Flocculation properties of a natural polyampholyte: The optimum condition toward clay suspensions

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ABSTRACT
Polyelectrolytes are commonly used as flocculants in drinking water treatment. However, the growing concerns about their toxicity have motivated the search for biocompatible flocculants. Here, we show that gelatin, a natural amphoteric polyelectrolyte, can be effectively adsorbed on clay surfaces and can potentially be a suitable substitute for existing flocculants. The adsorption of gelatin from its aqueous solution onto the mineral clay surfaces at different conditions was systematically investigated using the design of experiments methodology. The gelatin adsorption was found to vary considerably with pH variation showed a maximum adsorption at its isoelectric point. The amount of adsorbed gelatin increased with increasing pH from 3 to 5, attained a maximum at pH 5 and then decreased with increasing pH from 5 to 11. Similarly, the amount of adsorbed gelatin showed decreasing trends around salt concentration of 0.05 M and temperature 35°C. On the other hand, the adsorption was continuously increased with time and polymer concentration in the range of 0.1-0.9 mg/dL. Finally, the jar tests confirmed the ability of gelatin for using a natural flocculant for water treatment.

Keywords: Amphoteric polyelectrolytes, Clay, Flocculation, Surface adsorption, Water treatment

1. Introduction

Water quality is an important key to the quality of life. Drinking water supplies in a proper amount and of good quality are among the biggest problems of everyday life in the whole world [1-4]. There are several methods to produce potable water, in which flocculants are commonly used. Among various materials that have been utilized as flocculants, polyelectrolytes have received the most attention [5]. In comparison with aluminum sulfate (alum), the most commonly used flocculant, some of the advantages arising from the use of polymers in water treatment are: I) lower coagulant dose requirements, II) a smaller volume of sludge, III) smaller increase in the ionic load of the treated water, IV) reduced level of aluminum in treated water, and V) cost savings of up to 25-30% [6].

However, the major concerns with polyelectrolytes are the environmental impact and toxicity to aquatic life [7]. This has given rise to investigations into polymer toxicity, the analysis of residual polymer in the product water, and in wastes from water treatment processes and polymer degradation [6]. For these reasons, alternative coagulants and flocculants have been considered for environmental applications. Biopolymers may be of great interest since they are natural low-cost products, characterized by their environmentally friendly behavior. Among these biopolymers, natural polyampholytes, e.g. gelatin may be considered as one of the most promising coagulation/flocculation materials.

Due to biodegradable properties, biocompatibility, no toxicity, availability and low cost, gelatin as an amphoteric polyelectrolyte material can potentially be a suitable substitute for the existing flocculants. Piazza et al. measured the adsorption of proteins such as gelatin onto two different clays [4, 8]. They surveyed the adsorption process in a range of protein concentrations to determine correlations between the adsorption characteristics and sedimentation activities of the proteins [8, 9]. Adsorption of gelatin onto the Fuller’s earth surfaces [10], silver bromide [11], mica [12, 13], and polystyrene latex particle [14] have been investigated and the effect of polymer concentration, pH and electrolyte on gelatin adsorption have been explored [11, 12, 14]. For example, Bajpai et al. found that the thickness of adsorbed gelatin layer on Fuller’s earth surfaces decreased with increase in gelatin.
concentration [10] and Kawanishi et al. found that the thickness of the adsorbed gelatin layer onto mica decreased with increasing salt concentration [12].

Many other studies have investigated the adsorption of gelatin on various surfaces, however, all of these works have used conventional multifactor experiments, in which the optimization of the objective function, i.e., the amount of adsorbed gelatin, is carried out by varying a single factor while keeping all other factors fixed at a specific set of conditions; this method is time-consuming and incapable of effective optimization. Here, by exploiting experimental design and response surface methodology (RSM), we first focus on the adsorption of gelatin onto the mineral clay surfaces and investigate the simultaneous effect of various experimental factors including polymer concentration, pH, electrolyte concentration and temperature. By application of RSM, it is possible to evaluate the interactions of possible influencing factors with a limited number of planned experiments. Next, the potential of gelatin to be used as a flocculant in water purification by the use of RSM and jar test is studied.

2. Materials and Methods

2.1. Materials

All chemicals were of reagent grade and used without further purification. Gelatin was food grade with Mw ~ 70,000 gr/mol (as estimated by SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis) and isoelectric point of 5.00. Mineral clay was collected from Garmsar regional alluviums (Semnan Province, Iran). Hydrogen chloride (HCl), sodium hydroxide (NaOH), sodium chloride (NaCl) and n-propanol were obtained from Merck. Copper sulfate 5-hydrate and ethylene glycol were prepared from Kian Kaveh Azma (Iran). Deionized distilled water was used throughout the experiments.

2.2. Preparation of Solutions and Reagent

Gelatin solutions with different concentrations were prepared by mixing specified amount of dry gelatin in distilled water for 30 min at 60°C. The aqueous dispersion of clay, 0.04 g in 9 mL water, was prepared and sonicated (Labsonic, B. Braun: 400W, Germany) for 210 s before every use. To determine non-adsorbed gelatin, biuret reagent was prepared according to the procedure described in the literature (Table 1). All experiments were carried out in a random order.

