

# Optimization of coagulation-flocculation process for colour removal from synthetic dye wastewater using natural organic polymers: Response surface methodology applied.

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**ABSTRACT:** In this study, performance of natural polymer coagulants; *Vigna unguiculata* coagulant (VUC), *Telfairia occidentalis* coagulant (TOC), *Brachystegia eurycoma* coagulant (BEC), *Vigna subterranean* coagulant (VSC) and *Moringa oleifera* coagulant (MOC) for colour removal from crystal Ponceau 6R dye were investigated. For this purpose, response surface methodology (RSM) was applied to optimize four variables of the coagulation-flocculation process including pH, coagulant dosage, dye concentration and time. The results demonstrated that the decrease of pH was highly beneficial for enhancing colour removal and no re-stabilization occurred even at the maximum coagulant dosages used. It seems as the functional groups of the acid dyes are anionic, hydrolyses products of the organic biopolymer can neutralize the negative charges on dye molecules followed by polymer adsorption. Charge neutralization, sweep flocculation and polymer adsorption played a predominant role in the coagulation-flocculation process due to optimum pH and dosing values. The maximum colour removal efficiency obtained from the optimization analysis was 97.73%, 99.05%, 98.09%, 99.09% and 99.05%, for VUC, TOC, BEC, VSC and MOC, respectively. Optimal condition of pH 2, coagulant dosage 1000mg/l, dye concentration 20mg/l and time 420min was obtained from the compromise of the desirable responses. The verification experiments demonstrated a good agreement between experimental data and model values. The overlay contour plots show that multiple response optimization tool could be used to establish an optimum condition for multiple (five) responses. The response surface methodological approach was appropriate for optimizing the coagulation-flocculation process while minimizing the number of required experiments. Coagulants studied should be considered as an appropriate alternative for conventional coagulants that are widely used in dye wastewater treatment plants.

**Keywords:** Coagulation-flocculation, Crystal Ponceau 6R, optimization, response surface design, colour removal, organic polymer coagulants.

## 1. INTRODUCTION

Pollution and contamination of environment by wastewater discharge have caused several environmental, social, economic and public health problems [1]. Dye containing wastewaters are among these contaminants on the environment bodies because of their highly toxic characteristics. Therefore the removal of emerging contaminant of concern is now as ever important in the production of safe wastewater [2]. Presently, estimation has showed that over 10,000 of different commercial dyes and pigments are available and over  $7.11 \times 10^7$  kg/ yr is produced worldwide [3]. Dye production industries (such as textile, rubber, pulp, paper, plastic, cosmetics, food, pharmaceutical, leather tanning, printing, medicine, etc.) and many industries which use dyes and pigments generate wastewater characteristically high in colour, organic and inorganic contents. These dye wastewaters are toxic, carcinogenic, slow down self-purification of streams by reducing light penetration [4], retard photosynthetic activity and inhibit growth of biota [5]. Therefore, these wastewaters have to be treated to accord with discharge limit [6].

Dyes are classified differently as anionic (direct, acid and reactive dyes), cationic (basic dyes) and non-ionic (disperse dyes). Anionic dyes are the largest class of dyes used in the world [7]. In aqueous solution, anionic dyes carry a net negative charge due to the presence of sulphonate ( $\text{SO}_3^-$ ) groups. Acid dyes are anionic compounds and mostly belonged

to azo and anthraquinonic groups. Acid dyes are characterized by the existence of azo bond ( $\text{R-N=N-R}_2$ ), amino group. Removal of many acid dyes from dye containing wastewater is difficult because it contains highly soluble and semi-soluble dye contaminants [8]. This is also due to its complex aromatic structure, synthetic origin and difficult to be biodegraded [6]. Crystal ponceau 6R or ponceau 6R or acid red 44 is a red powdered azo dye. It is soluble in water and slightly soluble in ethanol and other compounds like xylene, acetone, glycerol, dioxane, etc. Also the application of crystal ponceau 6R is useful in history for staining fabrics, used as a food dye [9].

The techniques for colour removals from dye wastewater can be divided into three main categories; physical, chemical and biological. Physical treatments such as precipitation, ion exchange, membrane filtration, irradiation, ozonation and adsorption are widely used techniques. Physico-chemical treatments usually involve coagulation-flocculation, precipitation, photo-catalysis, oxidation and chemical sludge oxidation. Lastly, biological treatment techniques used are aerobic degradation, anaerobic degradation, and living/dead microbial biomass. Coagulation-flocculation is an already established technique for contaminant removal from most wastewater ranging from wastewater containing: BOD [10], colour [11], DOC [12], turbidity [13], COD [14], grease and oil [15], TSS [10], heavy metals [16], [17]. Coagulation-

flocculation is one of the most efficient unit process used for colour removal from industrial wastewater due to its efficiency and simple to operate [15]. It is considered a chemical treatment as it implies the addition of a coagulant. Stable colloids in water normally present negative charges all around their surface. Coagulant is able to cause the neutralization of these charges, so colloidal particles become unstable and tend to settle by gravity [18]. Typical coagulant agents are inorganic salt such as  $Al(SO_4)_3$  or  $FeCl_3$ , as well as synthetic organic polymer [19]. Although these chemicals are rather effective in removing dyes and suspended matters from the aqueous solution, several disadvantages have recently arisen, such as their impact on human diseases like Alzheimer's caused by inorganic salts [20]. Also, the synthetic organic polymers such as acrylic amide or polyacrylamides have neurotoxin and carcinogenic effects [21].

Natural polymer coagulants are concerned by many researchers because of their abundant source, low price, environmental friendly, multifunction, and biodegradable in water. The efficacies of using these plant seeds: cowpea (*Vigna unguiculata*), fluted pumpkin (*Telfairia occidentalis*), black timber (*Brachystegia eurycoma*), bambara nut (*Vigna subterranean*), horse radish (*Moringa oleifera*), as plant-based cationic coagulants have been studied here. The use of these plant-based precursors was established because animal-based precursors are expensive and difficult to source [22]. Crude extract from the seeds is believed to be a soluble cationic protein and have showed the ability to act as a natural polymer coagulant based on previous studies conducted [18], [23], [24], [25], [26], [27].

The mechanisms associated with different natural polymer coagulants are varied as well. Aggregation of particulates in a solution can occur via four classic coagulation mechanisms: (a) double layer compression; (b) sweep flocculation; (c) adsorption and charge neutralization; and (d) adsorption and interparticle bridging [28], [29], [30]. The presence of salts (or suitable coagulants) can cause compression of the double layer [29] which destabilizes the particulates. Sweep flocculation occurs when a coagulant encapsulates suspended particulates in a soft colloidal floc. Adsorption and charge neutralization refer to the sorption of two particulates with oppositely charged ions while interparticle bridging occurs when a coagulant provides a polymeric chain which sorbs particulates [29]. Polymeric coagulants are generally associated with mechanisms (b), (c) and (d) as their long-chained structures (especially polymers with high molecular weights) greatly increase the number of unoccupied adsorption sites. The mechanisms also provide underlying principles to the inner workings of plant-based coagulants as well.

