Desalination and Water Treatment
www.deswater.com

51 (2013) 442–447
January

1944-3994/1944-3986 © 2013 Desalination Publications. All rights reserved
doi: 10.1080/19443994.2012.714892

The fractionation of natural coagulant extracted from common bean by use of ultrafiltration membranes

Jelena M. Prodanović*, Mirjana G. Antov, Marina B. Šćiban, Bojana B. Ikonić, Dragana V. Kukić, Vesna M. Vasić, Darjana Ž. Ivetić

University of Novi Sad, Faculty of Technology, Blvd. Cara Lazara 1, 21000 Novi Sad, Republic of Serbia
Tel. +381 21 485 3813; Fax: +381 21 450 413; email: jejap@uns.ac.rs

Received 29 February 2012; Accepted 18 July 2012

ABSTRACT

In the age of rapid degradation of environment, the use of natural coagulants for water and wastewater treatment represents a promising idea which is in line with the global sustainable initiatives. Since these coagulants are of organic nature and have complex composition, it is important to separate components which show coagulation activity from those which do not show. In this work, ultrafiltration membranes with MW cut off of 10,000 Da and 30,000 Da were used for fractionation of natural coagulant extract obtained from common bean seed. Coagulation efficiency of these fractions was assessed by jar tests in model water. Coagulation conditions (pH and applied dose of coagulant) were optimized for each fraction. The fraction of extract with molecular weight lower than 10,000 Da was showed as the best coagulant with coagulation efficiency more than 40% at pH 10 and applied dose 2.5 ml/l.

Keywords: Natural coagulant; Common bean; Fractionation; Ultrafiltration

1. Introduction

Coagulation/flocculation is a commonly used method for removing particulates and organic matter from water and wastewater. Many coagulants/flocculants are widely used for this purpose and they can be inorganic (e.g. salts of aluminium and iron), synthetic organic (e.g. acrylamide), or those obtained from natural sources. It was reported that chemical coagulants/flocculants have several serious drawbacks, such as harmful influence on human health. There are studies which showed possible link between the residues of aluminium salts in the water and adverse neurological effects, such as Alzheimer's disease [1–3]. Also, there are studies that indicate that some of synthetic organic polymers, such as acrylamide, have strong neurotoxic and carcinogenic effects [4]. Moreover, the sludge remained after coagulation and flocculation cannot be used as an addition to fertilizers or feed, since it contains high concentrations of coagulants and flocculants. Besides, the alum sludges are gelatinous, acidic and difficult to dewater and to dispose of in the environment [5], and a biological posttreatment of this sludge might be problematic, since the residues of coagulants and flocculants can cause obstructions during this process. The lowering of pH of treated water and increase in conductivity are the additional disadvantages of alum [5].

Natural coagulants, which are intensively researched in recent years with an aim to replace chemical coagulants, can originate from animals,

---

*Corresponding author.

Presented at the International Conference on Desalination for the Environment, Clean Water and Energy, European Desalination Society, 23–26 April 2012, Barcelona, Spain

plants, or micro-organisms. It is believed that they are not harmful, and besides, they are biodegradable, so the resulting sludge can be disposed in the nature without any adverse influence. The most investigated plant in terms of natural coagulants derivation is *Moringa oleifera*, which is confirmed as good source of very efficient coagulant [6–10]. Considering the fact that *M. oleifera* is a plant that is commonly found in tropical areas, we investigated the possibility of extraction of natural coagulants from sources that are cheap and easily available in the region of the Balkan, as well as in Europe. Our previous investigations confirmed the fact that extracts of various strains of Leguminose could be used as natural coagulants [11–14].

The traditional experimental approach, one variable at a time, cannot be used to determine the relationships among all input factors and output responses, since it does not consider interaction among independent working parameters. The statistical design of experiments represents a good alternative to traditional approach since all factors are varied simultaneously over a set of experimental runs in order to determine the relationship between factors affecting the output response requiring minimum number of experiments. Using the design of experiments based on response surface methodology (RSM), the aggregate mix proportions can be arrived with the minimum number of experiments without the need for studying all possible combination experiments [15]. RSM is a collection of statistical tools and techniques that provides the functional relationship between a set of independent experimental variables (factors) and measured responses. The experiment is designed to allow researcher to estimate interaction and even quadratic effects, and therefore giving an idea of the (local) shape of the response surface investigated. It can be used for many purposes, but optimization (maximizing or minimizing a response) is a common goal [16]. The RSM has several classes of designs, and among them the most applied by the researchers are central composite designs, Box Behnken design and three-level factorial design.