Table 1. Factors and Levels Used in the Central Composite Design

| Factors          | Levels | Coded factors levels |
|------------------|--------|----------------------|
| Temperature, °C  | -2 2   | -1 1 0 1 2           |
| Time, min        | -2 2   | -1 1 0 1 2           |
| pH               | -3 3   | -2 2 1 0 1 2         |
| Salt Concentration, mg/dL | -0.01 0.01 | -0.03 0.03 0.05 0.07 0.09 |
| Polymer Concentration, mg/dL-Symbol | -0.01 0.01 | -0.03 0.03 0.05 0.07 0.09 |

2.3. Adsorption of Gelatin onto Clays

In all the experimental runs, the amount of ingredients used and the experimental conditions were determined based on the planned experimental design, described in the next section. First, the salt (NaCl) in gelatin solution in a defined amount, were mixed and the pH of solution adjusted to certain value using HCl and NaOH solutions. Then, the clay dispersion was added to the mixture and stirred for a period of time depending on experimental design runs. Once equilibrated, the mixture was centrifuged (model L5-65, Beckman, USA) at 35,000 rpm for 30 min to sediment the gelatin-coated clay particles. The absorbance of the supernatant was measured by UV/Vis at 542 nm and the free gelatin content was calculated using the calibration curve.

To study the morphology of the samples, the dried native mineral clay, and gelatin-adsorbed clay were set on a glass slide and then vacuum-coated with gold. Digital images of samples were acquired with the Zeiss DSM-960A scanning electron microscope (SEM) operating at 10-15 kV.

2.3.1. Experimental design and optimization

The optimization step was carried out exploiting of central composite design (CCD), the most popular class of second order designs. Variables for optimization were pH, temperature, time, polymer concentration, and salt concentration (NaCl). Maximum and minimum levels of each factor were established according to the works described in the literature (Table 1). All experiments were carried out in a random order.

2.4. Sedimentation Measurements

Coagulation and flocculation properties of gelatin to remove clay particles from the suspension were evaluated by jar test. For this test, the mixtures were prepared by addition of 0.125 g of clay and specified amount of NaCl into 50 mL water. Then, pH was adjusted to the specified value and the mixture was placed in a water bath for 20 min to reach the specified temperature. Next,
while stirring the mixture by a mechanical stirrer, gelatin solution (100 μL) was added and stirring continued in two stages, 150 rpm for 30 s and 50 rpm for 10 min. After that, the mixtures were left for 15 min until the sedimentation was completed. The transmittance of the upper solution was measured by UV-Vis at 550 nm.

The rheological behavior of flocs was investigated with oscillation rheometer (Physica MCR 300, Anton paar, Austria) and cone-plate geometry (diameter 25 mm; cone angle 2°). At first, stress-strain sweep of the sample was carried out at 25°C, then the dynamic viscoelastic modulus (\(G'\) & \(G''\)) measurements in the linear response were performed at the angular frequency of 0.1-100 rad/s. To identify thermal hysteresis, all samples were kept at the same condition of 25°C for 24 h. In order to avoid water evaporation during rheological tests, a suitable solvent trap, considering inertia effects, was designed and set on cone-plate geometry.

### 2.4.1. Experimental design and optimization

Box-Behnken design (BBD) was used for experimental design of sedimentation experiments. The variables for this design were pH, temperature, polymer concentration, and salt (NaCl) concentration. Maximum and minimum levels of each factor were established according to the works described in the literature (Table 2). All experiments were carried out in a random order.

| Variables                  | Coded factors levels |
|----------------------------|----------------------|
| Temperature, °C Symbol (A) | -1 25 35             |
| pH Symbol (B)              | 5 7 9                |
| Salt Concentration, M Symbol (C) | 0.00 0.05 0.10 |
| Polymer Concentration, g/dL Symbol (D) | 0.002 0.006 0.01 |

### 3. Results and Discussion

#### 3.1. Gelatin Adsorption on Clay Surfaces

##### 3.1.1. Response surface methodology (RSM)

RSM has been employed to optimize and understand the performance of complex systems [13, 16]. By application of RSM, it is possible to evaluate the interactions of possible influencing factors on the amount of adsorbed polymer with a limited number of planned experiments (Table 1 and Table S1, see the supplementary information). To find the most important effects and interactions, analysis of variance (ANOVA) was calculated using the software package Design-Expert 7.1.6 (Table S2).

For optimization of the adsorbed polymer, a fractional factorial design (2\(^{3-1}\)) was performed (Table S1). The central composite design plots for the effects of variables on adsorbed polymer are presented in Fig. 1. The experimental results were evaluated by the Design-Expert software using approximating functions of dependent variable, adsorbed gelatin (R), resulting from an adjustment of the quadratic model (Eq. (1)).

\[
R = 84.00 + 6.09 A + 4.52 B - 2.31 C + 1.86 D + \\
15.68 AB - 0.73 AC - 0.37 AD + \\
2.83 AE + 0.90 BC + 0.56 BD - 0.20 BE + \\
3.50 CD - 2.06 CE - 5.42 DE - 2.18 A^2 - 0.33 B^2 - \\
2.67 C^2 - 2.96 D^2 + 0.26 E^2
\] (1)

Eq. (1) indicates the dependence of response on temperature, pH, time, salt concentration and polymer concentration. Parameters of the equation were obtained by multiple regression analysis of the experimental data, where R is the predicted response in real value, and A, B, C, D, and E are the coded values of temperature, time, pH, salt concentration and polymer concentration, respectively. The mathematical model was evaluated by using ANOVA and the analysis of the residues generated between predicted values and observed values. Results demonstrated that the employed mathematical model suits well-obtained values. The model F-value of 42.19 infers that the model is significant. There is only a 0.01% chance that the large value of "Model F-value" is because of noise (Table S2). The values of "Prob > F" less than 0.0500 indicate that the model terms are significant and in that, the probability (p) value of the regression model was less than 0.0001, with no significant lack of fit (p = 0.4011). The determination coefficient (\(R^2 = 0.9871\)) was satisfactory, meaning a low experimental error according to ANOVA (Table S2).