Response surface methodology (RSM), a statistical design tool used for problem analysis in which a response of interest is influenced by several variables and the objective is to optimize this response. It is a combination of mathematical and statistical techniques useful for development, improving and optimizing processes and can be used for factor evaluation in complex interactions [31]. In this context, RSM makes process modelling simple, efficient, less time of operation and resource utilization. RSM has been widely used for optimization of the process variables in coagulation-flocculation [32], [33], [11] in dye removals. In this study, RSM is used to develop a

mathematical correlation between pH, coagulant dosage, initial dye concentration and settling time for the colour removal from the dye containing wastewater. A face-centered central composite design (CCD), which is a very efficient design tool for fitting the second-order models [31] was selected for use and design-expert software (version 9.0.1.0) was the software employed to achieve this purpose. The software was also used to demonstrate the analysis of variance (ANOVA), 3D surface plot, numerical optimization and multiple response optimization (MRO) using overlay contour plot.

Therefore, the potentials and effectiveness of using active coagulant proteins obtained from plant seeds for effective decolourization of acid red 44 (crystal ponceau 6R) in aqueous solution was studied. For this purpose, the response surface methodology (RSM) is used to develop a mathematical correlation between the pH, coagulant dosages, dye concentration, and time, for the colour removal from the dye containing wastewater. The main objective of using RSM is to determine the optimum operational conditions for the system and to determine a region that satisfies the operating specifications.

## 2.0 MATERIALS AND METHODS

### 2.1 Preparation of Coagulants

Dry seeds of five samples were bought from local market of Enugu city, Nigeria. Matured seeds showing no signs of discolouration, softening or extreme desiccation were used. The seeds were removed from the pod, dried under sun, and the external shells were removed. The seeds kernels were ground to fine powder (63-600 $\mu$ m) using an ordinary food processor. The seed powder was then used in each experiment.

### 2.2 Extraction of Active Component

The seed kernels were ground to fine powder to achieve solubilisation of active ingredients in the seeds. The active component from coagulants was extracted by adding distilled water to the fine powder to make 2% suspension (2g of fine powder fined samples in 100ml water). The suspension was stirred using a magnetic stirrer for 20min at room temperature to accomplish extraction and then filtered through a rugged filter paper (Macherey Nagel, MN 651/120). The resultant filtrate solutions were used as coagulants. Fresh solutions were prepared daily and kept refrigerated to prevent any ageing effects (such as change in pH, viscosity and coagulation activity). Also solutions were shaken vigorously before use. Fresh coagulants were prepared and used immediately for each sequence of experiment.

### 2.3 Characterization of the Coagulants

Moisture, protein, crude fat, crude fibre and ash contents of the seed powders were determined by the standard official methods of analysis of the A.O.A.C [34], while carbohydrate content was calculated by difference.

### 2.4 Buffered Solution

All assays were done in a pH-stable medium. Buffered solutions (pH 2, 4, 6, 7, 8, 10 and 12) were prepared by the standards established according to the National bureau of standards (NBS, US) and were standardized using a digital pH meter. All reagents used were of analytical purity grade.

### 2.5 Decolourization Procedures

#### 2.5.1 Absorption spectrum and stock solutions preparation

Acid Red 44 (water soluble dye) was provided by May & baker England with a molecular structures as shown in Fig. 1. The characteristics of acid red 44 (AR 44) are summarized in Table 1. Dye with commercial purity was used without further purification. The absorption spectrum of the dye was obtained as follows by dissolving 1000mg/l of AR 44 in distilled water. A sample of the solution was scanned against the blank of distilled water in the range of 200-850nm using UV-Vis spectrophotometer (Shimadzu, Model UV-1800). A maximum absorption spectrum was obtained against its reagent blank. Stock solution of 1000mg/l of dye was prepared by dissolving accurately weighed amounts of AR 44 in separate doses of 1L distilled water. The desirable experimental concentrations of 10-100mg/l were prepared by diluting the stock solution with distilled water when necessary. The wavelength of maximum absorbance ( $\lambda_{max}$ ) and its calibration curves at  $\lambda_{max}$  of the dye was determined.

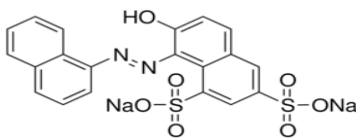


Fig. 1. Structure of Crystal Ponceau 6R dye (Acid Red 44).

Table 1: Physical properties of Crystal Ponceau 6R dye.

Property	Data
Chemical name	Crystal Ponceau
Chemical formula.	$C_{20}H_{12}N_2 O_7 S_2 Na_2$ .
Molecule Weight (g/mol)	502.43
CAS number	2766-77-0
EC number	E 126
UV/Visible Absorbance	Max (water): 511 +6nm
C.I number	16250
Class	AZ0
C.I name	Acid Red 44.

### 2.5.2. Decolourization determination

Colour measurement was determined with standard dilution multiple method [35] and by comparing absorbance to a calibration curve. AR 44 dye decolourization was determined by monitoring the decrease in the absorbance peak at the maximum wavelength of 511nm. Dye concentration of the supernatant (from coagulation experiment) was measured using UV-Vis spectrophotometer (Shimadzu, model 1800) at wavelength corresponding to the maximum absorbance ( $\lambda_{max}$ ) of the dye. The study was conducted by varying few experimental parameters which were pH, coagulants dosages, Initial dye concentrations, settling time and temperature. The pH was adjusted to the desired value using 0.1 M HCL and 0.1 M NaOH.

### 2.6 Coagulation Studies

The jar test was performed to evaluate the performance of the coagulants agent extracted from the various processes as described above based on standard methods [36], [27]. Coagulation-flocculation experiments were performed using a conventional jar test apparatus (Phipps and Bird, VA, USA) equipped with six beakers of 1 l capacity and six paddle stirrers. The coagulation-flocculation procedure involved 4min of rapid mixing at 100rpm. The mixing speed was reduced to 40 rpm for another 25 min. All the suspensions were left for settling (60-540min). The additional centrifuging (5000rpm for 5min) was performed to obtain clear liquid for all samples before analysis. After settling, supernatant sample was withdrawn for absorbance analysis using UV-Vis

spectrophotometer (Shimadzu model 1800) at maximum wavelength corresponding to the maximum absorbance of 511nm. Colour removal was measured as a decrease in optical density measurement at 511nm. Removal efficiency of colour was obtained according to the formula given below:

$$\text{Colour removal (\%)} = \left( \frac{C_0 - C}{C_0} \right) \times 100 \quad (1)$$

where  $C_0$  and  $C$  are the initial and final colour concentration (mg/l) in dye solutions before and after coagulation-flocculation treatment, respectively.