The aim of this study was the fractionation of crude extract obtained from common bean by ultrafiltration in order to get cleaner coagulant and optimization of coagulation process for obtained fractions using RSM in order to maximize their coagulation activity.

2. Materials and methods

2.1. Model water

The coagulation activity of 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 the sieve with pore size of 0.4 mm. The smaller fraction was then taken to prepare a 10 g/l suspension in tap water. The suspension was stirred for 60 min on a magnetic stirrer, and left for 24 h 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 100 NTU (nephelometric turbidity units).

2.2. Natural coagulant preparation

Natural coagulant was obtained in the following way: common bean seeds were ground and sieved through the sieve with pore size of 0.4 mm. An amount of a 10 g/l of the smaller fraction was suspended in 0.5 mol/l NaCl. This suspension was stirred for 10 min on a magnetic stirrer in order to extract active coagulant. After that, the suspension was filtered through filter paper Macherey-Nagel MN 651/120. The obtained filtrate, called crude extract, was stored in a refrigerator at +4˚C.

2.3. Fractionation of natural coagulant

One part of crude extract was subjected to ultrafiltration in Millipore Stirred Ultrafiltration Cell Model 8200. The ultrafiltration conditions were as follows: pressure of the inert gas 2.5 bar, stirring agility 80 rpm, and volume of coagulant that was filtered 100 ml. Ultrafiltration was carried out in the following way: 100 ml of crude extract was filtered through membrane with molecular weight cut-off 30,000 Da. The filtration was stopped when flow rate decreased significantly. The volume of retentate was 12 ml, and it was adjusted to 100 ml by adding distilled water. Volume of obtained permeate was adjusted to 100 ml in the same way. This permeate was subjected to filtration through membrane with molecular weight cut-off 10,000 Da. The filtration was stopped when flow rate decreased significantly. The volume of retentate was 5 ml. The volumes of obtained retentate and permeate were adjusted to 100 ml with distilled water.

Obtained fractions were marked as follows:

- I fraction—coagulant with molecular weight $M < 10{,}000$ Da.
- II fraction—coagulant with molecular weight $10{,}000 \text{ Da} < M < 30{,}000$ Da.
- III fraction—coagulant with molecular weight $M > 30{,}000$ Da.

The obtained fractions were stored in a refrigerator at +4˚C and as well as the crude extract were used as coagulants in coagulation tests.

2.4. Coagulation test

The coagulation activities were assessed by jar tests using model water of initial turbidity 100 NTU. The pH values of model water were adjusted by adding 33% NaOH just before performing coagulation tests. Jar tests were carried out by adding different amounts of fractions and crude extract 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 after that systems were left for 1 h for sedimentation. The same coagulation tests were conducted for each pH value with no coagulant as blanks. After sedimentation for 1 h, residual turbidity was determined in upper clarified liquid using turbidimeter WTW Turb 550/550IR and coagulation activity was calculated.

$$\text{Coagulation activity(KA) (\%)} = (T_b - T_s) \times 100/T_b \tag{1}$$

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

2.5. Optimization of parameters through response surface methodology

In this study a three-level factorial design was employed to obtain the relationship between the variables and the response and to optimize all affecting parameters. The variables selected were pH of the model water (v_1) and applied dose of coagulant (v_2), while the observed response was coagulation activity (Y). The variables (factors) and their levels in coded and actual values are presented in Table 1.

The behavior of the system is explained by second-order polynomial equation:

$$Y = b_0 + b_1v_1 + b_2v_2 + b_{12}v_1v_2 + b_{11}v_1^2 + b_{22}v_2^2 \tag{2}$$

where b_0, b_1, b_2, b_{12}, b_{11}, and b_{22} are the regression coefficients. The coefficients with one factor represent the effect of the particular factor, while the coefficients with two factors and those with second-order terms represent the interaction between the two factors and the quadratic effect, respectively.

