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Clarification of Pharmaceutical Wastewater with Moringa Oleifera: Optimization Through Response Surface Methodology

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ABSTRACT

Herbal pharmaceutical industrial wastewater contains a high amount of suspended solids and alkaline (pH > 8); therefore it requires appropriate coagulant and flocculant compounds for its wastewater treatment. The most widely used flocculant is a synthetic that has certain problems such as non-biodegradability and releases of toxic residual monomers. The use of eco-friendly flocculants as alternative materials for conventional flocculant in water and wastewater treatments is increasing. Numerous factors influence the performance of coagulation-flocculation process, such as coagulant dosage, flocculant dosage, initial potential of hydrogen (pH) and velocity gradient of coagulation-flocculation. The main aim of this research is to evaluate the capability and effectiveness of Moringa oleifera extract for removal of suspended solid in herbal pharmaceutical industry. A coagulation-flocculation test was done by performing jar test at various speeds, according to the variation of the conducted treatment research. In this study, response surface methodology (RSM) approach was used to optimize the concentration of coagulant dosage, flocculant dosage and flocculation velocity gradient (G), and the results were measured as maximum percentage of suspended solid removal. The wastewater used in this research originally came from the inlet of herbal pharmaceutical industry wastewater treatment plant, which was collected over 3 days. The wastewater has a total suspended solids of more than 1250 mg/L, and was alkaline (pH 9–10). The moringa extract was made from the extraction of a fat free moringa powder with a salt solution in a certain ratio. The percentage removal of suspended solid was 93.42–99.54%. The final results of the analysis of response surface showed that the variables of flocculant dosage and the flocculation velocity gradient (G) have a huge impact on the amount of suspended solid removal, compared with the coagulant dosage. The model generated from the response analysis is a quadratic model. The optimum point of the removal suspended solid quadratic model is at 10.6566 mg/L alum dosage, 13.8185 ml/L Moringa oleifera extract dosage, and G velocity of flocculation 84.845 sec⁻¹.

Keywords: flocculant, Moringa oleifera, response surface methodology

INTRODUCTION

In most cases, the source of herbal pharmaceutical industry wastewater disposal came from its raw material and production tank washing processes. The wastewater contains snuff-colored organic chemicals. The organic substance content, which was measured as Chemical Oxygen Demand (COD) amounted to 3000–6000 mg/L (Krzenińska et al., 2015). Wastewater treatment processes that were utilized in herbal pharmaceutical industry are coagulation-flocculation system, clarification with lamella, sedimentation and filtration. The coagulation-flocculation sys-

tem uses inorganic coagulants: Aluminum Sulfate (Alum) and Polyaluminum Chloride (PAC) (Bratby, 2016; Suopajarvi et al. 2013; Wang et al. 2014; Irfan et al. 2017).

In certain studies, inorganic coagulant like Aluminum Sulfate (Alum) has been widely used without any flocculants (Dassanayake et al. 2015; Subramonian et al. 2014). Aluminum Sulfate will carry out rapid hydrolyzing inside aqueous solution to form cationic species; further on, it will be adsorbed with negative charged colloid particles, creating microfloc formation. Smaller microfloc has low solidity that will be disintegrated if exposed by physical intensity. Low efficiency

process and metal residue found in supernatant shows the relevancy of flocculants applied in the coagulation-flocculation process (Lee et al. 2014).

Inorganic flocculants which are commonly used in wastewater treatment are synthetics, such as poly-acrylamides, poly-acrylic, polystyrene sulfonic acids and its derivatives. These flocculants are mostly linear water-soluble polymers with repeating units of various monomers, high molecular weight and can be ionic as polyelectrolyte or non-ionic. Flocculants synthetics caused some environmental and health issues regarding their non-biodegradable characteristic, and metal residue occurrence in water (Shak and Wu 2014; Bratby, 2016). Thus, the use of eco-friendly flocculants as alternative materials for conventional flocculants in water and wastewater treatments is increasing (Bhupathi et al. 2007).

