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Application of *Sclerocarya birrea* (Marula) Seed Kernel as a Natural Coagulant for the Treatment of Raw Water

Abubakar Zakari Zakari [1], Atuman Samaila Joel[1,2], Abdulkarim Yusuf *Abdulwadud*[1]

[1]*Department of Chemical Engineering, Abubakar Tafawa Balewa University, Bauchi, Nigeria*
[2]*School of Chemical and Metallurgical Engineering, Faculty of Engineering and Built Environment, Witwatersrand University, Johannesburg, South Africa*

Abstract
In this study, we aimed to explore the potential of marula seeds as a natural coagulant for treating raw water using the Jar test method. Our analysis, which included XRF, SEM, FTIR, and Proximate analysis, revealed some noteworthy findings. The cake produced during the process was found to have a significant composition of Al_2O_3 (17%) and SiO_3 (13%). SEM analysis indicated an irregular structure, which is conducive to the adsorption process, aiding in the sedimentation of flocs. Furthermore, our FTIR analysis showed distinct peaks at 1033.88 cm^{-1} and 1458.23 cm^{-1}, suggesting the presence of amines, alkenes, and hydroxyl groups. In addition, our proximate analysis indicated that the cake contained approximately 11.38% protein, indicating its potential as an effective coagulant. To optimize our process, we employed a two-factorial central composite design (CCD) across 13 experimental runs, resulting in the development of a second-order model. According to the model, the ideal coagulant dosage is 1g/l, and the optimal mixing time is approximately 7.4 minutes to achieve a turbidity of 1.070 NTU and maintain a slightly neutral pH of 7.107. Ultimately, our treatment process successfully met the stringent WHO standards for drinking water, with a turbidity of 1.055 NTU, a pH level of 7.08, a conductivity of 365 µs/m, total dissolved solids (TDS) at 174 mg/l, a colour measurement of 3.70 Pt-Co, and a total coliform bacteria (TCB) count of 1. Marula Seeds was found to be effective in removing color, turbidity, pH, and TDS from raw water. However, in terms of basic qualities, Marula seeds were more effective compared to Alum.
Key Words: *Marula seed, Natural coagulant, Jar test*

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I. Introduction

Industrialization has contributed to improved living Stringent standards have resulted in high levels of pollution in various water bodies. Turbidity and hardness emerge as key indicators of pollution in municipal water supplies. Industries such as tannery, textiles, food, pharmaceuticals, and electroplating contribute significantly to water pollution with their discharge of toxic pollutants (Mathew et al., 2017). In light of diminishing water resources, increasing wastewater costs, and stringent discharge regulations that reduce permissible contaminant levels in wastewater streams, the importance of raw water treatment has grown exponentially.

The significance of water quality cannot be overstated, as it directly impacts livelihoods and health. Surface water, sourced from rivers, rain-fed ponds, and lakes, has become a primary source of water supply (Anteneh and Sahu, 2014).

The global push for access to safe drinking water, previously under the Millennium Development Goals (MDG) and now part of the Sustainable Development Goals (SDG), has yielded progress, with 2 billion people gaining access to improved water sources. However, a staggering 780 million people still rely on unimproved sources such as surface water and unprotected wells for domestic use (WHO/UNICEF, 2014). The indiscriminate discharge of water containing suspended solids has led to elevated levels of pollution in natural water bodies.

Given the increasing pollution resulting from domestic and industrial water usage, the current water coagulation techniques appear inadequate. This insufficiency poses risks to public health and the environment (Bodlund, 2013).

Turbidity in water primarily stems from suspended particles and natural organic matter (NOM) present in the water. These particles are negatively charged, causing them to repel each other, hindering aggregation and settling. These particles often carry unwanted contaminants and pathogenic organisms (Bodlund, 2013). To reduce the turbidity of inlet water, positively charged coagulants (chemicals) are introduced. This neutralizes the negative charges on the particles, facilitating flocculation, which leads to the formation of larger flocs. Turbidity

is typically measured using a Turbidimeter and is expressed in Nephelometric Turbidity Units (NTU) (Zedan et al., 2022).

