Ultrafiltration as a simple purification method of a water extract of common bean seed as a natural coagulant

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Abstract

Natural coagulants from a crude water extract of common bean seed showed very good efficiency of turbidity removal from water of ~89 % under optimal coagulation conditions, which were determined using response surface methodology (RSM). However, they also increased the content of organic matter in treated model water by ~66 %, which is the main drawback of usage of natural coagulants, in general. Thus, ultrafiltration was applied for processing of the crude water extract in order to separate biomolecules, which exhibit the coagulation activity. Four fractions obtained by ultrafiltration were applied in coagulation tests under the same conditions as the crude extract, and the 4th fraction (molecules with molecular weights >30 kDa) with the predominant content of proteins with molecular weights 50 – 60 kDa, achieved almost as high efficiency of turbidity removal (75 %) as the crude extract. At the same time, the content of organic matter in treated water increased just for 16 % in comparison to the blank (model water processed in the same way but without coagulant). After optimization of process parameters by RSM for usage of the 4th fraction, the coagulation activity increased further to 80 %.

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

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1. INTRODUCTION

In the age of evident and rapid environmental degradation, research and application of novel technologies, which are less hazardous to nature, represent an important progress in line with global efforts for sustainable development. Use of natural coagulants for water and wastewater treatments is such kind of technique.

Although traditional coagulants used in water and wastewater treatments, such as salts of alum and iron as well as synthetic organic polyelectrolytes exhibit good efficiencies, many drawbacks are related to these applications [1-5].

In recent years, natural coagulants have been attracting worldwide attention of scientists, which is mainly motivated by several factors: natural coagulants originate from renewable sources, they are not harmful in general, and besides, the resulting biodegradable sludge can be simply anaerobically treated and disposed in the nature without any adverse influence or can be used as an addition to fertilizers or feed. Thus, natural coagulants are believed to be a good alternative to chemical coagulants and flocculants in water and wastewater treatments and are expected to play a more significant role in the future.

Recently, the most studied plant in terms of natural coagulants derivation is *Moringa oleifera* [6-9]. Considering the fact that *M. oleifera* is a tropical plant, we have investigated in our laboratory the possibility of extraction of natural coagulants from sources that are cheap and easily available in the Balkan region, as well as in Europe. These investigations confirmed the fact that common bean and other various strains of Leguminose could be used as sources of natural coagulants [10-14]. Beside its availability, common bean offers a few advantages over the *M. oleifera* seed.

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Presence of oil in the *M. oleifera* seeds could form an emulsion or film coating, which inhibits the surface where reaction takes place and thus reduces floc formation [15]. On the other hand, because of small quantities of oil present in common bean, a delipidation step can be avoided, which is beneficial for both economic and environmental reasons.

The major disadvantage of application of natural coagulants as crude extracts is an increase of organic matter in treated water. This adversely affects the water quality and complicates its further processing, but can be addressed by purification of the coagulant [16]. As reported in literature, natural coagulants are mostly purified by precipitation of proteins from crude extracts with salt (ammonium sulfate) followed by ion-exchange chromatography (batch or continuously operated column, single-step or two-step), or by using just one of these two methods [5,9,16]. On the other hand, this step may increase their processing costs. Hence, it is very important to research and find new, simple, eco-friendly and cost-effective techniques for purification of these coagulants. Ultrafiltration is a well known method for fractionation and purification, but to the best of our knowledge, it was not used for processing of natural coagulants, apart from our previous investigate-on [17], where it was successfully introduced for processing of a salt extract of common bean seed. It is known that different solvents (water, salt solutions, acidic and alkaline solutions, ethanol, *etc.*) produce extracts with different compositions that in turn may influence the coagulation process. Having this in mind, the aim of the present study was to investigate the coagulation behavior of a water extract of common bean seed by exploring the extracted protein fractions. In addition, we have investigated the possibility to maintain the coagulation performance and to minimize the concerns of residual organic matter by using ultrafiltration as a method of processing of the crude water extract of common bean. Also, the response surface methodology was used as a method for optimization of coagulation process influential parameters.

2. EXPERIMENTAL

2. 1. Natural coagulant

Natural coagulant was obtained according to the procedure described by Antov *et al.* [17]. The smaller fraction of the ground common bean seed was suspended in distilled water at the concentration of 50 g dm^{-3}.

