Malaysian Journal of Chemistry, 2020, Vol. 22 (1), 56-68
(Special Issue: *7th International Conference for Young Chemists - ICYC 2019*, Universiti Sains Malaysia)

Turbidity Removal of Kaolin Wastewater Using Polyacrylamide-Grafted Egg White†

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Polyacrylamide-grafted egg white (Egg-g-PAM) has been successfully synthesized by microwave-assisted graft copolymerization technique using ceric ammonium nitrate (CAN) as an initiator. The grafting process was optimized at various acrylamide weight, CAN weight, and microwave irradiation time. The grafting of polyacrylamide (PAM) chains on the egg white backbone was confirmed through percentage of grafting efficiency (%GE) and percentage water absorption (%WA). Egg white (control) and optimized Egg-g-PAM were further characterized by Fourier transform infrared (FTIR) spectroscopy, field emission scanning electron microscopy (FESEM) analysis, elemental analysis, thermogravimetric/derivative thermogravimetry (TG/DTG) analysis, and point of zero charge (pH_{zpc}). The turbidity removal for alum, egg white, acrylamide, and Egg-g-PAM was determined on 0.25 g/L kaolin suspension at pH 7 and 2 g/dL dosage. The % turbidity removal of Egg-g-PAM (95.55%) was greater than that of alum (94.58%) acrylamide (25.52%), and egg white (19.89%).

Key words: Albumin; graft copolymer; microwave-assisted technique; engineered flocculant; coagulant

Received: September 2019; Accepted: January 2020

Coagulation is a process of altering the physical state of suspended solids by addition of coagulants, while flocculation is a process that encourages primary floc to interact and progressively form larger agglomerates [1]. Alum ($Al_2(SO_4)_3$) has been used as a coagulant in wastewater treatment due to its low-cost of operation and high efficiency in the coagulation process. However, there are several issues related to the use of alum such as generation of huge volumes of sludge, possible neurotoxicity of aluminum residuals, and undesirable alteration of pH of the treated water [2-3]. A natural polymer such as egg white is more environmentally friendly and can be an alternative coagulant for water treatment.

Egg white contains high amounts of proteins such as ovalbumin (≥50% of the total protein in egg white proteins) that have high molecular weight (45 kDa) [4]. High molecular weight of ovalbumin will increase the coagulation/flocculation performance as it will help in the aggregation action thus facilitating the bridging mechanism [5]. However, egg white has a short shelf life as it contains hydrolysable in the polymer backbone, which are susceptible to biodegradation through the hydrolysis process [6]. As a result, causing a decrease in coagulation efficiency. In order to increase the shelf life of egg white, synthetic polymer (i.e., acrylamide) may be grafted into the egg white backbone.

Acrylamide is water-soluble and non-toxic in water as it is degraded by microorganisms within a few days, but highly mobile which may contaminate groundwater [7-9]. However, acrylamide will be counted as a contaminant in water when above 0.5 ppb [8]. Grafting of PAM chains onto egg white will produce a fairly stable construct and exhibit better flocculation characteristics as it has better approachability to colloidal particles in effluents [10]. The usage of acrylamide for grafting natural polymers has also been conducted on oatmeal [10] and barley [7].

In this study, graft copolymerization onto egg white was conducted using acrylamide (monomer) and CAN (initiator) under microwave radiation. The effects of reaction variables (i.e., weight of acrylamide, weight of CAN, and irradiation time) on the percentages of

†Paper presented at the 7th International Conference for Young Chemists (ICYC 2019), 14-16 August 2019, Universiti Sains Malaysia.

grafting efficiency (%GE) and water absorption (%WA) were investigated. Egg white and Egg-g-PAM were characterized by FTIR, FESEM, elemental analysis, and TGA. The % turbidity removal of alum, egg white, and Egg-g-PAM was investigated on kaolin suspension (0.25 g/L).

MATERIALS AND METHOD

Materials

Eggs were purchased from a local supermarket and the egg white was separated and then dried in an oven at 70°C for 24 h. The dried egg white was pulverized and sieved to <200 µm. Acrylamide (98.5%), CAN (99.0%), hydroquinone (99.5%), and acetone (99.0%) were supplied by R&M chemicals, Malaysia, and were used without further purification.

Synthesis of Egg White-grafted Polyacrylamide (Egg-g-PAM)

Graft copolymerization was optimized on the weight of acrylamide (g) and CAN (g), and microwave irradiation time (min). Egg white suspension was prepared by mixing 1 g of egg white powder in 40 mL of distilled water. Acrylamide was dissolved in 10 mL of distilled water at various weights, mixed with 0.3 g of CAN using magnetic stirrers and subjected to microwave irradiation at 800 W, around 65°C for 2 min. After the microwave irradiation process was completed, the mixtures were cooled for 24 h. The grafting reaction was ended by adding 10 mL of hydroquinone (10 M). The gel-like mass of Egg-g-PAM was washed with excess acetone and distilled water, dried at 70°C for 24 h, blended, sieved to <200 µm, and its final weight was determined with a calibrated analytical balance. The experiment was repeated at various CAN weights and irradiation time as in Table 1. The %GE was calculated according to Eq. 1 [12].

$$\%\text{GE} = \frac{Wt_1 - \text{Wt}_o}{Wt_2} \times 100\% \qquad \text{Eq. 1}$$

Where, Wt_1 is the weight of Egg-g-PAM, Wt_o is the weight of egg white, and Wt_2 is the weight of acrylamide.

