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NAME: Miss Manida Kaewphetsuwan

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR

(Miss Chalida Niamnuy, D.Eng.)

THESIS CO-ADVISOR

(Miss Nuttaporn Pimpha, Ph.D.)

GRADUATE COMMITTEE CHAIRMAN

(Associate Professor Thongchai Rohitatisha Srinophakun, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON

DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

PREPARATION AND CHARACTERIZATION OF TAPIOCA STARCH-GRAFT-POLY*(2-methacryloxyethyl trimethyl ammonium chloride)*AS ENVIRONMENTALLY FRIENDLY DUAL FUNCTIONAL FLOCCULANTS

MANIDA KAEWPHETSUWAN

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Water pollution arising from industrial processes is a matter of great concern. The effluents contain high level of suspended solid, turbidity, acidity or alkalinity and contaminants including dyes and heavy metal ions. These effluents will cause considerable damage to the environment and human health if discharged. Flocculation is an efficient and cost-effective method for wastewater treatment process. Natural flocculants become more attractive with their tailor-made, high flocculation ability and environmentally friendly. Tapioca starch is much highlighted to modify via conventional free-radical graft copolymerization with strongly positively charged momomer (*2-methacryloxyethyl trimethyl ammonium chloride)* (DMC) to enhance the flocculation ability. To optimize the graft copolymerization condition, the Box-Behken design (BBD) from Response surface methodology was applied. The successful of modified starch by grating was confirmed by physiochemical characterization. CHN elemental analysis and the FTIR spectra revealed the presence of N-content and characteristic peak of PDMC. Jar test is a conventional and popular method to find the approximately pH and flocculants dosage for the each flocculation system. It could be implied that the highly positively charged polymer was grafted to tapioca starch backbone enhanced their flocculation ability and their performance was equal to the commercial flocculants (PDADMAC) in any pH conditions. Hexavalent chromium ions (Cr(VI)) and Congo red dye could be accomplished by this innovate modified starch grafted copolymer.

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Manida Kaewphetsuwan
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LIST OF ABBREVIATIONS AND SYMBOL

ANOVA = Analysis of variance
BBD = Box-Behnken design
CCD = Central-composite design
Cr(VI) = Hexavalent Chromium
CR = Congo red dye
°C = Degree Celsius
M = Molar
mg = Milligram
nm = Nanometer
%w/v = Percent weight by volume
g = Gram
h = Hour
PDADMAC = Poly (*diallyldimethyl ammonium chloride)*
PDMC = Poly (*2-methacryloxyethyl trimethyl ammonium chloride*)
RSM = Response surface methodology
St = Tapioca starch

PREPARATION AND CHARACTERIZATION OF TAPIOCA STARCH-GRAFT-POLY*(2-methacryloxyethyl trimethyl ammonium chloride)*AS ENVIRONMENTALLY FRIENDLY DUAL FUNCTIONAL FLOCCULANTS

INTRODUCTION

According to the rapidly population growth now a day, the consumption of water in industrial process, agriculture, and human activities increase drastically in the opposite direction of available surface fresh water. A large number of wastewater and industrial effluence are produced and discharged into environment. It becomes a big problem around the world especially in Asia developing countries. Water impurities consist of dissolved and colloid natural organic matter (NOM), as dissolved salts, and suspended material such as clays silica, microbial cell or algae (Bolto and Gregory, 2007). These impurities are one of the causes of turbidity. The suspended solid particles are varies in any source; shape, density, particle size ranging from 0.04 mm for clay to 1 mm for sand and surface charge. Because suspended solid particle in water are negatively charged, they repel each other that stabilized or keep in suspension or colloidal by physical force. So, it is hardly to remove by conventional precipitation. Moreover, the high turbidity water is not suitable to use in industries processes. The suspended solid particles may clog or scour pipes and machinery. The effect to environment, higher turbidity increases water temperature resulting in decreasing of dissolved oxygen. Amount of light penetrating in the water are diminished affecting to photosynthesis and growing rates of egg and larval development. In drinking water, the turbidity can provide food and shelter for pathogen. Therefore, removing turbidity in water is importance aspect to concern in many countries. (EPA, 1999)

Besides, heavy metal is a highly contaminated material in wastewater effluence particularly wastewater from metal-plating facilities, electronic device manufacturing and mining process. Chromium is a well known toxic heavy metal and exist in the environmental both as trivalent (Cr (III)) and hexavalent forms (Cr (VI)).

Toxicity of hexavalent form is a hundred times more toxic than the trivalent form. They are tendency to accumulate in vital organs in human and animals. Researchers have indicated that Cr (VI) does not degrade into harmless products in the metabolic cycle but accumulate in the food chain causing great hazard to the living organs (Kotas, 2000; Maher *et al.*, 2009). Thus, Cr (VI) is considered to be highly toxic to the biological production systems and human health, and the US Environmental Protection Agency (EPA) recommends that amount of Cr (VI) in drinking water should be less than 0.05 mg/L due to its high toxicity to biological systems.

Congo red dye (CR) [1-napthalenesulfonic acid, 3, 3-(4, 4-biphenylene bis (azo) bis (4-amino-) disodium salt] is a benzidine-based anioncbisazo dye. This dye is known to metabolize to benzidine, a known human carcinogenic and mutagenic which is a potential danger problems. Here, it has been chosen as model dye because of its complex chemical structure, limited biodegradability and stability towards light as well as high solubility in aqueous solution. The way to remove all impurities from wastewater and industrial effluence in sustainability way is the great challenge for researchers and engineers.

The removal can be accomplished by gravitation (very slow), by coagulation (dependent on electric charge situation) and by flocculation (not dependent on electric charge and the fastest). Inorganic flocculants are used in very large quantities, strongly affected by pH changes, they leave large amount of sludge with is a secondary products which can be harmful to human health and caused the environmental problems, particularly aluminum. Hence, organic flocculants plays an important role in alternative flocculants. Both of natural and synthetic polymers are used as flocculants. Natural polymers are biodegradable, are effective at large dosages and are shear stable. Synthetic polymers are highly effective flocculants at small dosages and have high tailor ability but poor shear stability (Witold *et al*., 2009). The removal of Cr(VI) from aqueous effluent requires advanced technologies including chemical precipitation, reverse osmosis, electroplating and ion exchange (Barakat, 2011; Deng, and Bai, 2004) which these processes are not simplified and expensive. Although many research suggested that the adsorption by activated carbon is suitable, the problem is running out of coal-based resources nowadays. So, it is important to find other abundant and renewable resources.

The most abundant from renewable source besides cellulose is starch. It is polysaccharide which consists of repeating D-glucopyranose units, linked together by α-1, 4 and α-1, 6 glycosidic bonds. They are semi-crystalline structure which amylase is a crystalline region and amorphous region is amylopectin which have highly MW and branched structure. This reasons why we consider tapioca starch or cassava starch. A comparison of other types of commercial starch, cassava starch has higher amylopectin (83%) content. They are also lower impurities such as fat, protein, ash and phosphorus which simplify subsequent modification processes. Because one of the world's biggest exporter of cassava products is Thailand, so it is a great chance to look for newly modified products which biodegradable and added value.

The chemical modification which is convenience method to approach great properties of both organic flocculants is graft copolymerization induced by free radical initiation. The high performance flocculants not only should have rigid backbone and flexible branches or dangling polymer chains but also high MW to enhance the bridging mechanism. Polysaccharide grafted copolymer have been prepared in order to add new properties to the natural polymer with a minimum loss of native properties. Graft copolymer are prepared by first generating free radicals to serve as macro initiators for the vinyl or acrylic monomer polymerization (Lkhuoria, and Okieimen, 2010). Potassium persufate was used as radical initiator instead of ceric ammonium nitrate due to their cost effective. The most common used cationic starch graft copolymer with amino compounds, are as flocculating agents in water purification systems. (Dorota and Tharanathan, 2005) Many studies were successful prepared by a reaction of polysaccharide with various reagents which possessed positively charged groups such as quaternary ammonium chloride, carboxylate, xanthate, acrylamide and tertiary ammine phosphate (lkhuoria, 2010; Haradhan, 2012; Kaewtatip and Tanrattanakul, 2008; Yang, *et al*., 2013). Not only acts as flocculants, cationic polymer can be use in removal heavy metal with posses negatively charge in aqueous solution. However, only coagulation-flocculation can't treat the heavy metal wastewater completely. (Fu and Wang, 2011) Macromolecule heavy metal flocculants is new kind of flocculants could not only remove turbidity, but also remove heavy metal in wastewater, which this aspect we can reduce cost operation and consequence advanced process when less contaminated heavy metal ions or reducing amount of chemical adsorbent used in high concentrated one (Chang and Wang, 2007; Shening and Gao, 2010). To study the optimum condition and their ability, the statistic technique that mostly effective and commonly used in any research area; Response Surface Methodology (RSM) was applied. RSM can be used to determine the influence of individual factors and their interactive influences. They are useful to design experiments, building models, evaluating the effects for several factors, and searching optimum conditions for desirable responses. The RSM can reduce the experiment runs and provided sufficient information for statically acceptable results (Mohd *et al.*, 2012; Wang *et al.* 2007).

In this work, Response Surface Methodology (RSM), Box-Behnken design (BBD) was utilized to optimize the graft copolymerization conditions of 2-methacryloyloxyethyltrimethyl ammonium chloride monomer (DMC) onto tapioca starch backbone initiated by potassium persulfate, a water-soluble initiator. The final products are precipitated and purified by acetone and ethanol, respectively. This monomer was selected due to their water-soluble vinyl cationic monomer and molecular weight sufficient high introduced to tapioca starch which is the natural polymer; shear stable and high branches. Thus, introducing cationic properties, hydrophilicity, increasing dangling structure and molecular weight improve flocculation and anionic substance such as Cr (VI) and Congo red dye removal capacities of new product flocculants. Both of them were studied by standard method jar test in kaolin suspension compared to the commercial cationic flocculants polydiallydimethyl ammonium chloride (PDADMAC) and native tapioca starch. The presences of Cr (VI) and CR in aqueous solution were studied with quantitative spectroscopy method. We expect to present the newly longer sustainably macromolecule heavy metal flocculants which high performance and good for environment.

OBJECTIVES

In this work, we are looking for these objectives following;

1. To optimize the tapioca starch graft copolymerization and study the effect of initiator concentration, monomer concentration and copolymerization temperature to grafting parameter of tapioca starch–graft-Poly (2-methacryloyloxyethyltrimethyl ammonium chloride) (PDMC)

2. To study the physicochemical characteristic of tapioca starch-g-Poly (2-methacryloyloxyethyltrimethyl ammonium chloride) (PDMC)

3. To study flocculation ability of tapioca starch-g-PDMC compare to the commercial flocculants Poly (Diallyl Dimethyl Ammonium Chloride) Polymers or DADMAC or DMDAAC polymers and native tapioca starch.

4. To study the dual functional of tapioca starch-graft-PDMC copolymer to remove turbidity and anionic ion such as hexavalent chromium Cr (VI) and Congo red dye (CR) in low concentration solution.

Scope of Work

Our work was scoped by

1. Tapioca starch is food grade from Siam Quality Starch

2. Pre-gelatinization of tapioca starch at 65°C was used through all experiments

3. The overall solution concentration not over than 10% by weight because its viscosity were too high which reduced the diffusion of monomer to react. So, the ratio of tapioca starch to monomer by weight would be 3:1, 1:1, and 1:3, respectively.

4. The physicochemical characteristics were investigated by Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Elemental analysis (CHN), Scanning electron microscopy (SEM), Thermal gravimetric analysis (TGA) and zeta potential.

5. Solubility measurement using glacial acetic acid, distilled water, and NaOH 0.1M as medium.

6. The optimization of reaction conditions was applied by Response Surface Methodology (RSM); Box-Behnken Design from Minitab software version 16.0.2

7. The flocculation study was specified in kaolin suspension 0.25%wt in tab water, and the concentration of Cr(VI) and CR were 1 mg/l and 5 mg/l in tab water, respectively prepared from stock solution.

LITERATURE REVIEW

In this chapter, the literature review and theory were separated into 4 sections which explained about water impurity and wastewater treatment process, especially in coagulation-flocculation and Jar test, a conventional method to study the optimization of flocculation process. The reviews of tapioca starch modified by graft copolymerization with their useful as biodegradable flocculants were mention. In addition, hexavalentchromium ions (Cr (VI)) and Congo red dye (CR) as representation of anionic ions in wastewater was also mention in the scope of their properties and treatments. Finally, there are the literatures of the application of response surface methodology in chemical reaction.

1. Water Impurity

Water is one of the most abundant compounds found in nature, covering approximately three-fourths of the surface of the earth. In spite of this apparent abundance, several factors serve to limit the amount of water available for human use.

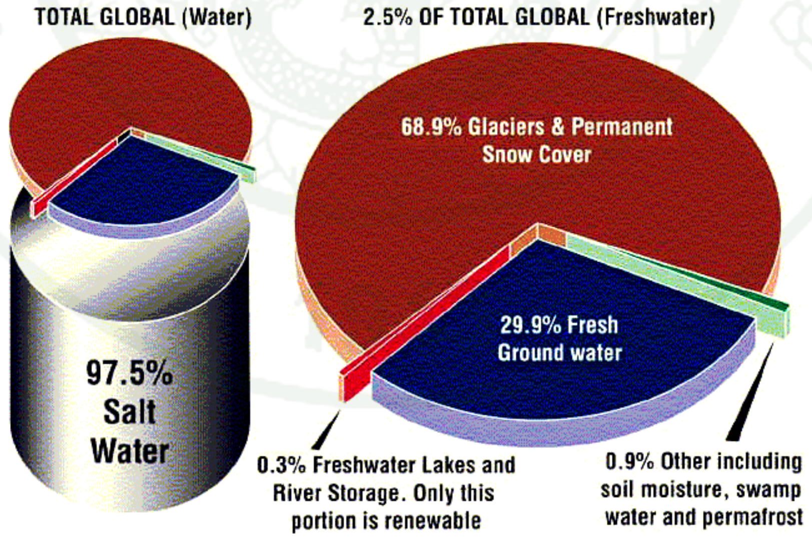


Figure 1 The total fresh water distribution

Source: Picture Image and Art from Our Friends (2014)

Over 97% of the total water supply is contained in the oceans and other saline bodies of water and is not readily usable for most purpose. Of remaining 3% of fresh water, not all of it is accessible for human use. A little over 67 % is tied up in ice caps and glaciers and inaccessible. Mostly 29% of fresh water is in groundwater which provides a critical reservoir that can be tapped for agricultural, industrial, and environmental uses as well as for drinking water supply.

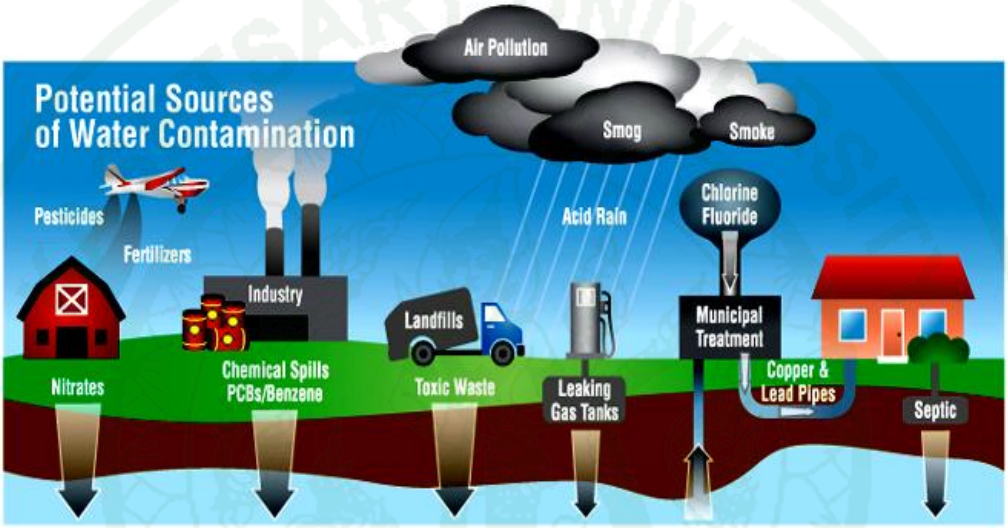


Figure 2 Man-made surface and groundwater contaminated by pollutions

Source: Ryan Andrew. 2006.