Many effective factors influence the performance of the coagulation-flocculation process, such as coagulant dosage, flocculant dosage, initial potential of hydrogen (pH) and velocity gradient of coagulation-flocculation. In previous studies on coagulation-flocculation, in terms of turbidity removal, optimization is mostly done with varying one factor while others remain constant (Ghebremichael et al. 2005; Hendrawati et al. 2016). This approach will surely consume more time and energy, which also leads to inaccurate optimization as regards the factor interaction. In order to overcome this issue, response surface methodology (RSM) can be considered as the right approach as it accounts for the impact from individual factors and their interactions. RSM consists of statistical and mathematic techniques which provide comprehension in mathematic correlation between effective factors. Thus, the RSM methodology will be effectively used to optimize coagulation-flocculation process in wastewater treatment system (Harfouchi et al. 2016; Nourani et al. 2016).

The studies on *Moringa oleifera*'s competence in water and wastewater treatment process had been undertaken by numerous scholars (Baptista et al. 2015, 2017; Santos et al. 2016; Amante et al. 2016; Pavankumar et al. 2014; Garde et al. 2017). A research on moringa seed extract (*Moringa oleifera* L.) in coagulation activity done by Aslamiah et al. (2013), indicates that the usage of moringa extract is more effective rather than alum. The moringa seed extract can reduce the amount of turbidity amount by 81%, while alum can only reduce 58%. The addition of moringa seed extract enables the pH level in the wastewater

to be within normal range. On the other hand, the addition of alum may cause acidification of water (lowering the amount of pH).

Many studies regarding natural coagulant *Moringa oleifera* (MO) only focused on its turbidity removal and wastewater suspended solids within the initial pH of 6–8. There is still not enough information about the effectiveness of moringa seed extract used as flocculants to lower the amount of wastewater suspended solids under alkaline conditions (pH > 9).

The main purpose of this research is to evaluate the capability and effectiveness of *Moringa oleifera* extract as flocculants to remove wastewater suspended solids in herbal pharmaceutical industry. The influence of flocculation dosage, coagulant dosage and velocity gradient of flocculation process were studied by determining the percentage of suspended solid removal.

MATERIAL AND METHOD

This research was done on a laboratory scale using batch system method. The research variable involved the dosage of flocculant *Moringa oleifera* extract (ml/L), coagulant dosage (mg/L) and flocculation gradient velocity (G , sec^{-1}).

Material

Herbal pharmaceutical industry wastewater

The wastewater used in this research originally came from the inlet of herbal pharmaceutical industry wastewater treatment process, which was collected over 3 days. During the preservation process, wastewater was being stored at 4°C.

Extract of *Moringa oleifera*

Moringa oleifera plant used in this research was obtained from local collectors in East Java, Indonesia. The part of the plant which was used included the white colored skinless seeds. The dried seeds were processed with coffee grinding mill, then crushed with mortar and sieved, until the size of Moringa powder was 40–60 mesh.

Moringa seeds contain fat by 0.1 gr/100 gr ingredients (Fahey, 2005). Fat can damage the protein solvability in water; hence, it has to be eliminated. The process of fat removal on Moringa seeds complies with the work procedure from Susanti et al. (2015) and Hidayat (2009), which gives Hexane solvent to Moringa powder with ra-

tio 1:4 (b/v). Hexane will dissolve fat and floats when centrifuged at 5000 rpm for 10 minutes at room temperature. The supernatant was disposed of, and the fat-free Moringa sediment was poured into beaker glass, and stirred to evaporate the remaining hexane.

The fat-free Moringa powder was then added with NaCl solution 1 M to extract the protein on Moringa according to the work procedure done by (Okuda et al. 2001; Sánchez-Martín et al. 2012; Fatehah et al. 2013; Aslamiah et al. 2013; Prihatinnyas, 2013), which is 5 gr MO powder plus 100 ml NaCl 1M solution and keep being stirred with 100 rpm speed for 1 hour. The physical quality of Moringa oleifera extract filtrate is characterized by pH 4.7, yellow colour, with a distinctive odor, and density of 1g/ml.