Temperature (A), time (B) and concentration of polymer (E) had a highly significant effect (p < 0.001) on the maximum response. Apart from that, salt concentration-polymer concentration (DE) had also a highly significant effect (p < 0.001). And pH (C), salt concentration (D), pH-salt concentration interaction term (CD) and temperature-concentration of polymer interaction term (AE) and the quadratic terms (\(A^2, C^2\) and \(D^2\)) had a significant effect (p < 0.05) on the maximum response (Table S2). The coefficient of variance (C.V.) which is defined as the ratio of the standard error of estimate to the mean value of the observed response (as a percentage) is a measure of reproducibility of the model. The C.V. value was calculated to be 4.17%. As a general rule, a model can be considered reasonably reproducible if its C.V. is not greater than 10% [17]. The most important source of error could be the determination of the concentration of free gelatin which demands a precise calibration based on UV/V measurements.

The obtained plots from three-dimensional (3D) central composite design and using Eq. (1), with one fixed variable at the central point while the others are allowed to vary, are shown in Fig. 1. The plots depict the interaction between effective parameters.

##### 3.1.2. Adsorption mechanism

The effect of polymers on colloidal stability is mainly due to adsorption of macromolecules on particle surfaces and the structure of adsorbed layers formed. The electrochemical nature of particle surface changes due to adsorption of polymers and polyelectrolytes. The adsorbed polymer chains introduce steric and bridging forces in addition to perturbing Van der Waals attraction and electrostatic repulsion. The Van der Waals attraction...
is affected because dielectric constant and refractive index of the polymer are different from those of the solvent. The surface potential increases or decreases depending on polymer charge density distribution. Moreover, the range over which particle-particle interaction occurs also changes depending on the thickness of the adsorbed layer, especially tail length in case of dangling polymer chains in the solution [18].

In the case of polyampholytes, polyelectrolytes that include anionic, cationic, and uncharged monomer residues [19], the adsorption depends on the Coulomb interaction between the net charge on the chain and the charged surface. If the excess charge on the chain has the same sign as the charges of the surface, the Coulomb repulsion may inhibit adsorption [20].

The configuration of the adsorbed chains will depend on a complicated balance between the attractive and repulsive electrostatic forces [21]. The adsorption of polyampholyte chains on a solid surface occurs via three-step mechanism: i. Transport of molecules towards the surface from the bulk of the protein solution, ii. Attachment of molecules at the active sites of the surface, and iii. Change in the conformation of the adsorbing macromolecule [6].

### 3.1.3. Effect of pH

The amount of adsorbed gelatin increases with increasing pH from 3 to 5 and attain a maximum 5 (Fig. 1(a)). By further increase in pH, from 5 to 11, adsorption decreases. These observations are consistent with the finding of other studies [10, 22, 23]. It is well known that gelatin molecules achieve different charge densities and conformations at different pHs. At pH lower than IEP, the overall charge on the chains is positive and they adapt more extended conformation. In another word, under this condition, the strong repulsion between positive charges on the gelatin chains decrease folding tendency of the chains. Therefore, the possibility of binding of positively charged segment of the chain to negatively charged face of the clay platelets decreases. At IEP, however, the negative charges on gelatin chains are balanced with the positively charged units and the gelatin chains become more flexible. Under this condition, maximum adsorption occurs. Beyond IEP, deprotonation of the carboxylic groups makes the net charge on the gelatin chains more negative and therefore electrostatic repulsion develops between clay surfaces and gelatin molecules. The higher is the net negative charge on the chains, the stronger is the repulsion forces and therefore the lower is the adsorbed gelatin on the clay surfaces [22-24].

### 3.1.4. Effect of time

The amount of the adsorbed gelatin increases linearly with time (Fig. 1(a)). At longer times, the probability of collision between gelatin and clay increases and results in increasing in the amount of adsorbed gelatin. Gelatin is a polyampholyte which means it is capable to develop cationic, anionic and hydrophobic interactions. This allows the gelatin molecules to associate with the interface by both electrostatic and hydrophobic interaction. At low polymer concentrations, the electrostatic interactions drive the adsorption of gelatin molecules on clay surfaces. At higher polymer concentration at which the clay surfaces are completely covered by gelatin, the hydrophobic interactions between the adsorbed gelatin segments and free gelatins in the solution leads to more adsorption.

### 3.1.6. Effect of salt concentration

The amount of adsorbed gelatin increased with salt (NaCl) concentration in the studied range of 0.01-0.09 M, however, beyond 0.05 M, the adsorption remains nearly unchanged (Fig. 1(c)). It seems, in the studied range of ionic concentration, the salt mostly screens the electrostatic repulsion between gelatin chains and clay platelets. Therefore, the overall interaction becomes stronger and consequently adsorption increase. By increasing the ionic strength, it is expected both electrostatic attraction and repulsion screen, weaker bindings form and lower amount of gelatin adsorb on clay surfaces. The same trend for the effect of salt concentration on the interaction between proteins and charged surfaces has been reported by Karimi et al. [25].