Water Absorption Capacity (%WA)

Water absorption capacity was determined by adding 0.2 g of dried Egg-g-PAM to 200 mL of distilled water and allowed to swell for 24 h at room temperature (25°C). The fully swollen Egg-g-PAM was then filtered through a sieve (100 mesh) for 1 h and weighed using a calibrated analytical balance. The %WA was calculated according to Eq. 2 [13].

$$\%\,WA = \frac{Wt_3 - Wt_1}{Wt_1} \times 100\% \qquad \text{Eq. 2}$$

Where, Wt_1 is the weight of the dry Egg-g-PAM (g) and Wt_3 is the weight of the swollen Egg-g-PAM (g).

Characterization

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of egg white, acrylamide, and Egg-g-PAM were recorded on KBr pellets, using a FTIR spectrometer (Perkin Elmer, Spectrum 400) at the wavenumber range of 450-4000 cm^{-1}.

Table 1. Synthesis parameters of Egg-g-PAM

Parameters	Weight of acrylamide (g)	Weight of CAN (g)	Irradiation time (min)
Effect of acrylamide weight	1, 5, 10, 15, 20	0.3	2
Effect of CAN weight	OP	0, 0.3, 0.6, 0.9, 1.2	2
Effect of irradiation time	OP	OP	0 to 5

OP: Optimum parameter

Field Emission Scanning Electron Microscopy (FESEM) Analysis

Surface morphology was analyzed by FESEM on gold-coated powder samples of egg white and Egg-g-PAM. The FESEM images were recorded at 1000× magnification.

Elemental Analysis

The elemental analysis (carbon, hydrogen, nitrogen and oxygen) of acrylamide, egg white, and Egg-g-PAM was carried out with an elemental analyzer (Thermo Scientific, Flash 2000 Elemental Analyzer).

Thermal Gravimetric/ Derivative Thermo-gravimetry (TG/DTG) Analysis

The TG/DTG analysis of egg white, acrylamide, and Egg-g-PAM was carried out with a TG analyzer (Perkin Elmer, Pyris) in nitrogen atmosphere at a heating rate of 10˚C/min up to a peak temperature of 700°C.

Point of Zero Charge (pH_{pzc})

pH_{pzc} of egg white and Egg-g-PAM was determined by using salt addition method [14]. The experiment was conducted in 250 mL conical flasks containing 100 mL of 0.01 M KNO_3 and the initial pH (pH_i) was adjusted by adding 0.1 M HCl or 0.1 M NaOH. Next, 50 mL of KNO_3 was added with 0.5 g of sample. The mixtures were then kept in a shaker for 48 h at 28˚C. The final pH (pH_f) for each of the suspensions was recorded by using a calibrated pH meter. The difference between initial and final pH values, ΔpH was plotted against initial pH. The pH_{pzc} was determined at the point of intersection at ΔpH = 0.

Turbidity Removal

The turbidity removal of alum, egg white, and Egg-g-PAM was evaluated at various initial pH of kaolin suspension at 2 g/dL dosage with 300 mL of 0.25 g/L of kaolin suspension, as in Table 2. The mixtures were stirred at 260 rpm for the first 5 min, then at 60 rpm for the next 15 min. The mixtures were allowed to settle for 1 h. The supernatant was collected at around 2 cm below the surface and the turbidity was determined by using a turbidity meter (Hach 2100Q). The experiment was repeated with various dosages at pH 7 and 1 h sedimentation time. The experiment was duplicated and carried out at room temperature (23–25°C). A baseline was established on an aqueous kaolin suspension without addition of any chemicals. The % turbidity removal was calculated according to Eq. 3 [12].

$$\%\text{Turbidity removal} = \frac{T_i - T_f}{T_i} \times 100\% \qquad \text{Eq. 3}$$

Where, T_i is the initial turbidity (NTU) and T_f is the final turbidity (NTU).

RESULTS AND DISCUSSION

Synthesis of Egg-g-PAM

Effects of Acrylamide Weight

Figure 1 shows that %GE for Egg-g-PAM increased with increasing weight of acrylamide (from 1 to 15 g) due to the large availability of grafting sites and accumulation of acrylamide molecules in close proximity to egg white backbone [15]. Beyond 15 g, %GE remained constant due to the reduction of active sites (radicals on egg white backbone) and formation of homopolymers [16]. The homopolymers could have hindered and decreased the penetration rate of acrylamide to the egg white-free radicals thus decreasing %GE as shown in Scheme 1 [17]. %WA increased as the weight of acrylamide increased (1 to 20 g), possibly due to the increase in the hydrophilic character of Egg-g-PAM in which greater amounts of the primary amine group was grafted on egg white protein. At 5 to 10 g, there was significant increase in %WA (372.70% to 1788.51%). The large increase of %GE at 10 g showed a high amount of PAM that successfully grafted on egg white and formed longer PAM chains that trapped and held more water molecules [18].