Highly civilization develops now a day, cause to water pollution. (Fig. 2) The impurities accumulated by water throughout the hydrologic cycle and as a result of human activities may be both suspended and dissolved form. Both of surface and ground water are contaminated because of improper sanitation system. Agriculture or industrial are also the major cause, the excess pesticide and fertilize, wastewater effluence from industrial process contains many inorganic matters. Because some surface and ground water reservoir are not renewable resources. It is clearly seen that the turbidity of surface water is the higher than ground water. Industrial owner and environmental engineer need some approaches to treat contaminated water in industrial process for reused or recycle, or wastewater effluence before discharge within the industrial effluent standard. It is the great challenge for industrial owner to meet their economic and respond to society also.

Table 1 Important anthropogenic sources of metals in the environment

Industry	**Metals**	**Pollutant arising**	**Reference**
Metalliferous mining	Cd, Cu, Ni, Cr, Co, Zn	Acid mine drainage, tailings, slag heaps	*Babich et al.(1985)* *Assawathanarayana(2003)*
Agricultural materials Fertilizers Manures sewage	Cd, Cr, Mo, Pb, U, V, Zn Zn, Cu, Ni, Pb, Cd, Cr, As, Hg	Run-off, surface and groundwater contamination, plant bioaccumulation Landspreading threat to ground and surface water	*Nicholson et al. (2003)* *Otero et al. (2005)* *Nicholson et al. (2003)* *Cheung and Wong (1983)* *Walter et al. (2006)*
Metallurgical industries Specialist alloys and steels	Pb, Mo, Ni, Cu, Cd, As, Te, U, Zn	Manufacture, disposal and recycling of metals. Tailings and slag heaps.	*Alloway and Ayres (1993)* *Rule et al. (2006)* *Cheng (2003*)
Waste disposal Landfill leachate	Zn, Cu, Cd, Pb, Ni, Cr, Hg	Landfill leachate, contamination of ground and surface water	*Kjeldson et al. (2002)* *Fernandez et al. (2005)*
Electronics	Pb, Cd, Hg, Pt, Au, Cr, As, Ni, Mn	Aqueous and solid metallic waste from manufacturing and recycling process	Veglio et al. (2003)

Table 1 (Continued)

Industry	Metals	Pollutant arising	Reference
Metal finishing industry			
Electroplating	Cr, Ni, Zn, Cu	Liquid effluents from plating processes.	*Castelblanque and Salimbeni (2004)* *Zhao et al. (1999)* *Alvarez-Ayuso et al.* (2003)
Miscellaneous sources			
Batteries	Pb, Sb, Zn, Cd, Ni, Hg	Waste battery fluid, contamination of soil and groundwater.	*ED Directorate general of the Environment (2004)* *Davis and Burns (1999)*
Paints and pigments	Pb, Cr, As, Ti, Ba, Zn	Aqueous waste from manufacture, old paint deterioration and soil pollution.	*Barnes and Davis (1996)* *Monken (2000*)

Source: Barakat (2011)

Water impurities consist of dissolved and colloid natural organic matter (NOM), as dissolved salts, and as suspended material such as clays silica, microbial cell or algae (Bolto and Gregory, 2007) which caused water turbidity. The suspended solid particles are varies in any source; shape, density, particle size ranging from 0.04 mm (clay) to 1 mm (sand) and surface charge. Turbidity is not direct measurement of suspended particles in water but, instead, a measure of scattering effect such particles have on light. Most of suspended solid particle in water are a negative charge, when they are come close together they repel each other and stabilized or kept in suspension or colloidal by physical force. In almost all water supplies, high levels of water turbidity are unacceptable for aesthetic reason and can interface with chemical and biological test. To environmental, higher turbidity increase water temperature, dissolved oxygen is decreased and also reduced amount of light penetrating in the water effect to photosynthesis and growing rates of egg and larval development.

Kaolin clay is one of turbidity causes. It is mostly used in cement production, ceramics, paint, paper filler, coating, pigment and rubber. Waste water effluence contain kaolin difficultly remove. Because of the colloidal, anisopholic shape and repulsive interaction between negatively charged basal faced.

Besides, heavy metal, elementals having atomic weight between 63.5 and 200.6 and a specific gravity greater than 5.0 (Fu and Wang, 2011), is a highly contaminated material in wastewater effluence. The sources of heavy metal and the toxicity to human health in the environment have been shown in the Table 1

Wastewaters from metal-plating facilities, electronic device manufacturing and mining process have been considerable for high pollution. Chromium is well-known toxic heavy metal which is carcinogenic for human health and accumulated into environmental. There are two main species of chromium existing both as trivalent [Cr (III)] and hexavalent chromium [Cr (VI)] forms. The toxicity of Cr (VI) form higher than Cr (III) form around five hundred times. When Cr (III) exist in the right environmental oxidation with the presence of oxidized manganese, the can be oxidized into Cr (VI) form over pH range from 3.0 to 10.1. Hence, the removal of both Cr(VI) and Cr(III) from the industrial wastewater to meet the regulation are great challenge for industrial management, the US Environmental Protection Agency (EPA) recommends that amount of Cr(VI) in drinking water should be less than 0.05 mg/L due to its high toxicity to biological systems (Kumar *et al.*, 2009). Chromium contaminated effluent are commonly in acidic solution. So, Cr (III) readily oxidizes to Cr (VI) form in environmental. The main Cr (VI) species in equilibrium at acidic pH range are anions, $HCrO_4^-$, CrO_4^{2-}.

There are many researcher groups studied about the removal of hexavalentchromium [Cr (VI)] by adsorption. Activated carbon is often useful as Cr (VI) or other heavy metal ions adsorbent due to its high specific surface area and porosity. However, the cost of activated is one of the limitation in using. So, the alternated activated carbon form biomass became attractive. Further, the natural polymer could be used as adsorbent also. Due to the negatively charged of Cr (VI) species, the natural cationic polyelectrolyte such as chitosan and starch and theirs derivative are very highlight due to their abundant, biodegradable and non-toxic. To adsorb of Cr (VI), the pH, initial concentration, absorbent type and dosage, contact time and temperature might be concerned. The tracing of Cr(VI) can be done by the pink colored complex formed through the reaction of DPC with Cr (VI) in acidic solution was able to be analyzed at 540 nm in the quantitative UV-spectroscopy method. The following literature showed the studied of many researchers in Cr (VI) adsorption:

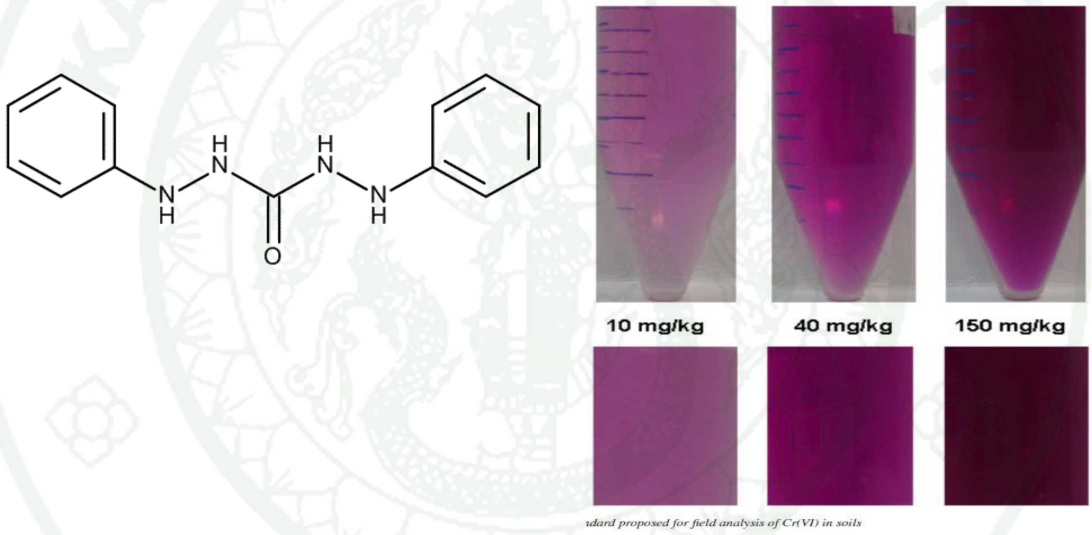


Figure 3 1, 5-diphenylcarbazide structure and red-violet form when the presence of Cr (VI).

Source: Wang *et al.* (2008)

Park *et al*. (2005) have been studied the biomass of brown seaweed, Ecklonia sp. The result revealed that acid-treatment showed the best performance with regards the improvement of Cr (VI) removal from aqueous phase. These results indicated that the amino and carboxyl group take part in the Cr (VI) removal from the aqueous phase. At low pH (below pH 2) the protonation of functional groups (e.g. carboxyl and amino groups) has been known to give an overall positive charge to the biomass, which is able to absorb negatively anionic metal ions. pH is the most important parameter in the practical use of nonliving biomass for Cr (VI) removal from aqueous phase. It is of significance that the pH of wastewaters containing chromium is generally very acidic. The biosorption system depends on solution pH, temperature, species of the biomass and Cr (VI) concentration.

Adsorption of chromium onto cross-linked chitosan studied by Rojas et al.(2005). They modified chitosan with chemical and physical method by cross linking or insertion of new functional groups to improve adsorption capacity of biopolymer as gel beads or microcrystalline chitosan. The results showed an increase in retention of Cr (III) when phosphate ions were present in concentrations less than 5 mg/L and the adsorption of Cr (VI) which cross-linked chitosan beads reached a maximum adsorption capacity of 76 mg/g. At pH <3 Cr(VI) is reduced to Cr(III), Ionic species Cr(III) are cation which adsorption mechanism in their interaction with cross-linked chitosan is chelating sites. On the other hand, the main Cr (VI) species in equilibrium at acidic pH range in our diluted solutions are anions, $HCrO_4^-$, CrO_4^{2-} and interacted effectively with protonated amine functional groups. The electrostatic is the adsorption mechanism in chromium removal. The presence of electrolytes as chlorides significantly reduce metal adsorption and thus based on pKa of chitosan at acidic pH anion removal is mainly by electrostatic interaction, while captions by chelating or covalent binding. The adsorption of chromium with cross-linked chitosan depends strongly on pH but not on particle size.

Removal of hexavalent chromium using a novel crosslinkedxanthated chitosan studied by Sankararamakrishnan *et al*. (2006).Cross linked chitosan, chemical modification, as glutaraldehyde was used as crosslink agent, after crosslinkedxanthate groups was grafted onto the back bone of chitosan. Sorption was found to be both pH and concentration dependent; with pH 3 being the optimum value, a pseudo-second-order kinetics and Langmuir isotherm model are fitted. Desorption studies revealed the reusability of sorbent for at least 10 cycles without any significant change in adsorption capacities.

Recent investigation reported the removal of both chromium ions by an amine-based polymer, aniline formaldehyde condensate (AFC) coated on silica gel. The optimum pH for removal of hexavalent chromium was observed as 3 and this acidic pH, amine group ($-NH_2$) of AFC polymer was in protonated form ($-NH_3^+$) and it removed negative charged hexavalent chromium (acid chromate ion ($HCrO_4^-$)] by electrostatic attraction.

A novel polyglycidylmethacrylate (PGMA) microspheres with high adsorption capacity of Cr(VI) was prepared by cerium(IV) initiated graft polymerization of tentacle-type polymer chains with amino group on polymer microspheres with hydroxyl groups was studied by Li ea al. (2012) Polymer microspheres had an average diameter of 5 micrometer with uniform size distribution. The Cr (VI) adsorption results indicated that the graft copolymerization of tentacle-type polymer chains on the polymer microspheres could produce adsorbents with high adsorption capacity. They have potential application in large-scale removal of Cr (VI) in aqueous solution.

Kim *et al*. (1999) have studied cornstarch was cross-linked by POCl3 (1%, v/w, starch db) and carboxymethylatedat a relatively low degree of substitution (DS), and its removal capacity for various divalent metal ions from their aqueous solutions. By cross-linking and carboxymethylation, corn starch could become an active and relatively insoluble metal scavenger for toxic divalent cations such as Cu, Pb, Cd, and Hg ions contaminated in water. The starch could be regenerated by a weak acidic washing. For effective metal removal, the metal solution should not be too acidic. By rising the levels of carboxymethylation and cross-linking, metal scavenging activity of the starch could be increased up to the level required for industrial applications.

Shi *et al*. (2003) successfully producedcrosslinked starches containing a quaternary ammonium cationic group and a carboxymethyl anionic group or amphoteric modified starch. They can be used to effectively remove Cr (VI) in wastewater, especially wastewater containing lower initial concentrations of Cr (VI). The adsorption capacity of cross linked amphoteric starch with the same DS of quaternary ammonium cationic groups decreased with the increasing DS o carboxymethyl anionic groups. The adsorption followed the Langmuir adsorption isotherm. Because the adsorption process was exothermic, lower temperatures facilitated the adsorption.

Recent investigation reported the removal of both chromium ions by an amine-based polymer, aniline formaldehyde condensate (AFC) coated on silica gel. The optimum pH for removal of hexavalent chromium was observed as 3 and this acidic pH, amine group ($-NH_2$) was in protonated form ($-NH_3^+$) and it removed negative charged hexavalent chromium (acid chromate ion ($HCrO_4^-$)] by electrostatic attraction. The dominant mechanism of Cr (VI) removal was adsorption between negatively charged Cr (VI) ions and the positively charged amine groups.

Dyestuff manufacturing industries and many other dye and pigment-using industries such as rubber, paper, textile, plastic and cosmetic generally highly colored and toxic effluent. Wastewater effluent that come from textile industry is around 200-300 m^3 per ton of finished products. Especially in dyeing operation, it generated a large portion of industry’s total wastewater about 10-15%. The major sources are dye bath and wash water. They generated wastewater about 150 m^3 per ton of product average in all dye types (disperse, reactive and direct dye) which they are classified shown in Table 2. Dyes usually have a synthetic origin and complex aromatic molecular structures which are more stable and more difficult to biodegrade. It is known that wastewater containing dyes are very difficult to treat, since the dyes recalcitrant molecules (particularly azo dye), resistance to aerobic digestion, and are stable to oxidizing agents.

Table 2 Characterization of different class of dyes mainly used in textile industry and its methods of application

Class	Characterization	Substrate (fiber)	Dye-fiber interaction	Method of application
Acid	Anionic, water-soluble	Nylon, wool, silk	Electrostatic, hydrogen bonding	Applied from neutral to acidic dye baths
Base	Cationic, water-soluble	Modified nylon, poly ester	Electrostatic attraction	Applied from acidic dye baths
Direct	Anionic, water-soluble	Cotton, rayon, nylon, leather	Intermolecular forces	Applied from neutral or slightly alkali bath containing additional electrolytes
Disperse	Very low water solubility	Polyester, polyamide, acetate, plastic, acrylic	Hydrophobic-solid state mechanism	Fine aqueous dispersion often applied by high temperature or lower temperature carrier method
Reactive	Anionic water-soluble	Cotton, nylon, silk, wool	Covalent bonding	Reactive sites on dye reacts with functional group on fiber

Table 2 (Contiuned)

Class	Characterization	Substrate (fiber)	Dye-fiber interaction	Method of application
				to bind dye covalently under influent of heat and pH (alkali)
Sulfur	Colloidal, water insoluble	Cotton, rayon	Covalent bonding	Aromatic substrate vetted with sodium sulfide and re-oxidized to insoluble sulfur containing products on fiber
Vat	Colloidal, water insoluble	Cotton, rayon	Impregnation and oxidation	Water insoluble dyes solubilised by reducing with sodium hydrosulfite, then exhausted on fiber and re-oxidized

Source: Verma *et al.* (2012)

Congo red (CR) dye [1-napthalenesulfonic acid, 3, 3-(4, 4-biphenylene bis (azo) bis (4-amino-) disodium salt] is a benzidine-based anioncbisazo dye. The structure of Congo red was shown in Figure 4. This dye is known to metabolize to benzidine, a known human carcinogenic and mutagenic, is a potential danger problem. Here, it has been chosen as model dye because of its complex chemical structure, limited biodegradability and stability towards light as well as high solubility in aqueous solution.