2. 2. Ultrafiltration

The coagulation active components from the crude extract were further processed by ultrafiltration in an Amicon stirred cell (model 8200, Millipore, USA) equipped with a regenerated cellulose membrane (Millipore, USA) having a cut-off 5,000 Da, and polyethersulfone membranes (Millipore, USA) having cut-offs 10,000 Da and 30,000 Da. Ultrafiltration was performed under the pressure of inert gas of 2.5·– 4·10^5 Pa and moderate magnetic stirring of 80 rpm at constant room temperature (294 K).

Fractionation of components was conducted using membranes in consecutive manner – ultrafiltration through a higher cut-off membrane was followed by the one with a lower cut-off. The initial volume of suspension was 150 cm^3. In all steps ultrafiltration was run until the volume of retentates reached 20 % of the initial volume. After each ultrafiltration step, volumes of permeates, as well as retentates were adjusted to the value of the initial volume by adding distilled water. The permeate obtained by ultrafiltration with the membrane cut-off 5,000 Da was assigned as the 1st fraction, the fraction containing components having approximate molecular weights between 5,000 and 10,000 Da was assigned as the 2nd fraction, the fraction containing components having approximate molecular weights between 10,000 and 30,000 Da was assigned as the 3rd fraction, and the retentate from ultrafiltration with the 30,000 Da cut-off membrane as the 4th fraction.

2. 3. Electrophoretic analysis

Molecular composition (in terms of molecular weights) of common bean crude extract and four fractions obtained by ultrafiltration was analyzed using sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli [18] at the omniPAGE Vertical Electrophoresis System (model CVS10DSYS, Cleaver Scientific Ltd., UK). For this purpose 12 % Precise™ Tris-HEPES-SDS Precast Polyacrylamide Mini Gels (Thermo Fisher Scientific Inc., UK) and unstained protein standards (Serva, Germany) with the range of molecular weights from 7 – 240 kDa were used. Gels were run at 100 V for 90 min and stained with Coomassie Brilliant Blue (Serva, Germany).

2. 4. Model water

Turbid waters of different initial turbidities were prepared for coagulation tests according to the procedure described by Antov *et al.* [17]. The initial pH of model waters was adjusted to 5.5, 6 or 6.5 by using concentrated HCl (Merck, Germany) just before the experiments.

2. 5. Coagulation test

Coagulation activity was assessed by jar tests according to the procedure described by Antov *et al.* [17]. Samples were added to beakers at different dosages: 0.4, 0.65 or 0.9 cm^3 dm^{-3} of model water. After sedimentation, upper clarified liquids were collected and residual turbidities, as well as permanganate demands were assessed. The same coagulation tests were performed for each initial turbidity and pH of the model water without addition of any coagulant and used as blanks. Coagulation activity (CA) was calculated using turbidity values (CA_T, Eq. 1) or by using permanganate demands (CA_{PD}, Eq. 2) as:

$$CA_T, \% = (T_b - T_s)\ 100/T_b \tag{1}$$

$$CA_{PD}, \% = (PD_b - PD_s)\ 100/PD_b \tag{2}$$

where T_b and T_s are the turbidity of the blank and the sample, respectively, and PD_b and PD_s are the permanganate demand of the blank and the sample, respectively.

All coagulation tests were run in triplicate and average values along with standard deviations are presented.

2. 6. Response surface methodology

Optimal levels of three variables (initial turbidity and pH of the model water, and dosage of coagulant) were determined using the Box-Behnken experimental design in order to obtain maximal coagulation activity of common bean crude extract and the 4th fraction.

The variables and their levels in coded and actual values investigated in this study are summarized in Table 1.

Table 1. Experimental factors and their levels

Factor (variable)	Level		
	Low (-1)	Zero (0)	High (+1)
v_1 – initial turbidity of model water, NTU	170	195	220
v_2 – pH of model water	5.5	6.0	6.5
v_3 – dosage of coagulant, cm^3 dm^{-3}	0.40	0.65	0.90

The range for initial turbidity of model water was chosen according to literature data, which show that natural coagulants achieve higher efficiencies of particle removal from more turbid waters [9,19-21]. The range of pH of model water was selected based on preliminary experiments in which influence of pH on coagulation activity (CA_T) of crude extract was investigated in a model water of initial turbidity 200 NTU. The applied dose of crude extract was 0.4 cm^3 dm^{-3}. Results of these experiments are presented in Supplementary Material (Fig. S-1).

