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Research Article

# Experimental design for the removal of Remazol Gelb – GR dye from aqueous solution by casein

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### *Abstract*

*In this study, batch removal experiments were carried out in order to evaluate the maximum removal conditions of the anionic dye remazol gelb gr from aqueous solutions by casein using a* $2^3$ *full factorial design. To our knowledge, the interaction of casein with such a reactive dye has not been reported. The three factors were selected such as temperature, pH, and the ionic strength of suspension in this study and the optimization of the factors to obtain maximum sorption was carried out by incorporating effect, normal probability and interaction plots, analysis of variance (ANOVA), pareto charts, surface and contour plots.*

***Keywords:*** *Reactive dye, full factorial design, statistical design, casein, removal*

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## 1. Introduction

Dyes (over $7\times10^5$ metric tons of synthetic dyes) are produced worldwide every year for dyeing and printing purposes and about 5–10% of this quantity is discharged with wastewater (N. Dafale *et al*, 2008). Reactive dyes are representing 20–30% of the total dye market and the most widely used dyes in the textile industry (Y.S. Al-Degs *et al*, 2008). Reactive dyes are anionic dyes that depend on a negative ion (O.D. Tyagi *et al*, 2002). Anionic dyes includes many compounds from the most varied classes of dyes, which exhibit characteristic differences in structure (e.g., azoic, anthraquinone, triphenylmethan and nitro dyes) but possess as a common feature, water-solubilizing, ionic substituents (K. Hunger, 2003). The release of reactive dyes into the environment is undesirable, because many reactive dyes are toxic to some organisms and may cause direct destruction of creatures in water (Y.J. Xue *et al*, 2009).

Caseins are also frequently used as additives in food, paint, glue, and coating colours for paper, chromatography and biological separations (P. Walstra and R. Jenness, 1984). Knowledge of the mechanisms by which dye adsorb and interact at surface or interfaces of casein particles is fundamental for applications as well as to understanding the structure of the dye or casein micelle. Caseins are the main protein elements of mammalian milk, forming colloidal aggregates that give milk its opaque consistency (D.S. Horne, 2006). Several isoforms have been identified in the heterogeneous casein protein family, including $\alpha_{S1}$-casein, $\alpha_{S2}$-casein, β-casein, and κ-casein. (L.K. Creamer *et al*, 1998; H.E. Swaisgood, 2003). It is relatively hydrophobic, making it almost no soluble in water. It is found in milk as a suspension of particles called "casein micelles" which show only limited resemblance with surfactant-type micelle in a sense that the hydrophilic parts reside at the surface and they are spherical. However, in sharp contrast to surfactant micelles, the interior of a casein micelle is highly hydrated. The caseins in the micelles are held together by calcium ions and hydrophobic interactions. The isoelectric point of casein is 4.8. Since milk's pH is 6.6, casein has a negative charge in milk (D.G. Dalgleish, 1998).

Optimization of parameters by the classical method involves changing one independent variable and keeping the other factors constant in the same time. This method investigating effect of one variable at a time may be effective in some cases, but it consumes extra time and material. It requires large number of experimental trials to find out the effects. Also, this method is unreliable and fails to consider the combined effects of all the factors involved. These limitations of the conventional method can be eliminated by optimizing all the affecting parameters collectively by statistical experimental design. Optimization of parameters of a process is usually carried out by factorial, or more commonly fractional factorial, design of experiment (DOE) (D.G. Montgomery, 2005). When the number of factors for studying is large, the factors are first screened using two level DOEs, which allows the study of the effect of a large number of factors (S.B. Imandi *et al*, 2008). Using such methodologies, significant and more important factors are identified.

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The objective of this study was to examine the interaction and soprtion of the main proteins in milk, caseins, with remazol gelb gr (RG-GR) textile dye under some selected conditions. To our knowledge, the interaction of casein with such a reactive dye has not been reported. This will increase our understanding of the system formed when casein is suspended in aqueous dye solution. To describe and explain the experimental results, a $2^3$ full factorial design was used to evaluate the importance of temperature, pH and ionic strength of the suspension on the sorption with Minitab® 16.0 software for Windows™.

## 2. Materials and Methods

### *2.1. Materials*

Remazol gelb gr (RG-GR) was obtained from Setaş and Eksoy Textile Co. (Bursa, Turkey). The computer generated model of RG-GR used is shown in Fig. 1. The casein used was obtained from Merck. All chemicals were obtained from Merck and Aldrich, and were of analytical grade. All water used was of Milli-Q quality or doubly distilled.