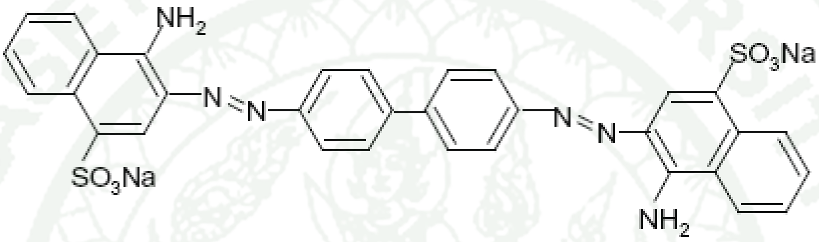


Figure 4 Congo Red dye structure

Source: Mittal (2009)

Recently, there are many researchers studied about how to removal dyes from wastewater effluent using both physical and chemical methods such as coagulation-flocculation, adsorption, Ion-exchanger, coronation, photochemicalsonolysis, Fenton-oxidation etc. However, operating cost and time required for the desired degree of treatment may be the major criteria for the selection of suitable methods it can observed from table 3 that each and every method is associated with some type of limitations.

Sommasekhara *et. al.* (2011) also have studied the use of an agriculture waste material, Jujuba seeds for the removal of anionic dye (Congo red) from aqueous medium. Due to its low cost and eco-friendly adsorbents and they reported that maximum color removal at pH 2. The equilibrium data was fitted to the Langmuir model, with maximum adsorption capacity of 55.56 mg/g.

As water contaminations both of Cr (VI) and Congo red dye, the specific properties of adsorbent are mostly influence by pH. How to choose the proper materials which can reduce operation cost, easy to use, safe for environmental and sustainability way is the great challenge for researchers and engineers.

Table 3 Congo red dye removal literature reviews

Dye	**Removal mechanism**	**Materials**	**Removal capacity**	**Reference**
Congo red	Adsorption-column operation	Waste materials-bottom ash and deoiled soya	96.95% and 97.14%	Mittal, A., J. et al., 2009
Congo red	Adsorption-batch	Biomass-nut shells charcoal and eichhonia charcoal	117.6 mg/g and 56.8 mg/g	Kaur et al., 2012
Congo red	Adsorption	Chitosan/montmorillonite nanocomposite	54.52 mg/g	Wang et al., 2007
Anionic and cationic dye	Flocculation	CMC-g-PAM	80 mg/g and 160 mg/g	Yang et al., 2013
C.I. Acid Black 210 dye	Flocculation	PDADMAC		Zahrim, C. et al., 2010

2. Waste water treatment process

Figure 5 and figure 6 showed wastewater treatment process, starting with physical process, screen the large objects out. Following by the chemicals process, coagulant is added in order to destabilize the suspended solid particles. The suspended solid particles are neutralized by adding opposite charge coagulants in rapid mixing for 1-3 minutes to promote and disperse coagulants collision. When the small flocs occur, flocculation is following to form flocs; the large flocs because of the small flocs come close together. Flocculation is the slow mixing of small particle to clump its up and heavy enough to settle down in sedimentation or setting basins. The water effluences from the sedimentation go through the filtration and disinfection for the next step. This water can be reuse after adjust pH and remove inorganic and organic compounds by ion exchange or activated carbon, the advanced process.

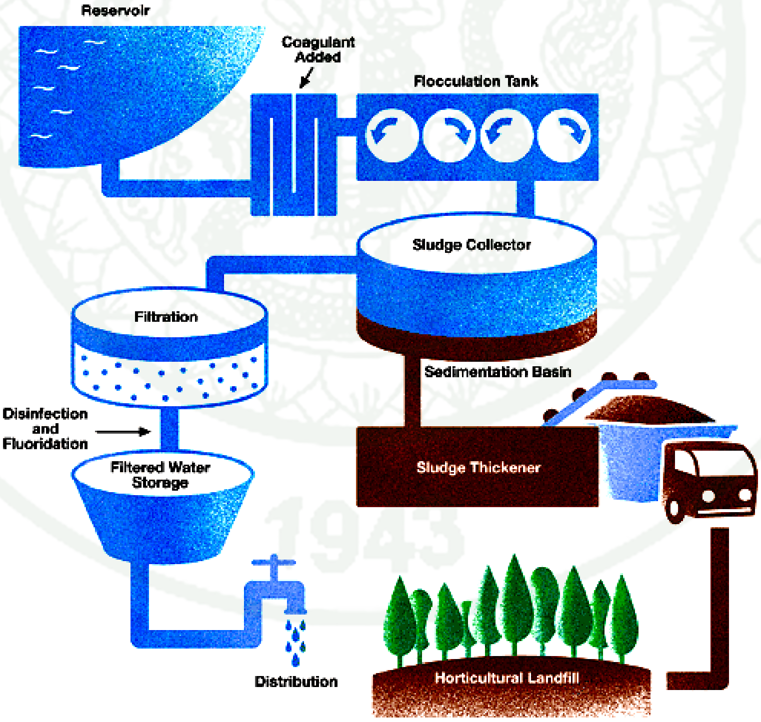
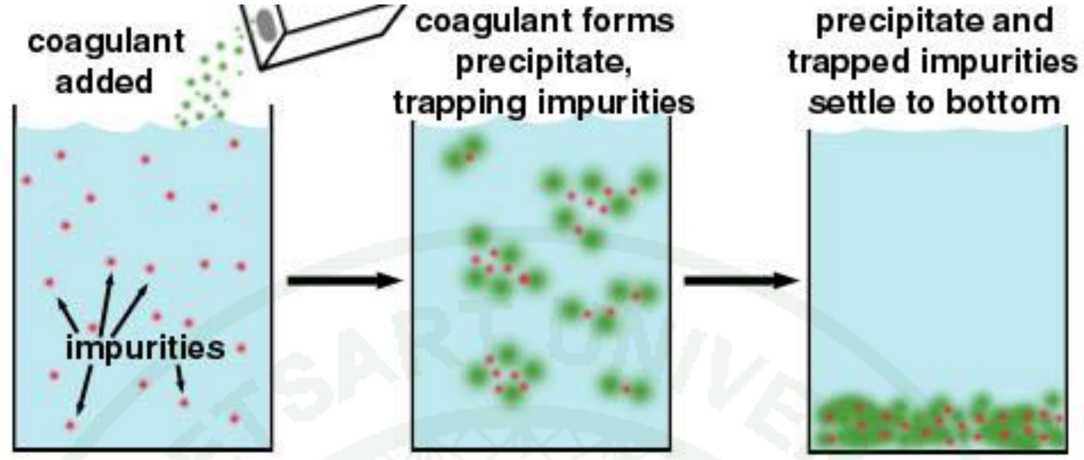


Figure 5 Wastewater treatment process

Source: Department of Civil Engineering, University of Memphis (2014



$$\text{Stable colloid} + \xrightarrow[\text{Coagulant}]{\text{Inorganic}} \text{Unstable colloid} \xrightarrow[\text{Time}]{\text{Conditioning}} \text{Flocs} + \xrightarrow[\text{Conditioning}]{\text{Polymer}} \text{Large flocs}$$

Figure 6 Coagulation-flocculation process

Source: Rosa-lee Cooke (2013)

3. Coagulation and flocculation

Coagulation-flocculation has been used for many years as main treatment or pretreatment due to its low capital cost. Correct application of coagulation and flocculation processes and selection of the coagulants depend upon understanding the interaction between these factors. Inorganic flocculants are used in very large quantities, strongly affected by pH changes, they leave large amount of sludge with is a secondary products which can be harmful to human health and caused the environmental problems, particularly aluminum. Hence, organic flocculants plays an important role in alternative flocculants. Both of natural and synthetic polymers are used as flocculants. A comparative advantage and disadvantage of inorganic and organic flocculants are showed in Table 4. Natural polymers are biodegradable, are effective at large dosages and are shear stable. Synthetic polymers are highly effective flocculants at small dosages and have high tailorability but poor shear stability (Witold *et al.* 2009). Although many research suggested that the sludge production are one of major limitation in flocculent usage, it can be minimized by optimizing process parameter and suitable selection of coagulant and flocculants. Due to scarcity of landfill sites, the disposal of sludge becomes more problematic and expensive. Therefore, recycle of sludge becomes the only viable option. In this regard, the use of sludge as building materials, a soil conditioner, or as a fuel has been studied by several researchers.

Table 4 A comparative advantage and disadvantage of inorganic and organic flocculants

Flocculants	Advantages	Disadvantages
Inorganic	Low cost Abundant	-High efficiency while using a lot of flocculants -Produce large amount of sludge which is secondary pollutants
Organics (natural polymer)	Low cost Abundant Non-toxic	Low efficiency
Organics (polymer synthetic)	lower dose usage High efficiency Reduce level of aluminum in treated water	Expensive High efficiency

Biopolymer is very effective method for treatment of industrial wastewater. The main reasons for the success of this biopolymer in wastewater treatment using coagulation/flocculation are the advantage of being a non-toxic material, no corrosive and safe to handle (non-hazardous product, not irritating for skin and eyes). In addition as biopolymer is biodegradable, hence, the sludge can be efficiently degraded by microorganisms

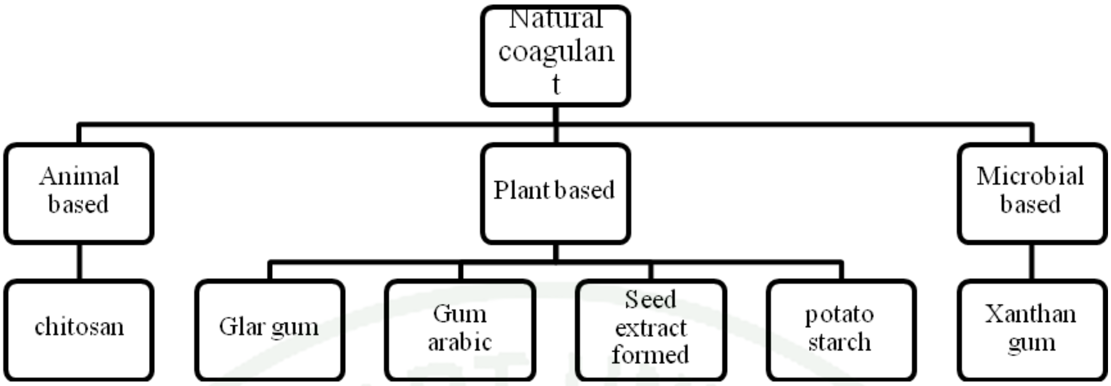


Figure 7 Classification of natural coagulant with their examples

Source: Verma *et al.* (2012)

The mechanism of coagulation-flocculation was explained in Figure 6. Addition of some chemicals (polyelectrolyte) enhances coagulation by promoting the growth of large, rapid settling of flocs. Polyelectrolyte is high-molecular-weight polymers, which contain absorbable groups and when small dosages of polyelectrolyte (1mg/l to 5 mg/l) are added in conjunction with coagulant, these are also referred as coagulants aids. The polyelectrolyte is substantially unaffected by pH variations and serves as a coagulant itself by reducing the effective charge on colloids. It produces a large amount of ions in water and shows properties of both polymer and electrolytes. The most practical benefit of polyelectrolyte is the formation of massive flocs. These massive flocs speed up the floc settling velocity, and also decrease the settled sludge volume.

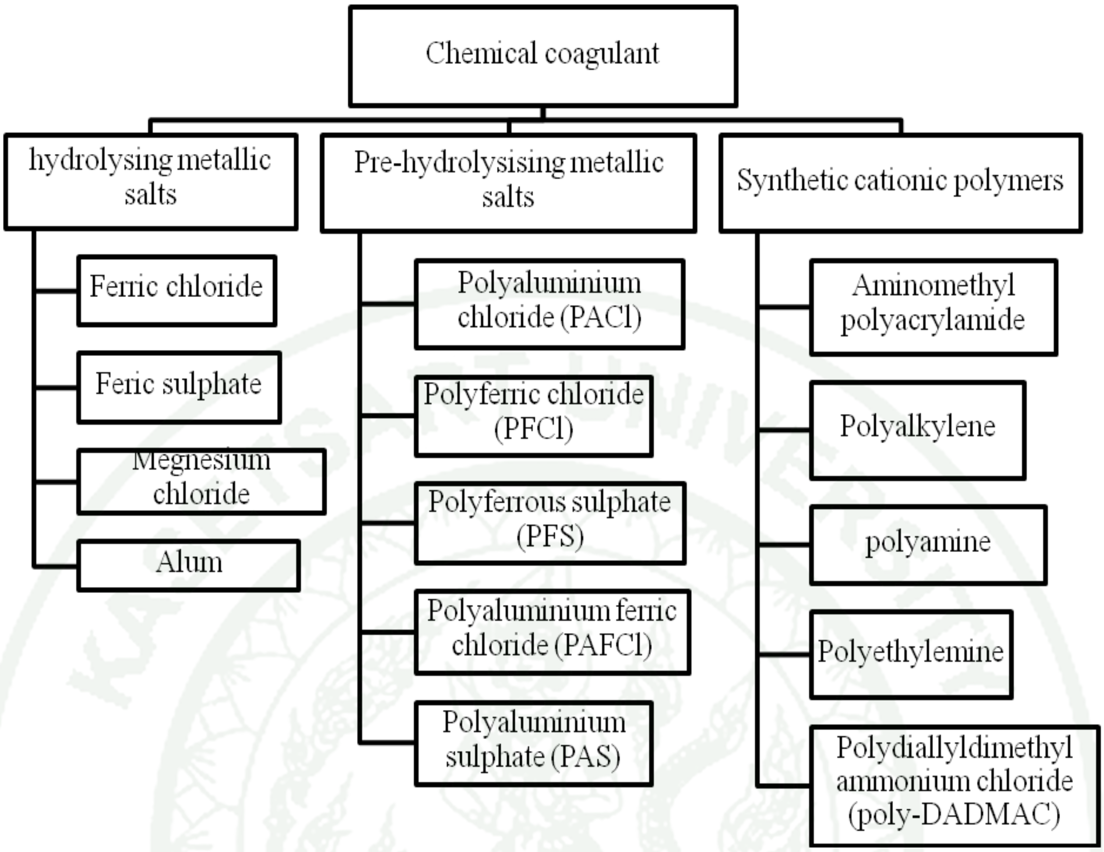


Figure 8 Classification of chemical coagulation with their examples

Source: Verma *et al.* (2012)

Jar test and turbidity measurement

Jar test is the conventional method using in the water treatment plant to find the best coagulant dosage under varying conditions and to measure the effect of coagulation, flocculation and sedimentation on turbidity.

In many plants require the operator to adjust coagulant dosage at interval to achieve optimal coagulation when water characteristic was changed. The source of water will have most impact on how often jar test performed. In ground water treatment plant, jar test is carried out less than in surface water treatment plant due to its low turbidity. However, in ground water treatment plant have a problem on the difficulty of removing the small suspended solid. In surface water treatment need to test frequently because of the characteristic of water change rapidly, especially after rain.