The experimental data were fitted to a second-order polynomial model:

$$Y = b_0 + b_1v_1 + b_2v_2 + b_3v_3 + b_{12}v_1v_2 + b_{13}v_1v_3 + b_{23}v_2v_3 + b_{11}v_1^2 + b_{22}v_2^2 + b_{33}v_3^2 \tag{3}$$

where b_0, b_1, b_2, b_3, b_{12}, b_{13}, b_{23}, b_{11}, b_{22} and b_{33} are the regression coefficients.

Results of the experimental design were studied by using Design-Expert® 7.1 Statistical Software (Stat-Ease, Inc., Minneapolis, USA).

2. 7. Analytical methods

Turbidity was measured using a turbidimeter (model Turb® 550 IR, WTW, Xylem Analytics, Germany) and it was expressed in nephelometric turbidity units (NTU). Permanganate demand was determined in an acid medium according to the Kübel-Tiemann method [22].

3. RESULTS AND DISCUSSION

In this paper design of experiment (DOE) based on response surface methodology (RSM) was used to develop polynomial regression equations and establish the relationship between the input factors (initial turbidity and pH of the model water, and dosage of coagulant) and response (turbidity removal, expressed as the coagulation activity, CA_T). In order to run the minimum number of experiments (without studying all possible experimental combinations), the Box-Behnken design was applied.

3. 1. Optimization of experimental factors for coagulation activity of the crude extract

The objective of the first part of experiment was to find optimum values of the above-mentioned process parameters to achieve the highest CA_T of the common bean crude extract. The Box-Behnken design, which included combinations of these three factors at three levels in random order, along with obtained responses is presented in Table 2.

Table 2. Box-Behnken experimental plan and responses (experimentally measured, n = 3) for crude common bean extract and the 4th fraction as coagulants

Run number	Independent variable (coded value)			CA_T, %	
	v_1	v_2	v_3	Crude extract	4th fraction
1	-1	-1	0	75.7±2.7	62.8±1.6
2	+1	-1	0	76.7±1.2	71.2±3.9
3	-1	+1	0	72.4±4.4	63.0±3.4
4	+1	+1	0	77.9±2.9	68.9±1.9
5	-1	0	-1	78.2±0.6	59.8±3.3
6	+1	0	-1	81.5±5.3	65.7±3.2
7	-1	0	+1	68.0±2.1	67.2±1.3
8	+1	0	+1	77.0±3.1	69.3±2.1
9	0	-1	-1	82.9±3.9	76.1±1.9
10	0	+1	-1	75.6±3.4	76.6±3.9
11	0	-1	+1	69.8±4.2	83.3±3.9
12	0	+1	+1	74.7±2.3	76.9±2.6
13	0	0	0	58.1±5.4	64.8±3.0
14	0	0	0	61.2±2.6	65.5±1.8
15	0	0	0	59.5±2.9	63.8±1.6

The response (CA_T) was correlated with the three investigated variables using multiple regression analysis and the second-order polynomial (Eq. 3). The values of coefficients of determination (R^2), as well as analysis of variance (ANOVA) of the quadratic regression model are shown in Table 3. Since the aim of the study was to develop the model for predicting the response variable (CA_T), the parameter termed as predicted R^2 (R^2_{pred}) should be close to the adjusted R^2 (R^2_{adj}) indicating good agreement of the model with experimental results.

According to the obtained results it can be concluded that v_1, v_3, v_2v_3, v_1^2, v_2^2, v_3^2 are the significant model variables. In this case the predicted values were found to be in good agreement with experimental values. Also, the model F-value of 51.03 and p-value of 0.0002 imply that the model is significant.

Graphical representation (3D surface graph) of the obtained model allows an estimation of the effects of experimental parameters on the response, and in the case of the crude extract as a coagulant it is presented in Figure 1. This surface graph illustrates from a qualitative point of view the influence of pH and coagulant dose on turbidity removal at the initial turbidity of model water of 220 NTU. As can be appreciated from Figure 1, working at lower pH values of model water and lower doses of crude extract leads to higher coagulation activity in terms of turbidity decrease.