The conventional water treatment process generally includes aeration, coagulation, flocculation, sedimentation, filtration, and disinfection. When particles cannot settle efficiently, or when settling occurs slowly, the addition of chemical coagulants (e.g., alum, polyaluminum chloride) expedites the process. These processes effectively remove fine suspended particles, which can serve as carriers for bacteria and viruses. The effectiveness of these chemicals as coagulants is well-established (Zedan et al., 2022).

Water serves a multitude of purposes for humans, and the purity of the water consumed significantly impacts health. The conventional water purification method employing aluminium sulfate (alum) and calcium hypochlorite places financial strain on many nations, especially in the developing world, as these chemicals are often imported. Consequently, treated water becomes prohibitively expensive for many rural populations.

Natural coagulants often comprise a combination of macromolecules, including carbohydrates, proteins, and lipids. In many cases, the key constituents are polymers of polysaccharides and amino acids (Adinolfi et al., 1996). The coagulation mechanism suggests that there is an interaction between these polymers and the dissolved particles in a solution, given that natural polymers contain multiple charged functional groups within their polysaccharide chains, such as –OH, –COOH, and –NH. Typically, coagulation processes involve four key steps: bridging mechanisms, charge neutralization, double-layer compression, and the sweep-floc mechanism (Renault et al., 2009). Cationic polyelectrolytes have shown promise as flocculants for neutralizing negatively charged contaminated particles. Electrostatic interactions play a pivotal role by strongly adsorbing and neutralizing the particles' surfaces. Furthermore, flocculation can occur as a result of the reduction in particle surface charge, diminishing electrical repulsion between them. When maximum charge density polyelectrolytes are absorbed on negatively charged surfaces with fewer potential sites, another phenomenon, known as the 'electrostatic patch mechanism,' can occur. In this case, adsorbed polyelectrolyte chains exhibit alternating positive and negative charges, which promote particle attraction and subsequent flocculation (Bolto and Gregory, 2007).

Numerous studies have delved into the utilization of natural coagulants for water and wastewater treatment. For instance, Ayangunna et al. (2016) conducted research on the coagulation-flocculation treatment of industrial wastewater using Tamarind seed powder. Their findings showcased an impressive turbidity removal rate of 97.01%. The optimal treatment conditions involved maintaining a pH of 7.25, employing a coagulant dosage of 400 mg/L, and mixing at 400 rpm for 15 minutes. Abdulsalam (2015) explored the potential of watermelon seeds as a coagulant for treating surface water. The results indicated that watermelon seeds were effective in reducing turbidity and colour, achieving a noteworthy 25% reduction in turbidity. Rajkumar (2013) focused on investigating natural coagulants' effectiveness in reducing turbidity in wastewater. The study examined the use of various natural coagulants and their impact on turbidity removal. Kumar et al. (2017) highlighted the applications of natural coagulants in wastewater treatment. Their research emphasized the advantages of using natural coagulants derived from diverse sources, including plants, animals, and biomass. Furthermore, Yadav et al. (2017) explored the application of natural coagulants for removing turbidity and hardness. Notably, they found that Moringa oleifera, a natural coagulant, exhibited increased efficiency in hardness removal with higher dosages.

II. Materials and Method

2.1 Sample Collection and Preparation

The Marula seeds (Scerocarya Birrea) were sourced from a tree in Turum village, Bauchi State. The seeds underwent a series of pretreatment steps, which included soaking in water for 24 hours, washing with distilled water to separate the seed from the pulp, sun drying, and grinding. The ground sample (kernel) was subsequently dried in an oven for 24 hours at 80°C and then sieved. Oil extraction was performed using a Soxhlet apparatus to obtain the cake. The residue or cake resulting from the extraction underwent five washes with distilled water and was then dried in an oven for 2 hours at 80°C. The washed and dried residue was stored in a tightly sealed container for use as a natural coagulant (Abood and Zhean, 2020).

For the coagulation process in treating raw water, Design Expert software was employed to design the experiment. The independent variables in this experiment were the coagulant dosage and mixing time, while the dependent variables were Turbidity and pH. The research considered a 2-level 2-factor experimental design, as indicated in Table 1.