### 2.7 Experimental Design and Data Analysis

Central composite design (CCD), a very efficient design tool for fitting the second-order models (Montgomery, 2001), is used as an RSM in the experimental design. The CCD was first introduced by Box Wilson in 1951, and is well suited for fitting quadratic surface, which usually works well for the process optimization [32]. In this research, the face-centred experimental plan was implemented as a CCD. A CCD is made face-centred by the choice of  $\alpha = 1$  [37]. Face-centre is having the position of the star points at the face of the cube portion on the design [31]. The choice of face-centred CCD was made considering that it is an option in the CCD design and due to the cumbersome nature of the design. Also face-centred option ensures that the axial runs will not be any more extreme than the factorial portion. The independent variables selected for this study were pH ( $X_1$  or A), coagulant dosage ( $X_2$  or B), dye concentration ( $X_3$  or C), and time ( $X_4$  or D). A  $2^4$  two-level factorial for four independent variables consisting of 16 factorial points coded to the usual  $\pm$  notation, 8 axial points and 6 replicate at centre point where conducted for each sample. A total of 30 experiments were conducted for each response. Mathematically, Eq. (2) was used to determine the total number of runs performed. The total number of experiments,  $N$  with  $k$  factors is:

$$N = 2^k + 2k + n \quad (2)$$

where  $k$  is the number of factors and  $n$  is centre points.

The experimental design table is presented in Table 2. For statistical calculations, the variables  $Z_i$  (the real value of an independent variable) were coded as  $X_i$  (dimensionless value of an independent variable) according to Eq. (3):

$$X_i = \frac{Z_i - Z_i^*}{\Delta Z_i} \quad (3)$$

where  $Z_i$  stands for the uncoded value of  $i$ th independent variables,  $Z_i^*$  stands for the uncoded value of  $i$ th independent variables at centre point and  $\Delta Z_i$  is a step change value.

Design-expert software 9.0 (State Ease, Minneapolis, USA) was used for regression and graphical analysis, fitting to a second-order polynomial model to optimize the variables in the coagulation-flocculation process. Each response was used to develop an empirical model which correlated the response to the dye coagulation-flocculation variables using a second degree polynomial equation as given by Eq. (4):

$$Y = b_0 + \sum_{i=1}^n b_i X_i + \sum_{i=1}^n b_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n b_{ij} X_i X_j + \epsilon \quad (4)$$

where  $Y$  is the predicted response,  $b_0$  the constant coefficient,  $b_i$  the linear coefficients,  $b_{ii}$  the quadratic coefficients,  $b_{ij}$  the

interaction coefficient,  $X_i X_j$  are the coded values of the variables,  $n$  is the number of independent test variables and  $\epsilon$  is the random error. Adequacy of the proposed model is then revealed using the diagnostic checking tests provided by analysis of variance (ANOVA). The quality of the polynomial fit model was expressed by the coefficient of determination ( $R^2$ ). The  $R^2$  values provide a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. These analyses are done by means of Fisher's 'F' test and P-value (probability). Model terms were evaluated by the P-value with 95% confidence level. Finally, the optimal values of the critical parameters were obtained by analyzing the surface and counter

plots and by solving the regression equation. The range and levels of the experimental design table is presented in Tables 2.

### 3.0 RESULTS AND DISCUSSION

#### 3.1 Characterization Result

The proximate analysis of coagulant precursors were determined and summarized in Table 3. The study shows a high protein contents. The results obtained from the proximate composition as shown in Table 3 also agrees with the literature results that the active coagulating agents are dimeric cationic peptides, which are capable of destabilizing the anionic dye particles. It justifies the use of these coagulants as potential source of coagulant in this work.

Table 2: Levels and range of the variables tested in the CCD design.

Variables	Factors	Unit	Range and levels				
			Lowest - $\alpha$	Low -1	Center 0	High +1	Highest + $\alpha$
pH	A, $X_1$	-	2	2	6	10	10
Coagulants dosage	B, $X_2$	mg /l	200	200	600	1000	1000
Dye concentration	C, $X_3$	mg/l	20	20	60	100	100
Time	D, $X_4$	min.	60	60	300	540	540

Table 3: Proximate compositions determination of the coagulant precursor

S/No.	Parameters	Values				
		Vigna unguiculata (Cowpea)	Telfaria occidentalis (fluted pumpkin seed)	Brachystegia eurycoma (Black timber)	Vigna subterranean (Bambara nut)	Moringa oleifera seed
1.	Yield	11.5	38.40	28.31	14.6	32.68
2.	Bulk density (g/mL)	0.299	0.354	0.235	0.241	0.425
3.	Moisture Content (%)	9.0	12.58	7.25	10.0	5.02
4.	Ash content (%)	3.48	1.52	3.48	2.97	2.12
5.	Protein content (%)	25.14	55.09	19.77	18.15	39.34
6.	Fat content (%)	0.53	17.17	10.53	6.30	19.47
7.	Fibre content (%)	6.78	0.87	2.20	1.64	1.16
8.	Carbohydrate (%)	55.07	12.77	56.76	60.94	32.89

#### 3.2 Absorption Spectra of Dye and Calibration Curve Analysis.

Figure 2 records the electronic absorption spectra of 1000mg/l stock solution of AR 44 in the wavelength range of 250-800nm. The maximum wavelength ( $\lambda_{max}$ ) of AR 44 obtained was 516nm. The  $\lambda_{max}$  values obtained from the spectra analysis were in agreement with the standard values obtained in the literature. The absorbance or calibrated curve carried at different initial dye concentrations of 10-100 mg/l for AR 44 was obtained as shown in Fig 3. Beer-Lambert's law was obeyed in the desired concentration range following the straight line graphs obtained from the plots [38]. The result of the calibration analysis validated spectrophotometric method as the most accurate method for determining the concentrations of colour in aqueous solution.

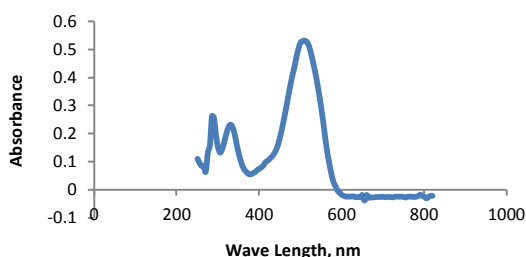


Fig. 2. Spectrum Peak Report for AR 44

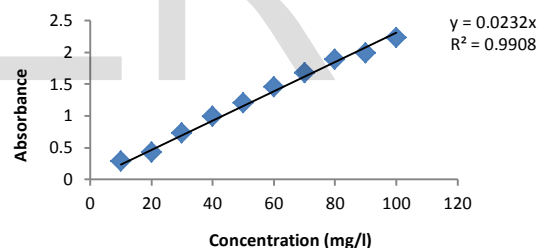


Fig. 3. Calibration Curve for for AR 44 at a Wave Length of 511 nm.