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Table 3. Coefficients in quadratic regression model, F- and p-values and coefficients of determination estimated for coagulation activity of the crude common bean extract

	Regression coefficient		
	Estimate*	F-value	p-value**
Linear			
b_0	1971.71350		
b_1	-5.74220	24.28	0.0044
b_2	-410.13500	1.39	0.2913
b_3	-378.37000	56.59	0.0007
2-way interaction			
b_{12}	0.09000	2.78	0.1562
b_{13}	0.22800	4.46	0.0883
b_{23}	24.40000	20.45	0.0063
Square			
b_{11}	0.013200	138.12	<0.0001
b_{22}	31.30000	124.26	0.0001
b_{33}	133.20000	140.64	<0.0001
Lack of fit		0.59	0.678
Model summary	R^2	R^2_{adj}	R^2_{pred}
	0.9892	0.9698	0.9061

*coefficients are given in terms of uncoded units
**p-values less than 0.05 indicate that model variables are significant

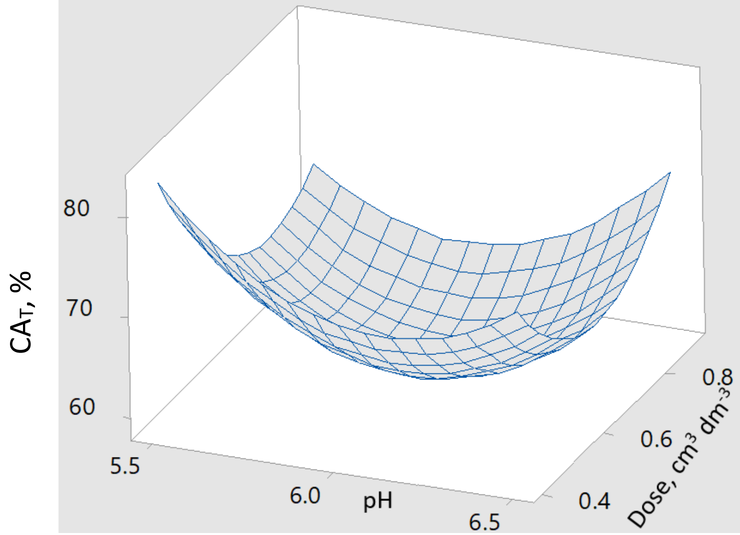

Figure 1. Effects of pH of the model water and dose of the crude extract on the coagulation activity at the initial model water turbidity of 220 NTU

The initial turbidity of 220 NTU, pH 5.5 and the dose 0.4 cm^3 dm^{-3} had been found to be the optimal conditions within the studied ranges for achieving the maximal turbidity removal of 91 % by using the crude extract as a coagulant. In order to verify the model fit, three individual coagulation tests were performed at these optimal conditions. The average turbidity removal of 88.5±3.1 % was achieved, which was close to the predicted value, and falls between the low and high 95 % PI (prediction interval). This coagulation activity can be considered as very good, since efficiencies reported in literature for the mostly investigated *M. oleifera*'s extract range from 74–100 % [19-20,23].

Contents of organic matter, expressed as permanganate demand, in water before and after the coagulation tests were determined too, in order to assess the increase in organic load, when the crude extract was added to the turbid water (at optimal process conditions). Organic load was expressed by the coagulation activity in terms of organic matter removal, calculated according to Eq. 2, and in the case of the crude extract as a coagulant, it amounted to -66.1±2.9 %, meaning that crude extract increased the organic matter content in treated water compared to the blank. This increase of organic load is considerably high and represents a drawback of application of natural coagulants in the form of crude extracts [17].

3. 2. Ultrafiltration of the crude extract

Since the aim of the study was to reduce or completely avoid the use of chemicals, and to simplify the purification process, ultrafiltration was applied as a method for fractionation of the crude extract. The working hypothesis was that some biomolecules, which do not exhibit coagulation activity, or it is negligible, will be removed by ultrafiltration membranes, thus leading to a decrease in organic load in treated water.

Ultrafiltration resulted in four fractions, which were used in coagulation tests under the same conditions as the crude extract. The obtained results expressed as turbidity removal and organic matter removal are presented in Table 4.