The step of jar test involve adding some chemicals coagulant or flocculant to the source water and mixing the water rapidly to completely dissolved the chemicals in water, then the water is mixed slowly for a longer period, finally the mixer stopped and floc is allowed to settle out. The supernatant is withdrawn to measure the turbidity. (Fig. 9)

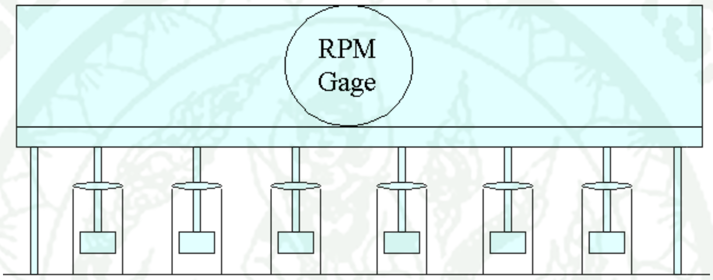
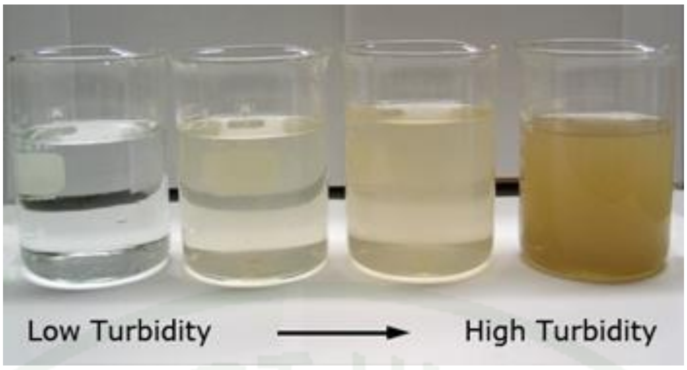


Figure 9 Jar test

Source: Water and Engineering (2014)

Turbidity is the measurement of the cloudiness of the water. The higher turbidity, the harder is to see through the water. Turbidity is usually reported in nephelometricturbidity unit (NTU) or Jackson Turbidity Unit (JTU) depending on which method is chosen to measure. The two units are roughly equal. With naked eye, an average person can begin to see turbidity level starting around 5 NTU and greater. The low and high turbidity is shown in Figure 10. Several methods for turbidity measurement and their advantages and disadvantages are summarized in Table. The most popular method is nephelometric method due to its high accuracy and can measuring at very low turbidity. However, the expensive costs, easily to damage and require the technician to deal with it become the limited using. In Table 8, the reviews of turbidity measurement are showed.



Nephelometric Turbidity Unit (NTU)

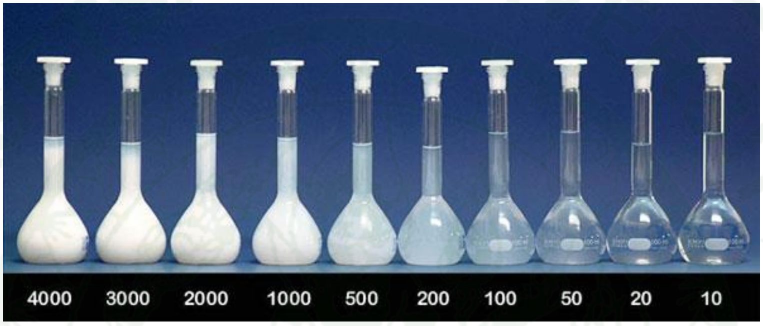


Figure 10 Turbidity levels

Source: SCCF RECON (2014)

Table 5 The summarize of turbidity measurement methods

Methods	**Advantage**	**Disadvantage**
Jackson Candle Tubidimeter	Historical methods	No, longer a standard methods Can't measure <25 JTU (NTU)
Turbidimeter (Nephelomenter)	Extremely accurate Some are portable Can measure very low turbidity	Expensive Easily damage Requires power source Requires calibration

Table 5 (Continued)

Methods	Advantage	Disadvantage
Secchi Disk	Low cost and portable No consumable Easy to learn	Less accurate Can't use in shallow water or swift current Not applicable to small size
Turbidity tube (Transparently Tube)	Low cost and portable No consumable Easy to learn Suitable for all water source	Less accurate Can't measure < 5 NTU
Transmittance	Medium to high turbidity value	

Source: EPA Guidance Manual Turbidity provision (1999)

There is several reported of measurement turbidity by using a transmittance design called 'absorbance unit (AU)' as measure the amount of light transmitted through a sample rather than the amount of light scattered like the mentioned methods above in Table 5 Light transmittance is measured by introducing a light source to a sample volume and measure transmitted through the sample volume to a photo detector located opposite the light source. Transmittance value is reported as 0-100 percent of incident light source transmitted through the sample. The higher %T is, the higher turbidity they are. However, the method is not approved by EPA. The several reports about the application of spectrophotometry in turbidity measurement are reviewed.

The different of turbidity measurement unit between NTU and AU is showed in Fig the position of incident light detector. For NTU, the detector are measured the scattering incident light at 90 degree while AU detected by measurement incident light in the opposite or 180 degree. The designation of NTU and AU signify the use of broad spectrum incident light in the wavelength range 400-680 nm. (Chauncey W. Anderson).

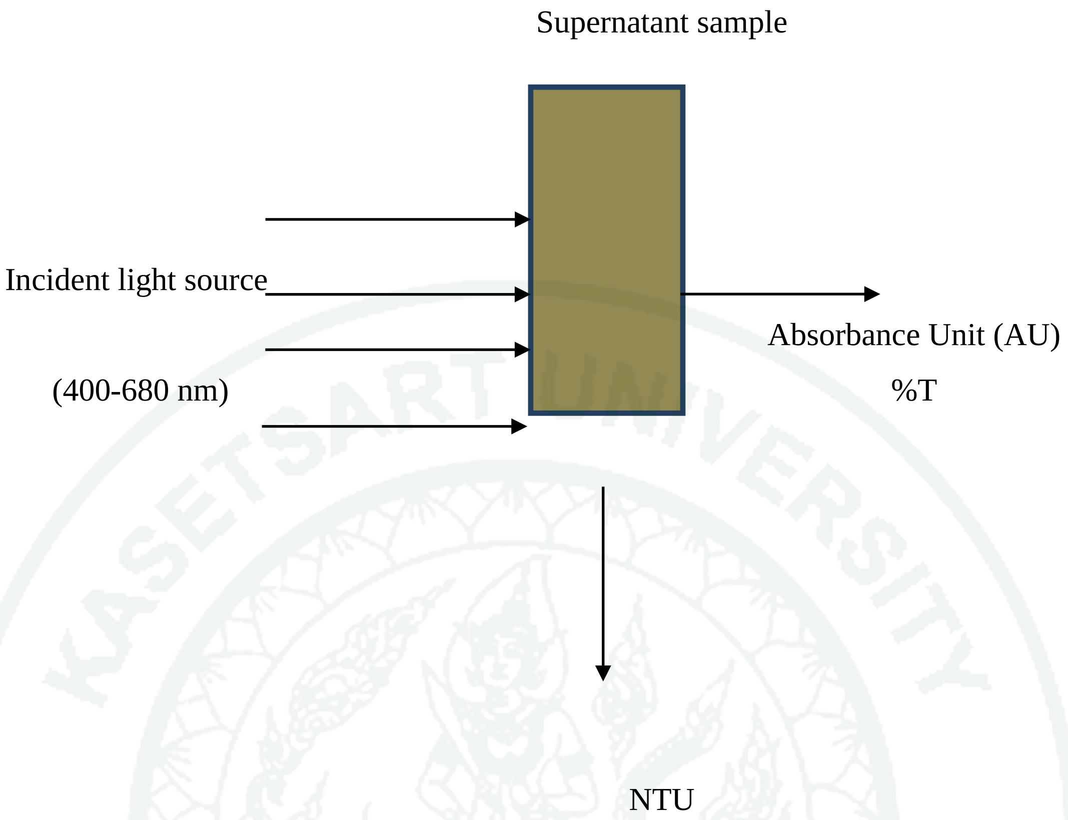


Figure 11 The different of detector position between NTU and AU

Table 6 The reviews of turbidity measurement by applied spectroscopy method

Coagulant/flocculant	**Sample solution**	**Wavelength (nm)**	**References**
Acrylamide-acryloyamino-2-hydroxypropyl trimethy ammonium chloride	Kaolin suspension	630 nm	*(Wang, L.-J. et al., 2009)*
Starch/Chitosan crosslinked copolymer	Kaolin suspension	560 nm	*(You, L., F. Lu, D. Li, Z. Qiao and Y. Yin, 2009)*
Starch-g-PDMC	Kaolin suspension	420 nm	*(Wang, J. P., S. J. Yuan, Y. Wang and H. Q. Yu, 2013)*
Sodium xanthate graftepolyethyleneimine	Kaolin suspension	680 nm	*(Chang, Q. andG. Wang, 2007)*

Tapioca starch

The most abundant from renewable source besides cellulose is starch. It is polysaccharide which consists of repeating D-glucopyranose units, linked together by α-1,4 and α-1,6 glycosidic bonds. They are semi-crystalline structure which amylose is a crystalline region and amorphous region is amylopectin which have highly MW and branched structure. Due to Thailand is one of the biggest world's cassava starch exporter, it would be a great opportunity to the specific functionalized of starch, new products are continuously being developed that find increasing use in wide variety of industrial application, leading to a significant value addition.

Tapioca starch is also known as cassava starch terms which are used interchangeably. Usually the product from the grinding of cassava tuber (dry milling) is called tapioca flour, while starch extracted by wet milling is known as cassava starch. A comparison of the physical properties and composition of several commercial starches is presented in Table 7. Cassava starch has higher amylopectin content compared to other starches. This contributes to the formation of soft gels upon gelatinization compared to the more firm gel from other starches. As a consequence, gelatinized cassava starch is flow able during processing. Another factor that differentiates cassava starch from other starches is a low level of contaminants (fat, protein, ash, and phosphorous material), which simplifies subsequent modification processes. The high paste viscosity, high paste clarity and high freeze-thaw stability of cassava starch after cooling, which are favorable properties for many industrial application. Although cassava starch has these advantages compared to other starches, the use of cassava starch for graft polymerization is still limited, although increasing in recent years. Other native starches were more extensively studied for graft copolymerization, like maize (corn) and potato starch.

Table 7 Tapioca starch in comparison to some other commercial starches

source	Granule diameter (μm)	Average diameter (μm)	Amylose content (%)	Amylose Average DP	T_g range (°C)	Ash (%)	P (%)	Protein (%)	Lipid (%)
Tapioca	4.35	15	17	3000	65-70	0.2	0.01	0.10	0.1
Potato	5-100	27	21	3000	60-65	0.4	0.08	0.06	0.05
Norma corn	2-30	10	28	800	75-80	0.1	0.02	0.35	0.7
Waxy corn	2-30	10	0	Not applicable	65-70	0.1	0.01	0.25	0.15
Wheat	1-45	Bimodal	28	800	80-85	0.2	0.06	0.40	0.8

Source: Witono *et al*. (2012)

There are many approaches to improve and enhance properties of native starch for using in many industrials such as food, textile, pharmaceutical, etc. as shown in Table 8.

Table 8 Properties and applications of modified starches

Modification	Functional group induced	Properties and applications
Pre-gelatinization	-	Cold water dispersibility-useful in instant convenience foods
Dextrinization	-	White/yellow dextrins-useful in baked goods
Anionic substitution	Carboxylmethyl- phosphate-acetate-	Freeze-thaw stability-useful in frozen foods

Table 8 (Continued)

Modification	**Functional group induced**	**Properties and applications**
Cationic substitution	Quaternary ammonium-	Lower gelatinization temperature-useful as a pigment retention aid in paper, sizing-coating, textile-wrapping
Hydroxypropylation	Hydroxypropyl-	Stabilizing agent-useful in processed foods, candies, low temperature stability
Cross-linking	Phosphate ester-epichlorohydrin-	Granule stability, resistance to retrogradation, gelling-useful in soups, puddings, bakery products
Oxidation	Dialdehydes or dicarboxylic acid	Cross-link with amino/imino groups-useful in leather tanning, water resistance to adhesives
Partial acid or enzyme hydrolysis	Glycosidic bond cleavage	Increased retrogradation and set back-useful in confectionery, HFCS
Graft co-polymerization	Vinyl- acrylic-	Film making-biodegradability-useful in biodegradable packaging films
Dual modification	-	Combination of properties-useful in bakery and frozen foods, salad dressings

Source: Tharanathan (2005)

The chemical modification which is convenience method to approach great properties of both organic flocculants is graft copolymerization induced by free radical initiation. The high performance flocculants not only should have rigid backbone and flexible branches or dangling polymer chains but also high MW to enhance the bridging mechanism. Polysaccharide grafted copolymer have been prepared in order to add new properties to the natural polymer with a minimum loss of native properties. Graft copolymer are prepared by first generating free radicals to serve as macro initiators for the vinyl or acrylic monomer polymerization (Lkhuoria and Okieimen, 2010). Potassium persufate was used as radical initiator instead of ceric ammonium nitrate due to their cost effective. The most common used cationic starch graft copolymer with amino compounds, are as flocculating agents in water purification systems. (Ziolkowswa and Tharanathan, 2005) Many studies were successful prepared by a reaction of polysaccharide with various reagents which possessed positively charged groups such as quaternary ammonium chloride, carboxylate, xanthate, acrylamide and tertiary ammine phosphate (lkhuoria, 2010; (Haradhan, 2012; Kaewtatip and Tanrattanakul, 2008a; Yang et al., 2013a). Not only acts as flocculants, cationic polymer can be used in removal heavy metal with possess negatively charge in aqueous solution. However, only coagulation-flocculation can't treat the heavy metal wastewater completely. (Fu and Wang, 2011) Macromolecule heavy metal flocculants is new kind of flocculants could not only remove turbidity, but also remove heavy metal in wastewater, which this aspect we can reduce cost operation and consequence advanced process when less contaminated heavy metal ions or reducing amount of chemical adsorbent used in high concentrated one (Chang and Wang, 2007; Shening and Gao, 2010). The graft copolymerization of monomer onto different kind of polysaccharide as flocculants are over viewed in Table 9.

Table 9 Graft copolymerization of monomer onto different kind of polysaccharide as flocculants. An overview research articles in literature reviews

Polysaccharide	Monomer	Initiator	Reference
Starch	Amide	CAN	Xing et al.
Cassava starch	Styrene	KPS	Kaewtatip and Tanrattanakul (2008)
Cassava starch	Acrylonitrile	CAN	Lkhuoria et al. (2010)
Maize starch	N-tertbutylacrylamide (BAM)	CAN	Mohammad et al. (2003)
Xanthagum	N,N-dimethylacrylamide (DMA) Acrylamide (AM)	CAN	Kolya (2012)
Starch	PDMC	KPS	Wang et al. (2013)

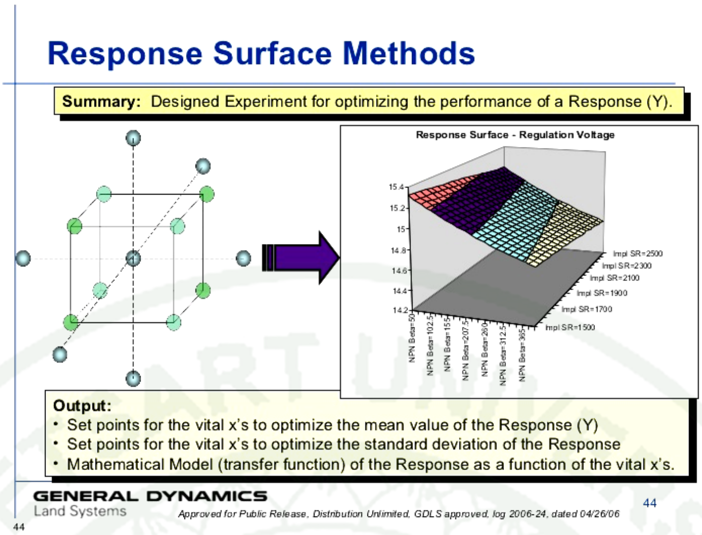
Table 9 (Continued)

Polysaccharide	Monomer	Initiator	Reference
Carboxymethyl Chitosan (CMC)	Acrylamide (AM)	CAN	Yang et al. (2013)
Cassava	PolyDADMAC	CAN	Razali et at. (2012)
Chitosan	PDMC	KPS	Wang et al. (2009)

Note: CAN : ceric ammonium nitrate
KPS : potassium persulphate

4. Response surface methodology (Box-Behnken Design)

Response surface methodology is one of statistically approach to study about the relationship of variables to response. They are widely used in many industrial to assess the objectives especially, in the quality controlled, they used these approach to optimize the production process and controlled product quantities and qualities. Also in the chemical industries, the R&D, they used to optimize the reaction rate and predicted the products if increase or decrease the substance, temperature or pressure, etc. These are only few examples. Not only used to predict, they are also used to finding the variable interaction and used ANOVA (analysis of variance), the statistically method to find the variance significance and their effect to the response and then the regression model will be formed. From the ANOVA result, we can conclude that which variable and their interaction affect to our response. So, with it easy to compute by statistic software, these methods are popular to apply. The two models are mostly used in chemical reaction design are Box-Behnken Design (BBD), and Central-composite design (CCD). BBD is considered as an efficient option in RSM and an ideal alternative to CCD (Sudamalla et al., 2012).