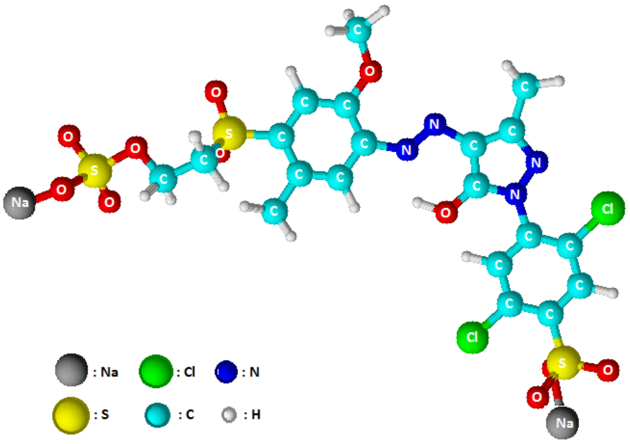


**Fig. 1** Computer generated model for Remazol Gelb GR dye

### *2.2. Experimental procedure*

The sorption of the dye from aqueous solutions was performed by batch experiments in volume and concentration of the dye in the initial solution and adsorbent mass were kept constant at 100 mL of $3.5\times10^{-4}$ M and 0.50 g. All of the dye solution was prepared with ultra pure water. As seen in Figure 2, agitation was made for 3 h., which is more than sufficient time to reach equilibrium at a constant agitation speed of 400 rpm. The pH was adjusted using 0.1N NaOH and 0.1N HCl solutions by using an Orion 920A pH-meter with a combined pH electrode. pH-meter was standardized with NBS buffers before every measurement. After 3 h, the samples were then centrifuged for 15 min at 5000 rpm and the left out concentration in the supernatant solution were analyzed using UV–Vis. spectrophotometer (Cary 1E UV–Vis. spectrophotometer, Varian) by monitoring the absorbance changes at a wavelength of maximum absorbance. Calibration curves were plotted between absorbance and concentration of the dye solution. The adsorbed amounts $q_m$ were calculated from the concentrations differences. The effect of pH was studied at pH 2 and 4 because of below the isoelectrical pH for casein. The sorption studies were also carried out at 18 and 45 °C. The effect of ionic strength was studied using 0.0 and 0.1 M NaCl.

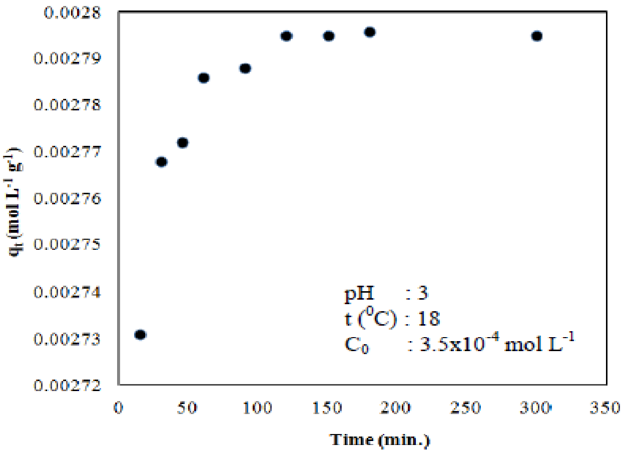


**Fig. 2** The effect of contact time to the sorption rate of dye by casein

### *2.3. The full factorial design*

The high and low levels defined for the $2^3$ full factorial design were listed in Table 1. The low and high levels for the factors were selected according to some preliminary experiments. The factorial design matrix and $q_m$ measured in each factorial experiment is shown in Table 2, with the low (−1) and high (+1) levels as specified in Table 1. $q_m$ was determined as average of three parallel experiments. The order in which the experiments were made was randomized to avoid systematic errors. Fig. 3 illustrates the mean of the experimental results for the respective low and high levels of temperature, initial pH, and ionic strength of suspension.