Table 2: Conditions for turbidity removal

Parameters	pH	Dosage (g/dL)
Effect of initial pH	3, 5, 7, 9, 11	2
Effect of dosage	7	1, 2, 3, 4, 5

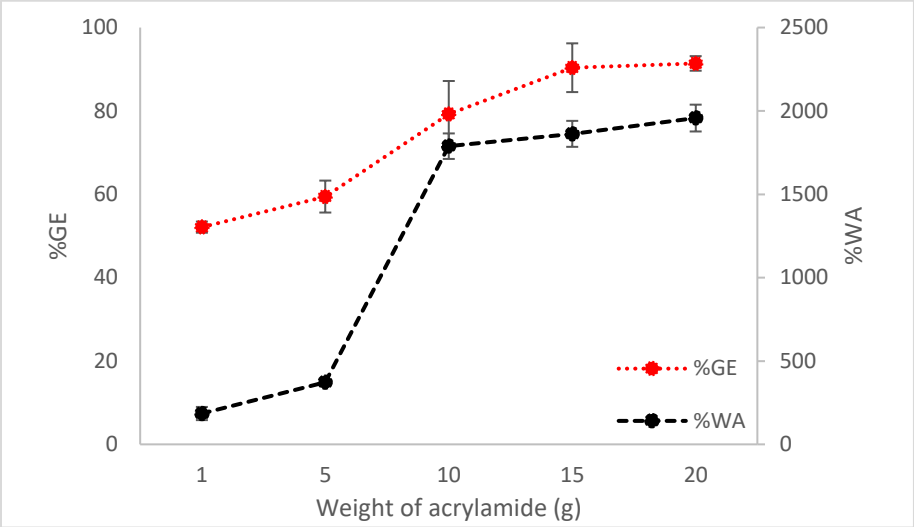


Figure 1. %GE and %WA as functions of acrylamide weight (g) [Weight of egg white: 1 g; weight of CAN: 0.3 g; microwave irradiation time: 2 min]

Effects of CAN Weight

As shown in Figure 2, %GE and %WA increased when CAN weight was increased from 0.3 to 0.6 g, then decreased when CAN weight increased beyond 0.6 g. The initial increase of %GE and %WA was due to the larger availability of free radicals on the egg white backbone that readily reacted with PAM to form more grafts [19]. %GE decreased subsequently (0.9 to 1.2 g), probably due to the termination of the grafting process by ceric ion that resulted in short chains of PAM grafted on egg white and initiation of homopolymers by excess ceric ions [13,17,19]. The decrease in %WA (from 0.9 to 1.2 g) was due to desorption of water molecules due to decrease in PAM grafts. The effect of CAN weight on %WA was lower as compared to the effect of acrylamide weight as 1g of acrylamide was used for the grafting process for this parameter [18].

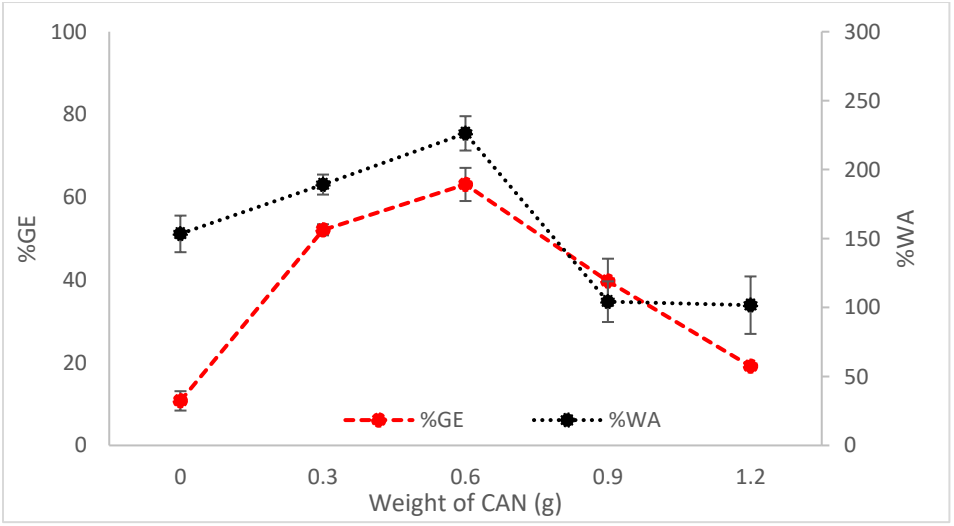
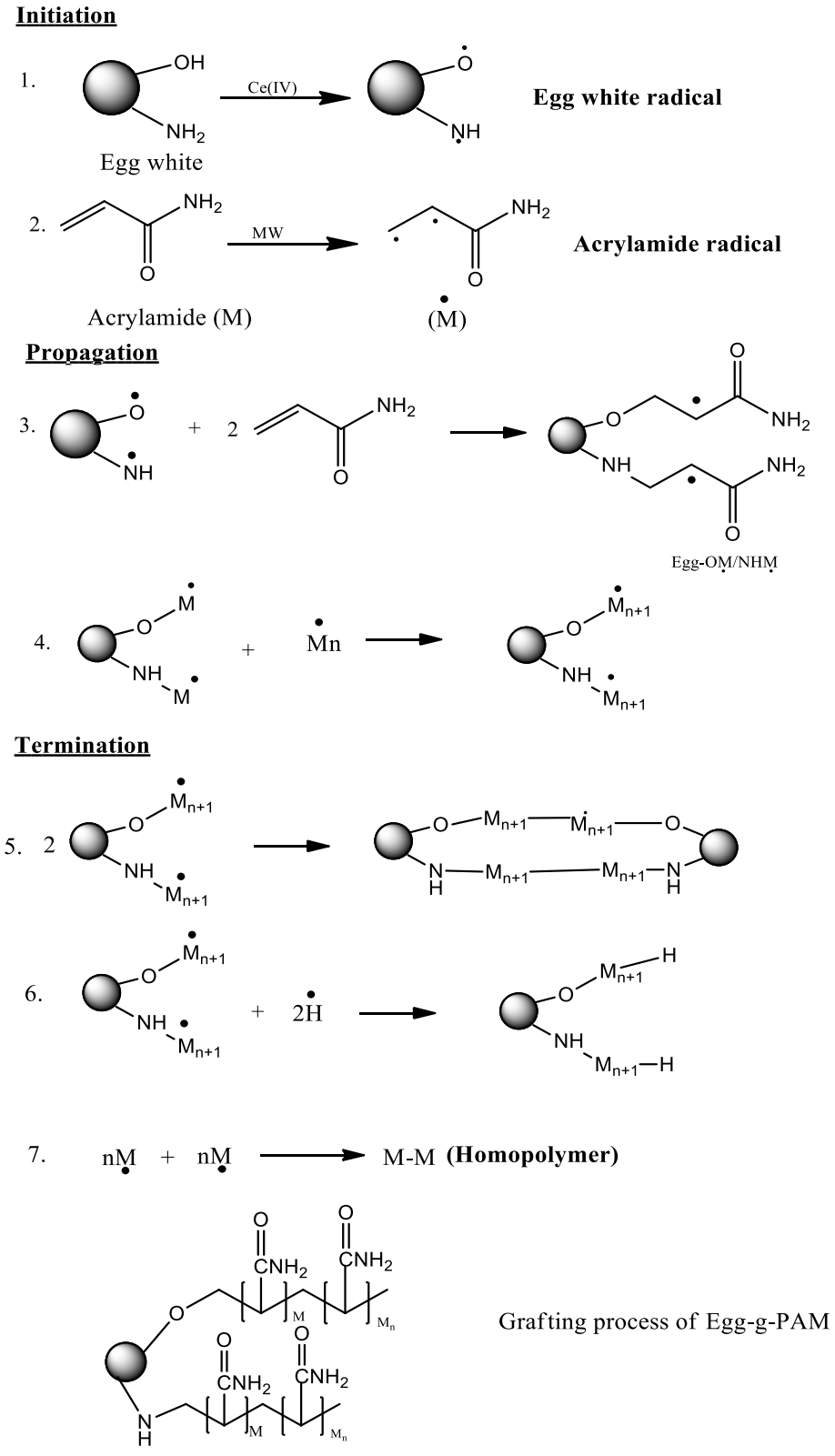