Table 4. Coagulation activities (experimentally measured, n=3) of fractions obtained by ultrafiltration of the common bean seed crude extract. Coagulation process conditions: initial turbidity of the model water 220 NTU, pH 5.5, dose of fractions 0.4 cm^3 dm^{-3}

Coagulant	CA_T, %	CA_{PD}, %
1st fraction (M<5,000 Da)	18.9±0.9	-18.1±2.2
2nd fraction (5,000<M<10,000 Da)	19.5±0.4	-22.0±2.9
3rd fraction (10,000<M<30,000 Da)	43.2±3.1	-18.1±0.5
4th fraction (M>30,000 Da)	74.9±3.8	-16.0±1.0

As can be seen in Table 4, the 1st, 2nd and even 3rd fraction showed low coagulation activities in terms of turbidity removal (19, 20 and 43 % respectively), while the 4th fraction showed the highest coagulation activity (75 %) close to that of the crude extract. This result leads to the conclusion that molecules, predominantly exhibiting coagulation activity in the crude extract are those of molecular weights over 30 kDa, while molecules of lower molecular weights do not have significant abilities for removal of particulates. In our previous work [17] we have investigated coagulation efficiencies of fractions obtained by ultrafiltration of a common bean crude extract, which was obtained by using 0.5 mol dm^{-3} NaCl solution. Those fractions showed different coagulation behaviors as compared to fractions investigated in the present study, *i.e.* they all achieved similar coagulation maxima, with the 1st fraction (components having approximate molecular weights below 10 kDa) exhibiting the highest one. These dissimilarities in behavior can be assigned to different solvents used for extractions, as it was shown that water and salt extracts may be of different nature [24].

Although the 4th fraction in the present study was not as efficient in turbidity removal as the crude extract, its organic load (16 %) to the treated water was considerably lower than that of the crude extract (66 %) under the same operating conditions. This was also the lowest organic load induced and the highest coagulation activity achieved among the fractions.

3. 3. Electrophoretic characterization

The common bean crude extract and four fractions obtained by ultrafiltration were subjected to electrophoresis and the obtained results are presented in Figure 2.

According to literature data, *Phaseolus vulgaris* seed contains 213 – 313 g of crude protein/kg DM, the major components of which are globulins (54 – 79 % of total protein), glutelins (20 – 30 % of total protein) and albumins (12 – 30 % of total protein) [25]. As can be seen in Figure 2, the predominant protein bands in the crude extract are those of molecular weights of 50 – 60 kDa. These bands represent subunits of the main storage protein of common bean, phaseolin. Phaseolin is a glycoprotein containing neutral sugars, with a large variation in molecular weights of its subunits [25]. According to Montoya *et al.* [25-26] phaseolins are composed of 2 – 6 polypeptides differing regarding their molecular weight (ranging from 40 – 54 kDa) and isoelectric point. Other emphasized bands (Fig. 2) are those of 25 kDa (which correspond to phytohemagglutinins) and 10 kDa. When compared to the electrophoregram of a crude extract obtained by extraction with 0.5 mol dm^{-3} NaCl (unpublished data), differences in protein pattern of the water extract can be observed. This finding can be the explanation for different coagulation behaviors of the crude water extract and its fractions obtained by ultrafiltration in comparison to those of samples obtained by NaCl extraction reported previously [17].

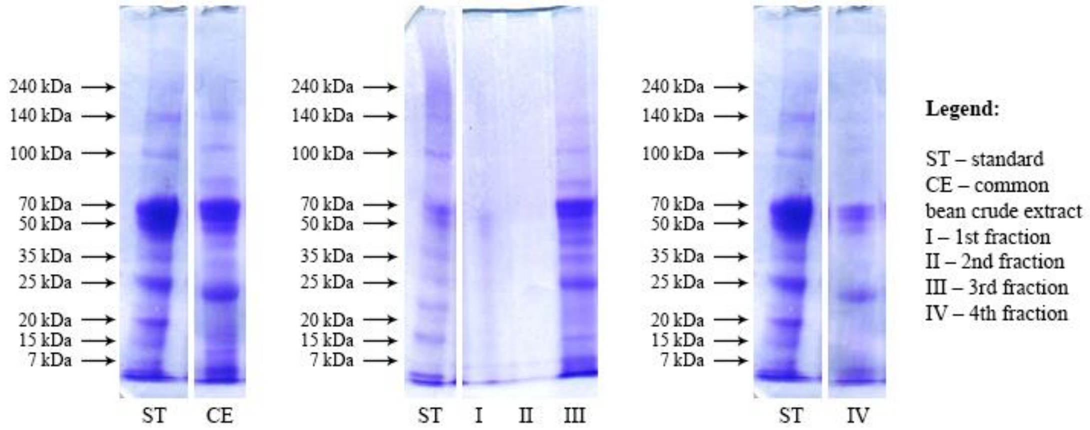

Figure 2. SDS-PAGE of the common bean crude extract and the 1st, 2nd, 3rd and 4th fraction obtained by ultrafiltration of the crude extract