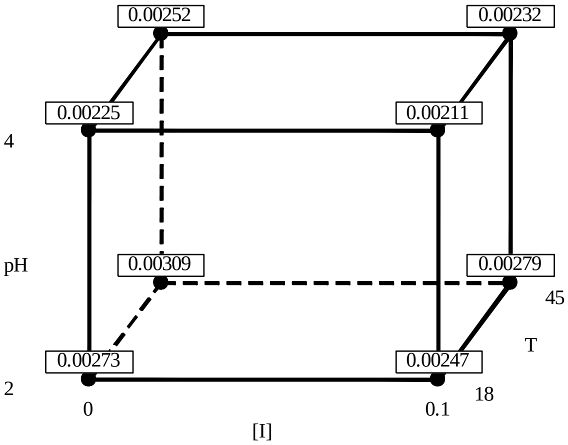


**Fig. 3** Cube plots for $q_m$

**Table 1**The high and low levels of experimental factors**.**

| Factor | Low level (-1) | High level (+1) |
|---|---|---|
| Ionic strength of the suspension, *mol* $L^{-1}$ (A) | 0 | 0.1 |
| pH of the dispersion (B) | 2.0 | 4.0 |
| Temperature, $^0C$ (C) | 18 | 45 |

**Table 2** Experimental design matrix and results

| Factor | | | | $q_m$ (mol/g)x$10^3$ | | | |
|---|---|---|---|---|---|---|---|
| Run no. | A | B | C | Trial 1 | Trial 2 | Trial 3 | Average |
| 1 | -1 | -1 | -1 | 2.730 | 2.755 | 2.704 | 2.729 |
| 2 | -1 | -1 | 1 | 3.090 | 3.088 | 3.092 | 3.090 |
| 3 | -1 | 1 | -1 | 2.251 | 2.231 | 2.271 | 2.251 |
| 4 | -1 | 1 | 1 | 2.518 | 2.509 | 2.527 | 2.518 |
| 5 | 1 | 1 | -1 | 2.104 | 2.110 | 2.104 | 2.106 |
| 6 | 1 | -1 | -1 | 2.466 | 2.472 | 2.46 | 2.466 |
| 7 | 1 | 1 | 1 | 2.334 | 2.334 | 2.297 | 2.321 |
| 8 | 1 | -1 | 1 | 2.788 | 2.793 | 2.784 | 2.788 |

## 3. Results and Discussion

Factors that influence the quantity of dye sorbed by casein were evaluated by using factorial plots: main effect, interaction effect, the pareto chart plot, normal probability plots, the surface plot, and the contour plot. ANOVA and *P*-value significant levels were used to check the significance of the effect on $q_m$. The main effect and interactions were also observed in the pareto chart plot.

### *3.1. Anova*

The results were displayed in Tables 3 and 4. Main, interaction effect, coefficients of the model, standard deviation of each coefficient, and probability for the full $2^3$ factorial designs were presented in Table 3 and 4. The significance of the regression coefficients was determined by applying a Student's t-test. As seen in Table 3, with the exception of ABC (*P*-value = 0.458), all other effects were significant with 95% confidence level. In addition, the model presented an adjusted square correlation coefficient $R^2$ (adj) of 99.60%, fitting the statistical model quite well. In this way, the dye uptake by casein could be expressed using the following equation:

$$q_m = (25.35 - 1.13A - 2.35B + 1.46C + 0.28AB - 0.11AC - 0.25BC)\ x10^{-4} \qquad (1)$$

This function describes how the experimental variables and their interactions influence the dye removal (T. Lundstedt *et al,* 1998). The initial pH of the solution (B) had the greatest effect on $q_m$, followed by temperature (A), ionic strength (C), ionic strength–pH interaction (AB), temperature –pH –interaction (BC), and ionic strength –temperature interaction (AC). The positive values of these effects reveal that the increase of these parameters increased $q_m$. Conversely, negative values of the effects decreased the response ($q_m$). According to Eq. (1), the ionic strength and pH had a negative effect on $q_m$, while temperature of the dispersion had a positive effect. In order to ensure an appropriate model, the test for the significance of regression was performed by applying a variance analysis (ANOVA). According to the ANOVA table, *P*-value<0.05 for the main factors and their 2-way interactions, and the $R^2$ value for $q_m$ was 0.99, which was a desirable figure. Table 4 shows the sum of squares being used to estimate the factors' effect and the *F*-ratios, which are defined as the ratio of the respective mean-square-effect to the mean-square-error. The significance of these effects was evaluated using the *t*-test, and had a significance level of 5%; i.e., with a confidence level of 95%. The R-squared statistic indicated that the first-order model explained 99.85% of $q_m$'s variability. The results revealed that the studied factors (A, B and C) and their 2-way interaction (AB, AC, and BC) were statistically significant to $q_m$. Notably, 3-way interaction (ABC) had no effect at the 95% confidence level.