#### 3.3 Development of Regression Model

In order to study the combined effect of the factors, experiments were performed for different combinations of the parameters using statistical tool. Table 4 presented the experimental design matrix together with the experimental (exp) and predicted (pre) decontamination efficiencies for VUC, TOC, BEC, VSC and MOC. The experiment was conducted to compute the coagulation-flocculation model for VUC, TOC, BEC, VSC and MOC. As a result, the responses of the colour removal efficiencies were correlated with the four independent variables (pH, coagulant dosage, dye concentration and time), using the second-order polynomial of Eq. (4).



Table 4: CCD in coded unit and results obtained for colour removal from AR 44.

Run	Factors				Responses									
	A: X <sub>1</sub>	B: X <sub>2</sub>	C: X <sub>3</sub>	D: X <sub>4</sub>	Y <sub>vuc</sub>		Y <sub>toc</sub>		Y <sub>bec</sub>		Y <sub>vsc</sub>		Y <sub>moc</sub>	
	-	mg/l	mg/l	min	Y <sub>exp</sub>	Y <sub>pre</sub>	Y <sub>exp</sub>	Y <sub>pre</sub>	Y <sub>exp</sub>	Y <sub>pre</sub>	Y <sub>exp</sub>	Y <sub>pre</sub>	Y <sub>exp</sub>	Y <sub>pre</sub>
1	0	-1	0	0	19.2	19.95	8.3	9.20	34	28.70	34.9	35.78	54.3	47.94
2	1	1	-1	-1	33	33.90	9.3	12.61	31.7	31.70	36.2	37.36	66.7	64.58
3	-1	1	1	1	95.1	93.86	77.7	79.70	78.7	79.98	95.7	93.85	95.8	96.73
4	0	0	-1	0	22.7	23.39	16.9	17.22	43.9	41.12	37.1	38.67	63.9	61.50
5	-1	-1	-1	-1	62.5	60.78	57.1	58.68	31.6	33.09	62.3	60.87	45.1	47.73
6	-1	1	1	-1	65.6	64.28	47.9	50.06	48.7	49.60	65.2	63.77	65.8	67.26
7	1	1	1	1	58.9	60.16	26.3	25.12	54.1	53.01	63.9	63.60	88.3	86.62
8	1	1	1	-1	30	30.73	3.7	3.89	23.5	23.03	34.2	35.98	58.5	56.86
9	0	0	0	0	13.9	13.04	12.4	14.11	39.8	42.90	29.8	27.78	59.7	61.90
10	1	-1	1	1	54.7	55.43	19.5	24.27	43.3	44.68	52.7	56.91	78.5	77.51
11	1	-1	-1	1	59.3	60.15	27.6	25.84	56.7	56.20	62.9	62.60	85.6	85.09
12	0	1	0	0	24.6	25.57	18.9	14.58	45.2	44.30	38.3	41.45	63.8	65.57
13	1	0	0	0	24.9	18.48	24	17.79	52.5	52.97	29.6	20.81	67.4	79.94
14	-1	1	-1	1	98.6	97.73	99.5	99.57	86.5	86.79	99.3	99.09	99.5	102.1
15	0	0	0	0	13.9	13.04	12.4	14.11	39.8	42.90	29.8	27.78	59.1	61.90
16	-1	-1	-1	1	91.8	91.11	89.4	89.66	61.9	63.52	92.5	91.44	75.1	76.94
17	-1	-1	1	-1	57.3	57.06	50.3	48.66	23.4	24.33	58.8	58.33	37.3	40.36
18	-1	0	0	0	42.6	50.74	70	72.79	76.6	69.92	36.3	49.13	98.2	81.07
19	0	0	0	0	13.9	13.04	12.4	14.11	39.8	42.90	29.8	27.78	59.7	61.90
20	0	0	0	1	35.4	36.45	33.5	30.15	60.3	56.70	49.4	50.62	79.4	76.65
21	0	0	0	0	13.9	13.04	12.4	14.11	39.8	42.90	29.8	27.78	59.7	61.90
22	0	0	0	0	13.9	13.04	12.4	14.11	39.8	42.90	29.8	27.78	59.7	61.90
23	0	0	0	0	13.9	13.04	12.4	14.11	39.8	42.90	29.8	27.78	59.7	61.90
24	0	0	1	0	18.5	19.52	10.9	7.17	35.6	32.17	32.5	34.96	56.6	54.40
25	-1	-1	1	1	87.9	86.54	81.2	78.30	53.9	54.30	88.3	85.41	67.4	70.47
26	-1	1	-1	-1	68	67.30	72.9	68.58	56.2	55.97	69	65.51	72.7	73.89
27	1	-1	1	-1	25.7	26.11	2.7	3.03	15	15.11	33.8	32.29	49.1	47.10
28	1	-1	-1	-1	28.7	29.97	4.8	3.25	26.3	26.18	31.9	34.47	56.3	55.57
29	0	0	0	-1	5.9	6.57	4.1	4.04	29.1	26.50	18.7	21.52	49	47.16
30	1	1	-1	1	63.9	64.17	33.1	35.19	61.9	62.13	67.3	68.49	96.3	93.44

**3.4 Analysis of Variance (ANOVA) for Response Surface Quadratic Model.**

The adequacy of the model was further justified through ANOVA as shown in Table 5. The quadratic regression for colour removal efficiency shows that all the models were significant at 95% confidence level by the Fisher's test as shown in Table 5. These were confirmed having obtained F-values of 181.29, 133.11, 47.58, 44.37 and 12.37 for VUC, TOC, BEC, VSC and MOC, respectively. In addition, the P-values for the quadratic models for the five coagulants was less than 0.05, (P-values of regression  $\leq 0.05$ ) indicating models were statistically significant. In addition, the models did not exhibit lack-of-fit indicating insignificant lack-of-fit. The lack-of-fit test measures the failure of the model to represent data in experimental domain at points that are not included in the regression. If a model is significant and not suffers from lack-of-fit, does not necessarily mean it is a good one. If the experimental environment is quite noisy or some important variables are left out of the experiment, then it is possible that the portion of the variability in the data not explained by the model, also called the residual could be large [39]. Thus, a measure of the model's overall performance referred to as the coefficient of determination ( $R^2$ ) must be considered. At the same time, adjusted  $R^2$  allowing for the degrees of freedom

associated with the sums of the squares is also considered in the lack-of-fit test which should be an approximate value of  $R^2$ . When predicted  $R^2$  and adjusted  $R^2$  differ dramatically, there is a good chance that non-significant terms have been included in the model [37]. A high  $R^2$  value, close to 1, is desirable and ensures a satisfactory adjustment of the quadratic model to the experimental data. The  $R^2$  values of 99.41%, 99.20%, 97.80%, 97.64% and 92.03% for VUC, TOC, BEC, VSC and MOC indicate that the models could not explain 0.59%, 0.8%, 2.2%, 2.36% and 7.97% of the total variations, respectively. The values of predicted  $R^2$  and adjusted  $R^2$  are not significantly different as shown in Table 5. These close values of predicted  $R^2$  and adjusted  $R^2$  confirm the accuracy of the model. It was also observed from the table that for the coefficients terms such as pH (A), coagulant dosage (B), dye concentration (C) and settling time (D), whose  $P < 0.05$  were significant whereas some of the interaction terms (AB, AC, AD, BC, BD and CD) and the square terms ( $A^2$ ,  $B^2$ ,  $C^2$  and  $D^2$ ) were also significant to the response. Nevertheless, the interactive and square terms with P-value  $> 0.05$  could be considered to have no effect on the colour removal.