Table 1: Experimental Design Factors

Factor	Unit	Coded Factor	Low Level	High Level
Coagulant Dosage	mg/l	X_1	1	10
Mixing Time	min	X_2	2	10

1.3 SAMPLE COLLECTION AND PREPARATION

Scerocarya birrea (Marula) was obtained from available tree in Turum village of Bauchi State. It was pretreated which included soaking in water for 24 h, washing with distilled water to separate the seed from the pulp, sun drying and grinding. The grounded sample (kernel) was then dried in an oven for 24 h at 80°C and then sieved. Oil extraction was done to obtain the cake using a Soxhlet apparatus. The residue/cake from the extraction was washed five times with distilled water and then dried in an oven for 2 h at 80°C. The washed and dried residue was kept in a tight container for use as natural coagulants (Abood and Zhean, 2020).

A design expert was used to design the experiment for the coagulation process in the treatment of raw water. The independent variables are the coagulant dosage and mixing time while the dependent variables are Turbidity and pH.

2-levels 2 factors were considered in this research as shown in Table 1.

Table 1: 2 – Level – 2– Factors Experimental Design Plan

Level	Unit		-1	+1
Coagulant Dosage	mg/l	x_1	1	10
Mixing Time	min	x_2	2	10

2.2 Methods

The process flow diagram depicted in Figure 1 illustrates a seven-stage process for water treatment using Marula seeds:

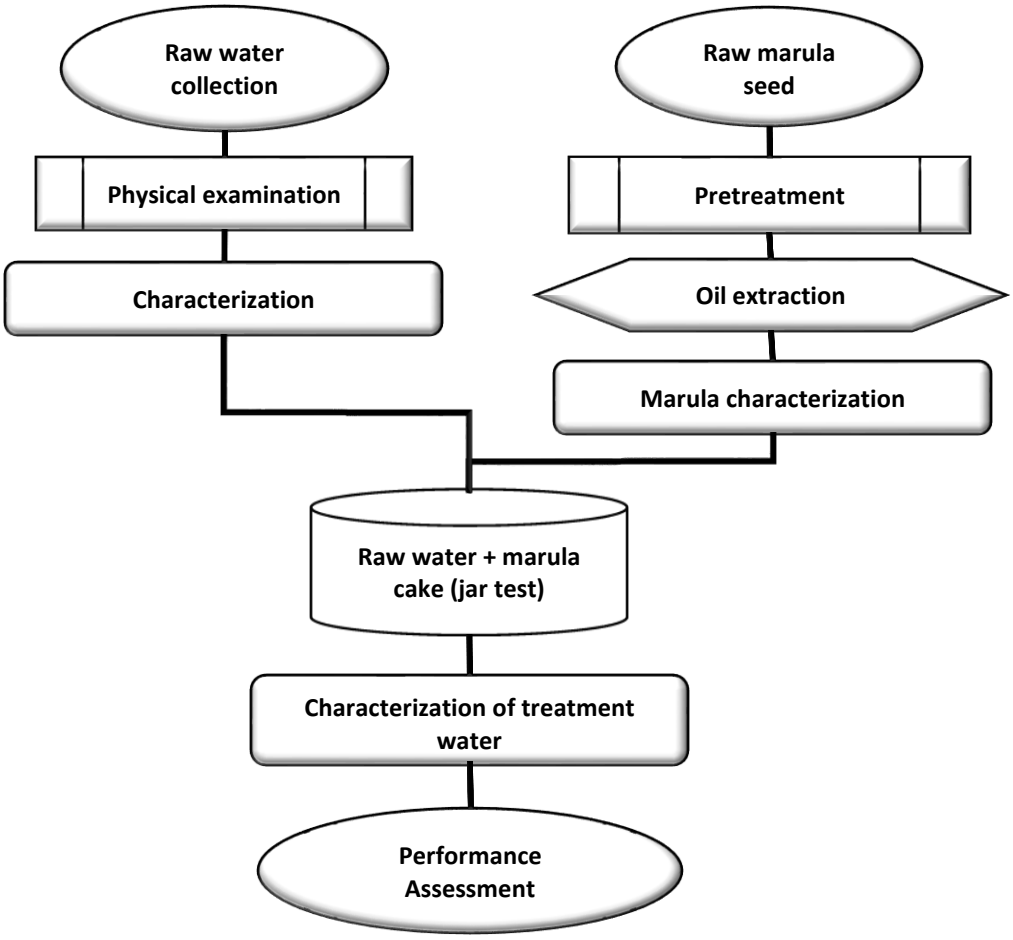

Figure 1: Process Flow Diagram of Water Treatment using Marula Seed

The methodology outlines the systematic approach taken in sample collection, preparation, and the experimental design for the coagulation process in water treatment using Marula seeds. The process involved seven stages which include.

1. Raw Materials Collection
2. Pretreatment
3. Oil Extraction

4. Characterization
5. Jar Test
6. Physicochemical Analysis of Treated Water

Performance Assessment

2.3 Statistical Analysis and Responses

Design Expert version 12 was used to analyze the transesterification data for developing response equations. Multiple linear regression analysis will be employed to estimate t-values, p-values, and F-values to evaluate the adequacy and consistency of the models and screen out the effect on the water treatment. The software generated 13 experimental runs.

III. Result and Discussion

The Marula seed kernel analyzed produced the image displayed, which shows the transmission frequencies of the functional groups present on its surface.

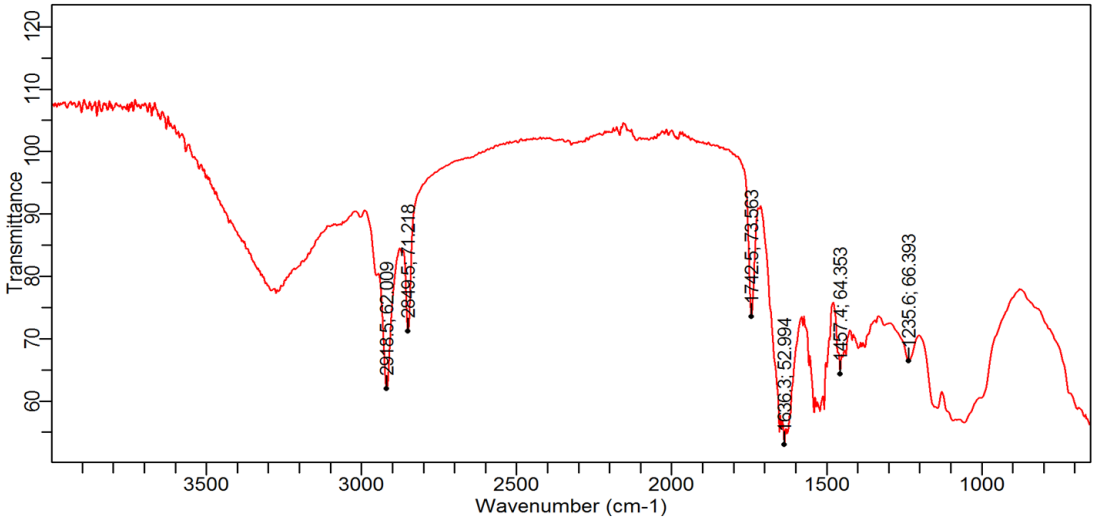

Figure 2: Various functional groups present in the sample of Marula seed kernel.

The FTIR results were assessed by scrutinizing different wave numbers and were interpreted based on their positions within distinct regions of the mid-IR spectrum, as elucidated by Kamara (2019):

I. The Single Bond Region (2500-4000 cm^{-1}): This area represents vibrations associated with single bonds.
II. The Triple Bond Region (2000-2500 cm^{-1}): Vibrations related to triple bonds manifest within this range.
III. The Double Bond Region (1500-2000 cm^{-1}): This section corresponds to vibrations tied to double bonds.
IV. The Fingerprint Region (600-1500 cm^{-1}): Vibrations in this region are recognized as characteristic "fingerprint" vibrations indicative of specific chemical groups.