Response Surface Methods

Summary: Designed Experiment for optimizing the performance of a Response (Y).

Output:
- Set points for the vital x's to optimize the mean value of the Response (Y)
- Set points for the vital x's to optimize the standard deviation of the Response
- Mathematical Model (transfer function) of the Response as a function of the vital x's.

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Figure 12 The Response surface methodology

Source: Wasiloff (2006)

Box-Behnken design is one of the most powerful and efficient experimental design among the other response surface designs (Central composite, Doehlert matrix, and three-level of full factorial designs), due to its ability to estimate the variables of the quadratic model, constructing of sequential design, detection of lack of fit of the model, use of blocks. Moreover it needs fewer run and has specific positioning of the design point caused it more interest in cost-effective. In recent years, Box-Behnken design is used in different field of chemistry that is spotlighted by many researchers for its feasibility and efficiency as shown it Table 10.

Minitab 16.2.0 statistical software was used for design of experiment. The sequential model fitting test was carried out in order to choose a suitable model. A full quadratic model has been used to identify all possible interactions of selected factors obtained from the Box-Behnken model:

$$Y = \beta_0 0 + \sum_{i=0}^{4} \beta_i X_i + \sum_{j=0}^{4} \beta_{ii} X_i^2 0 + \sum_{i=0}^{4} \sum_{j=0}^{4} \beta_{ij} X_i X_j$$

Where Y is the percentage of add-on and the grafting efficiency (%GE), b_0 is the intercept parameter and b_i,b_{ii} and b_{ij} are the variables for linear, squared and interaction factors effects, respectively

The data were subjected to the analysis of variance and the coefficient of regression (R^2) was calculated to find out the goodness of fit of the model.

The RSM is a statistical technique for designing experiments, building models, evaluating the effects of several factors, and searching optimum conditions for desirable responses. With RSM, the interactions of possible influencing parameters on treatment efficiency can be evaluated with a limited number of planned experiments. RSM is widely used in various fields, e.g. preparation. The design procedure of response surface methodology is as follows:

(i) Designing of series of experiments for adequate and reliable measurement of the response of interest.

(ii) Developing a mathematical model of the second order response surface with the best fittings.

(iii) Finding the optimal set of experimental parameters that produce a maximum or minimum value of response.

(iv) Representing the direct and interactive effects of process parameters through two and three dimensional plots.

In this study, The RSM was employed to find the graft copolymerization conditions under which a certain process attains the optimal results and establish the functional relationships between the some grafting parameters starch content, initiator concentration and reaction temperature on grafting parameters.

Table 10 The application of RSM

Objectives	**Model**	**Reference**
Optimization of biopolymerization rate	Central composite design (CCD)	(Aktaş, N., 2005)
Optimization of ultrasolnicextration	Box-Behnken design (BBD)	(Zhong, K. andQ. Wang, 2010)
Optimization of fluoride adsorption	Box-Behnken design(BBD)	(Mourabet, M. et al., 2012)
Removal hexavalent chromium	Central composite faced -center	(Garg, U. K., M. P. Kaur, D. Sud and V. K. Garg, 2009)
Optimizing Cr(VI) removal	Box-Behnken design	(Kiran, B., A. Kaushik and C. P. Kaushik, 2007)
Optimization of heavy metal ions biosorption	Box-Behnken design	(Kumar, R., R. Singh, N. Kumar, K. Bishnoi and N. R. Bishnoi, 2009)
Optimization of Cr(VI) removal	Central composite design (CCD)	(Cao, J., Y. Wu, Y. Jin, P. Yilihan and W. Huang, 2014)
Optimization of Cr(VI) removal	Box-Behnken design	(Jain, M., V. K. Garg and K. Kadirvelu, 2011)
Screening factors	Two-leveled and three-leveled factorial design	(Yördem, O. S., M. Papila and Y. Z. Menceloğlu, 2008)

MATERIALS AND METHODS

There are 4 main experimental steps on this study consist of optimization of tapioca starch graft PDMC, characterization of the optimum starch grafted copolymer, flocculation ability and the dual functional flocculants in removing of kaolin suspension and anionic substance such as hexavalent chromium ions (Cr(VI)) and Congo red dye (CR). To find out the optimization of tapioca starch graft copolymer, 17 runs 2 replicates from3 factors 3 levels of Box-Behnken design was applied. Then, the modified cationic starch grafted copolymers were investigated by FT-IR, XRD, TGA, SEM and CHN analysis to confirm the existing of DMC monomer. In the flocculation study, jar test was conducted to study the flocculation ability of the modified cationic starch grafted copolymer compared to Poly (*diallyldimethyl ammonium chloride)*, the commercials flocculants (PDADMAC) and the controlled non-modified starch. Finally, the dual functional flocculation was studied by synthetic wastewater containing Cr(VI) 1 mg/l and Congo red dye 5 mg/l in kaolin suspension 0.25% w/v.

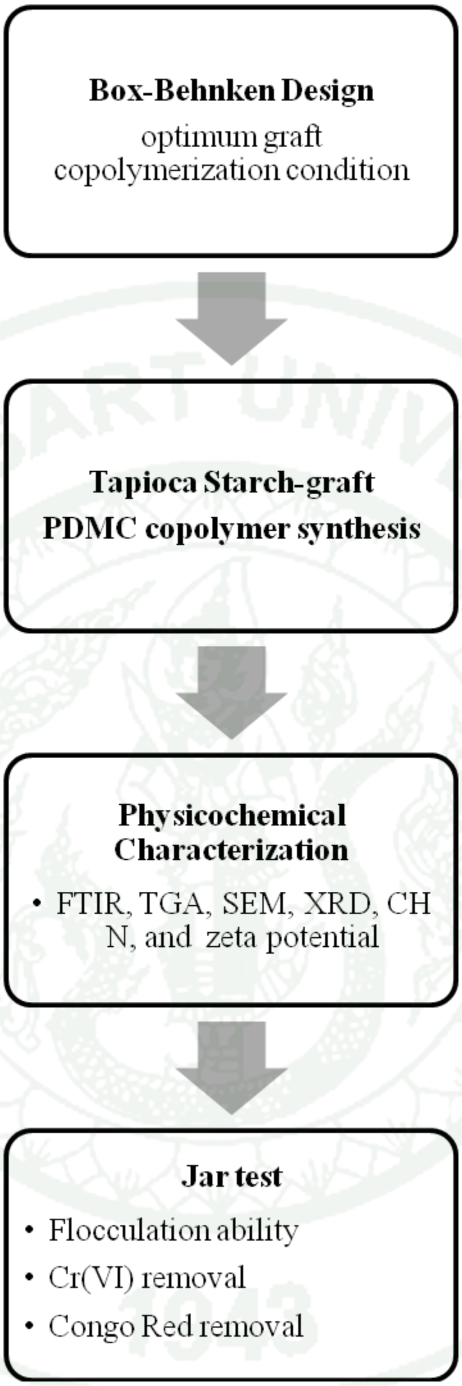

Figure 13 Schematic of overall works

Materials and Equipments

1. Materials and chemicals

1. Tapioca starch (food grade) was kindly supplied by Siam Quality Starch Co.,Ltd., Thailand. It is a native tapioca starch. The starch was dried at 100°C for 48 h in an oven and kept in desiccators prior to using.

2. 2-methacryloyloxyethyl trimethyl ammonium chloride (DMC) monomer and KPS from Sigma-Aldrich Co., USA.

3. Acetone, ethanol, HNO_3, NaOH 1M are analytical reagent grade from Thermo Fisher Scientific lnc., UK.

4. Kaolin suspended (Acros Organics) was kindly supported from Department of Chemical Engineering, Faculty of Engineering, Thumasat University.

5. Congo Red dye and potassium dichromate ($K_2Cr_2O_7$) are analytic reagent grade form Carlo Erbra and Sigma-Aldrich Co., USA, respectively.

6. Poly (*diallyldimethyl ammonium chloride)* was kindly supported by Nanotechnology center laboratory.

2. Equipments

1. The Fourier-transform infrared spectroscopy (FTIR) spectrometer (Nicolet 6700, NicoletInstrumentCo.,USA).

2. A scanning electron microscope(SEM, Quanta 450 FEI)

3. Thermal properties were evaluated by thermogravimetric analysis (Mettle Toledo, TGA851e/1600).

4. A wide angle X-ray diffractometer

5. Elemental analysis was performed on an automatic elemental analyzer (CHN628, Leco instrument Ctd., USA)

6. Zetasizer (Nanosizer, Malvern).

7. Mechanic stirrer

Methods

1. Optimization of tapioca starch-graft-PDMC copolymer

1.1 Experimental design

A three level, three variable Box-Benhken factorial designs (BBD) (Minitab software version 16.2.0) was applied to determine the best combination of graft copolymerization variables for the grafting parameter %add-on and %GE. Three graft copolymerization variables considered for this research were starch content (X_1), concentration of KPS initiator (X_2) and reaction temperature (X_3). These three variable concern of the great influence to the starch graft copolymerization from the recent studied by many researcher(Lanthong *et al.*, 2006); (Kaewtatip and Tanrattanakul, 2008; Mohd *et al.*, 2012; Witono *et al.*, 2012)). The proper ranges of three variables were determined on the basis of literature reviews for the starch graft copolymerization in chapter 2. Table 1. Listed of the whole design consisted of 15 runs combination experiment, five replicates at the center of the design were used to allow for the estimation of a pure error sum of squares. The 2 replicates were performed at all design points in randomized order.

1.2 Statistical analyses

Experimental data were fitted to a quadratic polynomial model and regression coefficients obtained. The non-linear computer-generated quadratic model used in the response surface was as follows:

$$Y = \beta_0 0 + \sum_{i=0}^{4} \beta_i X_i + \sum_{j=0}^{4} \beta_{ii} X_i^2 0 + \sum_{i=0}^{4} \sum_{j=0}^{4} \beta_{ij} X_i X_j$$

Eq.2

Where Y is the measured response associated with each factor level combination;β_0 is an intercept;β_i is regression coefficient computed from the observed experimental values of Y; and X_i is the code levels of independent variables. The terms X_i, X_j and X_i^2 represented the interaction and quadratic terms, respectively.

The statistical analysis system was computer program used for performing the analysis of variance (ANOVA) and response surface studied. The quality of the fit of the quadratic model equation was expressed by the coefficient of determination R^2, and the significances of the regression coefficient were checked by F-test and p-value.

All three-dimensional response surface graphs and two-dimensional contour plots were generated using Statistic software (Minitab version 16.0.2).

2. Tapioca starch-Graft-PolyDMC copolymer preparation

A 3 factors 3 levels response surface Box-Behnken design with requires 15 runs was used for the synthesis. The synthesis parameters were the weight ratio of tapioca starch and DMC monomer (X_1), concentration of KPS (X_2) and reaction temperature (X_3). These three variables and with their respective ranges, were chosen based on the preliminary studied, as shown in Table 13.

Table 11 Independent variables and their coded levels

Variables (factors)	Code	Code variable levels		
		low	**medium**	**high**
		-1	**0**	**1**
Tapioca starch content (g)	X_1	2.5	5	7.5
Concentration of KPS (M)	X_2	0.074	0.111	0.174
Reaction temperature (°C)	X_3	40	50	60

The synthesis procedures and grafting parameter %add-on and grafting efficiency (%GE) for this research was based on the procedured by (Lkhuoria, and Okieimen, 2010; Kaewtatip and Tanrattanakul, 2008)

Figure 17 explained the graft polymerization procedure. First, dried tapioca starch and distilled water 80 ml were mixed in a 250 ml of three-neck flash under nitrogen atmosphere and stirred with 240 rpm through the experiment. To obtain homogeneous slurry (pre-gelatinization), starch solution had been stirred at 65°C for 45 min. After added amount of KPS with 20 ml of distilled water for 15 min, 2-methacryloyloxyethyl trimethyl ammonium chloride (DMC) monomer was added. The total amount of starch and DMC monomer was 10 g in total water 100 ml. Graft copolymer was completed at a various conditions followed Table 14. When reaction was over, modified grafted starch was precipitated and purified with acetone and ethanol, respectively and dried in an oven at 60°C. Soxhlet extraction with ethanol for 24 h was required to eliminate DMC homopolymer from the dried modified grafted starch copolymer. Finally, the tapioca starch-graft-PDMC was dried in an oven at 60°C until a constant weigh and kept in the desiccators. In order to test reproducibility of graft copolymerization, three run of polymerization were carried out for all samples.

Table 12 The treatment combination from Box-Behnken Design using Minitab 16.2.0 software

Run	Coded variable levels		
	Tapioca starch content (X_1)	**Concentration of KPS initiator (X_2)**	**Reaction temperature (X_3)**
1	0	1	1
2	0	1	-1
3	0	0	0
4	0	-1	1
5	-1	0	-1
6	0	0	0
7	1	-1	0
8	-1	-1	0
9	-1	0	1
10	0	0	0
11	1	0	1
12	1	0	-1
13	0	0	0
14	0	0	0
15	1	1	0
16	-1	1	0
17	0	1	-1

To calculate grafting parameters, the products before and after Soxhlet extraction were weighed and characterized by various techniques. The percentage of add-on (%add-on) and grafting efficiency (%GE) were calculated from Eqs. (2) and (3), respectively.

(A) Grafting efficiency (%GE):

$$\%\mathrm{GE} = \frac{\text{weight of grafted copolymer}}{\text{weight of grafted copolymer before soxhlet}} \times 100$$

(Eq.3)

Grafting efficiency express the selectivity of the main reaction with respect to the most important byproduct homopolymer

(B) Add-on

$$\text{Add} - \text{on} = \frac{\text{weight of grafted copolymer}}{\text{weight of monomer}} \times 100$$

(Eq.4)

The add-on or yielding is explained about grafted polymer with respect to the amount of monomer.

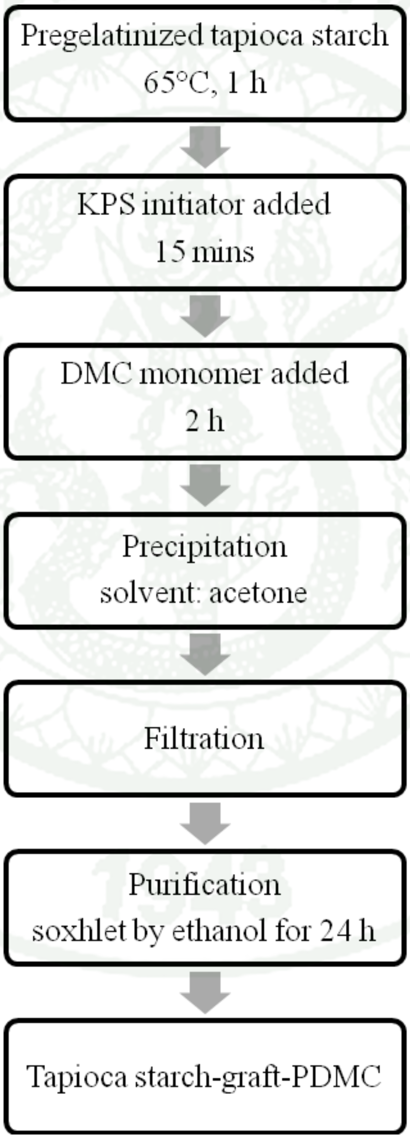

Figure 14 The steps of tapioca starch grafting copolymerization by introducing PDMC

3. Characterization of tapioca starch-Graft-PDMC copolymer

The physicochemical characterization was used to confirm the successful of grafted copolymerization of DMC monomer onto tapioca starch backbone. In this study, Fourier transforms infrared spectrometer (FTIR), Thermogravimetric analysis (TGA), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Elemental analysis (CHN), and Zeta potential was studied.