Positive sign in front of Eq. (5-9) indicates synergistic effect of the factors, whereas negative sign indicates antagonistic factor effect. Therefore, the overall quadratic models as expressed in

Eq. (5-9) for the responses measured are significant and adequate.

Table 5: ANOVA results for the five responses:  $Y_{vuc}$ ,  $Y_{toc}$ ,  $Y_{bec}$ ,  $Y_{vsc}$  and  $Y_{moc}$ .

$Y_{vuc}$	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		R- Squared
	Model	22382.85	14	1598.77	181.29	< 0.0001	significant	
	A-pH	4681.89	1	4681.89	530.89	< 0.0001		
	B-Dosage	142.24	1	142.24	16.13	0.0011		
	C-Dye concentration	67.28	1	67.28	7.63	0.0145		
	D-Time	4017.07	1	4017.07	455.50	< 0.0001		
	AB	6.76	1	6.76	0.77	0.3951		
	AC	0.022	1	0.022	2.551E-003	0.9604		
	AD	0.022	1	0.022	2.551E-003	0.9604		
	BC	0.49	1	0.49	0.056	0.8168		
	BD	1.000E-002	1	1.000E-002	1.134E-003	0.9736		
	CD	0.72	1	0.72	0.082	0.7786		
	A^2	1204.99	1	1204.99	136.64	< 0.0001		
	B^2	244.57	1	244.57	27.73	< 0.0001		
	C^2	183.50	1	183.50	20.81	0.0004		
	D^2	185.69	1	185.69	21.06	0.0004		
	Residual	132.28	15	8.82				
	Lack of Fit	132.28	10	13.23				
	Pure Error	0.000	5	0.000				
	R - Squared							0.9941
	Adjusted R - Squared							0.9890
	Pred R - Squared							0.9749

$Y_{toc}$	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		R- Squared
	Model	24179.47	14	1727.10	133.11	< 0.0001	significant	
	A-pH	13612.50	1	13612.50	1049.17	< 0.0001		
	B-Dosage	130.14	1	130.14	10.03	0.0064		
	C-Dye concentration	454.01	1	454.01	34.99	< 0.0001		
	D-Time	3068.06	1	3068.06	236.47	< 0.0001		
	AB	0.30	1	0.30	0.023	0.8807		
	AC	96.04	1	96.04	7.40	0.0158		
	AD	70.56	1	70.56	5.44	0.0340		
	BC	72.25	1	72.25	5.57	0.0323		
	BD	0.000	1	0.000	0.000	1.0000		
	CD	1.82	1	1.82	0.14	0.7131		
	A^2	2519.83	1	2519.83	194.21	< 0.0001		
	B^2	12.70	1	12.70	0.98	0.3382		
	C^2	9.49	1	9.49	0.73	0.4058		
	D^2	23.10	1	23.10	1.78	0.2020		
	Residual	194.62	15	12.97				
	Lack of Fit	194.62	10	19.46				
	Pure Error	0.000	5	0.000				
	Cor Total	24374.09	29					
	R - Squared							0.9920
	Adjusted R - Squared							0.9846
	Pred R - Squared							0.9547

$Y_{bec}$	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		R- Squared
	Model	8124.12	14	580.29	47.58	< 0.0001	significant	
	A-pH	1292.01	1	1292.01	105.94	< 0.0001		
	B-Dosage	1095.12	1	1095.12	89.79	< 0.0001		
	C-Dye concentration	360.01	1	360.01	29.52	< 0.0001		
	D-Time	4104.18	1	4104.18	336.52	< 0.0001		
	AB	301.02	1	301.02	24.68	0.0002		
	AC	5.29	1	5.29	0.43	0.5201		
	AD	0.16	1	0.16	0.013	0.9103		
	BC	5.76	1	5.76	0.47	0.5024		
	BD	0.16	1	0.16	0.013	0.9103		
	CD	0.20	1	0.20	0.017	0.8992		
	A^2	890.86	1	890.86	73.05	< 0.0001		
	B^2	106.36	1	106.36	8.72	0.0099		
	C^2	101.43	1	101.43	8.32	0.0114		
	D^2	4.43	1	4.43	0.36	0.5559		
	Residual	182.94	15	12.20				
	Lack of Fit	182.94	10	18.29				
	Pure Error	0.000	5	0.000				
	Cor Total	8307.05	29					
	R - Squared							0.978
	Adjusted R - Squared							0.9574
	Pred R - Squared							0.9276

Y <sub>vsc</sub>	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		R- Squared
	Model	14790.96	14	1056.50	44.37	< 0.0001	significant	
	A-pH	3609.67	1	3609.67	151.60	< 0.0001		
	B-Dosage	144.50	1	144.50	6.07	0.0263		
	C-Dye concentration	61.98	1	61.98	2.60	0.1275		
	D-Time	3810.64	1	3810.64	160.04	< 0.0001		
	AB	3.06	1	3.06	0.13	0.7249		
	AC	0.12	1	0.12	5.145E-003	0.9438		
	AD	6.00	1	6.00	0.25	0.6229		
	BC	0.64	1	0.64	0.027	0.8720		
	BD	9.00	1	9.00	0.38	0.5479		
	CD	12.25	1	12.25	0.51	0.4842		
	A^2	133.76	1	133.76	5.62	0.0316		
	B^2	304.17	1	304.17	12.77	0.0028		
	C^2	211.50	1	211.50	8.88	0.0093		
	D^2	177.85	1	177.85	7.47	0.0154		
	Residual	357.16	15	23.81				
	Lack of Fit	357.16	10	35.72				
	Pure Error	0.000	5	0.000				
	Cor Total	15148.11	29					
	R - Squared							0.9764
	Adjusted R - Squared							0.9544
	Pred R - Squared							0.8954