Under the applied staining protocol, protein bands are not visible in the 1st and 2nd fractions, which can explain their poor coagulation activity. The 3rd fraction consists of proteins with the molecular weights around 10, 11, 25 and 30 kDa, but bands of higher molecular weight proteins are also observable. Predominant proteins in the 4th fraction have molecular weights of 50 – 60 kDa, same as in the crude extract, and taking into account similar coagulation activities of these two samples (the crude extract and the 4th fraction), it can be concluded that these proteins are the most potent in the water extract of common bean seed regarding coagulation. Beside these, proteins of 11 and 25 kDa are present too, which can be explained by the fact that ultrafiltration was run to some predetermined volume of retentate, so it was expected that some lower molecular weight molecules remained in the retentate. Smaller number of bands in the electrophoregram of the 4th fraction as compared to those of the crude extract and the 3rd fraction indicates that certain purification of the 4th fraction was accomplished by ultrafiltration, which is in correlation with its lower organic matter load in the treated model water.

3. 4. Optimization of experimental factors for coagulation activity of the 4th fraction

In order to maximize turbidity removal, optimization of process parameters was performed for the 4th fraction as a coagulant using the Box-Behnken experimental design again. The experimental plan along with the obtained responses is presented in Table 2.

As in the case of the crude extract as a coagulant, the response (CA_T) was correlated to the investigated variables using the second-order polynomial (Eq. 3). The obtained values of coefficients of determination (R^2), as well as ANOVA analysis of the quadratic regression model are presented in Table 5. According to the results, five factors have the p-value below significance limit of 0.05, so they are statistically significant model terms. These are v_1, v_3, v_1^2, v_2^2 and v_3^2. Considering the model F-value of 34.18 and p-value of 0.0006, it can be said that the model is significant.

The response surface plot of this model is shown in Figure 3 illustrating influences of pH and the 4th fraction dose on turbidity removal at the initial model water turbidity of 220 NTU. The highest coagulation activity of ~81 % can be achieved at the lowest investigated pH of the model water by adding the highest dose of the 4th fraction (Fig. 3). This result is opposite to the results obtained with the crude extract as a coagulant where the lowest dose was the most efficient. The common bean crude extract is a complex matrix, which consists not only of proteins but also of other polyelectrolytes such as polysaccharides, soluble fibers and phytic acid. All these components can influence the coagulation behavior having as a result a synergistic or antagonistic effect. Considering the changed composition of the 4th fraction compared to that of the crude extract, its different coagulation behavior regarding the dose and pH value was expected.

Coagulation tests were performed in order to verify the obtained model, and the coagulation activity of 79.8±2.3 % was achieved with the 4th fraction dose of 0.9 $cm^3\ dm^{-3}$ in the model water with the initial turbidity of 220 NTU and pH 5.5. The obtained value falls between the low and high 95 % PI (prediction interval), and once again it confirmed the applicability of the obtained model. Also, it is important to point out that this value is just by 9.8 % lower as compared to that obtained by the crude extract as a coagulant.

Table 5. Coefficients in the quadratic regression model, F- and p-values and coefficients of determination estimated for coagulation activity of the 4th fraction

	Regression coefficient		
	Estimate*	F-value	p-value**
Linear			
b_0	670.67900		
b_1	3.92670	31.85	0.0024
b_2	-331.28000	4.10	0.0988
b_3	-8.83000	21.92	0.0054
2-way interaction			
b_{12}	-0.050000	0.80	0.4119
b_{13}	-0.15200	1.85	0.2319
b_{23}	-13.80000	6.10	0.0566
Square			
b_{11}	$-8.76000 \cdot 10^{-3}$	56.71	0.0007
b_{22}	29.00000	99.45	0.0002
b_{33}	100.40000	74.50	0.0003
Lack of fit		3.79	0.216
Model summary	R^2	R^2_{adj}	R^2_{pred}
	0.9840	0.9552	0.7770