Figure 2. %GE and %WA as functions of CAN weight (g) [Weight of egg white; 1g, weight of acrylamide: 1g; irradiation time: 2min].



Grafting process of Egg-g-PAM

Scheme 1. Schematic representation of the mechanism for microwave-assisted synthesis of Egg-g-PAM

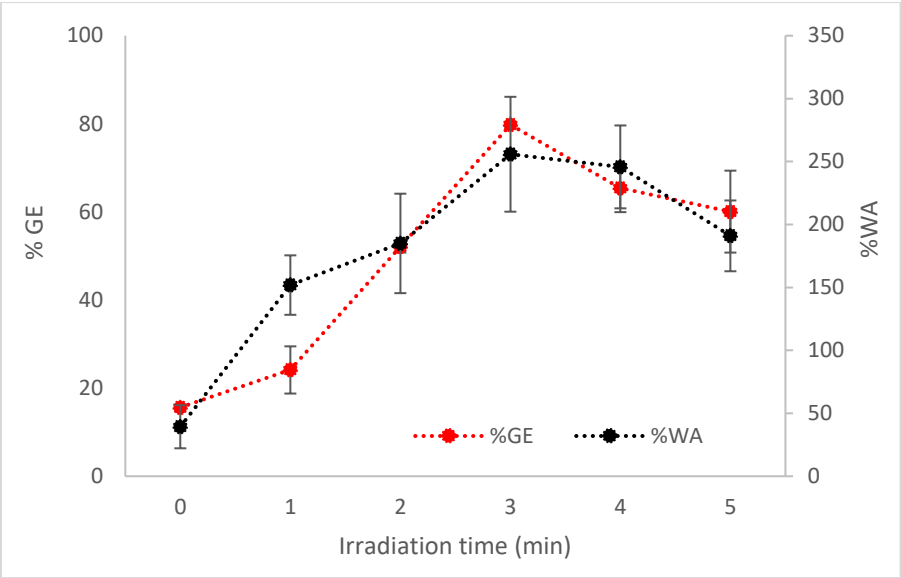


Figure 3. %GE and %WA as functions of microwave irradiation time (min) [Weight egg white: 1 g; weight of acrylamide: 1 g; weight of CAN: 0.3 g]

Effects of Microwave Irradiation Time

%GE and %WA increased with increasing irradiation time from 1 to 3 min (Figure 3). Increase in the interaction between microwave irradiation, acrylamide, CAN, and egg white would increase the generation of free radicals on the egg white backbone [19]. The decrease of %GE (3 to 5 min) was possibly due to the depletion and loss in concentration of egg white and acrylamide during the reaction process [18-19]. %WA was dependent upon %GE. The decrease in %GE reflected the decrease in the length of PAM chains and resulted in the decrease in %WA [20].

Characterization

FTIR Analysis

Based on Figure 4, the FTIR spectrum for egg white showed broad peaks at 3400 cm^{-1}, which corresponded to O-H and N-H stretching bands. Next, bands at 1650 cm^{-1} and 1533 cm^{-1} were due to the stretching of C=O from amide I and C-N stretch with N-H bending mode from amide II band, respectively, from the protein structure (albumin) of egg white [7, 21, 22]. The acrylamide spectrum showed a broad and intense band at 3356 cm^{-1}, due to the presence of N-H stretching of amine (NH_2) group. Two strong peaks at 1673 and 1615 cm^{-1} were due to C=O stretching of amide group and N-H bending, respectively [13]. The peak at 1421 cm^{-1} was attributed to C-N [23]. A sharp peak at 1280 cm^{-1} was due to NH_2 rocking [24]. N-H wagging vibrations occurred at 841 and 709 cm^{-1} [13].