The FTIR spectra of the marula cake are showcased in Figure 2. Notably, the FTIR spectrum of marula displayed distinct bands at varying wave numbers, each bearing unique chemical significance.

An observable band at 1033.88 cm^{-1} was attributed to cyanide stretch, signifying the likely presence of cyanide-related chemical groups in the sample. Remarkably, this peak is linked to water that is physiosorbed on the surface of the marula. This bonding is likely associated with protons and oxygen atoms that coordinate with the marula's octahedral structural layer (Burany, 2003).

Another significant band emerged at 2360.95 cm^{-1}, ascribed to Si–O–Si bond deformation. This suggests the existence of silicon-oxygen-silicon bonds in the sample.

Furthermore, a band at 3450 cm^{-1} was observed, potentially linked to the stretching of inner surface hydroxyl groups within the marula's octahedral layers (Panda et al., 2010). These FTIR findings offer valuable insights into the chemical composition and molecular structure of the marula cake, aiding in the identification of specific chemical groups and bonds present in the sample.

3.1 Proximate Analysis

The proximate analysis results reveal important nutritional information about both the raw Marula seed and its cake. The raw Marula seed exhibited a composition of 5.79% moisture, 2.72% ash, 0.08% crude fibre, 3.10% crude lipid, 7.88% protein, 80.43% carbohydrates, and an energy value of 384.14 kcal. In comparison, the Marula seed kernel displayed composition values of 0.55% moisture, 4.45% ash, 0.20% crude fibre, 0.10% crude lipid, 11.38% protein, 83.32% carbohydrates, and an energy content of 379.7 kcal.

Notably, the moisture content of both the raw Marula seed powder and the raw seed residue was relatively low, recorded at 4.17% and 4.00%, respectively. These values were below the 7.50% w/w moisture content recorded in the Lophira lanceolata seed kernel (Eromosele et al., 1991) and also lower than the 5.5% w/w in the seed kernel of Zizyphus sonorensis (Montiel-Herrera et al., 2005). High moisture content can lead to increased microbial activity during storage; therefore, it is advisable to thoroughly dry seed kernels with high moisture content before storage.

The ash content in both the raw Marula seed powder and raw seed residue was noteworthy, measuring 4.63% and 6.77%, respectively. This indicates the presence of nutritionally important mineral elements in the seeds. These ash content values were higher than the 2.0% dry weight (DW) reported for the seed kernel of Zizyphus sonorensis (Montiel-Herrera et al., 2005).

The crude fiber content was notably low at 0.08% in the raw Marula seed, differing from other reports. In comparison, the crude fiber content in both the S. birrea kernel and Sclerocarya birrea kernel residue was 2.47% and 6.77%, respectively. These values were considerably lower than the 21.20% DW in the kernel of baobab seed (Renault et al., 2009) and the 36.33% DW in the seed of sugar apple (Annona squamosa) reported by Francis and Tahir (2016).

The crude lipid content was 3.10% in the raw seed, while the protein content was 7.88% for the raw seed and 11.38% for the cake, which was somewhat different from other reports (Umar, 2004).

The carbohydrate content was found to be 80.43%, and the energy content was 381.14 kcal. Carbohydrates primarily serve as an energy source, suggesting that raw seeds could be used to supplement carbohydrates, especially in rural areas.

In conclusion, the proximate analysis was conducted on both the raw Marula seed and its cake kernel, providing essential nutritional insights. The results are summarized in Tables 3 and 4.

Table 3: Result of Proximate Analysis of Marula Seed

Parameters	Value
Moisture content (%)	5.79
Ash content (%)	2.72
Crude fiber (%)	0.08
Crude lipid (%)	3.10
Protein content (%)	7.88
Carbohydrate (%)	80.43
Energy (kcal)	381.14

Table 4: Result of Proximate Analysis of Marula Cake

Parameter	Value
Moisture content (%)	0.55
Ash content (%)	4.45
Crude fiber (%)	0.20
Crude lipid (%)	0.10
Protein content (%)	11.38
Carbohydrate (%)	83.32
Energy (kcal)	379.7

3.2 Preliminary Optimization

To establish a robust relationship between the variables and the response, the study employed Response Surface Methodology (RSM). A factorial design involving two factors and five replicates at central points was utilized to construct a second-order factorial model. This model facilitated the generation of thirteen experimental runs, as outlined in Table 5 through the software.