*coefficients are given in terms of uncoded units

**p-values less than 0.05 indicate that model terms are significant

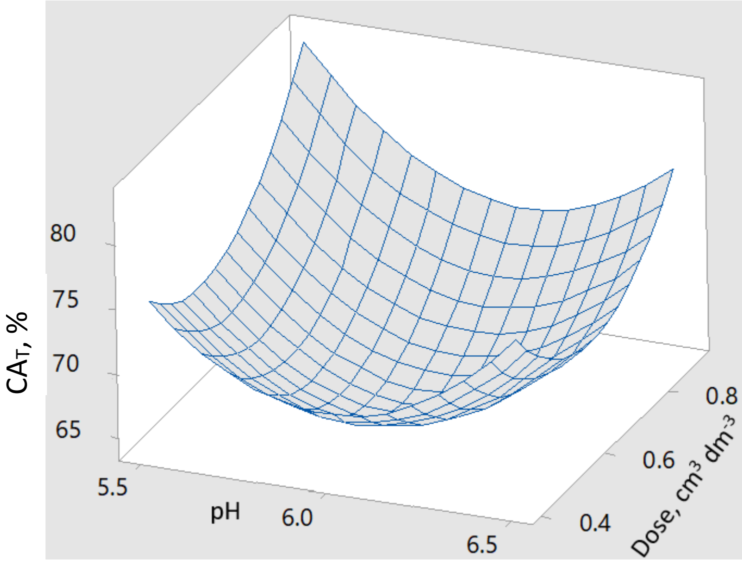

Figure 3. Effects of pH of the model water and the 4th fraction dose on the coagulation activity at the initial model water turbidity of 220 NTU

4. CONCLUSION

In order to minimize the organic load that the crude extract from common bean (*Phaseolus vulgaris*) add to the treated water, ultrafiltration was applied to remove molecules that do not exhibit, or exhibit a low coagulation activity in terms of turbidity removal. Organic load in the treated water decreased for 50 % in the case of the use of the 4th fraction as a coagulant as compared to that of the crude extract, while the capability of turbidity removal for these two coagulants was similar. Taking into account simplicity of the procedure in comparison to other multistep purification techniques and environmental aspects of omitting the use of additional chemicals, ultrafiltration appears to be a promising technique for purification of natural coagulants. To the best of our knowledge, this is among the first papers considering and recognizing the potential of ultrafiltration as the method of choice for processing and purification of natural coagulants.

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SAŽETAK

Jednostavno prečišćavanje prirodnog koagulanta iz vodenog ekstrakta semena pasulja ultrafiltracijom

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(Stručni rad)

Prirodni koagulanti koji se nalaze u sirovom vodenom ekstraktu dobijenom iz semena pasulja su pokazali veoma dobru efikasnost uklanjanja mutnoće iz vode od oko 89 % pod optimalnim uslovima koagulacije, koji su utvrđeni primenom metode odzivne površine (engl. *Response Surface Methodology*, RSM). S druge strane, oni su povećali sadržaj organskih materija u obrađenoj vodi za ~66 %, što je generalno osnovni nedostatak primene prirodnih koagulanata. Stoga je u ovom radu primenjena ultrafiltracija za frakcionisanje sirovog vodenog ekstrakta kako bi se prečistili biomolekuli koji poseduju koagulacionu aktivnost. Četiri frakcije dobijene ultrafiltracijom su ispitane u testovima koagulacije pod istim uslovima kao i sirovi ekstrakt, i 4-ta frakcija (molekuli molekulske mase > 30 kDa) u kojoj preovlađuju proteini molekulske mase 50 – 60 kDa, je postigla sličnu efikasnost (75 %) uklanjanja mutnoće kao sirovi ekstrakt, dok je u isto vreme povećala sadržaj organskih materija u tretiranoj vodi za samo 16 % u poređenju sa slepom probom. Nakon optimizacije procesnih parametara za 4-tu frakciju kao koagulanta primenom RSM, koagulaciona aktivnost ove frakcije se dodatno povećala na 80 %.

K*ljučne reči*: bistrenje vode, koagulaciona aktivnost, proteini, opterećenje organskim materijama