**Table 3** Estimated effects and coefficients for $q_m$ (mol/g).

| Term | Effect | Coefficient | Standard error of coefficient | *T*-value | *P*-value |
|---|---|---|---|---|---|
| Constant | | 0.002534 | 0.000003 | 852.84 | 0 |
| [I] | -0.000227 | -0.000113 | 0.000003 | -41.45 | 0 |
| pH | -0.00047 | -0.000235 | 0.000003 | -78.54 | 0.001 |
| T | 0.000291 | 0.000146 | 0.000003 | 52.12 | 0 |
| [I]*pH | 0.000056 | 0.000028 | 0.000003 | 11.47 | 0.001 |
| [I]*T | -0.000023 | -0.000011 | 0.000003 | -2.78 | 0.001 |
| pH*T | -0.00005 | -0.000025 | 0.000003 | -9.51 | 0 |
| [I]*pH*T | -0.000003 | -0.000002 | 0.000003 | -0.45 | 0.458 |

S = 0.0000142513 R-Sq = 99.25% R-Sq(pred) = 99.33% R-Sq(adj) = 99.60%

### *3.2. The main effects*

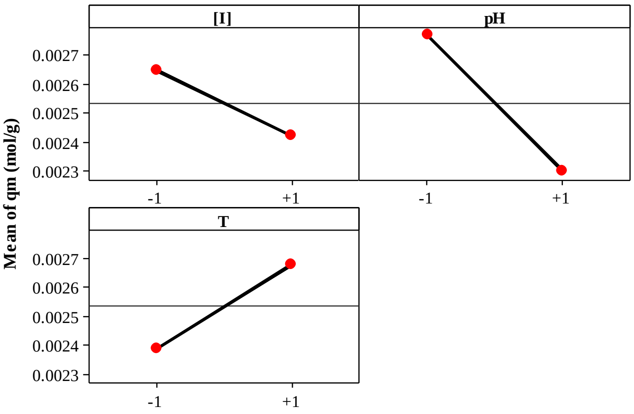


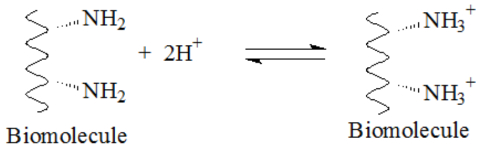
**Fig. 4** Main effects plot for $q_m$

The main effects of each parameter on the dye removal are shown in Fig. 4. The main effect plots were generated to represent the results of the regression analysis. It shows only the factors that were significant at the 95% confidence interval. The main effects represent deviations of the average between the high and low levels for each factor. When the effect of a factor is positive, $q_m$ increases as the factor changes from low to high levels. In contrast,

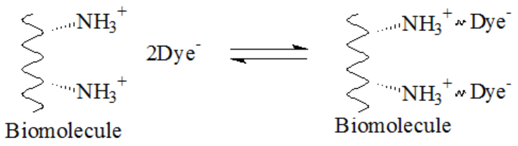
**Table 4** Analysis of variance for $q_m$ (mol/g).

| Source | | Degrees of freedom | Sum of squares | Adj. Sum of squares | Adj.Mean squares | *F*-ratio | *P*-value |
|---|---|---|---|---|---|---|---|
| Main Effects | | 3 | 0.00000214 | 0.00000214 | 0.00000071 | 3211.56 | 0 |
| | A | 1 | 0.00000031 | 0.00000031 | 0.00000031 | 1654.87 | 0 |
| | B | 1 | 0.00000132 | 0.00000132 | 0.00000132 | 7200.45 | 0 |
| | C | 1 | 0.00000051 | 0.00000051 | 0.00000051 | 2354.25 | 0 |
| 2-Way Interactions | | 3 | 0.00000004 | 0.00000004 | 0.00000001 | 61.25 | 0 |
| | AB | 1 | 0.00000002 | 0.00000002 | 0.00000002 | 125.54 | 0 |
| | AC | 1 | 0 | 0 | 0 | 22.45 | 0.001 |
| | BC | 1 | 0.00000002 | 0.00000002 | 0.00000002 | 68.45 | 0 |
| 3-Way Interactions | | 1 | 0 | 0 | 0 | 0.66 | 0.458 |
| | ABC | 1 | 0 | 0 | 0 | 0.59 | 0.458 |
| Residual Error | | 16 | 0 | 0 | 0 | | |
| Pure Error | | 16 | 0 | 0 | 0 | | |
| Total | | 23 | 0.00000218 | | | | |

