су исталожени додавањем амонијум-сулфата. Након таложења, одвајања талога и поновног растварања протеина, даље пречишћавање је изведено на анјонској јоноизмењивачкој смоли Amberlite IRA 958 Cl у шаржном поступку. Делимично пречишћени коагулант који је добијен елуирањем са 2 mol L^{-1} раствором NaCl је показао највећу коагулациону активност од 53,3 % при дози од 1 mL L^{-1} иако је та фракција садржала најмању концентрацију протеина. Незнатно нижу коагулациону активност од 49,8 % је иста фракција постигла при 5 пута нижој дози. Повећање садржаја органских материја у обрађеној води је било око 4 пута ниже у случају примене ове пречишћене фракције као коагуланта у поређењу са сировим екстрактом.

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