The observed increased adsorption in the initial range of added salt may be explained by the fact that the added salt may cause neutralization of protein charge and therefore fold of the protein molecules, which results in a compact structure of gelatin. Thus, because of a reduced radii of gyration of the gelatin macromolecule, fewer active sites are occupied by the adsorbing protein molecules onto clay surface and, therefore, the gelatin adsorption increases [10]. However, at higher salt concentrations, that is, beyond 0.05 M of added salt, due to the lack of enough positive charges in gelatin, the adsorption of gelatin onto negatively charged clay surfaces decreases.

### 3.1.7. Effect of temperature

The influence of temperature on the adsorption of gelatin was studied by accomplishing the adsorption experiments in the temperature range 5-45°C. The results clearly reveals that the adsorption increases from 5°C to 35°C and then decreases with further increase in temperature (Fig. 1(c)). These results may be explained as follows; it is well known that the chains can be adsorbed onto surface in two stages; transferring of chain from solution onto the surface and rearrangement of the adsorbed chain on the surface. Since, the segmental motion of chains enhances with thermal actuators. Therefore, rearrangement of adsorbed chains takes place easily with an increase in temperature. This phenomenon gives an opportunity to other chains in solution to be adsorbed quickly onto the surface, meaning increase in the number of adsorbed chains.

From a thermodynamics point of view, when the chains are in interaction with the surface, i.e. negative enthalpy and at lower temperatures, the Bibbs free energy of adsorption (ΔG° = ΔH° - TΔS°), is negative and the adsorption process is spontaneous. However, at higher temperatures the interactions become weaker (smaller enthalpy) and at the same time TΔS° becomes larger, leading to ΔG° > 0 and positive free energy. Under this condition, chain desorption phenomenon occurs and the amount of adsorbed polymer decreases [26].
Fig. 1. The 3D central composite design plots for the effects of variables on the amount of adsorbed polymer: (a) Effect of the time and pH, (b) Effect of the polymer concentration and the salt concentration, and (c) Effect of the temperature and the salt concentration.
3.1.8. Morphology of adsorbed gelatin onto clay particles
The morphological features of the mineral clay and gelatin-adsorbed clay are presented in Fig. 2(a), Fig. 2(b), respectively. The average size of the clay particles varies in the range 1 to 10 μm, whereas for the gelatin-adsorbed clay, the size varies in the range 3 to 15 μm. Thus, big-sized particles of protein adsorbed clay may be attributed to the fact that the molecules of gelatin flocculate the mineral clay suspension due to a bridging mechanism, thus resulting in a formation of intercalation composite. Aggregation takes place as a result of particle-polymer chains collisions, thus resulting in the formation of bigger particles.

3.1.9. Optimized condition
The aim of this work was finding optimum conditions for flocculation of clay suspension by gelatin which is crucial for water treatment. For several responses and factors, all goals get combined into one desirability function. The main aim of optimization is to find a good set of conditions that will meet all the goals, not necessarily getting to a desirability value of 1.0. Desirability is a simply mathematical method to find the optimum and the numerical optimization finds a point that maximizes the desirability function (Table 3).

This optimized condition was repeated four times and the results of the adsorbed gelatin (121.50, 115.42, 120.38 and 108.22 mg/g) were in good agreement with the design of experiment’s result i.e., 114.53 mg/g (Table 3). The error difference percentage between the mean of the result of the adsorbed gelatin (116.38 mg/g) and the design of experiment’s result i.e., 114.53 mg/g is 1.62% which is within the acceptable range of 5%.

3.2. Sedimentation Experiments
3.2.1. Response surface methodology (RSM)
The Box–Behnken experimental design was exploited for finding the relationship between the response functions and variables for three different size fractions [27]. For the three-level four-factorial Box–Behnken experimental design, a total of 29 experimental runs is needed (Table 2). The design matrix and the responses are shown in Table S3. ANOVA was calculated using the software package Design-Expert 7.1.6 (Table S4) for finding the most important effects and interactions. The Box-Behnken design can be described with Eq. (2), resulting from an adjustment of the quadratic model.

\[ R = 94.36 - 2.16 A - 2.50 B - 3.68 C - 3.46 D - 2.46 AB - 1.47 AC + 3.11 AD - 0.26 BC - 0.73 BD - 1.13 CD - 4.91 A^2 - 0.97 B^2 - 0.011 C^2 - 3.46 D^2 \] (2)

Eq. (2) defines the response as a function of temperature, pH, salt concentration, and polymer concentration. Parameters of the equation were obtained by multiple regression analysis of the experimental data, where R is the predicted response in real value, and A, B, C, and D are the coded values of temperature, pH, salt concentration and polymer concentration, respectively. Synergistic or antagonistic effects of a factor were indicated by the positive or negative signs in front of that factor term, respectively. The mathematical model was evaluated by using ANOVA and the analysis of the residues generated between predicted values and observed values. The model F-value of 10.84 infers that the model is significant. There is only a 0.01% chance that the large value of "Model F-Value" is because of noise (Table S4). As mentioned before values of "Prob > F" less than 0.0500 indicate model terms are significant and in that, the probability (p) value of the regression model was less than 0.0001, with no significant lack of fit (p = 0.5896). The determination coefficient (R² = 0.9156) was satisfactory, meaning a low experimental error according to ANOVA (Table S4). The salt concentration (C) and concentration of polymer (D) had a highly significant effect (p < 0.001) on the maximum response. Aside from that, the quadratic term (A²) had a highly significant effect (p < 0.001). The temperature (A), pH (B), temperature-pH interaction term (AB) and temperature-concentration of polymer interaction term (AD) as well as quadratic terms (D²) had a significant effect (p < 0.05). The C.V. value for this experimental design is 2.42%.