if the effects are negative, a reduction in ($q_m$) occurs for high level of the same factor. From Fig. 4, it is inferred that the larger the vertical line, the larger the change in $q_m$ when changing from level −1 to level +1. It should be pointed out that the statistical significance of a factor is directly related to the length of the vertical line (K. Palanikumar and J.P. Dawim, 2009). The effects of ionic strength and pH factors are negative, that is, a decrease of $q_m$ is observed when the factor changes from low to high. Ionic strength and pH factors result in a higher mean $q_m$ at their low level, compared to that at the high level. For the temperature factor, the opposite is true. In addition, pH had a greater effect on $q_m$, as is evident by the longer vertical line. Maximum removal occurred at pH 2. Figure demonstrates that the removal increases with decreasing pH because of the electrostatic attraction between the chromophore groups of dye and the positively charged casein surface. The higher sorption of RG-GR by casein at low pH may result due to the neutralization of the positive sites at the surface of casein. The isoelectric point of casein is approximately 4.6, and thus, at pH 2 and 4 it has a positive net charge. At pH above isoelectrical point at approximately 4.6, the removal of the anionic dye is not favoured due to electrostatic repulsion. At lower pH (pH 2), the surface of casein particles may become more positively charged because of amine groups, which enhances the negatively charged RG-GR anions through electrostatic interactions. In this case, it can be written as following equations (3) and (4):



(3)



(4)

The positively charged sites favour the interaction of dye anions due to electrostatic interactions (M. Alkan *et al*, 2004). $q_m$ decreased with increasing ionic strength. An increase in ionic strength will reduce the lateral repulsion between like charges, as the interaction between casein molecules, but it will also screen the attractive force between the opposite charged dye and the casein molecules. The screening of attractive forces between the dye and the casein molecules by counter-ions is probably the major reason for the observation of decreased removal at high ionic concentration (M. Alkan *et al*, 2004;M. Lundin *et al*, 2010; M. Alkan *et al.*, 2005). As a result of, the sorbed amount of dye to casein particles decreases with increasing ionic strength as a result of the electrostatic screening of the protein/dye substrate attraction at high electrolyte concentrations. According to the Fig.4, it is observed that at higher temperature the removal is higher, and the removal process was endothermic process. Increasing the temperature is known to increase the rate of diffusion of the dye molecules across the external boundary layer and in the internal pores of the casein particle, owing to the decrease in the viscosity of the solution. In addition, as can be seen clearly in Figure 5, casein and the dye-covered casein after dyeing shows time-dependent morphological changes. Casein surface morphology completely changed after treatment. It can be concluded that the images are consistent with experimental data.

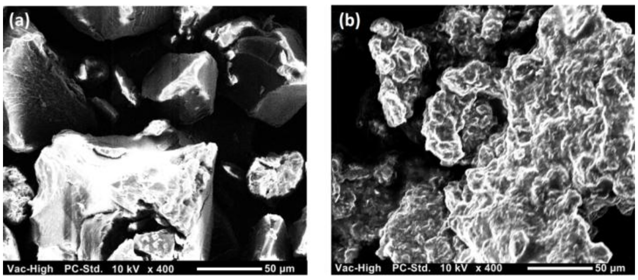


**Fig. 5** Pure casein (a) and dye-covered casein (b)

### *3.3. The interaction effects*

An interaction (Fig. 6) is effective when the change in the response from low to high levels of a factor is dependent on the level of a second factor, i.e. when the lines do not run parallel (T. Mathialagan and T. Viraraghavan, 2005). The interaction effect plots showed that interaction of pH, ionic strength and temperature played major role in removal. Fig. 6 shows the significant interactions between the parameters (AB, BC, and AC). The interaction plots were also generated with ANOVA. All the interactions of the factors were statistically significant in determining $q_m$.