For Egg-g-PAM, bands at 3254 and 1650 cm^{-1} indicated O-H and C=O stretching, respectively. The intensity of O-H and C=O (amide I) bands decreased as one of the active sites for radical formation as in Scheme 1. The C-N stretch band (1474 cm^{-1}) shifted to a lower wavenumber. A sharp peak of N-H_2 appeared at 1208 cm^{-1} which indicated the increase in NH_2 concentration in Egg-g-PAM. At 1054 cm^{-1}, the intensity of C-O band decreased as compared to egg white. This was due to the change from hydroxyl to ether group after grafting. The small peaks which appeared at 830 and 757 cm^{-1} indicated the presence of N-H wagging vibrations that only appeared in the acrylamide spectrum [11].

FESEM Analysis

Based on Figure 5, the surface of egg white showed a smooth and flaky structure while Egg-g-PAM had rough and irregular surface. Grafting of PAM onto egg white led to a denser embedment and non-uniform distribution of acrylamide on the egg white surface that might improve the natural characteristics of egg white [19].

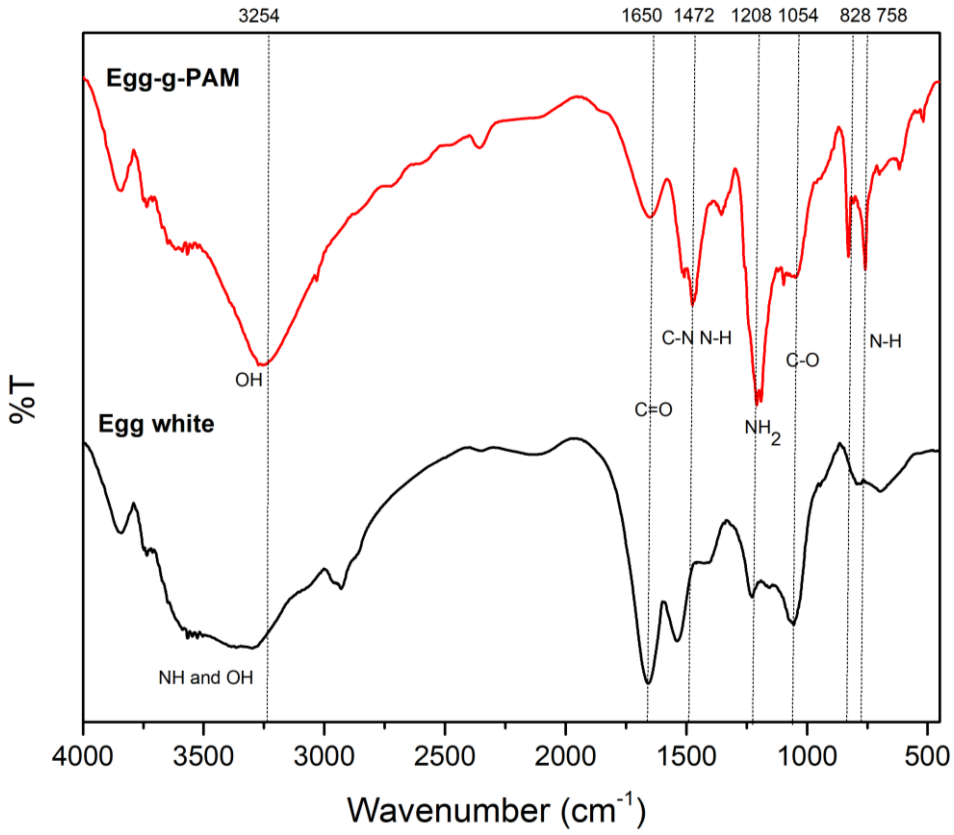


Figure 4. FTIR spectra of egg white and Egg-g-PAM

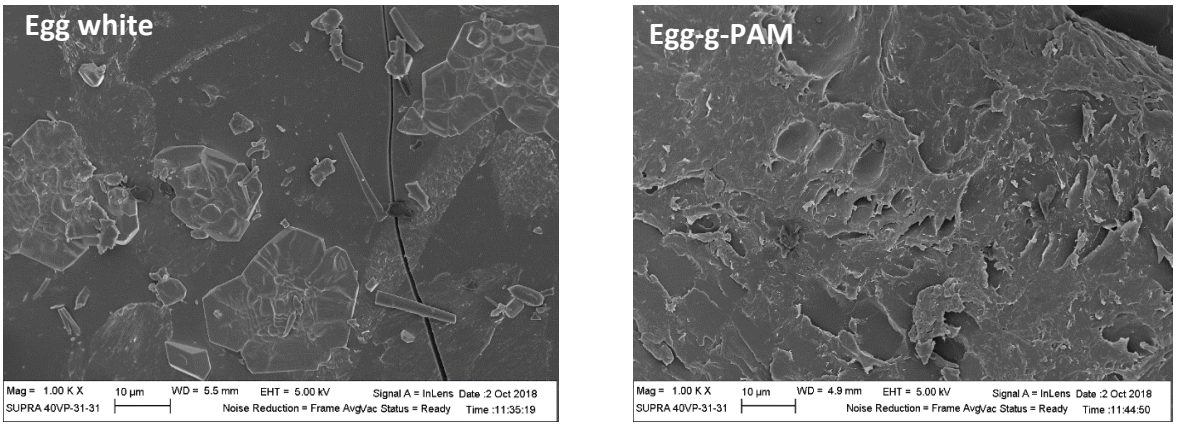


Figure 5. FESEM images of egg white and Egg-g-PAM (1000× magnification)

Elemental Analysis

There were increases in %N (1.08%) and %C (3.31%) for Egg-g-PAM as compared to egg white (Table 3). Although the %N and %C increases were not significant, it could be accounted to the presence of grafted PAM on egg white as supported by the presence of C-N peak in the Egg-g-PAM FTIR spectrum in Figure 4.