In order to solve Eq. (2), 13 experimental runs were required for each coagulant: eight experimental runs represent all possible combinations of two factor levels and five experimental runs represent replicates of center point. The regression and graphical analysis with statistical significance were done using Statistica software (version 10, StatSoft, Inc., USA).

Table 1
Experimental factors and their levels

Factors	Real values of coded levels		
	Low level (−1)	Intermediate level (0)	High level (+1)
pH	8	9	10
Coagulant dose (ml/l)	0.5	1.5	2.5

3. Results and discussion

3.1. Crude extract as a coagulant

As stated earlier, the RSM has been applied to develop the polynomial regression equations and find out the relation between the output response (coagulation activity) and the input factors (pH and coagulant dose). According to the RSM, six factors are considered in terms of their significance. ANOVA analysis (analysis of variance) showed that all of these factors had the *p*-value above 0.05 (which is significance limit). This means that no one of them affects significantly coagulation activity of crude extract. Besides, the *p*-value of regression was 0.07, which means that the model was not statistically significant, and the value of R^2 was a bit low—0.797. Therefore, this statistical method was not appropriate in this case.

3.2. I Fraction as a coagulant

The regression coefficients and their significance, presented through *t*- and *p*-values are given in Table 2.

As can be seen from Table 2, *p*-values of three coefficients are lower than 0.05, so they are statistically significant. The regression coefficients b_2, b_{22}, and the interaction term b_{12} were found to be negligible. Therefore, in Eq. (2) only the significant coefficients

Table 2
Regression coefficients and their *t*- and *p*-values for I fraction as coagulant

Regression coefficients	Estimate	*t*-value	*p*-value
b_0	−861.630	−4.06605	0.026831
b_1	194.955	4.13512	0.025667
b_2	−33.580	−1.82022	0.166285
b_{11}	−10.822	−4.14091	0.025572
b_{22}	6.738	2.57842	0.081892
b_{12}	3.000	1.62345	0.202954

were maintained, the final empirical model in terms of actual parameters was determined as follows:

$$Y = -861.630 + 194.955v_1 - 10.822v_1^2 \tag{3}$$

The positive sign in front of the terms indicates synergistic effect, while negative sign indicates antagonistic effect. The response surface model was validated statistically for adequacy by analysis of variance. These results are presented in Table 3.

The statistical significance of the second-order regression model was determined by p-value (Table 3). As can be seen, in this case, according to ANOVA, the p-value was found to be lower than 0.05, meaning that the obtained response model was validated from a statistical standpoint and was a good predictor of the experimental data. The R^2-value was found to be 0.975, which implied that 97,5% of the experimental results can be explained by obtained model.

Table 3
Analysis of variance for coagulation activity of the I fraction

	Sum of Squares	Mean Squares	F-value	p-value
Regression	4848.864	808.1440	59.16455	0.003323
Residual	40.978	13.6593	/	/
Total	4889.842	/	/	/
Corrected total	1648.058	/	/	/
Regression vs. corrected total	4848.864	808.1440	3.92289	0.039565

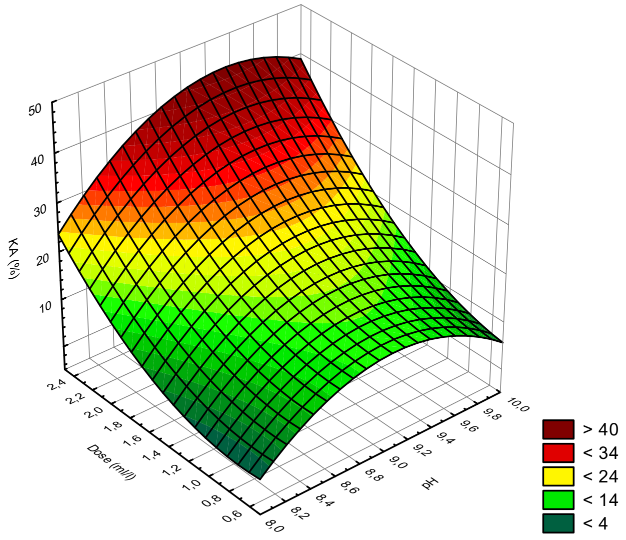

Fig. 1. Effect of pH of model water and dose of coagulant on coagulation activity of I fraction.