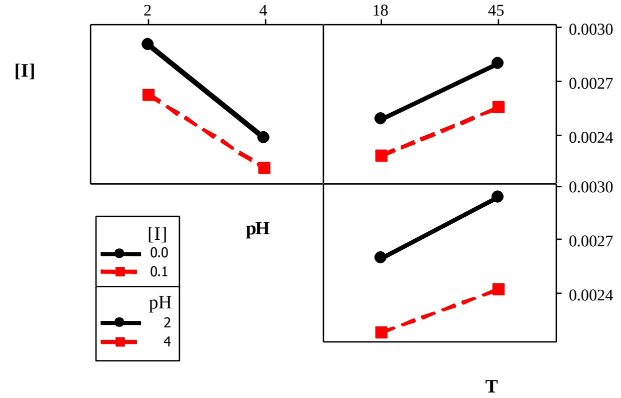


**Fig. 6** Interaction plots for $q_m$

These plots clearly indicated that interaction between ionic strength and pH (AB) was stronger than between pH and temperature (BC). The interaction between ionic strength and temperature (AC) was statistically significant but much smaller. The effect of pH and temperature was more significant at lower ionic strength. The interaction effects between the factors AB, BC, and AC revealed that the amount adsorbed was higher at lower ionic strength (A), initial pH (B) and temperature of the suspension (C).

### *3.4. The Pareto chart*

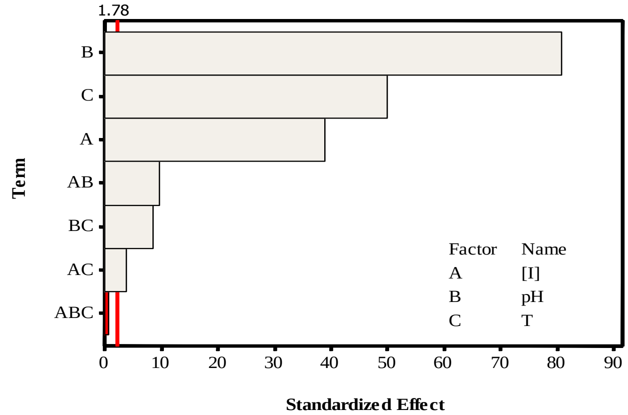


**Fig.7** Pareto chart of the standardized effects (Alpha = .05)

The relative importance of the main effects and their interactions was also observed on the Pareto chart (Fig. 7). For the 95% confidence level, the t-value is 1.78. As shown in Fig. 7, some values are positioned around a reference line. According to Fig. 7, the main factors (A, B, and C) and their interactions (AB, BC, and AC) that extend beyond the reference line were significant at the level of 0.05. The pH represented the most significant effect on $q_m$. The pH (B), ionic strength interaction (A), and temperature (C) had greater effects on $q_m$ while, except for the interaction effect between ionic strength, pH, and temperature (ABC) all other factors and their interactions had smaller effects and were statistically significant at 95% confidence.