Three-dimensional (3D) central composite design plots of the response, using Eq. (2) when one of the variables is fixed at the central point and the other two are allowed to vary, are shown in Fig. 3. The plots depicted interaction between effective parameters.

3.2.2. Effect of pH
The transmittance (%) of samples decreases with increasing of pH (Fig. 3(a)). When pH is 5, the number of positive charges on gelatin chains is more than when pH is 7 and 9. Therefore, gelatin can effectively causes flocculation of clay particles, larger

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Table 3. The Optimized Condition for Gelatin Adsorption on Clay Particles

| Run | Temperature, °C | Time, min | pH | Salt Concentration, M | Polymer Concentration, mg/dL | Adsorbed Polymer, mg/g | Desirability* |
|-----|-----------------|-----------|----|----------------------|-----------------------------|------------------------|---------------|
| 1   | 35              | 20        | 5  | 0.03                 | 0.7                         | 114.53                 | 1.00          |

* This is the desirability value for this solution.
clots, quickly settled and thus reduced in the turbidity of the sample. And, when pH raises, the number of the negative charges on gelatin chain increases and due to less number of the active site (positively charge), less adsorption takes place. Hence, the small formed flocs lead to a greater stability and high turbidity.

3.2.3. Effect of polymer concentration

The turbidity of the samples decreased with gelatin concentration from 0.002 to 0.004 g/dL, and beyond 0.004 g/dL, the turbidity increases (Fig. 3(a)). In sedimentation/flocculation area, an essential requirement for bridging flocculation is the existence of sufficient unoccupied surface on a particle for attachment of polymer segments which has been previously adsorbed on other particles. And since the number of connections through the bridging between particles depends on the amount of adsorbed polymer, the optimal conditions is needed to attain maximum efficiency of flocculation. Due to this trend, with increasing of gelatin concentration up to 0.004 g/dL, bridging connections becomes possible. Thus, a greater number of particles can be connected with each other by gelatin chains. This means a better flocculation, reduction in turbidity and increase in transmittance. With further increase in gelatin concentration, the number of adsorbed chains on particle surfaces increases, so there is no sufficient unoccupied surface on particle surface for bridging between the particles. Thus, the size of flocs becomes smaller and turbidity increases.

Measuring the rheological behavior of the flocs is necessary in order to characterize their internal structure. So, the linear viscoelastic properties of the gelatin-clay flocs were measured by using low-amplitude oscillatory shear rheometry in frequency sweep mode. Dynamic rheology behavior for all samples containing different concentrations of the polymer was studied. Significantly, all samples have a storage modulus ($G'$) greater

Fig. 3. The 3D Box-Behnken design plots for the effects of variables on % transmittance: (A) effect of pH and polymer concentration, and (B) effect of the temperature and salt concentration on the amount of % transmittance.
than the loss modulus ($G''$), where the gel-like behavior of the
flocs is indicated. However, the flocs formed at the optimum
copolymer concentration have denser structure and higher storage
modulus.

3.2.4. Effect of salt concentration

The transmittance of samples decreases with salt (NaCl) concen-
tration in the studied range of 0-0.1 M (Fig. 3(b)). The reason
lies in the fact that gelatin active sites are screened by salt ions,
resulting in fewer active sites for adsorption on the surface of
clay particles and polymeric bridging. As well, the repulsive forces
between the remained negatively charge of gelatin and negatively
charge of clay particles cause to reduction in gelatin adsorption.
As a result, the size of formed flocs decreases, turbidity increase
and transmittance of the samples decreases.

3.2.5. Effect of temperature

Temperature effect on the gelatin flocculation was studied by
turbidity tests in the temperature range of 15-35°C. The results
show that the turbidity increases from 15°C to 25°C and then
decreases with further increases in temperature (Fig. 3(b)). As
it is mentioned above, the rearrangement of adsorbed chains in-
creases with temperature, which in turn leads to adsorption of
more gelatin chains onto the clay surfaces and creating oppor-
tunities for bridging between gelatin-clay particles. Thereby, big-
ger flocs increase turbidity. At higher temperatures, however,
the gelatin desorption process is dominated by entropic term and
therefore bridging of particles becomes weaker and smaller
gelatin-clay flocs form. This leads to decrease in turbidity.