3.1 Fourier transforms infrared spectrometer (FTIR)

An Infrared spectra of tapioca starch and the grafted copolymer were investigated by The Fourier-transform infrared spectroscopy (FTIR) spectrometer (Nicolet 6700, NicoletInstrumentCo.,USA) using potassium bromide (KBr) disc technique. The samples were scanned at a frequency range of 4000-400 cm^{-11} with 64 consecutive scan in 4 cm^{-1} resolution. All spectra were baseline corrected and normalized

3.2 Thermogravimetric analysis (TGA)

Thermal properties were evaluated by thermogravimetric analysis (Mettle Toledo, TGA851e/1600) operating at a heating rate 10°C/min from 50 to 950 °C under nitrogen atmosphere.

3.3 X-ray diffraction (XRD)

Semi-crystalline structure of starch granules was detected by using a wide angle X-ray diffractometer.

3.4. Scanning Electron Microscopy (SEM)

The surface morphology of the native starch and the grafted copolymer examined with a scanning electron microscope (SEM, Quanta 450 FEI).The sample was coated with goal prior to observation.

3.5 Elemental analysis (CHN)

Elemental analysis was performed on an automatic elemental analyzer (CHN628, Leco instrument Ctd., USA).

3.6. Zeta potential

Surface charges of tapioca -graft-PDMC copolymer, PDADMAC flocculants and kaolin suspension were tested by Zeta potential (Nanosizer, Malvern). Experiment were performed by using 5 mg/l of flocculant dosage. pH of flocculant solutions was adjusted by adding HCl (0.1M) or NaOH (0.1M). After 5 minutes of gentle stirring, the samples were analyzed and the data were recorded.

4. The flocculation ability of tapioca starch-graft-PDMC compares to commercial flocculants (PDADMAC) and non-modified tapioca starch

The comparative of flocculation ability of tapioca starch-graft-PDMC to the commercial flocculants PDADMAC and non-modified tapioca starch within the same conditions was studied.

Jar test was used to study each three flocculants performances. The jar test procedure was conducted in this study shown in Figure 18.

200 ml of wastewater sample were added to a 500 ml beaker. The pH of the waters sample were adjust to 4.0, 7.0, and 10.0 with 1 M of HCl and NaOH solution, respectively monitored by a pH meter. The beakers was placed on joint-stirrers and agitated by stirring blades in the solution. The flocculant solution was added in different dosage ranging from 0 to 6 mg/l to the different beakers, which were stirred at uniform speed of 200 rpm for 2 min, then at 60 rpm for 15 min. After allowing settling to occur, about 25 ml of the supernatant was withdrawn using syringe from a height of about 3 cm below the liquid surface in each jar. The supernatant was detected by measurement the absorbance of solution at a wavelength of 630 nm. The turbidity values of the flocculation experiment in the present work are expressed by

the %Transmittance. Experimental were all duplicated to test their reproducibility. All experimental were conducted at room temperature (20-25°C). Effects of pH and flocculants dosage to %Transmittance were studied.

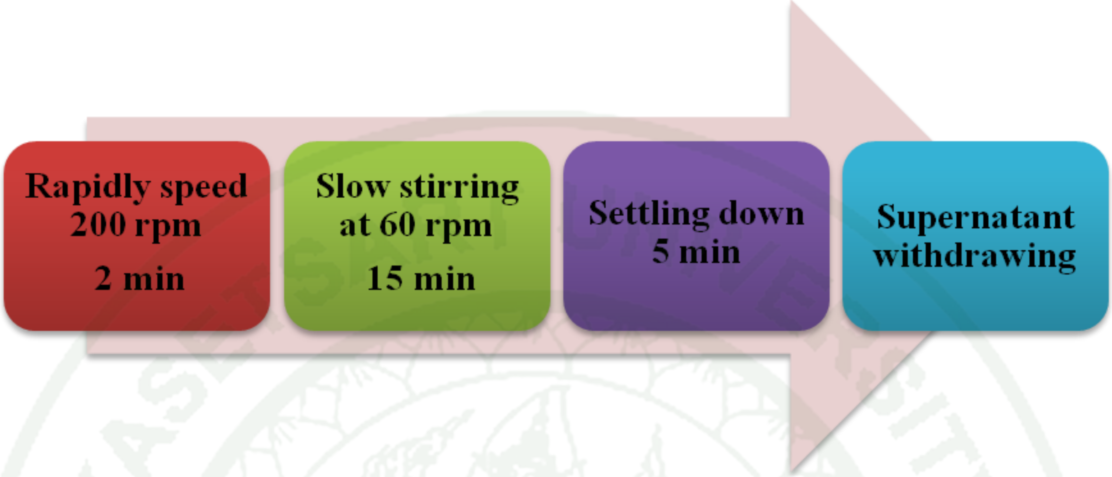

Figure 15 Jar test procedures

5. Dual functional of tapioca starch-graft-PDMC flocculants study

To study the dual functional of tapioca starch-graft-PDMC flocculant in both removal of anion substance and reduction of turbidity, the 2 separated experiments were carried out. First, the flocculation ability in the solution consisted of hexavalent chromium (Cr(VI)) and kaolin suspension and the second was the flocculation ability in the solution consisted of Congo red dye (CR) and kaolin suspension. The effect of kaolin suspension to the removal efficiency was studied. The measurements by UV-vis spectroscopy of all supernatants were shown in Figure 19. The calibration curve of Cr(VI) and Congo red dye concentration was prepared to convert the absorbance into mg/l.

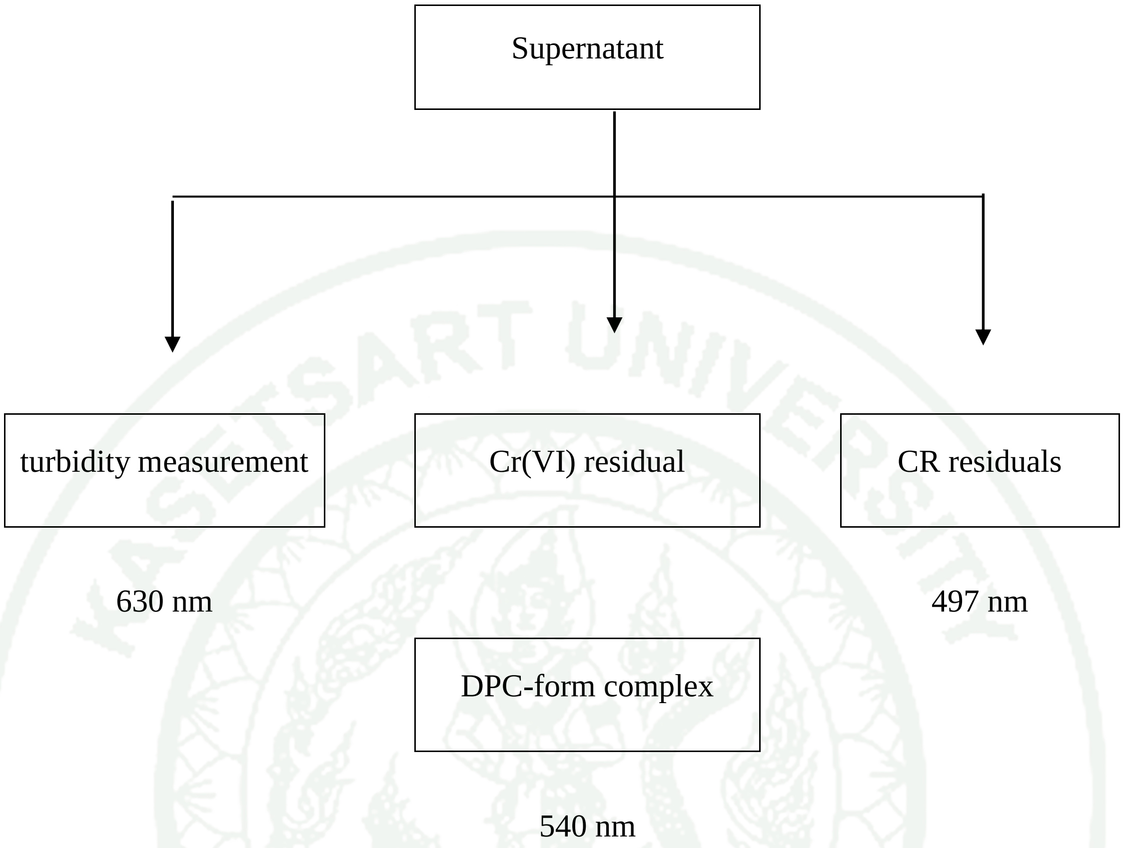

Figure 16 The supernatant measurement by UV-vis spectroscopy

5.1 Turbidity reduction and Cr(VI) removal efficiency of tapioca starch-graft-PDMC in synthetic wastewater contained 0.25%wt kaolin suspension and 1 mg/l Cr(VI).

Preparation of calibration curve of hexavalent chromium (VI)

Tapioca starch-graft-PDMC (10 mg/l) solution and the wastewater contained 5 mg/l of Cr(VI) was prepared with $K_2Cr_2O_7$ as stock solution. 1,5-diphenylcarbazide (DPC) reagent was prepared by dissolving 250 mg 1,5-diphenylcarbazide in 50 ml of acetone. Sulfuric acid was prepared by sulfuric acid 10 ml added to 100 ml of distilled water (10% v/v).

Pipet a chromium stock solution in measured volumes into 100 ml of volumetric flask to generate Cr(VI) standard solutions ranging from 0.2 to 1 mg/l Cr (VI) when diluted to the appropriate volume. The concentrations of chromium were traced by 1,5- diphenylcarbazide (DPC) complex quantitative spectroscopy.

Let stand for full color development same as sample. Transfer an appropriate portion of the solution to 1-cm absorption cell and measure its absorbance at 540 nm using UV-vis Spectrophotometer (Lambda 650 UV/Vis spectrometer, Perkin Elmer). Use distilled water as reference. Correct the absorbance reading of the sample by subtracting the absorbance of blank carried through the method. Calibration curve was constructed by plotting correct absorbance value against mg/l of Cr(VI).If the reading of absorbance was greater than 1.0, then necessary dilution was made. The samples should be stored 4°C until analyzed. The maximum holding time prior to analysis of the samples is 24 h. *(method 7196A)(Epa-600/4-82-055, December 1982)*

Jar test

The efficiency of tapioca starch-graft-PDMC flocculant to remove turbidity and hexavalent chromium (VI) was carried out following the previous jar test procedure. The effect of pH solution, flocculant dosage, and kaolin suspension to turbidity reduction and Cr(VI) removal efficiency was studied.

200 ml of wastewater sample contained kaolin suspension 0.25% w/v which prepared from stock solution of 10 mg/l of tapioca starch-graft-PDMC flocculant solution and Cr(VI) concentration of 1 mg/l were added to a 500 ml beaker. The pHs of the water sample were adjust to 2.0, 4.0, 7.0, and 10.0 with 1 M of HCl and NaOH solution, respectively, monitored by a pH meter. The beakers was placed on joint-stirrers and agitated by stirring blades in the solution. The tapioca starch –graft-PDMC solution was added in different dosage ranging from 0 to 6 mg/l to the different beakers. Each beaker was stirred at uniform speed of 200 rpm for 2 min, then at 60 rpm for 15 min. The flocs were allowed to settle for 5 min and the

supernatant were taken to test %Transmittance and to trace amount of Cr(VI) in solution.

The determination of the residual Cr(VI) in sample solution had done by following steps.

After filtration with syringe filter,

1. 2.0 ml the sample solution was added into 5 ml of volume-flask.

2. 2.0 ml of DPC were added and mixed,

3. H_2SO_4 added following to give solution pH as 2±0.5 and diluted to 5 ml with diluted water.

4. Let stand for 5 to 10 min for full color development.

5. Transfer an appropriate portion of the solution to 1-cm absorption cell and measure its absorbance at 540 nm. Use distilled water as reference.

6. Correct the absorbance reading of the sample by subtracting the absorbance of blank carried through the method.

7. The initial and final concentration of Hexavalent chromium (Cr(VI)) in mg/l were determined by linear regression equation Eq.4 obtained by plotting calibration curve

$$Cr(VI)\ removal\ (\%) = 100 \times \frac{C_i - C_f}{C_i} \qquad Eq.5$$

When C_i is the initial Chromium (VI) concentration and C_f is the final concentration.

Figure 17 Blade and mechanical stir using in jar test for study flocculation ability of tapioca starch-graft-PDMC in this present work

5.2 Turbidity reduction and Congo red dye removal efficiency of tapioca starch-graft-PDMC in synthetic wastewater contained 0.25%wt kaolin suspension and 5 mg/l Congo red dye

Preparation of calibration curve of Congo red dye (CR)

Tapioca starch-graft-PDMC (10 mg/l) solution and the wastewater contained 50 mg/l of Congo Red dye was prepared as stock solution.

Pipet a Congo Red dye (CR) stock solution in measured volumes into 100 ml of volumetric flask to generate CR standard solutions ranging from 0.5 to 50 mg/l. Transfer an appropriate portion of the solution to 1-cm absorption cell and measure its absorbance at 497 nm. Use distilled water as reference. Correct the absorbance reading of the sample by subtracting the absorbance of blank carried through the method. Calibration curve was constructed by plotting correct absorbance value against mg/l of CR. If the reading of absorbance was greater than 1.0, then necessary dilution was made.

Jar test

The efficiency of tapioca starch-graft-PDMC flocculant to remove turbidity and Congo red dye (CR) was carried out followed the previous jar test procedure. The effect of pH solution, flocculant dosage, and kaolin suspension to turbidity reduction and Congo red dye removal (CR) efficiency was studied.

200 ml of wastewater sample which contained kaolin suspension 0.25% w/v and CR concentration of 5 mg/l were added to a 500 ml beaker. The pHs of the water sample were adjust to 2.0, 4.0, 7.0, and 10.0 with 1 M of HCl and NaOH solution, respectively, monitored by a pH meter. The beakers was placed on joint-stirrers and agitated by stirring blades in the solution. The tapioca starch –graft-PDMC solution was added in different dosage ranging from 0 to 6 mg/l to the different beakers. The beakers were stirred at uniform speed of 200 rpm for 2 min, then at 60 rpm for 15 min. The flocs were allowed to settle for 5 min and the supernatant were taken to test %Transmittance and absorbance of CR at 630 nm and at 497 nm, respectively. The initial and final concentration of Congo red dye (CR) in mg/l were determined by linear regression equation Eq.5 obtained by plotting calibration curve.

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$$\text{Dye removal } (\%) = 100 \times \frac{C_i - C_f}{C_i} \qquad Eq.6$$

When C_i is the initial dye concentration and C_f is the final concentration.

5. Place

Nanotechnology Center of National Science and Technology Development Agency, Pathumthani, 12120, Thailand

6. Duration

April 2013 to January 2014

RESULTS AND DISCUSSIONS

This chapter is separated into 4 parts consisting of (4.1) optimization of Tapioca starch-graft-PDMC copolymerization by using response surface methodology (RSM); Box-Behnken design (BBD) and the effect of tapioca starch content, concentration of KPS initiator and reaction temperature to %add-on and %GE, (2) physicochemical characterization of Tapioca starch-graft-PDMC copolymer prepared from optimum condition, (3) turbidity reduction efficiency of the starch grafted copolymer flocculants compare to commercials flocculants (PDADMAC) and native tapioca starch, and (4) the dual functional study in both of turbidity reduction efficiency and the removal of anionic substances such as heavy metal ion (hexavalent chromium or Cr(VI) and Congo red dye.