The graphical representation (3D surface graph) of the model (Eq. 3) allows an estimation of the effects of the experimental parameters on the response, and in the case of I fraction as a coagulant it is illustrated in Fig. 1.

As it can be appreciated from Fig. 1, working at higher pH values of model water leads to higher coagulation activity. The maximum point appeared around the maximal applied dose of I fraction (2.5 ml/l) and around pH value of model water 9.4, and it was predicted to be almost 45%.

3.3. II Fraction as a coagulant

The II fraction, applied as coagulant, showed in general very low coagulation efficiency. The highest obtained coagulation activity was around 22%. The

Table 4
Regression coefficients and their t- and p-values for III fraction as coagulant

Regression coefficients	Estimate	t-value	p-value
b_0	91.5409	0.40387	0.713372
b_1	−4.8495	−0.09617	0.929452
b_2	−52.9475	−2.68326	0.074841
b_{11}	−0.3400	−0.12164	0.910877
b_{22}	−3.5600	−1.27358	0.292530
b_{12}	6.5575	3.31765	0.045133

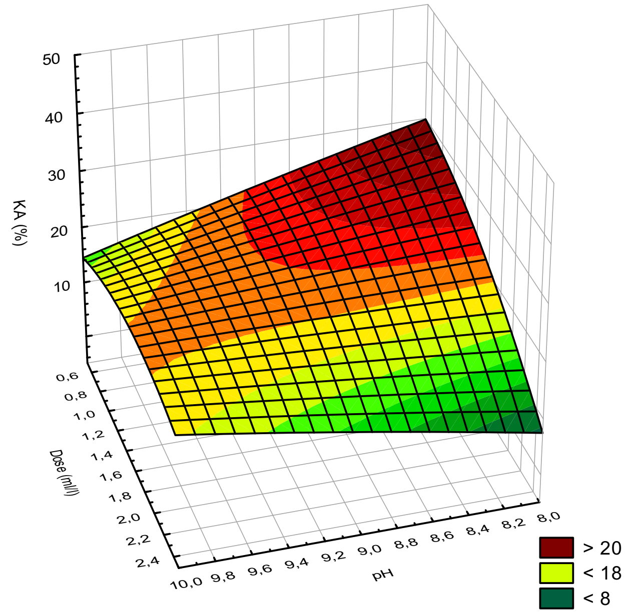

Fig. 2. Effect of pH of model water and dose of coagulant on coagulation activity of III fraction.

Table 5
Analysis of variance for coagulation activity of the III fraction

	Sum of squares	Mean squares	*F*-value	*p*-value
Regression	3530.703	588.4506	37.65609	0.006460
Residual	46.881	15.6270	/	/
Total	3577.584	/	/	/
Corrected total	379.682	/	/	/
Regression vs. corrected total	3530.703	588.4506	12.39882	0.001134

multiple regression analysis showed that all regression coefficients, as well as regression itself, had *p*-values greater than 0.05, so there was no statistically significance.

3.4. III Fraction as a coagulant

When the experimental results were analyzed through RSM, the regression coefficients and their *t*- and *p*-values were obtained, and they are given in Table 4.

According to these results, only the interaction term b_{12} was found to be statistically significant, since its *p*-value was 0.045. In this sense, the regression equation (Eq. (2)) can be transformed as follows:

$$Y = 6.5575 v_1 v_2 \qquad (4)$$

The response surface model was validated statistically for adequacy by analysis of variance. These results are presented in Table 5.

Considering *p*-value of regression, it can be said that the model is significant. This is in correlation with obtained R^2-value, which was found to be 0.877.

The response surface plot of this model is shown in Fig. 2. As can be seen from Fig. 2, the decrease of both pH of model water and applied dose of III fraction leads to increase of coagulation efficiency, hence the maximal coagulation activity (approximately 30%) was achieved at minimal applied coagulant dose (0.5 ml/l) and minimal pH (8), but it was significantly lower compared to I fraction.