Table 3. Elemental contents of acrylamide, egg white, and optimized Egg-g-PAM

Polymer grade	% C	% H	% N	% O
Acrylamide	47.25	7.92	18.12	26.71
Egg white	43.01	7.05	10.08	39.86
Egg-g-PAM	46.32	6.50	11.13	36.05

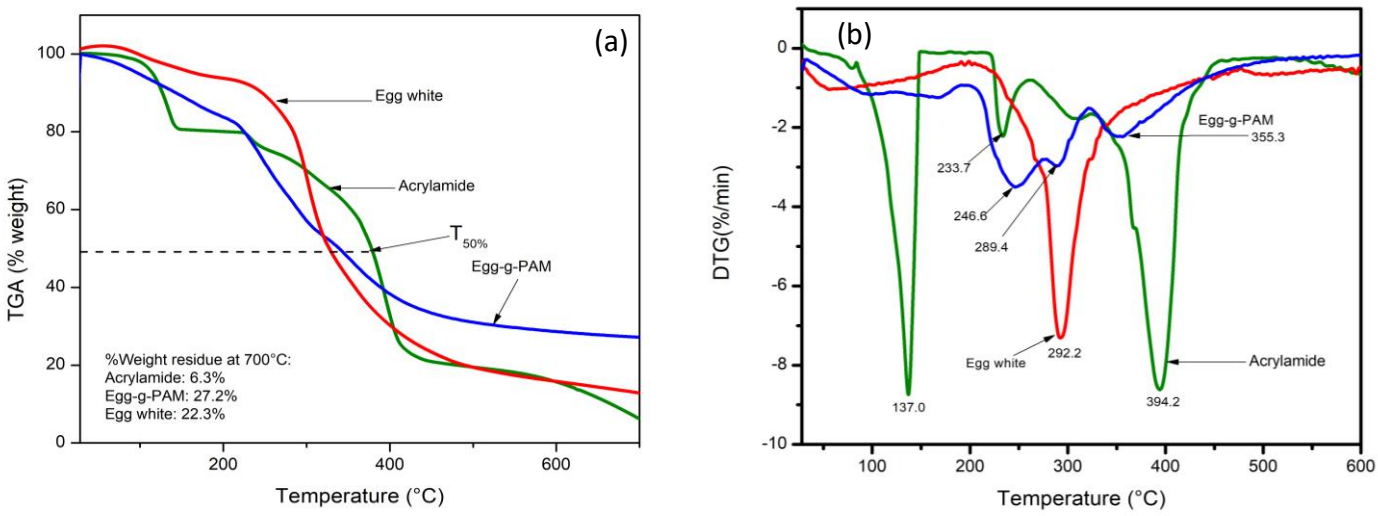


Figure 6. (a) TG and (b) DTG thermograms for egg white (red), acrylamide (green), and Egg-g-PAM (blue)

TG/DTG Analysis

From the TGA curve in Figure 6a, 50%-weight-loss temperature ($T_{50\%}$) and % weight residue at 700°C for Egg-g-PAM were higher and exhibited higher thermal stability compared to egg white and acrylamide. This may due to the noncompact stereo structure and less carbonization of Egg-g-PAM [19]. The % weight residue difference between Egg-g-PAM and egg white was 4.9%, comparable with % weight residue of acrylamide. This confirmed the successful grafting of acrylamide chains on the backbone of egg white via a free radical mechanism.

The DTG curve (Figure 6b) indicated a two-stage decomposition for egg white and three-stage decomposition behavior for Egg-g-PAM and acrylamide. A sharp and broad band at 246.6°C for Egg-g-PAM was due to a combination of 233.7°C (acrylamide) and 292.2°C (egg white). Egg-g-PAM exhibited a band at 355.3°C which resembled the acrylamide band at 394.2°C.

Point of Zero Charge (pH_{zpc})

From Figure 8, pH_{zpc} of egg white was at pH 7.61. When pH > pH_{zpc}, the surface of egg white was negatively charged. When pH < pH_{zpc}, the surface was positively charged [16]. Under high temperature and presence of NaOH, exposed sulfhydryl groups (cysteine) oxidized to form disulfide bonds [25]. The sulfide group changed to sulfoxides or sulfones as in Figure 7 [26].

pH_{zpc} of Egg-g-PAM was at pH 2.43. When pH > pH_{zpc}, the surface of Egg-g-PAM was negatively charged. Egg-g-PAM was oxidized with the presence of microwave radiation and CAN. Oxidation may take place at the sulfide functional group present in egg white, as shown in Figure 7 [26].