1. Optimum condition of tapioca starch graft copolymerization and effect of tapioca starch content, concentration of KPS initiator and reaction temperature to %add-on and %GE

1.1 Response surface methodology approach

1.1.1 Response surface methodology (Box-Behnken Design) evaluations

The factors mostly effect to grafting parameters; %add-on and %GE are starch content, concentration of KPS initiator and reaction temperature. Table 15 showed the overall Box-Behnken experimental design with the response %add-on and %GE. Data were also analyzed to check the normality of the residuals was showed in Figure 21. A normal probability plot or a dot diagram of these residuals showed the data points on this plot lie reasonably close to a straight line for both of two grafting parameter responses. This implied that the assumption models of the analysis are desirable.

When fitting the model, various statistical analysis technique are employed to analyze the experimental error, the approximately of model and statistical significance of the terms in model. Some of the model items may be insignificant for a good representation of experimental data so usually a subset of model with fewer terms is selected. There are often several subsets that lead to similar results, so care must be taken when trying to interpret single model term.

Table 13 Box-Behnken design matrix for the three variables together with the observed responses.

Run	Coded variable levels			Response	
	Starch content (X_1)	[KPS] (X_2)	Reaction Temperature (X_3)	%add-on	%GE
1	0	1	1	41.4	29.28
2	0	1	-1	16.6	14.24
3	0	0	0	34	25.37
4	0	-1	1	13.4	11.82
5	-1	0	-1	111.3	60.38
6	0	0	0	42.6	29.87
7	1	-1	0	10	3.23
8	-1	-1	0	16.7	33.33
9	-1	0	1	63.3	65.52
10	0	0	0	34	25.37
11	1	0	1	78	43.82
12	1	0	-1	27.2	8.31
13	0	0	0	54	35.06
14	0	0	0	34	25.37
15	1	1	0	61.6	17.04
16	-1	1	0	34.4	50.79
17	0	1	-1	18.4	15.54

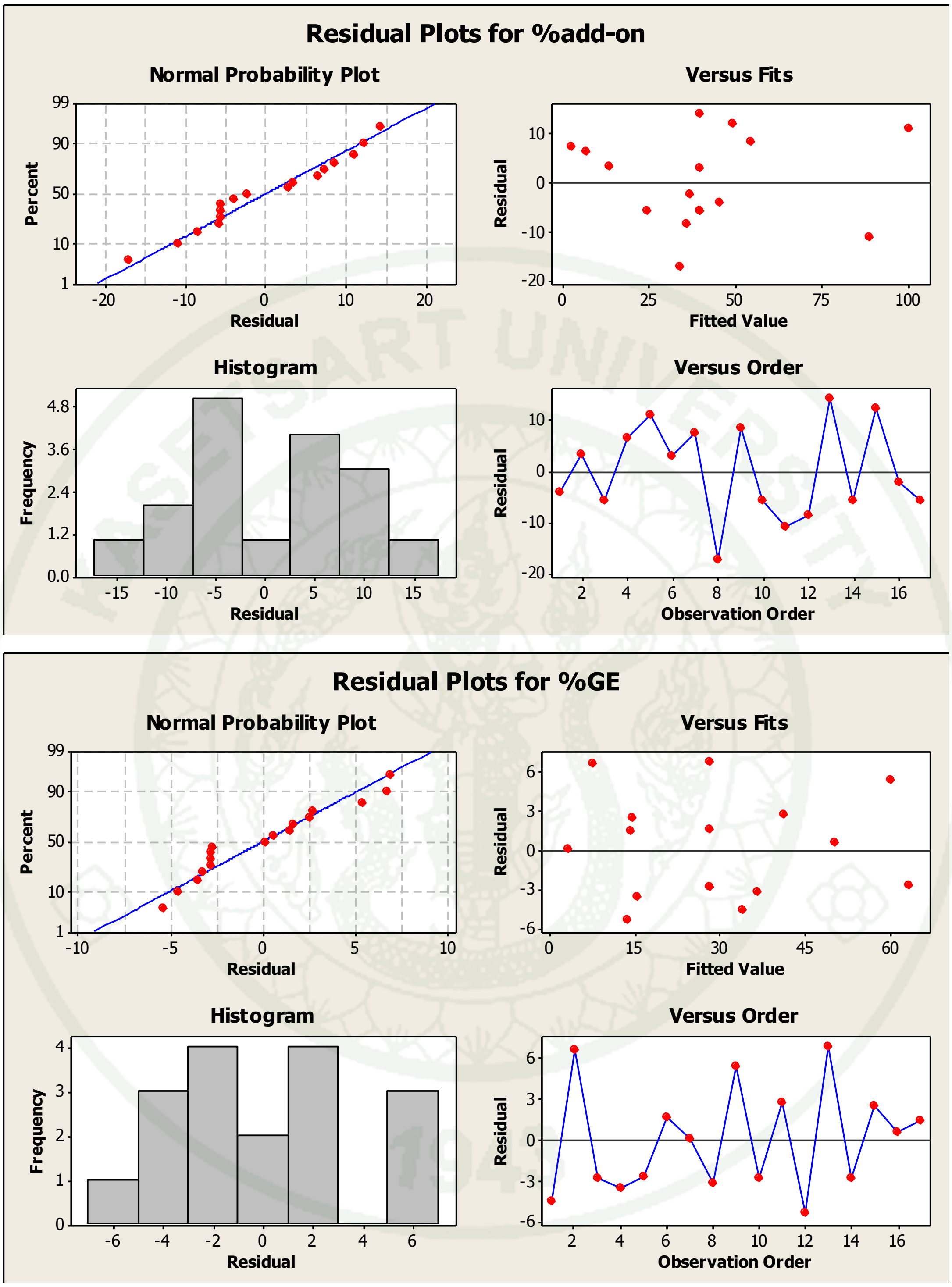


Figure 18 The plots for checking data of %add-on and %GE before analyzed

1.1.2 Experimental design

A regression analysis result of %add-on and %GE was showed in Table 14 and 15 The final models can be use to create graphical representations of parameter dependencies, e.g. as contour plots to see the relative influence of parameters to find optimum parameter combination and to predict experimental results for other parameter combinations.

Table 14 Estimated regression model of relationship between responses response (%add) and independent variables (X_1, X_2, X_3).

Term	**Coef**	**SE Coef**	**T**	**P**	**comment**
Constant	536.4	201.97	2.656	0.033	-
X_1	-93	18.59	-5.002	0.002	Significant
X_2	2470.4	1040.98	2.373	0.049	Significant
X_3	-17.3	7.01	-2.464	0.043	Significant
X_1^2	3.1	1.07	2.881	0.024	Significant
X_2^2	-13510.6	2913.77	-4.637	0.002	Significant
X_3^2	0.1	0.07	1.648	0.143	-
X_1X_2	88.2	53.88	1.637	0.146	-
X_1X_3	1	0.27	3.607	0.009	Significant
X_2X_3	13.7	13.47	1.02	0.342	-

S = 13.6954

R-Sq = 88.28% R-Sq(adj) = 73.21%

Table 15 Estimated regression model of relationship between responses response (%add) and independent variables (X_1, X_2, X_3).

Term	**Coef**	**SE Coef**	**T**	**P**	**Comment**
Constant	168.08	87.01	1.932	0.095	-
X_1	-41.27	8.01	-5.154	0.001	Significant
X_2	1559.57	448.46	3.478	0.01	Significant
X_3	-5.53	3.02	-1.831	0.11	Significant
X_1^2	1.97	0.46	4.291	0.004	Significant
X_2^2	-6896.17	1255.26	-5.494	0.001	Significant
X_3^2	0.04	0.03	1.378	0.211	-
X_1X_2	-4.57	23.21	-0.197	0.85	-
X_1X_3	0.3	0.12	2.574	0.037	Significant
X_2X_3	5.97	5.8	1.029	0.338	-

S = 5.90003

R-Sq = 95.16% R-Sq(adj) = 88.94%

Predicted response Y for the %add-on and %GE of starch-graft-PDMC copolymerization could be expressed by the following second order polynomial equation in terms of coded values:

$$Y_{\%add-on} = 536.4 - 93X_1 + 2470.4X_2 - 17.3X_3 + 3.1X_1^2 - 13510.6X_2^2 + 0.1X_3^2 + 88.2X_1X_2 + 1X_1X_3 + 13.7X_2X_3$$

(Eq. 7)

$$Y_{\%GE} = 168.08 - 41.27X_1 + 1559.57X_2 - 5.53X_3 + 1.97X_1^2 - 6869.17X_2^2 + 0.04X_3^2 - 4.57X_1X_2 - 0.3X_1X_3 + 5.97X_2X_3$$

(Eq. 8)

Where Y are the %add-on and %GE of tapioca starch graft copolymer , and X_1, X_2, X_3 are the coded variables for starch content, concentration of KPS initiator and reaction temperature, respectively.

The P-values were used as a tool to check the significance of each coefficient, which in turn might indicate the pattern of the interactions between the variables. The smaller the value of P was, the more significant the corresponding coefficients were. Since Table 14 and 15, the coefficients of all independent variables X_1, X_2, X_3, and two square terms X_1^2, X_2^2 and one interaction term X_1X_3 were significant because of very small P-values ($P < 0.05$) for %add-on. Meanwhile %GE, the coefficient of independence X_3 was found insignificant term. Consequently, the quadratic model to explain the effect of each factor to %add-on and %GE could be expressed without the insignificant terms as follows:

$$Y_{\%add-on} = 536.4 - 93X_1 + 2470.4X_2 - 17.3X_3 + 3.1X_1^2 - 13510.6X_2^2 + 1X_1X_3$$

Eq. 9

$$Y_{\%GE} = -41.27X_1 + 1559.57X_2 + 1.97X_1^2 - 6869.17X_2^2 - 0.3X_1X_3$$

Eq. 10

1.1.3 Analysis of variance

ANOVA was used to check the significance and fitness of the model. The applied ANOVA for the regression model of %add-on and %GE were presented in Table 17. Values of Prob>F less than 0.0500 indicated that model terms were significant. The model terms were insignificance when p-value are greater than 0.05.

From Table 16, the model F-value for the regression of %add-on was 5.86 and Prob>F showed less than 0.05. It meant that the regression model was significant and suitable to explain the relationship between graft copolymerization factors and %add-on response. Tapioca starch content, KPS initiator concentration showed square effect. So, there was curvature in the response. There was interaction effect between tapioca starch and reaction temperature which meant tapioca starch effect to %add-on depended on reaction temperature. Tapioca starch, KPS initiator concentration and reaction temperature had linear effect to the response.

The regression model of %GE was analyzed by ANOVA was determined in Table 17 The model F-value was 15.3 and the Prob>F showed less than 0.05. It meant that this regression model was significant and suitable to explain the relationship between graft copolymerization factors and %GE response. In addition, ANOVA resulted tapioca starch content and KPS initiator concentration had square effect which created the curvature in the response surface. There was only one interaction effect between tapioca starch and reaction temperature. Hence, in this study tapioca starch content effect to %GE depended on reaction temperature. There were only tapioca starch content and KPS initiator concentration had linear effect to %GE response.

The lack of fit F-value of both %add-on and %GE response were 4.31 and 3.06, respectively. It meant that there was no significance relative to the pure error. So, the model equation was adequate for predicting the grafting parameters under any combination of values of the variables. R^2_{adj} (adjusted determination coefficient) is the correlation measurement for testing the goodness-of-fit of the regression equation. The higher values it is, the better degree of correlation between the observed and predicted values are. [(Zhong, K. andQ. Wang, 2010)]. The value of R^2_{adj} for Eq. 6 and 7 were 0.7321 and 0.8894, which was reasonably close to 1. It meant that only less than 27% for %add-on and 11% for %GE of the total variations were not explained by model.

Table 16 Analysis of variance for the fitted quadratic polynomial modes of tapioca starch graft copolymerization for % add-on

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**	**Comment**
Regression	9	9889.7	9889.7	1098.86	5.86	0.015	Significant
Linear	3	951	6984.2	2328.06	12.41	0.003	Significant
X_1	1	298.9	4693.4	4693.4	25.02	0.002	Significant
X_2	1	588.2	1056.3	1056.31	5.63	0.049	Significant
X_3	1	63.8	1138.5	1138.51	6.07	0.043	Significant
Square	3	5800.6	5800.6	1933.54	10.31	0.006	Significant
X_1^2	1	1394.8	1556.6	1556.62	8.3	0.024	Significant
X_2^2	1	3896.1	4032.6	4032.63	21.5	0.002	Significant
X_3^2	1	509.7	509.7	509.71	2.72	0.143	-
Interaction	3	3138.1	3138.1	1046.03	5.58	0.028	Significant
X_1X_2	1	502.6	502.6	502.56	2.68	0.146	-
X_1X_3	1	2440.4	2440.4	2440.36	13.01	0.009	Significant
X_2X_3	1	195.2	195.2	195.18	1.04	0.342	-
Residual Error	7	1312.9	1312.9	187.56			
Lack-of-Fit	3	1002.6	1002.6	334.19	4.31	0.096	
Pure Error	4	310.4	310.4	77.59			
Total	16	11202.6					

Table 17 Analysis of variance for the fitted quadratic polynomial modes of tapioca starch graft copolymerization for % GE

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**	**Comment**
Regression	9	4794.3	4794.3	532.7	15.3	0.001	Significant
Linear	3	2853.84	1532.35	510.78	14.67	0.002	Significant
X_1	1	2367.41	924.86	924.86	26.57	0.001	Significant
X_2	1	148.82	420.99	420.99	12.09	0.01	Significant
X_3	1	337.61	116.74	116.74	3.35	0.11	-

Table 17 (Continued)

Source	DF	Seq SS	Adj SS	Adj MS	F	P	Comment
Square	3	1671.67	1671.67	557.22	16.01	0.002	Significant
X_1^2	1	579.3	641	641	18.41	0.004	Significant
X_2^2	1	1026.31	1050.64	1050.64	30.18	0.001	Significant
X_3^2	1	66.06	66.06	66.06	1.9	0.211	-
Interaction	3	268.8	268.8	89.6	2.57	0.137	-
X_1X_2	1	1.35	1.35	1.35	0.04	0.85	-
X_1X_3	1	230.58	230.58	230.58	6.62	0.037	Significant
X_2X_3	1	36.87	36.87	36.87	1.06	0.338	-
Residual Error	7	243.67	243.67	34.81			
Lack-of-Fit	3	169.8	169.8	56.6	3.06	0.154	
Pure Error	4	73.87	73.87	18.47			
Total	16	5037.98					

1.1.4 Response surface estimation for maximum grafting parameters (%add-on and %GE)

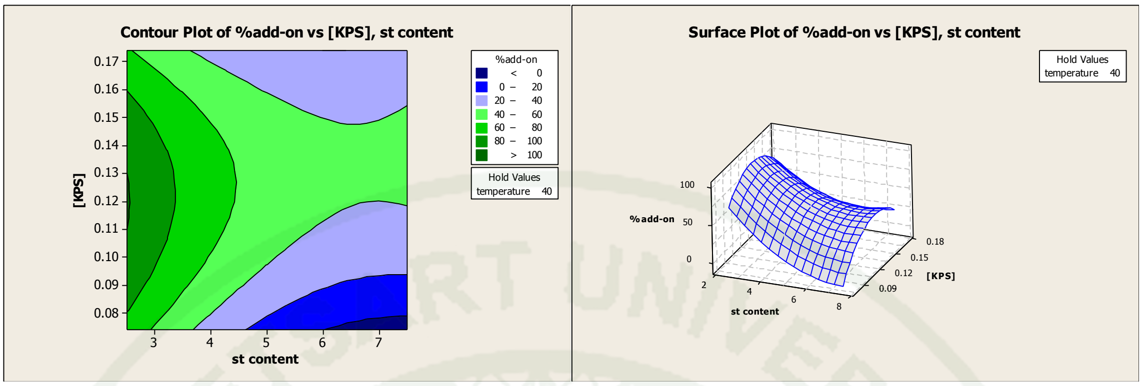
The 3D response surface and 2D contour plots are graphical representations of regression equations. Figure 19 and 20 provided a visualization of the relationship between responses and experimental levels of each variable and the type of interaction between two test variables.