Equipment

Jar test equipment used included Jar Test Model JT 203/6 with six 14 l vats, and pH (pH meter-PG 1800 GEHAKA). A qualitative analysis to reveal the specific surface functional groups on Moringa oleifera extract was performed by Fourier Transform Infrared (FTIR) transmission spectra using KBr technique. The analysis was carried out on MAGNA-IR 560, in the wave number of 400–4000 cm^{-1} . The element analysis was conducted by Energy dispersive X-ray spectrometer (EDX) Quantax 75.

Method

Coagulation – flocculation test

Coagulation – flocculation test was done by conducting the jar test at various speeds according to variation of conducted treatment research. Flash mixing performed with G factor 390 sec^{-1} for 1 minute, followed by slow mixing for 9 minutes with G factor variation 50–100 sec^{-1} , and settling at room temperature for 30 minutes. Removal of suspended solids was selected as the dependent variable.

Experimental design and data analysis

The central composite design (CCD), which is one of the important designs of RSM, was selected for the optimization of the parameters. Since different variables are usually expressed in different units and/or have different limits of variation, the importance of their effect on the response can only be compared after they are coded (Nourani, 2016). Input variable pointed into a

code as $x = (x_1, \dots, x_k)$. CCD consists of 3 (three) sections comprising eight factorial point (coded to the usual ± 1 notation), six axial points ($\pm \alpha, 0, 0$), $(0, \pm \alpha, 0)$ and $(0, 0, \pm \alpha)$, and six replicates at center points $(0, 0, 0)$. The number of points in cube portion of design (N_p) affects the α value, which is given in Eq. 1:

$$\alpha = (N_p)^{1/4} \quad (1)$$

where: $N_p = 2^k$,

k is the number of factor. According to Eq.1,

α is equal to $(2^3)^{1/4} = 1.682$. The relationship between the code and actual values of factors and range of levels of variables tested in CCD are given in Table 1.

RESULTS AND DISCUSSION

Wastewater of herbal pharmaceutical industry

In this study, the concentration of suspended solids (SS) of herbal pharmaceutical industrial wastewater was 1375 mg/L, average initial pH 9–10 and settling time of sedimentation – 30 minutes.

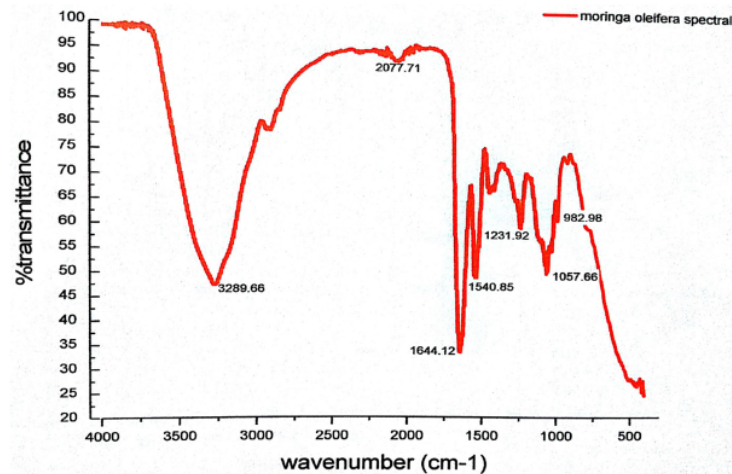
Characteristic of Moringa oleifera extract

Identification of coagulation function cluster has been measured with FTIR method (Fourier Transform Infra Red). The FTIR method was used to quantitatively identify organic/ inorganic compounds. The final check of spectral cluster from Moringa oleifera active extract was displayed in Figure 1.