### *3.5. Normal probability plots*

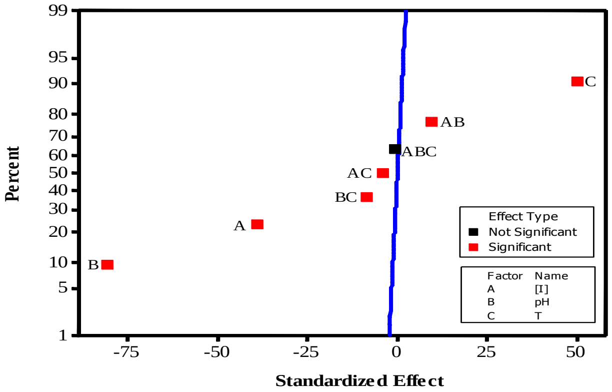


**Fig.8**. Normal probability plot of the standardized effects (Alpha = .05)

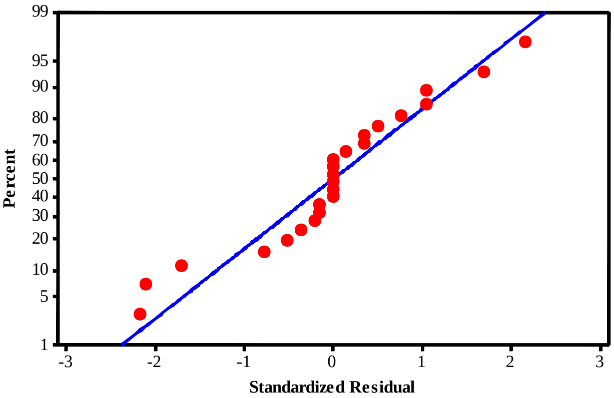


**Fig. 9** Normal probability plot of standardized residuals

The normal probability plot was given in Fig. 8. According to the normal probability plots, the points which are close to a line fitted to the middle group of points represent those estimated factors that do not demonstrate any significant effect on the response variables. The main factors (A, B, and C) and their interactions (AB, AC, and BC) are far away from the straight line. Because A, B, BC, and AC lie to the left of the line, their contribution had a negative effect, C and AB on the right had a positive effect. The pH (B) had largest effect because its point lies farthest from the line. These results confirm the previous Pareto chart analysis and the values of Table 3. The normal probability plot of residuals for $q_m$ (Fig. 9) showed how closely the set of observed values followed the theoretical distribution. Generally, experimental points are reasonably aligned, suggesting a

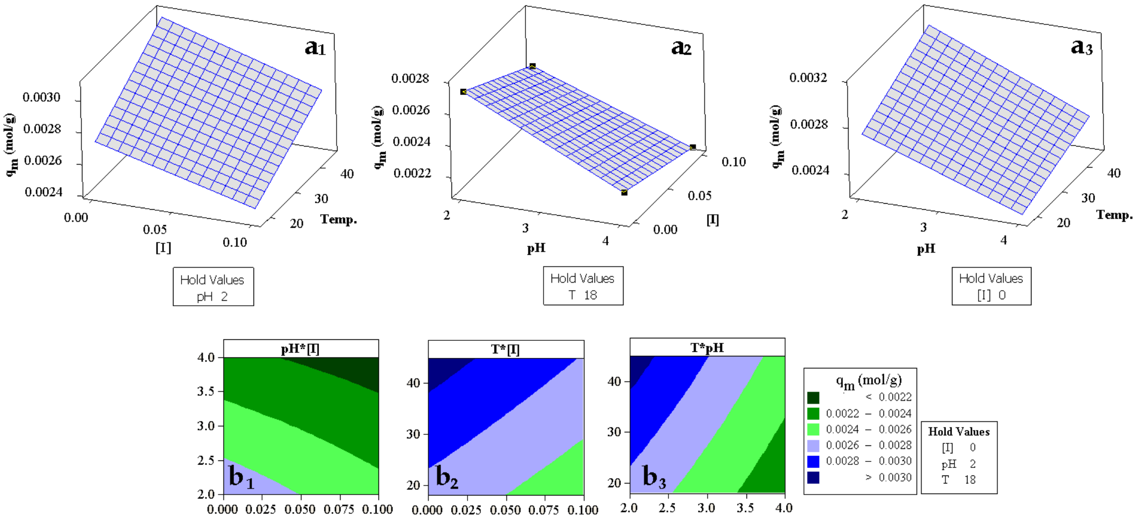


**Fig.10.** Surface plots ($a_1$, $a_2$ and $a_3$) and contour of the estimated response surface ($b_1$, $b_2$ and $b_3$) for $q_m$

normal distribution. The selected model adequately described the observed data, explaining approximately 99% (due to $R^2$=0.98) of the variability of $q_m$.

### *3.6. Response surface and contour plots*

The surface plots of the response functions are useful in understanding both the main and interaction effects of the factors (C. Cojocaru and G.Z. Trznadel, 2007). The response surface plots are reported in Fig. 10 ($a_1$,$a_2$,$a_3$) for the average $q_m$. Fig. 10 ($b_1$,$b_2$,$b_3$) illustrates the response surface contour plots when one parameter for each graph is at a hold value. This figure also shows the estimated $q_m$ parameter as a function of the normalized independent variables, the height of the surface represents the value of $q_m$. From three surface plots, maximum values of $q_m$ required lower ionic strength (A), pH (B), and higher temperature of the suspension (C) in agreement with the interaction graphs.

## 4. Conclusions

The following conclusions are drawn from this investigation:

The statistical design of the experiments combined with techniques of regression was applied in optimizing the conditions of maximum sorption of the dye by casein. Using a full factorial and laboratory scale experiments, significant process factors influencing removal were identified and the interactions between factors were highlighted. Three sorption parameters (pH, temperature and ionic strength) were tested by using full factorial design criterion and all of them showed significant effect on sorption process. This mathematical model was used to develop contour plots for various factors' effects. It was observed that the initial pH of the suspension exerted the greatest influence on the amounts of dye sorbed $q_m$. Temperature had positive effect but ionic strength and pH had a negative influence on $q_m$, is the validity of this study was limited to temperatures between 18 and 45 °C, pH between 2 and 4, and ionic strength of less than 0.1 M NaCl. The interactions between pH, temperature and ionic strength showed significant effect on sorption process.