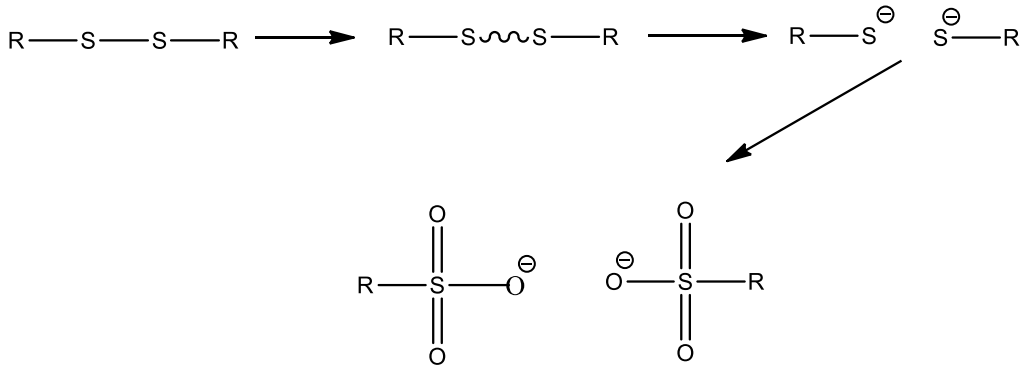


Figure 7. Oxidation at sulfide functional group

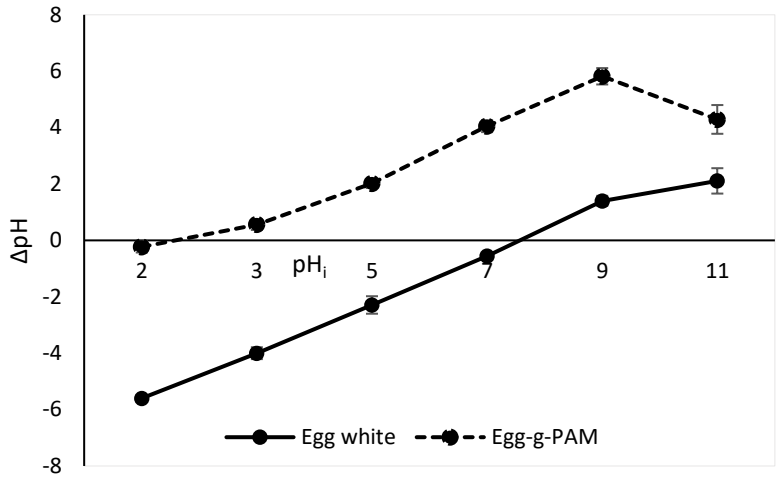


Figure 8. Point of zero charge (pH_{pzc}) of egg white and Egg-g-PAM

Turbidity Removal

Effects of pH

Figure 9 shows % turbidity removal for alum, egg white, acrylamide, and Egg-g-PAM decreased with increasing initial pH (pH 3 to 11). Egg-g-PAM was able to perform effectively at a broad pH range without pH adjustment. Acrylamide and egg white worked effectively only at pH 3. At pH 11, % turbidity removal for alum, egg white, and acrylamide was below the baseline.

In acidic conditions, alum will dissociate to form positively charged monomeric species which is conducive for the neutralization and adsorption of negative charges of kaolin suspension [27]. The positively-charged surface of egg white was neutralized and absorbed kaolin particles through charge neutralization mechanism [28]. As the initial pH increased, egg white exhibited negatively-charged surface (COO-). The repulsion between protein molecules and kaolin particles resulted in lower % turbidity removal [23]. Acrylamide only worked at pH 3, which may due to the addition of strong acid that might change the chemical structure and ionic strength for charge neutralization to occur. At high pH, negatively-charged Egg-g-PAM will undergo a bridging mechanism. This is due to the flexibility of PAM graft chains, as it is able to coil and aggregate kaolin particles through bridging effect and form larger net-like flocs [3].

From Figure 10, pH changes for egg white, acrylamide, and Egg-g-PAM did not show drastic changes after the treatment and required no pH adjustment before proceeding to the next treatment. Alum caused a significant pH shift towards the acidic region to around pH 3.8 and it could be treated with the addition of lime [29-30].

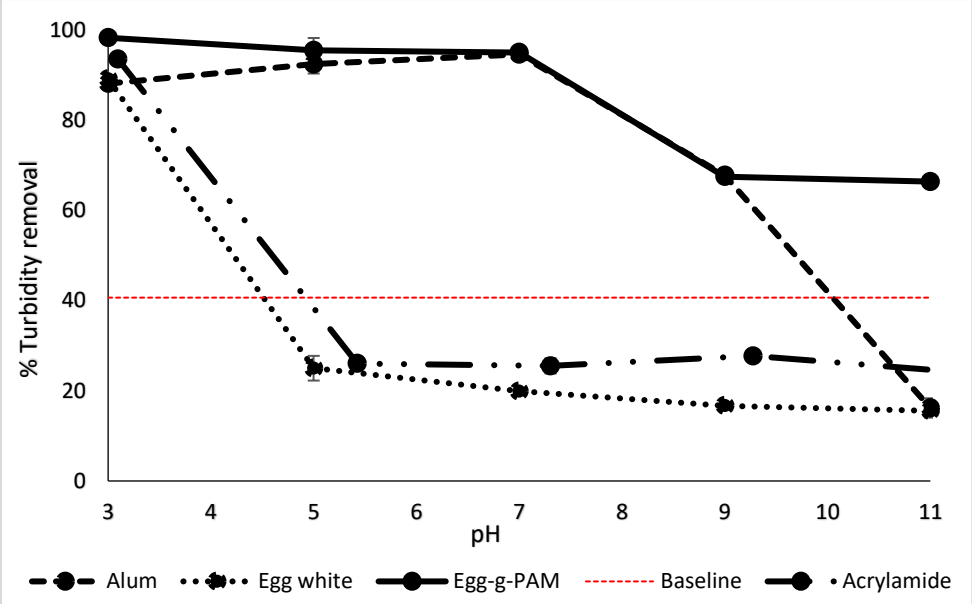


Figure 9. % turbidity removal for alum, egg white, acrylamide, and Egg-g-PAM on 0.25 g/L kaolin wastewater at various pH (pH 3-11). Coagulation/flocculation: 2 g/dL dosage; 1 h sedimentation time