4. Conclusions

In this work, the adsorption of gelatin from its aqueous solution
onto the mineral clay surfaces at different conditions was studied.
Design of expert software was used for optimization of various
parameters, including mixing time, temperature, pH, salt (NaCl)
and polymer concentration. The temperature, time and salt had
a dual effect on gelatin adsorption and an optimum value was
observed for each of them. Polymer adsorption was continuously
increased with time and polymer concentration. The results were
interpreted based on the electrostatic interaction between the
clay-gelatin as well as gelatin-gelatin. Morphological observations
also confirmed an increase in particle size and formation of flocs
in the presence of the gelatin. Finally, the effectiveness of gelatin
in water treatment process was investigated by lab-scale jar
experiments. The jar tests confirmed the proficiency of gelatin
as a new class of low cost, biocompatible, non-toxic and bio-
degradable flocculent for water treatment.

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**References**

1. Barkacs K, Bohuss I, Bukovsky A, Varga I, Zamy G. Comparison
   of polyelectrolytes applied in drinking water treatment. Micro-
   roc. J. 2000;67:271-277.
2. Valipour M. Surface irrigation simulation models: A review. Int.
   J. Hydrol. Sci. Technol. 2015;5:51-70.
3. Yannopoulos SI, Lyberatos G, Theodossiou N, et al. Evolution
   of water lifting devices (pumps) over the centuries worldwide.
   Water 2015;7:5031-5060.
4. Valipour M. Sprinkle and trickle irrigation system design using
   tapered pipes for pressure loss adjusting. J. Agr. Sci. 2012;4:125-133.
5. Kawamura S. Considerations on improving flocculation. J.
   Am. Water. Works Ass. 1976;68:328-336.
6. Boltoa B, Gregoryb J. Organic polyelectrolytes in water
   treatment. Water Res. 2007;41:2301-2324.
7. Overview of the effects of residual flocculants on aquatic re-
   ceiving environments [Internet]. Technical Publication No
   226. Available from: http://www.aucklandcouncil.govt.nz/EN/
   planspoliciesprojects/reports/technicalpublications/Pages/
   technicalpublications201-250.aspx.
8. Piazza GJ, Garcia RA. Proteins and peptides as renewable
   flocculants. Bioresour. Technol. 2010;101:5759-5766.
9. Piazza GJ, Garcia RA. Meat & bone meal extract and gelatin
   as renewable flocculants. Bioresour. Technol. 2010;101:781-787.
10. Bajpai AK, Vishwakarma N. An adsorption study of gelatin
    onto the Fuller’s earth surfaces. J. Appl. Polym. Sci. 2005;98:42-52.
11. Maternaghan TJ, Bunghan OB, Ottewill RH. The adsorbed
    layer of gelatin on silver bromide - its nature and photographic
    significance. J. Photogr. Sci. 1980:28:1-13.
12. Kawanshi N, Christenson HK, Ninham BW. Measurement of
    the interaction between adsorbed polyelectrolytes: Gelatin
    on mica surfaces. J. Phys. Chem. 1990:94:461-4617.
13. Khani R, Shemirani F, Majidi B. Combination of dispersive
    liquid–liquid microextraction and flame atomic absorption
    spectrometry for preconcentration and determination of copper
    in water samples. Desalination 2011;266:238-243.
14. Cosgrove T, Hone JHE, Howe AM, Heenan RK. A small-angle
   neutron scattering study of the structure of gelatin at the surface
   of polystyrene latex particles. Langmuir 1998;14:5376-5382.
15. Howe AM, Clarke A. Viscosity of emulsions of polydisperse droplets with a thick adsorbed layer. *Langmuir* 1997;13: 2617-2626.
16. Korbalti BK, Aktas N, Tanyolac A. Optimization of electrochemical treatment of industrial paint wastewater with response surface methodology. *J. Hazard. Mater.* 2007;148:83-90.
17. Ahmadi M, Vahabzadeh F, Bonakdarpour B, Mofarrah E, Mehranian M. Application of the central composite design and response surface methodology to the advanced treatment of olive oil processing wastewater using Fenton's peroxidation. *J. Hazard. Mater.* 2005;123:187-195.
18. Runkana V. A population balance model for flocculation of colloidal suspensions by polymer bridging. *Chem. Eng. Sci.* 2006;61:182-191.
19. Ciferri A, Kudaibergenov S. Natural and synthetic polyampholytes, 1 theory and basic structures. *Macromol. Rapid Commun.* 2007;28:1953-1968.
20. Dobrynin AV, Rubinstein M. Adsorption of a polyampholyte chain on a charged surface. *Macromolecules* 1997;30:4332-4341.
21. Khan MO, Chan DYC. Monte carlo simulations of stretched charged polymers. *J. Phys. Chem. B.* 2003;107:8131-8139.
22. Lee JF, Tseng DH, Wen PT. Behavior of organic polymers in drinking water purification. *Chemosphere* 1998;37:1045-1061.
23. Turner SF. Adsorption of gelatin to a polystyrene/water interface as a function of concentration, pH, and ionic strength. *Langmuir* 2005;21:10082-10088.
24. Schrieber R, Gareis H. Gelatine handbook: Theory and industrial practice. Wiley-VCH GmbH & Co.; 2007, p. 61-62.
25. Karimi F, Taheri Qazvini N, Namivandi-Zanganeh R. Fish gelatin/Laponite biohybrid elastic coacervates: A complexation kinetics-structure relationship study. *Int. J. Biol. Macromol.* 2013;61:102-113.
26. Tekin N, Dincer A, Demirbas O, Alkan M. Adsorption of cationic polyacrylamide onto sepiolite. *J. Hazard. Mater.* 2006;134:211-219.
27. Ahmad AL, Wong SS, Teng TT, Zuhairi A. Optimization of coagulation-flocculation process for pulp and paper mill effluent response surface methodological analysis. *J. Hazard. Mater.* 2007;145:162-168.
Table S1. Design Matrix and the Results of the Central Composite Fractional Factorial Design