Y <sub>moc</sub>	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		R- Squared
	Model	7089.21	14	506.37	12.37	< 0.0001	significant	
	A-pH	5.78	1	5.78	0.14	0.7123		
	B-Dosage	1399.20	1	1399.20	34.18	< 0.0001		
	C-Dye concentration	226.85	1	226.85	5.54	0.0326		
	D-Time	3913.18	1	3913.18	95.60	< 0.0001		
	AB	294.12	1	294.12	7.19	0.0171		
	AC	1.21	1	1.21	0.030	0.8658		
	AD	0.090	1	0.090	2.199E-003	0.9632		
	BC	0.56	1	0.56	0.014	0.9082		
	BD	0.42	1	0.42	0.010	0.9204		
	CD	0.81	1	0.81	0.020	0.8900		
	A^2	896.69	1	896.69	21.91	0.0003		
	B^2	68.62	1	68.62	1.68	0.0150		
	C^2	40.35	1	40.35	0.99	0.3365		
	D^2	3.190E-005	1	3.190E-005	7.793E-007	0.9993		
	Residual	613.98	15	40.93				
	Lack of Fit	613.68	10	61.37				
	Pure Error	0.30	5	0.060				
	Cor Total	7703.20	29					
	R - Squared							0.9203
	Adjusted R - Squared							0.8459
	Pred R - Squared							0.7459

Quadratic empirical models of Eq. (5-9) were obtained from experimental data for AR 44 colour removal efficiency using Y<sub>vuc</sub>, Y<sub>toc</sub>, Y<sub>bec</sub>, Y<sub>vsc</sub> and Y<sub>moc</sub> in terms of actual significant factors as:

$$Y_{vuc} = +118.76483 - 19.93597 * pH - 0.064143 * Dosage - 0.67759 * Dye concentration - 0.040550 * Time + 1.34786 * pH^2 + 6.07237E-005 * Dosage^2 + 5.25987E-003 * Dye concentration^2 + 2.61290E-004 * Time^2 \quad (5)$$

$$Y_{toc} = +103.18513 - 30.43166 * pH + 0.031812 * Dosage + 0.017060 * Dye concentration + 0.048607 * Time + 0.015313 * pH * Dye concentration - 2.91667E-003 * pH * Time - 1.32813E-004 * Dosage * Dye concentration + 1.94912 * pH^2 \quad (6)$$

$$Y_{bec} = +31.46276 - 14.14977 * pH + 0.081235 * Dosage + 0.36028 * Dye concentration + 0.10419 * Time - 2.71094E-003 * pH * Dosage + 1.15894 * pH^2 - 4.00439E-005 * Dosage^2 - 3.91064E-003 * Dye concentration^2 \quad (7)$$

$$Y_{vsc} = +101.94834 - 8.59368 * pH - 0.075789 * Dosage - 0.5897563 * Time + 0.44907 * pH^2 + 6.77193E-005 * Dosage^2 - 3.81094E-003 * Dye concentration^2 - 4.23400E-005 * Time^2 \quad (8)$$

$$Y_{moc} = +47.02516 + 0.076557 * Dosage + 0.20302 * Dye concentration + 0.080716 * Time - 2.67969E-003 * pH * Dosage + 1.16272 * pH^2 - 3.21656E-005 * Dosage^2 \quad (9)$$

### 3.5 Model Adequacy Checking

#### 3.5.1 Actual and predicted results of the percentage colour removal.

For a model to be reliable, the response should be predicted with a reasonable accuracy when compared with the experimental data. Figure 4 compares experimental colour removal efficiencies (%) with the predicted values obtained from the model. The figure indicated good agreements between the experimental and predicted values. The observed points on

these plots reveal that the actual values are distributed relatively near to the straight line in most cases, indicating that the regression model is able to predict these removal

efficiencies. A close relationship between predicted and experimental data indicates a good fit.

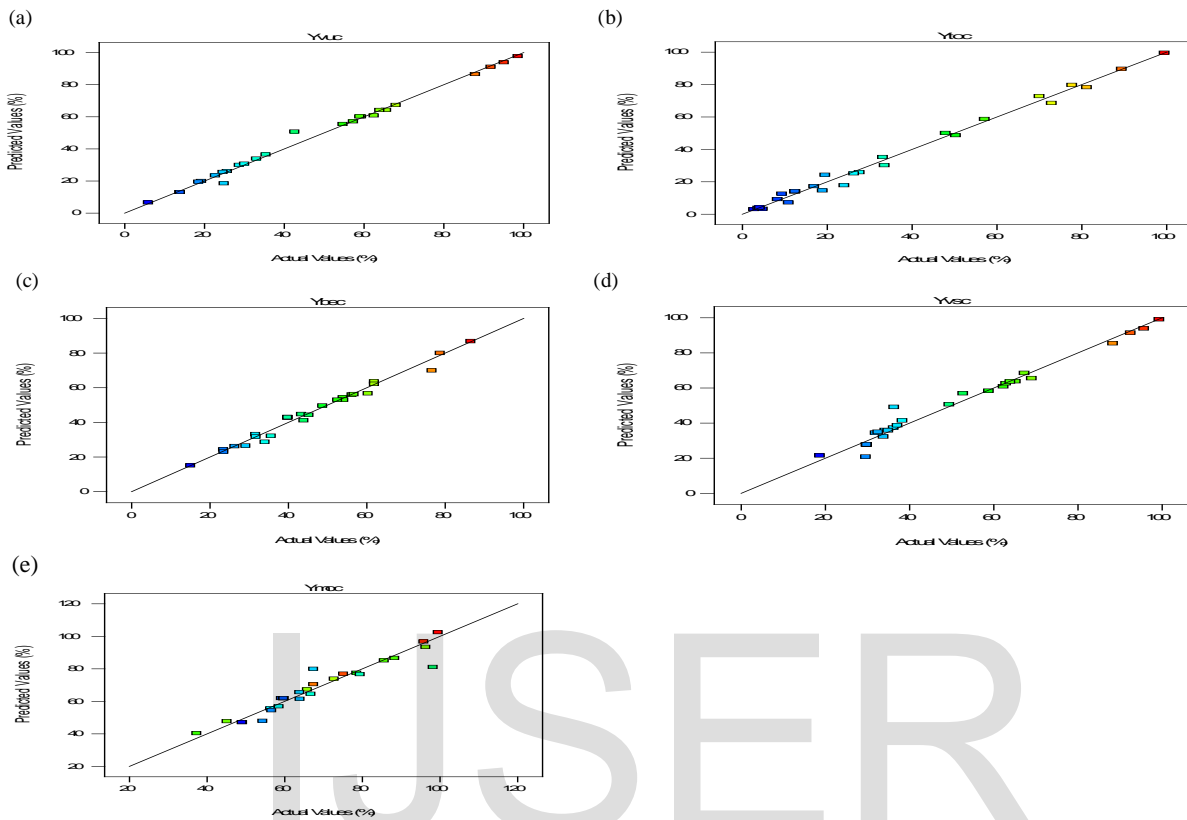


Fig. 4. Parity plot for the actual values and predicted values of AR 44 colour removal: (a)  $Y_{vuc}$ ; (b)  $Y_{toc}$ ; (c)  $Y_{bec}$ ; (d)  $Y_{vsc}$ ; (e)  $Y_{moc}$ .