4. Conclusions

This research demonstrates that common bean extract as well as its fraction containing molecules with molecular weight lower than 10,000 Da (I fraction) can be used as coagulants since they show the capability to reduce water turbidity. The response surface methodology was used to evaluate the effects of pH of model water and applied coagulant dose on the turbidity removal and to obtain the optimum value of the process variables. I fraction showed as the best coagulant, achieving the highest coagulation activity of approximately 45% at pH 9.4 and applied dose 2.5 ml/l.

Acknowledgments

This research was supported by the grant number III 43005 from the Ministry of Education and Science of the Republic of Serbia.

References

[1] A. Becaria, D.K. Lahiri, S.C. Bondy, D.M. Chen, A. Hamadeh, H. Li, R. Taylor, A. Campbell, Aluminium and copper in drinking water enhance inflammatory or oxidative events specifically in the brain, J. Neuroimmunol. 176 (2006) 16–23.

[2] J.R. Walton, M.-X. Wang, APP expression, distribution and accumulation are altered by aluminium in a rodent model for Alzheimer's disease, J. Inorg. Biochem. 103 (2009) 1548–1554.

[3] S.C. Bondy, The neurotoxicity of environmental aluminium is still an issue, NeuroToxicology 31 (2010) 575–581.

[4] V. Chico Galdo, C. Massart, L. Jin, V. Vanvooren, P. Caillet-Fauquet, G. Andry, P. Lothaire, D. Dequanter, M. Friedman, J. Van Sande, Acrylamide, an *in vivo* thyroid carcinogenic agent, induces DNA damage in rat thyroid cell lines and primary cultures, Mol. Cell. Endocrinol. 257–258 (2006) 6–14.

[5] A. Ndabigengesere, K.S. Narasiah, Quality of water treated by coagulation using *Moringa oleifera* seeds, Water Res. 32 (1998) 781–791.

[6] T. Ocuda, A.U. Baes, W. Nishijima, M. Okada, Isolation and characterization of coagulant extracted from *Moringa oleifera* seed by salt solution, Water Res. 35 (2001) 405–410.

[7] J. Beltrán-Heredia, J. Sánchez-Martín, A. Delgado-Regalado, Removal of dyes by *Moringa oleifera* seed extract. Study through response surface methodology, J. Chem. Technol. Biotechnol. 84 (2009) 1653–1659.

[8] J. Beltrán-Heredia, J. Sánchez-Martín, A. Delgado-Regalado, C. Jurado-Bustos, Removal of Alizarin Violet 3R (anthraquinonic dye) from aqueous solutions by natural coagulants, J. Hazard. Mater. 170 (2009) 43–50.

[9] J. Beltrán-Heredia, J. Sánchez-Martín, Removal of sodium lauryl sulphate by coagulation/flocculation with *Moringa oleifera* seed extract, J. Hazard. Mater. 164 (2009) 713–719.

[10] R. Krishna Prasad, Color removal from distillery spent wash through coagulation using *Moringa oleifera* seeds: Use of optimum response surface methodology, J. Hazard. Mater. 165 (2009) 804–811.

[11] M. Šćiban, M. Klašnja, J. Stojimirović, Investigation of coagulation activity of natural coagulants from seeds of different Leguminose species, Acta Period. Technol. 36 (2005) 81–87.

[12] M.B. Šćiban, M.A. Vasić, J.M. Prodanović, M.G. Antov, M.T. Klašnja, The investigation of coagulation activity of natural coagulants extracted from different strains of common bean, Acta Period. Technol. 41 (2010) 141–147.

[13] M.G. Antov, M.B. Šćiban, N.J. Petrović, Proteins from common bean (*Phaseolus vulgaris*) seed as a natural coagulant for potential application in water turbidity removal, Bioresour. Technol. 101 (2010) 2167–2172.

[14] J.M. Prodanović, M.B. Šćiban, M.G. Antov, J.M. Dodić, Comparing the use of common bean extracted natural coagulants with centrifugation in the treatment of distillery wastewaters, Rom. Biotechnol. Lett. 16 (2011) 6638–6647.

[15] S. Bhatia, Z. Othman, A.L. Ahmad, Coagulation–flocculation process for POME treatment using *Moringa oleifera* seeds extract: Optimization studies, Chem. Eng. J. 133 (2007) 205–212.

[16] NIST/SEMATECH e-Handbook of Statistical Methods, http://www.itl.nist.gov/div898/handbook/,(accessed19 January 2012).