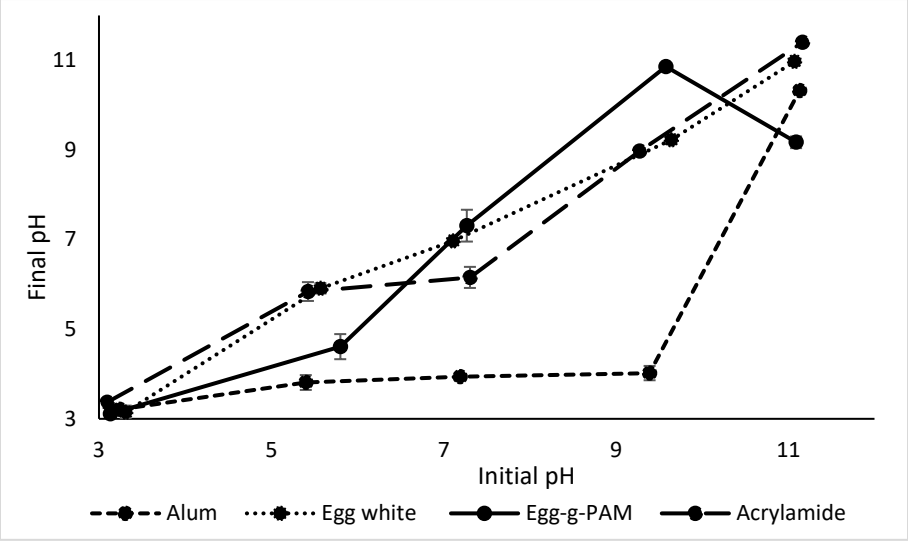


Figure 10. pH change of kaolin suspension after the treatment

Effects of Dosage

Figure 11 shows % turbidity removal decreased with increasing of dosage for egg white, acrylamide, and Egg-g-PAM. This might be due to overdosing that lead to critical coagulation concentration (insignificant effect of coagulation when coagulant dosage is applied beyond this point) and destabilization (resuspension of aggregated particles) [31]. The destabilized kaolin particles with alum were through charge neutralization. Positively-charged monomeric species (Al^{3+}, $AlOH^{+2}$ and $Al(OH)^{+2}$) will absorb negatively-charged of kaolin particles [27]. Grafting copolymerization technique has increased % turbidity removal as Egg-g-PAM was able to remove 95.55% turbidity and comparable to alum (94.58%) at optimum dosage (2 g/dL). Egg white and acrylamide showed low % turbidity removal (below the baseline) and may not be used as coagulants without undergoing any modification.

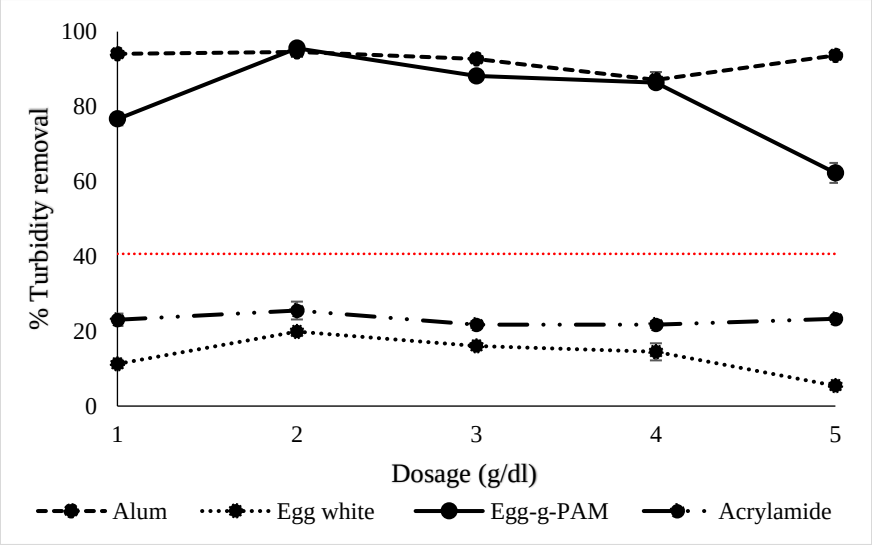


Figure 11. % turbidity removal for alum, egg white, acrylamide, and Egg-g-PAM on 0.25 g/L kaolin wastewater under dosage effect. Coagulation/flocculation: pH 7; 1 h sedimentation time

CONCLUSION

The graft polymerization of acrylamide onto egg white using microwave-assisted technique was optimized using 1 g acrylamide, 0.3 g CAN, and 2 min of irradiation time. The results showed high flocculation efficiency of Egg-g-PAM (95.55% turbidity removal) at pH 7 and 2 g/dL dosage by undergoing bridging mechanism and showed comparable results with alum. Grafting of a synthetic polymer (acrylamide) onto egg white can enhance the efficiency of treatments as well as eliminates the effect of pH and decreasing the dosage used.

ACKNOWLEDGEMENT

The authors acknowledge the financial assistance supported by the Fundamental Research Grant Scheme (FRGS) 600-IRMI/FRGS 5/3 (024/2017). Many thanks to Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam for the support on research facilities.

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