Table 5: Experimental Results for the Central Composite Design

Std	Run	A: Coagulant dosage	B: Mixing time	Turbidity	pH
		g/l	Min	NTU	
4	1	10	10	4.4	7.05

1	2	1	2	1.38	7.1
3	3	1	10	1.1	7.14
5	4	1	6	1.2	7.11
6	5	10	6	4	7
13	6	5.5	6	1.24	7.02
2	7	10	2	4.48	6.85
9	8	5.5	6	1.24	7.02
11	9	5.5	6	1.25	7.02
10	10	5.5	6	1.24	7.03
7	11	5.5	2	1.35	7
8	12	5.5	10	1.56	7.05
12	13	5.5	6	1.24	7.01

3.3 ANOVA for Quadratic Model

The analysis of variance (ANOVA) was employed to examine the interaction between the process variables and the response. According to the ANOVA results (as presented in Table 6), factor A exhibited high significance, with a p-value less than 0.01, while factor B was not found to be significant, with a p-value greater than 0.05. The Model F-value of 282.65 indicates the overall significance of the model. The likelihood of obtaining such a large "Model F-Value" purely by chance is only 0.01%. Model terms with "Prob > F" values less than 0.05 are considered significant, and in this case, A, AB, A^2, and B^2 are significant model terms.

The "Curvature F-value" of 195.52 suggests the presence of significant curvature in the design space, as measured by the difference between the averages of the center points and the averages of the factorial points. The chance of observing a "Curvature F-value" this substantial due to random noise is only 0.01%.

The "Lack of Fit F-value" of 0.001 indicates that the Lack of Fit is not significant relative to the pure error. There is a 2% chance that a "Lack of Fit F-value" of this magnitude could occur due to noise. This aligns with the findings of previous research regarding the quadratic models for turbidity removal in relation to coagulant dosage and mixing time (Trinh & Kang, 2010).

The "Predicted R-Squared" value of 0.9499 is close to the "Adjusted R-Squared" of 0.9916, with a difference of less than 2%. This consistency in results is in line with prior studies (Wynberg et al., 2012; Zainal-Abideen et al., 2012).

Significant model terms were determined based on P-values less than 0.0500, with A, A^2, and B^2 being identified as essential model terms. Model terms with values exceeding 0.1000 were considered unimportant. If there are many insignificant model terms, model reduction may be beneficial, provided that the hierarchy is maintained.

Table 6 further supports the significance of the model, with all p-values of regression less than 0.05. Additionally, the lack of fit test did not indicate any significant lack of fit ($P > 0.05$). This test evaluates the model's ability to represent data in regions not included in the regression. In this case, the model is both significant and free from lack of fit.

Table 12 also shows that the P-values of coagulant dosage, the quadratic effect of coagulant dosage, the quadratic effect of mixing time, and the interaction effect between coagulant dosage and mixing time were all less than 0.05. This indicates that these factors indeed affect the response variables, further affirming the model's validity.

Table 6: ANOVA Table
Response 1: Turbidity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	21.12	5	4.22	282.65	< 0.0001	**Significant**
A-Coagulant dosage	14.11	1	14.11	943.90	< 0.0001	
B-Mixing time	0.0038	1	0.0038	0.2509	0.6318	
AB	0.0100	1	0.0100	0.6691	0.4403	
A^2	5.19	1	5.19	347.38	< 0.0001	
B^2	0.1411	1	0.1411	9.44	0.0180	

Residual	0.1046	7	0.0149			
Lack of Fit	0.1045	3	0.0348	1742.27	< 0.0001	**Not significant**
Pure Error	0.0001	4	0.0000			
Cor Total	21.23	12				