| Run | Temperature (°C) | Time (min) | pH | Salt Concentration (M) | Polymer Concentration (mg/dL) | Adsorbed Polymer (mg/g) |
|-----|------------------|------------|----|------------------------|------------------------------|------------------------|
| 1   | 1                | –1         | 1  | 1                      | 1                            | 70.21                  |
| 2   | 1                | –1         | 1  | –1                     | 1                            | 95.11                  |
| 3   | 2                | 0          | 0  | 0                      | 0                            | 84.08                  |
| 4   | 1                | –1         | –1 | 1                      | 1                            | 97.87                  |
| 5   | 0                | 0          | 0  | 0                      | 0                            | 87.09                  |
| 6   | 0                | 0          | 0  | 0                      | 0                            | 80.01                  |
| 7   | 1                | 1          | –1 | –1                     | 1                            | 114.20                 |
| 8   | 0                | 0          | 0  | 2                      | 0                            | 74.88                  |
| 9   | 0                | 0          | 0  | 0                      | –2                           | 55.23                  |
| 10  | –1               | –1         | –1 | 1                      | –1                           | 54.77                  |
| 11  | 1                | 1          | 1  | 1                      | –1                           | 70.13                  |
| 12  | –1               | 1          | 1  | –1                     | 1                            | 84.39                  |
| 13  | 0                | 0          | 0  | –2                     | 0                            | 69.88                  |
| 14  | –1               | –1         | 1  | –1                     | –1                           | 39.99                  |
| 15  | 0                | 0          | 0  | 0                      | 2                            | 114.50                 |
| 16  | 1                | 1          | 1  | 1                      | –1                           | 55.27                  |
| 17  | 0                | 0          | –2 | 0                      | 0                            | 79.10                  |
| 18  | 0                | 0          | 0  | 0                      | 0                            | 84.03                  |
| 19  | 0                | 0          | 0  | 0                      | 0                            | 87.99                  |
| 20  | 1                | 1          | 1  | 1                      | 1                            | 100.11                 |
| 21  | 0                | 0          | 0  | 0                      | 0                            | 81.06                  |
| 22  | –2               | 0          | 0  | 0                      | 0                            | 65.24                  |
| 23  | 0                | –2         | 0  | 0                      | 0                            | 69.88                  |
| 24  | –1               | 1          | –1 | –1                     | –1                           | 55.08                  |
| 25  | –1               | –1         | 1  | 1                      | 1                            | 73.58                  |
| 26  | –1               | 1          | 1  | 1                      | –1                           | 75.33                  |
| 27  | 0                | 0          | 0  | 0                      | 0                            | 83.32                  |
| 28  | 0                | 0          | 0  | 0                      | 0                            | 89.07                  |
| 29  | –1               | –1         | –1 | –1                     | 1                            | 84.32                  |
| 30  | –1               | 1          | –1 | 1                      | 1                            | 95.11                  |
| 31  | 0                | 2          | 0  | 0                      | 0                            | 67.20                  |
| 32  | 0                | 0          | 2  | 0                      | 0                            |                       |

* All experiment were done in one block.

Table S2. Analysis of Variance Table (ANOVA) for Response Surface Quadratic Model

| Source          | Sum of Squares | df | Mean Square | F Value | p-value | Prob > F |
|-----------------|----------------|----|-------------|---------|---------|----------|
| Model           | 8961.00        | 20 | 448.05      | 42.19   | < 0.0001| significant |
| A               | 888.78         | 1  | 888.78      | 83.70   | < 0.0001|          |
| B               | 490.42         | 1  | 490.42      | 46.18   | < 0.0001|          |
| C               | 128.11         | 1  | 128.11      | 12.06   | 0.0052  |          |
| D               | 83.14          | 1  | 83.14       | 7.83    | 0.0173  |          |
| E               | 5971.25        | 1  | 5971.25     | 555.36  | < 0.0001|          |
| AB              | 42.29          | 1  | 42.29       | 3.76    | 0.0774  |          |
| AC              | 8.63           | 1  | 8.63        | 0.81    | 0.3867  |          |
| AD              | 2.20           | 1  | 2.20        | 0.21    | 0.6580  |          |
| AE              | 128.20         | 1  | 128.20      | 12.07   | 0.0052  |          |
| BC              | 12.94          | 1  | 12.94       | 1.22    | 0.2932  |          |
| BD              | 4.94           | 1  | 4.94        | 0.47    | 0.5093  |          |
| BE              | 0.06           | 1  | 0.06        | 0.002   | 0.8077  |          |
| CD              | 195.51         | 1  | 195.51      | 18.41   | 0.0013  |          |
| CE              | 67.61          | 1  | 67.61       | 6.37    | 0.0283  |          |
| DE              | 470.78         | 1  | 470.78      | 44.33   | < 0.0001|          |
| A2              | 138.90         | 1  | 138.90      | 13.08   | 0.0041  |          |
| B2              | 3.19           | 1  | 3.19        | 0.30    | 0.5946  |          |
| C2              | 208.49         | 1  | 208.49      | 19.63   | 0.0010  |          |
| D2              | 257.40         | 1  | 257.40      | 24.24   | 0.0005  |          |
| E2              | 2.02           | 1  | 2.02        | 0.19    | 0.6708  |          |
| Residual        | 116.81         | 11 | 10.62       |         |         |          |
| Lack of Fit<sup>d</sup> | 70.82 | 6  | 11.80       | 1.28    | 0.4011  | not significant |
| Pure Error      | 45.99          | 5  | 9.20        |         |         |          |
| Cor Total       | 9077.81        | 31 |             |         |         |          |