Figure 1 shows that specific peak number of Moringa oleifera extract occurs in the wavelength region 3495.26–455.13 cm^{-1} . A broad stretching peak at around 3289 cm^{-1} indicated the presence of (N–H) group. The weak peak at 1644 cm^{-1} indicated the presence of C=O and N–H₂ group. Another peak at 1448 was the effect of bonding C–H₃ group. The effect of bonding O–H group was shown at the peak 1231 cm^{-1} . These results confirmed the specific peak indicates that MO extract has aliphatic primary amides and primary aliphatic alcohol function cluster (Al-Anizi et al. 2014; Barrado-Moreno et al. 2016; Garde et al. 2017; Nordmark et al. 2016). Amide cluster indicates that flocculant is positively charged, and

Table 1. The code and actual values of factor and range of variable tested in CCD

Code (X_i)	Value of factor (X_i)	Moringa oleifera extract (flocculant) dosage (ml/L), X_1	Alum (coagulant) dosage (mg/L), X_2	G flocculation (sec^{-1}), X_3
$-\alpha$	X_{min}	3.00	5.00	50
-1	$\frac{(\alpha - 1)X_{max} + (\alpha + 1)X_{min}}{2\alpha}$	6.45	7.03	60
0	$\frac{X_{max} + X_{min}}{2}$	11.50	10.00	75
1	$\frac{(\alpha - 1)X_{min} + (\alpha + 1)X_{max}}{2\alpha}$	16.55	12.97	90
$+\alpha$	X_{max}	20.00	15.00	100

**Figure 1.** IR spectral of Moringa oleifera extract

hydroxyl cluster is charged negatively. Figure 1 also shows that in Moringa oleifera extract, many polypeptides are found, including polyamide, poly(N-methyl acrylamide), phthalamide polymer, pantothenyl compound (Prihatinnytyas, 2013; De Paula et al. 2014).

Moringa oleifera extract was tested by energy dispersive X-ray spectrometer (EDX) and the results are represented in Figure 2. The presence of aluminium and chloride confirmed that moringa oleifera have the same component as poly-aluminium chloride (PAC)

Modelling and statistical analysis

The percentages of suspended solid removal from CCD structure were 93.42–99.54%. These results indicate that concentration of coagulant dosage, flocculant dosage and G flocculation have a significant impact on the removal of suspended

solid (Table 2). The adequacy and significance of the quadratic model was justified by the analysis of variance (ANOVA).

The 2nd order polynomial equation was developed to correlate the percentage of suspended solid removal ($Y_{ss}, \%$) as function of coagulant dosage (X_1), flocculant dosage (X_2) and gradient velocity of flocculation (X_3). The following equation is a regression model with experimental results:

$$Y = 99.439 + 1.0873X_1 - 0.1515X_2 + 1.2165X_3 - 1.1050X_1^2 - 0.3361X_2^2 - 0.8964X_3^2 + 0.276X_1X_2 - 0.239X_1X_3 + 0.259X_2X_3 \quad (2)$$

where: Y is the suspended solid removal efficiency predicted response, X_1 the flocculant dosage (mg/L), X_2 the coagulant dosage (mg/L), X_3 the gradient velocity of flocculation (sec^{-1}).

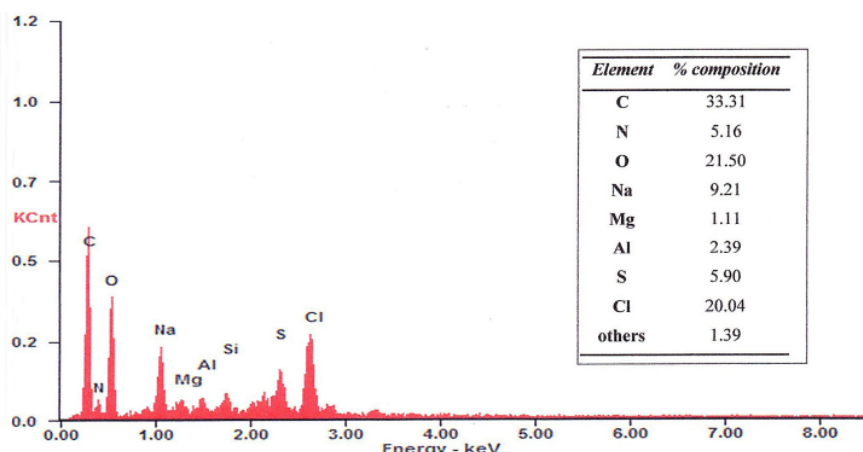


Figure 2. Energy-dispersive X-ray spectroscopy (EDX) analysis

Table 2. Central composite design (CCD) of suspended solid removal (%)