### 3.6 Response Surface Plotting for Evaluation of Operational Parameters

Figures 5(a-e) shows the 3D response surface plots of quadratic models for colour removal efficiency using VUC, TOC, BEC, VSC and MOC respectively. As can be seen from Fig. 5a, the maximum colour removal efficiency using VUC is in the region where the pH ranged from 2-4, coagulant dosage ranged from 800-1000mg/l at 60mg/l dye concentration and time 240min. Also Fig. 5b shows that the maximum colour removal efficiency using TOC is in the region where the pH ranged from 2-4, coagulant dosage ranged from 600-800mg/l at 60mg/l dye concentration and time 240min. In addition, Fig. 5c shows that the maximum colour removal efficiency using BEC is in the region where the pH ranged from 2-4, coagulant dosage ranged from 600-1000mg/l at 60mg/l dye concentration and time 240min. Furthermore, Fig. 5d shows that the maximum colour removal efficiency using VSC is in the region where the pH ranged from 2-4, time from 330-420min at coagulant dosage of 600mg/l and dye concentration of 60mg/l. Lastly, Fig. 5e shows that the maximum colour removal efficiency using MOC is in the region where the pH ranged from 2-4, coagulant dosage ranged from 800-1000mg/l at 60mg/l dye concentration and time 240min. In general, the response surface plots indicate that the maximum colour removal efficiencies are located inside the design boundary.

pH plays an important role in the coagulation-flocculation process because it must be controlled in order to establish optimum conditions for coagulation-flocculation. The effectiveness of the polymers in colour removal from AR 44 dye are highly dependent on pH as shown in Fig. 5. It was observed that the polymers showed higher colour removals at low pH values and maximum removal was obtained when initial pH of the solutions were 2. In other words, colour removal efficiency decreased with increasing pH. The highest removal efficiency was observed in MOC followed by VUC giving efficiencies of 92.1% and 91.7%, respectively. Charge on the hydrolysis products and precipitation of polymeric hydroxides are both controlled by pH variations [40]. As the functional groups of the acid dyes are anionic, hydrolyses products of the organic biopolymer can neutralize the negative charges on dye molecules followed by polymer adsorption.

The result illustrated in Fig. 5 indicates that with the increase of coagulant dosages, the removal efficiencies increased and maximum colour removal efficiency was achieved at maximum coagulant dosages (800 mg/l) with MOC efficiency of 92.2% followed by VUC with efficiency of 91.9%. The high removal efficiencies of >70% was observed in all the coagulants for the 800mg/l doses. With the increase of coagulant dosages, the removal efficiency steadily increased and no “re-stabilization zones” with negative dye removals



were found. The higher removal could be due to the sweep flocculation mechanism, which is inclined to occur at high dosages. The coagulant apparently served as condensation nuclei and the dye particles were enmeshed as the precipitate was settled. The high dosages of the organic polymer could also give rise to chain bridging and adsorption mechanism [14]. In general, charge neutralization, sweep flocculation and polymer adsorption played a predominant role in the coagulation-flocculation process due to its pH and dosing values. However, the acidic condition (pH 2-4) was in favour of the improvement of cationic charge density as well as the extension of the grafting chain in the solution. This brought about the fourth mechanism “inter-particle bridging” which could be present due to high polymer dosages.

At high pH values which yielded low efficiencies for the colour removal studied, there is a competition between OH<sup>-</sup> and

organic anions contaminants for polymer hydrolysis products. Adsorption of the natural organic contaminants (NOC) or NOC-polymer contaminant complexes onto polymer hydroxide precipitate forming at high pH is also limited. As pH increases, natural organic compounds become more negatively charged and polymer hydrolysis species become less positively charged, resulting in less adsorption propensity. For these reasons, coagulation-flocculation of NOC in wastewater is mainly performed under low pH conditions along with the presence of soluble cationic polymer hydrolysis species. These species react with anionic functional groups on NOC to precipitate as a polymer-NOC. Conclusively, high removal efficiency at low pH values are predominant in organic contaminants removal from acid dyes. Similar results were reported by [17], [32].