3.4 Effect of Coagulant Dosage

Figures 3 and 4 depict 3D response surface plots representing the quadratic models for turbidity removal concerning coagulant dosage and mixing time, aligning closely with findings by Trinh and Kang (2011). Figure 3 demonstrates that as the coagulant dosage of marula powder increased, turbidity removal also increased. The highest removal efficiency, reaching 98%, was achieved at a coagulant dosage of 1 g/l of Marula seed powder and a pH of 8.78. However, when the marula seed powder exceeded the optimal range, turbidity removal efficiency decreased. This trend, resembling similar observations made by Künzle et al. (2011), indicates an overdose in the reaction solution, resulting in increased water turbidity, as illustrated in Figures 3 and 4.

The influence of Marula seed amount on the removal of pollutants, specifically turbidity and pH, from raw water, is visually presented in Figures 3 and 4. An evident steep increase in turbidity removal efficiency is observed at a dosage of 10 g/l and a mixing time of 5 minutes, as shown in Figures 7 and 8. Similarly, a rapid increase in turbidity removal efficiency is noted with a coagulant dosage of 1 g/l and a mixing time of 10 minutes. Beyond a dosage of 1 g/l of Marula seed powder and 10 minutes of mixing time, turbidity removal efficiency diminishes. This suggests an excess of cations in the wastewater sample compared to anions. Lower mixing speeds may enhance turbidity removal due to reduced shearing of flocs during initial formation, in agreement with the findings of N. Muhammad et al. (2016).

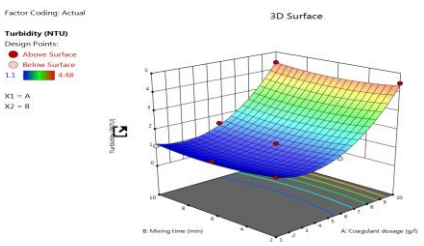

Chart 3: Effect of Coagulant Dosage and Mixing Time on Turbidity

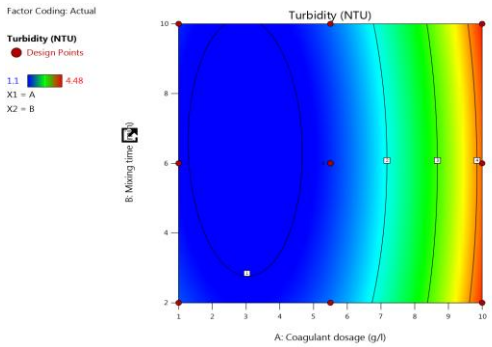

Chart 4: Contour Plot Showing the Interaction Effect of Coagulant Dosage and Mixing Time on Turbidity

Response 2: pH

In this study, under the experimental conditions employed, a significance level of 95% for the p-value indicates that all the variables, as well as their interactions, exert a significant effect on pH ($p < 0.05$). Moreover, the model does not display a lack of fit ($P > 0.05$). The lack of fit test evaluates the model's ability to represent data in an experimental domain at points that are not included in the regression. If a model is significant, it means that the model contains one or more important terms, and it does not suffer from a lack of fit.

It's worth noting that in environments with substantial noise or when critical variables are not included in the experiment, there's a possibility that a significant portion of the data variability, known as the residual, remains unexplained by the model. However, as indicated in Table 7, the P-values of mixing time and coagulant dosage were both less than 0.05, affirming that these factors indeed impact the response variables.

Table 7: ANOVA Table for Response 2: pH

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0542	3	0.0181	32.99	< 0.0001	Significant
A-Coagulant dosage	0.0338	1	0.0338	61.67	< 0.0001	
B-Mixing time	0.0140	1	0.0140	25.61	0.0007	
AB	0.0064	1	0.0064	11.69	0.0076	
Residual	0.0049	9	0.0005			
Lack of Fit	0.0047	5	0.0009	18.90	0.0069	Not significant
Pure Error	0.0002	4	0.0001			
Cor Total	0.0591	12				

The 3D surface and contour plot illustrated in Figure 10 provide insights into the reduction in pH, which notably increases when low-speed mixing is conducted over a span of 2 to 10 minutes. The response surface depicted in Figure 10 exhibits curvature, indicating that the interaction effect of mixing time on residual pH is quite pronounced, a conclusion that aligns with the results of significant testing.