StdOrder	RunOrder	PtType	Blocks	Coded value			Suspended solid removal (%)		
				Moringa oleifera extract (flocculant) dosage (X_1)	Alum (coagulant) dosage (X_2)	Gradient velocity of flocculation (X_3)	Actual	Predicted	Residual
4	1	1	1	1	1	-1	97.04	97.08	-0.0367
13	2	-1	1	0	0	-1.681	95.05	94.86	0.1928
17	3	0	1	0	0	0	99.54	99.44	0.1013
1	4	1	1	-1	-1	-1	95.47	95.25	0.2249
2	5	1	1	1	-1	-1	97.11	97.34	-0.2347
20	6	0	1	0	0	0	99.33	99.44	-0.1087
16	7	0	1	0	0	0	99.54	99.44	0.1013
7	8	1	1	-1	1	1	97.32	97.30	0.0199
14	9	-1	1	0	0	1.681	99.06	98.95	0.1109
15	10	0	1	0	0	0	99.31	99.44	-0.1287
5	11	1	1	-1	-1	1	97.46	97.64	-0.1781
3	12	1	1	-1	1	-1	93.42	93.87	-0.4520
19	13	0	1	0	0	0	99.32	99.44	-0.1187
8	14	1	1	1	1	1	99.11	99.55	-0.4397
18	15	0	1	0	0	0	99.54	99.44	0.1013
11	16	-1	1	0	-1.681	0	98.61	98.74	-0.1329
10	17	-1	1	1.681	0	0	98.32	98.14	0.1782
9	18	-1	1	-1.681	0	0	94.61	94.48	0.1255
12	19	-1	1	0	1.681	0	98.67	98.23	0.4366
6	20	1	1	1	-1	1	99.02	98.78	0.2372

Statistical testing of the model was performed with analysis of variance (ANOVA). The results of ANOVA for suspended solid removal are shown in Table 3. The P_{value} determines whether F_{value} is large enough to indicate statistical significance or lack thereof. The P_{value} less than α (or 0.05) indicates that the model is significant (Turányi, 2015). Table 3 shown that the quadratic model has a very

low probability value ($P_{\text{value}} < 0.0000$), it indicates that the model is very significant. Another test procedure that has been done regarding the model feasibility is Lack of Fit test. The statistical hypothesis is suitable regression model (lack of fit is not found) if $P_{\text{value}} > 0.05$. Table 3 shows that lack of fit score has $P_{\text{value}} = 0.108 (> 0.05)$, thus there is no model gap. A R^2 value greater than 98.53%

indicates the aptness of the model (Harfouchi et al. 2016; Teh et al. 2014)

Response surface and contour plots

Surface plots and its corresponding contour plots are represented in Figure 3. The elliptical shape in the contours shows significant interaction between factors. The ellipse central point in the contour diagram represents the highest predictive value for the response in the selected intervals. The ellipse pattern indicates several interactions between independent variables.

In Figure 3 (a), it is seen that by increasing MO extracts to a certain amount, the percentage of suspended solid removal reaches to maximum and then decreases. This indicates that the coagulant and flocculant disturb the stability of colloidal ionic of wastewater, thus forming flocs that can precipitate. In Figure 3 (a) it can also be seen that suspended solid removal is more dependent on flocculant dosage. Therefore, increasing or decreasing the coagulant dosage (alum) has no influence on the suspended solid removal. The P_{value} coagulant dosage (X_1) is 0.148 (> 0.05), indicated that coagulant has an insignificant effect on the suspended solid removal model.