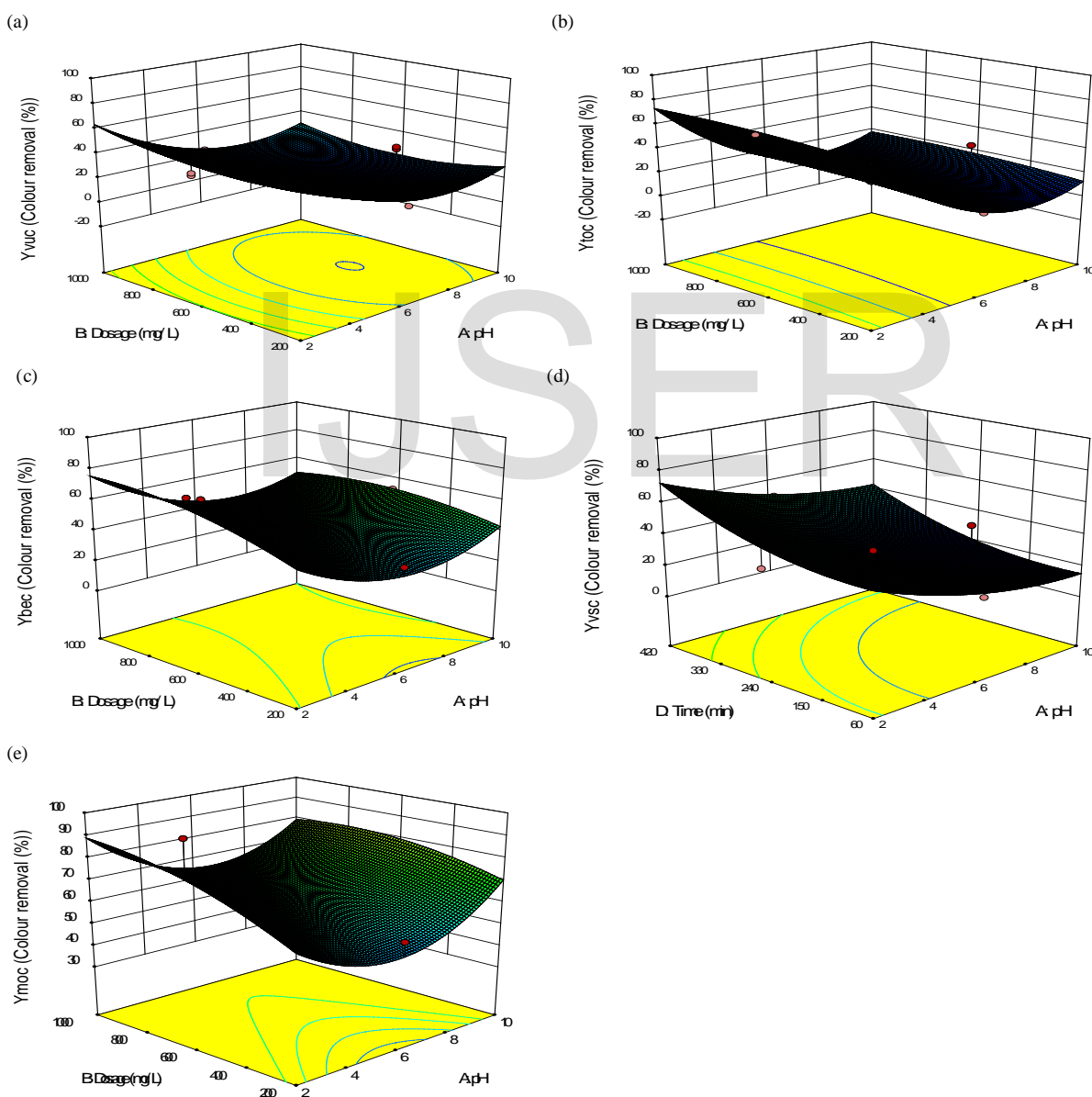


Fig. 5. 3D Surface plots for AR 44 colour removal as a function of: (a) pH and dosage at dye concentration 60 mg/l, time 240 min for  $Y_{vuc}$ ; (b) pH and dosage at dye concentration 60 mg/l, time 240 min for  $Y_{loc}$ ; (c) pH and dosage at dye concentration 60 mg/l, time 240 min for  $Y_{bec}$ ; (d) pH and time at dosage 600mg/l, dye concentration 60 mg/l for  $Y_{vsc}$ ; (e) pH and dosage at dye concentration 60 mg/l, time 240 min for  $Y_{moc}$ .

Table 6: Confirmation analyses of the model predicted using optimum values for colour removal from dye.

Colour	pH	Dosage (mg/l)	Dye Concentration mg/l)	Time (min)	Predicted value (%)	Experimental value (%)	Error (%)
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AR 44	$Y_{vuc}$	2	1000	20	420	97.73	95.02	2.77
	$Y_{toc}$	2	1000	20	420	99.57	98.77	0.80
	$Y_{bec}$	2	1000	51.19	419.99	90.09	88.25	2.04
	$Y_{vsc}$	2	1000	20	420	99.09	98.86	0.23
	$Y_{moc}$	2	999.79	45.80	419.99	99.05	97.50	1.56

### 3.7 Optimization Analysis

In order to maximize the contaminant removal efficiencies, optimization analysis was done using Design expert 9.0 software. Process optimization searches for a combination of factor levels that simultaneously satisfy the criteria placed on each responses and factors. Numerical optimization was employed and the desired maximum goal was set for each factor and responses. These goals are combined into an overall desirability function, for effective maximization of the function. Optimal conditions and the optimization results are shown in Table 6.

#### 3.7.1 Model validation and confirmation experiments.

The optimum predicted values were further validated by carrying out the experiment at the optimal predicted conditions and the results of the experimental values were also shown in Table 6. The experimental checking for the optimum conditions confirms good agreements with RSM results. The verification experiments demonstrated a good agreement between the experimental and model, indicating RSM approach adopted in this study was appropriate for optimizing the coagulation-flocculation process. Also as a consequence, the maximum error (%) between the predicted values and the experimental values were less than 4% indicating that the models adopted could predict experimental results well. The adequacy of the model was once again verified effectively by the experimental data validation

#### 3.7.2 Multiple response optimization (MRO)

Removal efficiencies of the colour using VUC, TOC, BEC, VSC and MOC yielded five individual responses, and their optimizations were achieved under different optimal conditions. Thus, a compromise among the conditions for the five responses is desirable. The desirability function approach together with graphical optimization was used to achieve such goal. With multiple responses, the optimum conditions where all parameters simultaneously meet the desirable treatment level can be visualized graphically by superimposing the contours of the response in an overlay plot [31]. By defining the desired limits of 88%, 89%, 75%, 89% and 88% for VUC, TOC, BEC, VSC and MOC, respectively, the optimum condition can be visualized graphically by superimposing the contours of the five responses in an overlain plot, as shown in Fig. 6. The shaded portion of the overlay plot defined clearly the permissible values of the independent variables. The regions that do not fit the optimization criteria were shaded gray. The yellow shade ("sweet spot") satisfies the goal for every response. The values for different responses shown in Fig. 6 indicate the minimum desirable efficiency value to be overlaid. As a result, the each colour removal efficiency was optimized and the best conditions for the responses were determined. Graphical optimization of Fig 6 displays the area of feasible response values in the factor space and the regions that fit the optimization criteria was shaded yellow. Using the overlain plot for optimal region further confirms the relevant of numerical optimization done and it offers a greater flexibility

in MRO [39]. Also, it is often applied when there is an emergency because it reduces preparation time and cost of experiment.

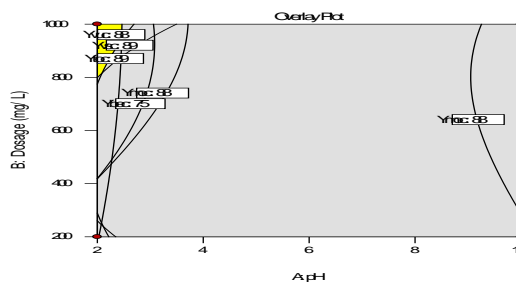


Fig. 6. Overlay plots of the optimal regions for the AR 44 colour removal at optimum dye concentration of 20mg/l and time of 420min.

### CONCLUSION

In this study, coagulation-flocculation process was optimized using VUC, TOC, BEC, VSC and MOC. The response surface methodology using face-centered central composite design was used to investigate the effect changes in the level of pH, coagulant dosages, dye concentration and time on the percentage colour removal. The optimum combination of operating parameters that yields the maximum colour removal was determined. The results clearly showed that the colour removal efficiency was severely influenced by pH, coagulant dosage and time. It was found that apart from the charge neutralization/adsorption which is the primary mechanism in the process, sweep-flocculation and interparticle bridging played important roles in enhancing colour removal process. From the optimization analysis, the maximum colour removal efficiency was obtained as 97.73%, 99.05%, 98.09%, 99.09% and 99.05% for VUC, TOC, BEC, VSC and MOC, respectively and the optimal conditions for optimum efficiencies were established. An optimal condition of pH 2, coagulant dosage 1000mg/l, dye concentration 20mg/l and time 420min was obtained from the compromise of the five desirable responses. The confirmation experiments demonstrated a good agreement between experimental data and model values, indicating RSM approach can be successfully applied for modelling and optimizing the coagulation-flocculation process. It is an economical way of obtaining the maximum result in a short period of time and with the least number of experiments.

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