Technologies for the sorption of dyes are generally expensive. Thus, it may be conducted that casein may be used for sorption of RG-GR from wastewater since it is a low-cost, abundant and available sorbent.

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## References

C. Cojocaru, G.Z. Trznadel (2007), Response surface modeling and optimization of copper removal from aqua solutions using polymer assisted ultrafiltration, *J. Membr. Sci.* 298, 56–70.

D.G. Dalgleish (1998), Casein micelles as colloids. Surface structures and stabilities, *J Dairy Sci.* 81 (11), 3013–8.

D.G. Montgomery (2005), Design and Analysis of Experiments, 6th edn, *John Wiley Sons, Inc.*, Arizona, USA.

D.S. Horne (2006), Casein micelle structure: models and muddles, *Curr. Opin. Colloid Interface Sci.* 11, 148–153.

H.E. Swaisgood (2003), Chemistry of the caseins, in: P.F. Fox, P.L.H. McSweeney (Eds.), Advanced Dairy Chemistry, vol. 1, *Kluwer Academic Plenum Publishers*, pp. 139–150.

K. Hunger (2003), Industrial Dyes, Chemistry, Properties, Applications, *Wiley-VCH, Weinheim*, Germany, 2003, p. 1–10.

K. Palanikumar, J.P. Dawim (2009), Assessment of some factors influencing tool wear on the machining of glass fibre-reinforced plastics by coated cemented carbide tools, *J. Mater. Process. Technol.* 209, 511–519.

L.K. Creamer, J.E. Plowman, M.J. Liddell, M.H. Smith, J.P. Hill (1998), Micelle stability:kappa-casein structure and function, *J. Dairy Sci.* 81, 3004–3012.

M. Alkan, O. Demirbas, S. Celikcapa, M. Dogan (2004), Sorption of acid red 57 from aqueous solution onto sepiolite. *J. Hazard. Mater.*, 116, 135–145.

M. Alkan, O. Demirbas, S. Celikcapa, M. Dogan (2005), Removal of reactive blue 221 and acid blue 62 anionic dyes from aqueous solutions by sepiolite, *Dyes Pigm.* 65, 251–259.

M. Lundin, U.M. Elofsson, E. Blomberg, M.W. Rutland (2010), Adsorption of lysozyme, β-casein and their layer-by-layer formation on hydrophilic surfaces: Effect of ionic strength, Colloids and Surfaces B: *Biointerfaces*. 77(1), 1-11.

N. Dafale, N.N. Rao, S.U. Meshram, S.R. Wate (2008), Decolorization of azo dyes and simulated dye bath wastewater using acclimatized microbial consortium – biostimulation and halo tolerance, *Bioresour. Technol.* 99, 2552–2558.

O.D. Tyagi, M.S. Yadav, M. Yadav (2002), A Textbook of Synthetic Dyes, *Anmol-PVT. LTD.*, p.67.

P. Walstra, R. Jenness (1984), Dairy Chemistry and Physics; *Wiley-Interscience*: New York.

S.B. Imandi, V.V.R. Bandaru, S.R. Sumalanka, S.R. Bandru, H.R. Garapti (2008), Application of Statistical Experimental Designs for the Optimization of Medium Constituents for the Production of Citric Acid from Pineapple Waste, *Bioresour. Technol.* 99, 4445–4450.

T. Lundstedt, E. Seifert, L. Abramo, B. Thelin, A. Nyström, J. Pettersen, R. Bergman (1998), Experimental design and optimization, *Chemom. Intell. Lab. Syst.* 42, 3–40.

T. Mathialagan, T. Viraraghavan (2005), Biosorption of pentachlorophenol by fungal biomass from aqueous solutions: a factorial design analysis, *Environ. Technol.* 6, 571–579.

Y.J. Xue, H.B. Hou, S.J. Zhu (2009), Adsorption removal of reactive dyes from aqueous solution by modified basic oxygen furnace slag: isotherm and kinetic study, *Chem. Eng. J.* 147, 272–279.

Y.S. Al-Degs, M.I. El-Barghouthi, A.H. El-Sheikh, G.A. Walker (2008), Effect of solution pH, ionic strength, and temperature on adsorption behavior of reactive dyes on activated carbon, *Dyes Pigm.* 77, 16–23.