Figure 3 (b) and (c) show that the gradient velocity of flocculation (G) has a significant effect on suspended solid removal. The G values that are too low or too high will decrease the percent-

age of removal, although both the alum and MO extracts are increased. Determining the optimum G value is very important in the suspended solid clarification process. The interaction values of X_1X_3 (0.051) and X_2X_3 (0.0032) showed that the interaction of G-alum and G-MO extracts had a significant effect on the suspended solid removal.

Response optimization of suspended solid removal

Optimum conditions for the removal of suspended solid process were searched by applying desirability function. As shown in Table 4, the optimum point (100%) of decreasing suspended solid quadratic model is at 10.6566 mg/L alum dosage, 13.8182 ml/L Moringa oleifera extract dosage, and G flocculation 84.8485 sec⁻¹.

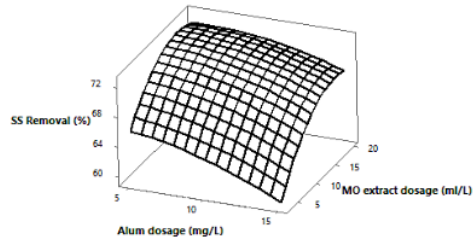
Mechanism of flocculation with MO extract

Wastewater from herbal pharmaceutical industry is a stable and alkaline colloid (pH > 9). Under alkaline conditions, alum cannot work properly; therefore, no charge neutralization in the suspension occurs. Moringa oleifera can function as flocculant because it conceives soluble protein in the water with low molecule (Fahy, 2005). Protein will be positively charged when it is dissolved in the water. Protein will act as positive synthesis substance. The most pos-

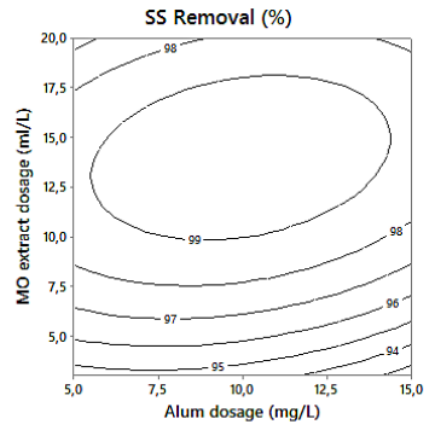
Table 3. Analysis of variance for suspended solid removal

Source	Df	Sum of squares	Mean square	F-ratio	P-value
Model	9	65.2335	7.2482	74.59	0.000
Linear	3	36.6711	12.2237	125.80	0.000
X_1	1	16.1462	16.1462	166.17	0.000
X_2	1	0.3135	0.3135	3.23	0.148
X_3	1	20.2114	20.2114	208.00	0.000
Square	3	26.9602	8.9867	92.49	0.000
X_1^2	1	17.5980	17.5980	181.11	0.000
X_2^2	1	1.6276	1.6276	16.75	0.002
X_3^2	1	11.5812	11.5812	119.19	0.000
2-way Interaction	3	1.6021	0.5340	5.50	0.017
X_1X_2	1	0.6105	0.6105	6.28	0.031
X_1X_3	1	0.4560	0.4560	4.69	0.051
X_2X_3	1	0.5356	0.5356	5.51	0.0041
Error	10	0.9717	0.0972		
Lack of fit	5	0.8989	0.1798	12.35	0.108
Pure Error	5	0.0728	0.0146		
Total	19	66.2052			
Model Summary	S	R ²	Adj R ²	R ² predicted	
	0.311719	98.53%	97.21%	88.64%	

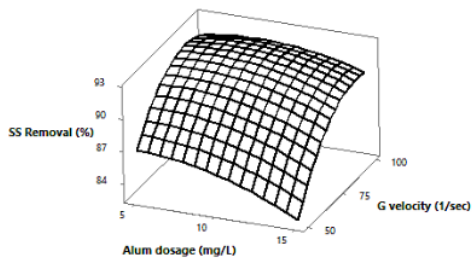
a1)