Low-speed mixing plays a critical role, particularly in the flocculation process, where the characteristics of particles and fluid mixing conditions are pivotal. In flocculation, mixing serves the purpose of moving particles to enable them to encounter each other after a coagulant has been employed to neutralize their natural repulsion, as previously reported by Montiel-Herrera et al. (2005). The flocculation of small particles primarily occurs through diffusion, and the rate of flocculation is directly related to how quickly particles diffuse towards each other.

Figure 10 further reveals the impact of low-speed mixing and coagulant dosage through its 3D surface response and contour plots. The plot clearly demonstrates that doses ranging from 0.1 to 10 g/L result in reduced residual turbidity. Consequently, the dosage factor is highly significant and influences residual turbidity up to a certain threshold, beyond which higher dosages lead to an increase in residual turbidity due to destabilization.

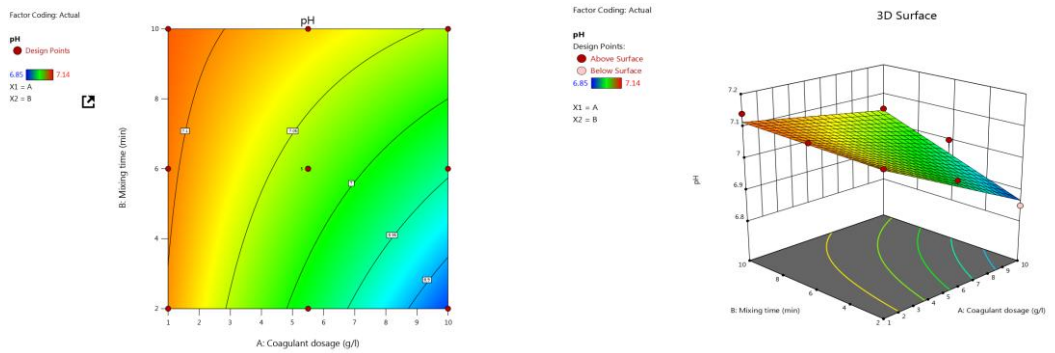

Chart 5: Contour and 3D plots for Interaction Effect of Mixing Time and Coagulant Dosage on pH.

3.5 Numerical Optimization

An optimization study was carried out to identify the optimal combination of operational parameters aimed at achieving the minimum turbidity. During this study, the coagulant dosage was minimized, and the mixing time was also kept at a minimum. The pH was adjusted within the specified range, and the mixing time was set accordingly.

The results obtained from the experimental design pinpointed the optimal conditions, which include a low coagulant dosage of 1 g/L and a short mixing time of 7.4 minutes. Under these specific conditions, the predicted (theoretical) values for turbidity and pH were 1.077 and 7.109, respectively.

IV. Conclusion

In this research, the response was analyzed using a quadratic model and the coefficient of regression, which correlated the two parameter settings. The significance of coagulant dosage and mixing time on turbidity and pH was assessed. It's important to note that when single parameters are considered, they reflect the effect of that individual factor. In contrast, when two variables are involved, they represent the interaction between those two factors, and the second-order terms indicate their quadratic effects. The positive and negative signs indicate synergistic and antagonistic effects of the terms, respectively. When compared with Alum, the results obtained fall within WHO standards. Marula Seeds was found to be effective in removing color, turbidity, pH, and TDS from raw water. However, in terms of basic qualities, Marula seeds were more effective compared to Alum. Marula seeds increased the pH from 6.98 to 7.05, while Alum made the water more acidic, reducing the pH from 6.98 to 4.14. Regarding turbidity reduction, Marula seeds reduced turbidity to 1.055 NTU, while Alum achieved a turbidity of 0.9 NTU. The sedimentation rate was faster when using Marula seeds as a natural coagulant compared to Alum, as the former formed heavier flocs. Additionally, there was a rapid drop in electrical conductivity when water was treated with Marula seeds compared to Alum

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