<sup>a</sup> Degrees of freedom.
<sup>b</sup> Test for comparing model variance with residual (error) variance.
<sup>c</sup> Probability of seeing the observed F-value if the null hypothesis is true.
<sup>d</sup> The variation of the data around the fitted model.
### Table S3. Design Matrix and the Results of the Box-Behnken Design

| Run | Temperature (°C) | pH | Salt Concentration (M) | Polymer Concentration (g/L) | Transmittance (%) |
|-----|------------------|----|------------------------|-----------------------------|-------------------|
| 1   | 0                | 0  | −1                     | 1                           | 93.16             |
| 2   | 0                | 0  | −1                     | 1                           | 97.86             |
| 3   | 1                | 0  | 0                      | −1                          | 82.30             |
| 4   | 1                | 0  | 0                      | 1                           | 83.87             |
| 5   | 0                | 1  | 0                      | 0                           | 90.67             |
| 6   | 1                | 1  | 0                      | −1                          | 80.25             |
| 7   | 0                | −1 | 0                      | −1                          | 96.61             |
| 8   | 1                | 0  | 0                      | 1                           | 83.06             |
| 9   | 0                | 1  | 1                      | 0                           | 90.07             |
| 10  | 0                | 0  | 0                      | 0                           | 93.81             |
| 11  | 0                | −1 | 0                      | 1                           | 91.28             |
| 12  | −1               | 0  | 0                      | 1                           | 81.27             |
| 13  | 1                | 0  | −1                     | 0                           | 92.63             |
| 14  | −1               | 0  | 0                      | −1                          | 90.29             |
| 15  | −1               | 0  | 0                      | −1                          | 94.50             |
| 16  | 0                | 1  | 0                      | −1                          | 91.16             |
| 17  | 0                | −1 | −1                     | 0                           | 95.54             |
| 18  | −1               | −1 | 0                      | 0                           | 91.29             |
| 19  | 0                | 1  | −1                     | 0                           | 95.30             |
| 20  | 0                | 0  | 1                      | −1                          | 95.70             |
| 21  | −1               | 1  | 0                      | −1                          | 89.65             |
| 22  | 0                | 1  | 0                      | 1                           | 82.90             |
| 23  | 0                | 0  | 1                      | 1                           | 90.39             |
| 24  | 0                | 0  | 0                      | −1                          | 96.33             |
| 25  | −1               | 0  | −1                     | 0                           | 94.75             |
| 26  | 0                | 0  | 0                      | 0                           | 95.69             |
| 27  | 0                | −1 | −1                     | 0                           | 95.55             |
| 28  | 1                | −1 | 0                      | 0                           | 92.74             |
| 29  | 0                | 0  | −1                     | 0                           | 91.11             |

* All experiments were done in one block.

### Table S4. Analysis of Variance Table (ANOVA) for Response Surface Quadratic Model

| Source | Sum of Squares | df | Mean Square | F Value | p-value | Prob > F |
|--------|----------------|----|-------------|---------|---------|----------|
| Model  | 728.21         | 14 | 52.01       | 10.84   | < 0.0001| significant |
| A      | 55.90          | 1  | 55.90       | 11.65   | 0.0042  |
| B      | 75.05          | 1  | 75.05       | 15.65   | 0.0014  |
| C      | 162.51         | 1  | 162.51      | 33.88   | < 0.0001|          |
| D      | 143.87         | 1  | 143.87      | 29.99   | < 0.0001|          |
| AB     | 24.26          | 1  | 24.26       | 5.06    | 0.0412  |
| AC     | 8.61           | 1  | 8.61        | 1.80    | 0.2016  |
| AD     | 38.56          | 1  | 38.56       | 8.04    | 0.0132  |
| BC     | 0.27           | 1  | 0.27        | 0.055   | 0.8175  |
| BD     | 2.15           | 1  | 2.15        | 0.45    | 0.5144  |
| CD     | 5.11           | 1  | 5.11        | 1.06    | 0.3196  |
| A2     | 156.59         | 1  | 156.59      | 32.64   | < 0.0001|          |
| B2     | 6.10           | 1  | 6.10        | 1.27    | 0.2785  |
| C2     | 7.613E-004     | 1  | 7.613E-004  | 1.587E-004 | 0.9901  |
| D2     | 77.63          | 1  | 77.63       | 16.18   | 0.0013  |
| Residual| 67.15         | 14 | 4.80        |         |         |
| Lack of Fit| 46.70       | 10 | 4.67        | 0.91    | 0.5896  | not significant |
| Pure Error| 20.45         | 4  | 5.11        |         |         |
| Cor Total| 795.39        | 28 |             |         |         |

a Degrees of freedom.
b Test for comparing model variance with residual (error) variance.
c Probability of seeing the observed F-value if the null hypothesis is true.
d The variation of the data around the fitted model.