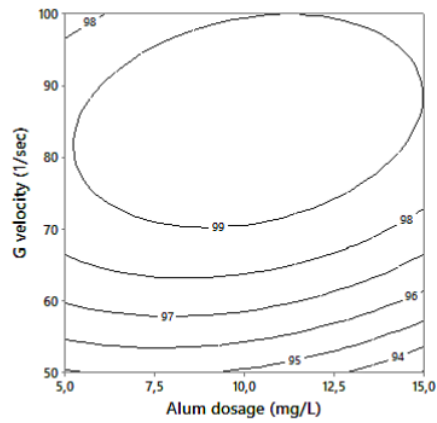
a2)



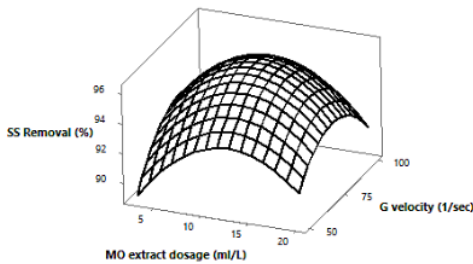
b1)



b2)



c1)



c2)

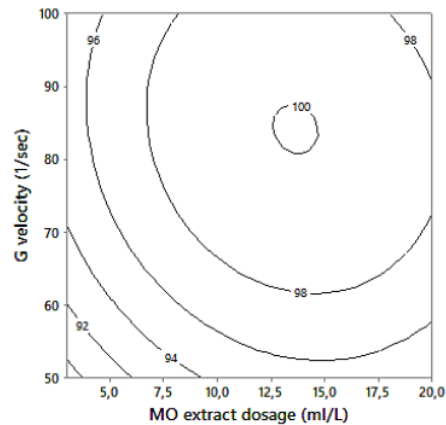


Figure 3. Surface plots and contour plots of suspended solid removal showing the effect of variables, (a) Alum dosage vs MO dosage at G 75 sec^{-1} (b) Alum dosage vs G velocity at MO dosage 11.5 ml/L (c) MO dosage vs G velocity at Alum dosage 10 mg/L

Table 4. Response optimization of percentae suspended solid removal

Parameter	Results
Goal	maximum response suspended solid removal (%)
Lower	93.42
Target	99.54
Weight	1
Importance	1
Solution: Alum dosage (mg/L)	10.6566
MO dosage (ml/L)	13.8182
G velocity (sec ⁻¹)	84.8485
SS removal (%), Fit	100
SS removal (%), SE Fit	0.127

sible mechanism that occurred on the moringa flocculation process is adsorption as well as voltage neutralization or adsorption and unstable particle bonds. It is hard to determine which mechanism possibly occurs, they are simultaneous (De Paula et al., 2014).

The positive charge released into the solution will react with the hydroxyl ions in the suspension, so the pH of the suspension becomes neutral. On the other hand, the negative charge will react with the metal in colloids, to form heavier flocs which are able to precipitate (Hendrawati et al. 2016; Jiang, 2015; Barrado-Moreno et al. 2016).

CONCLUSION

Flocculant active cluster from Moringa oleifera extract consists of Aliphatic Primary Amides and Primary Aliphatic Alcohol. The amide cluster shows that polyelectrolyte flocculant is positively charged, while polyelectrolyte hydroxyl cluster is charged negatively. The mechanism that occurs during the MO flocculant process is adsorption and voltage neutralization or adsorption and unstable particle bond.

The test results of analysis response surface showed that the variables of flocculant dosage and the flocculation velocity gradient (G) exert a huge impact on the amount of suspended solid removal, compared with the coagulant dosage. The model generated from the response analysis is a quadratic model. The optimum point of the removal suspended solid quadratic model is 10.6566 mg/L alum dosage, 13.8185 ml/L Moringa oleifera extract dosage, and G flocculation velocity of 84.8485 sec⁻¹.

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