Analysis of response surface of %add-on

From graft copolymerization data which presented previous analysis indicated that starch content (X_1) and concentration of KPS initiator (X_2) had quadratic effects on add-on, while reaction temperature (X_3) only had a linear effect. Therefore, the interpretation of the contour plot could be done by holding reaction temperature (X_3) fixed at its low and high levels as described below:

(a) 2D contour plot of low reaction temperature 40°C

(b) 3D surface plot for low reaction temperature 40°C



(c) 2D contour plot of high reaction temperature 60°C

(d) 3D surface plot of high reaction temperature 60°C

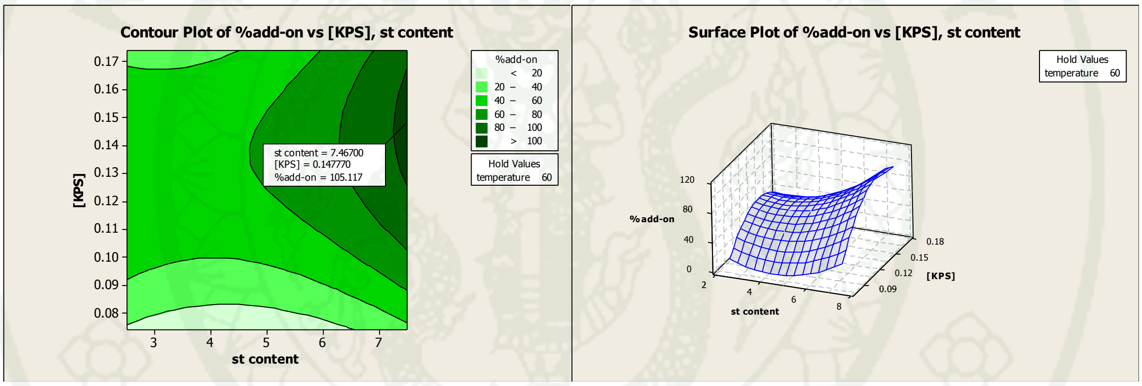


Figure 19 2D contour plot (a), (c) and 3D surface plot (b), (d) surface plot of % add-on. The reaction temperature was hold at low (40°C) and high (60°C), respectively

The plot in Figure 22, 2D (a) demonstrated how starch content and initiator concentration ([KPS]) related to %add-on when reaction temperature was at its low level of 40°C. The darkest green area indicated the contour where the response was the highest value more than 100%.

To maximize add-on, we would choose settings for starch content and concentration of KPS initiator in the slightly higher than the center of the left side of the plot. For instance, we could choose a starch content around 2.5 g and concentration of KPS initiator around 0.12 M.

Fig. 19, 2D (c) plot shows how starch content and concentration of KPS initiator related to add-on when reaction temperature was at its high level of 60°C. The darkest green area indicated the contour where the response was the highest value more than 105%. To maximize cleaning efficiency, we would choose settings for starch content and concentration of KPS initiator in the slightly higher the center of the right side of the plot. For example, we could choose a starch content around 7.5 g and concentration of KPS initiator around 0.145 M.

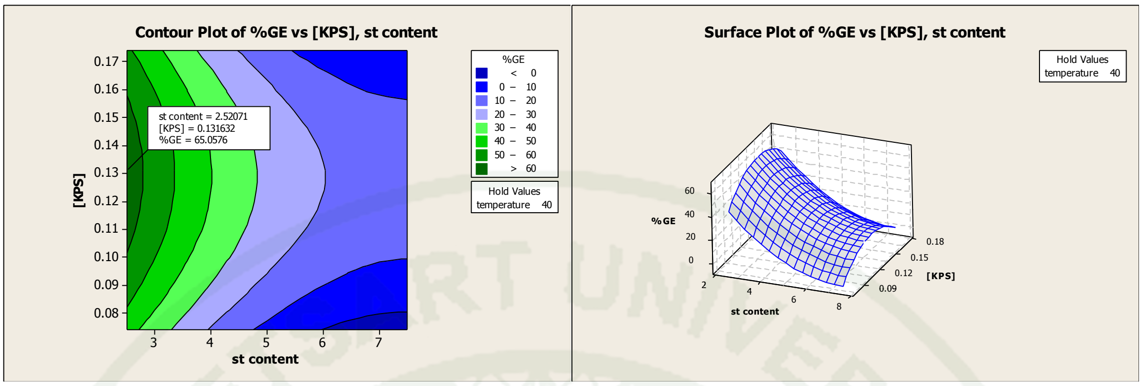
The plots indicate that as reaction temperature increased from its low to high level (40°C to 60°C), %add-on also increasing. Maximum add-on of approximately 105% occurred around starch content was 7.5, concentration of KPS initiator was 0.145 M, and reaction temperature was 60°C. Therefore, the contours might correspond to a minimax response surface.

Analysis of response surface of %GE

For graft copolymerization data, previous analysis showed that starch content and [KPS] had quadratic effects on grafting efficiency (%GE), while reaction temperature had no linear effect and interaction effect were only found. Therefore, the interpretation of the contour plot could be done by holding reaction temperature (X_3) fixed at its low and high levels as described below:

(a) 2D contour plot of low reaction temperature 40°C

(b) 3D surface plot for low reaction temperature 40°C



(c) 2D contour plot of low reaction temperature 60°C

(d) 3D surface plot of low reaction temperature 60°C

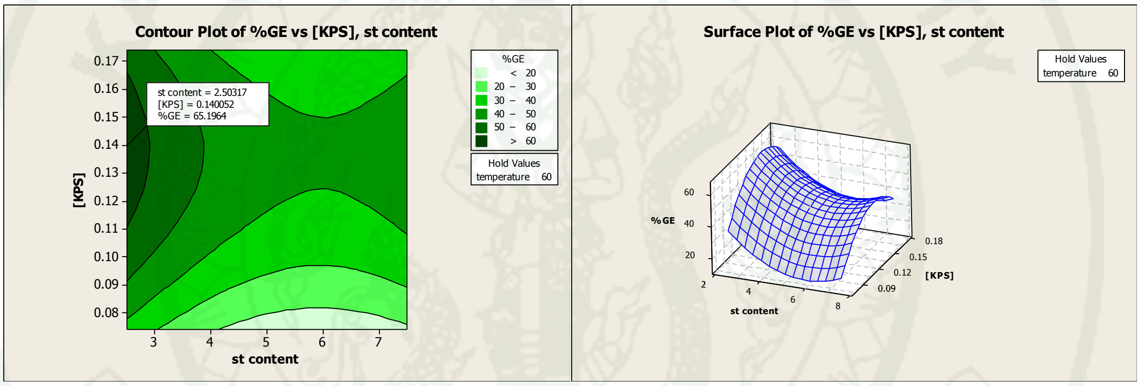


Figure 20 2D contour plot (a), (c) and 3D surface plot (b), (d) surface plot of % GE. The reaction temperature was hold at low (40°C) and high (60°C), respectively

This plot in Figure 20, 2D (a.) showed how starch content and concentration of KPS initiator were related to %GE when reaction temperature was 40°C. The darkest green area indicated the contour where the response was the highest value around 63 %. To maximize add-on, we would choose settings for starch content and concentration of KPS initiator around the center of the left side of the plot. For instance, we could choose a starch content around 2.5 g and [KPS] around 0.13 M.

The plot in Figure 20 2D (c) showed how starch content and concentration of KPS initiator related to %GE when reaction temperature was at its high level of 60°C. The darkest green area indicated the contour where the response was the highest value around 64%. To maximize cleaning efficiency, we would choose settings for starch content and concentration of KPS initiator in the slightly higher the center of the left side of the plot. For example, we could choose a starch content around 2.5 g and concentration of KPS initiator around 0.14 M.

The plots were also indicated that when reaction temperature increased from its low to high level (40°C to 60°C), %GE also increasing. Maximum %GE of approximately 64% occurred around starch content was 2.5, concentration of KPS initiator was 0.14 M, and reaction temperature was 60°C. Therefore, the contours might correspond to a minimax response surface.

1.1.5 Optimization of tapioca starch-graft-PDCM copolymerization

One of the main objectives of response surface methodology (RSM) is the determination of the optimum setting of control variables that result a maximum (or minimum) response over a certain region of interest (I. Khuri, Adre, et al, 2010). To ensure the predicted result was not bias the practical value, experiment rechecking was performed using this modified optimal conditions: tapioca starch content 2.5 g, DMC monomer 7.5 g, concentration of KPS 0.12 M , reaction temperature 40°C and reaction was carried out for 2 h. A mean values which obtained from real experiments (N=3) agree with the predicted value significantly (p>0.05). It was demonstrated that the validation of the RSM model was satisfactory and accurate.

Table 18 Optimum conditions and the predicted experimental values of response at the optimum conditions.

	Tapioca stanch content (g)	Concentration of KPS initiator (M)	Reaction temperature (°C)	Grafting parameters	
				%add-on	%GE
Optimum conditions	2.5	0.1195	40	101.3368	64.7923
Modified condition	2.5	0.12	40	111.3	60.38

The maximum predicted yield and experimental grafting parameter were given in Table 18. Additional experimental using the predicted optimum conditions for tapioca starch graft copolymer were carried out as using starch content of 2.5 g, concentration of KPS initiator of 0.1195 M and the reaction temperature of 40°C. The model had predicted a maximum %add-on and %GE response of 101% and 65%, respectively.

1.2 Effect of graft copolymer factors to %add-on and %GE

Figure 24 can be interpreted that when tapioca starch content increase, the grafting parameters both of %add-on and %GE decline. The concentration of KPS initiator increase, %add-on and %GE increase but after the optimum value the %add-on and %GE decrease drastically. The reaction temperature increase moving %add-on decrease far from the target while the decrease on %GE was slightly.

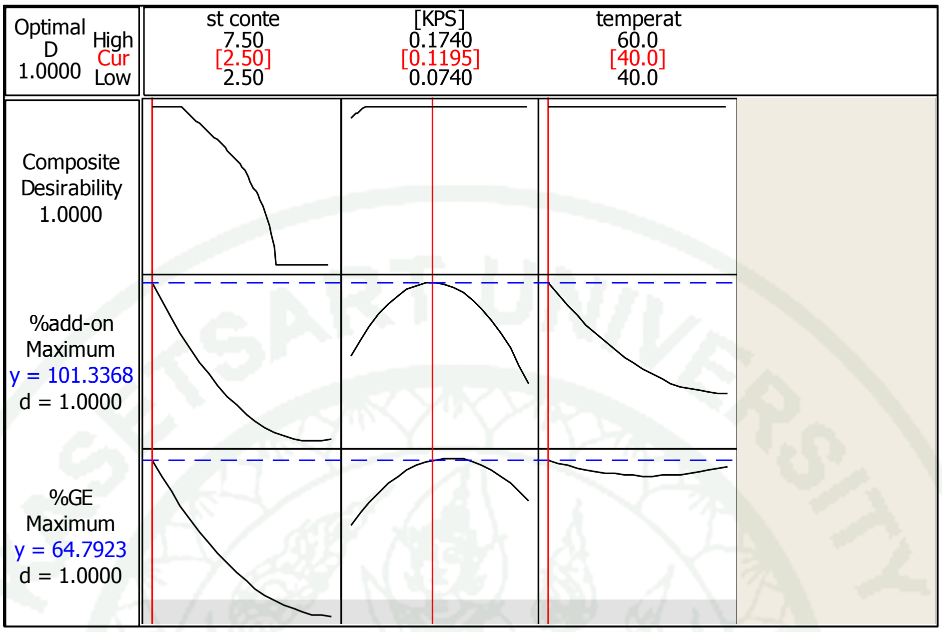


Figure 21 The optimum conditions resulted from Minitab 16.2.0 software

It is well known that free-radical graft copolymerization initiated by water-soluble initiator such as KPS, are dissociated in water and attacked the OH group of starch causing free-radicals on the starch molecules called “ macro radicals” and these macro radicals will be attacked the double bonds of vinyl monomer resulting in graft copolymer, the mechanism was demonstrated in Figure 21.

$$S_2O_8^{-\bullet} \longrightarrow 2SO_4^{-\bullet}$$

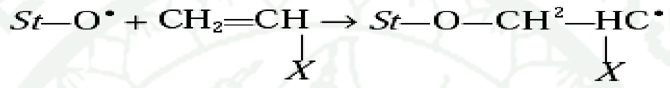
$$SO_4^{-\bullet} + H_2O \longrightarrow HSO_4 + HO^{\bullet}$$

$$2HO^{\bullet} \longrightarrow HOOH$$

$$HO^{\bullet} + HOOH \longrightarrow H_2O + HO_2^{\bullet}$$

$$S_2O_8^{-\bullet} + HO_2^{\bullet} \longrightarrow HSO_4^{-} + SO_4^{-\bullet} + O_2$$

$$St\text{—}OH + R^{\bullet} \longrightarrow St\text{—}O^{\bullet} + RH$$



$$St\text{—}O^{\bullet} + CH_2{=}\underset{\displaystyle X}{\underset{|}{CH}} \rightarrow St\text{—}O\text{—}CH^{2}\text{—}\underset{\displaystyle X}{\underset{|}{HC}}{}^{\bullet}$$

Figure 22 The starch graft copolymerization via KPS initiator in water media

Beginning with the KPS formed reactive radical to sulfate ion ($S_2O_8^{2-}$) and initiated on a starch backbone, then DMC monomer were simultaneously to form polymer chain and finally the starch macro radicals was reacted with the growing polyDMC chains. Therefore, it was believed that synthesis of tapioca starch-graft-PDMC copolymer under the present system could be capable.

1.2.1 Effect of tapioca starch content

In this current work, the tapioca starch content was proportion of the DMC monomer content. Due to the overall concentration of substance should not over than 10% w/w, if the starch content was 2.5 g, the DMC monomer would be 7.5 g. The overall levels studies of starch content and DMC monomer was shown in Table. 19

Table 19 Tapioca starch and DMC content used in this study

Levels	Tapioca starch content	DMC monomer content	Total
Low	2.5 g	7.5 g	
Medium	5 g	5 g	10 g
High	7.5 g	2.5 g	

The result from ANOVA indicated that the amount of tapioca starch and DMC monomer showed significantly affecting on %add-on and %GE. The highest DMC monomer content (7.5g) offered highest %add-on and %GE. Therefore, the DMC monomer-rich system was preferable in this study. The %add-on and %GE increasing with monomer concentration and the optimal condition obtained from the monomer-rich system agree with the report by other researchers ((Kaewtatip, K. andV. Tanrattanakul, 2008; Witono, J. R., I. W. Noordergraaf, H. J. Heeres and L. P. Janssen, 2012)). The more tapioca starch content, the more viscosity in system which implied that the less mobility of monomer penetrated to graft copolymerization. In monomer-rich system or less tapioca starch content, there was more opportunity for graft copolymerization. In general, there would be an optimum monomer concentration for each system. The low monomer concentration leads to decrease in active sites on the growing chains, in opposition to high concentration that leading to more homopolymerization. Another reason for the low %GE was the tapioca starch properties, there was highly amylopectin content which might reduce the mobility of the polymer chains and increased viscosity. So, they effect to the decreasing in grafting ration and grafting efficiency as same as the studied of (Zou, *et al*., 2012)

1.2.2 Effect of initiator concentration

As the interpretation in Figure 21, the %add-on and %GE increased with the increasing of concentration of KPS that agree with statistical study, the initiator was significant grafting parameter. The highest values were obtained when concentration of KPS was 0.1195 M, and then trended to decrease after higher concentration of KPS. The higher concentration of KPS, the more homopolymerization of PolyDMC were promoted. From other researchers studied, they have been said that the decreasing in grafting parameter after the optimum might be due to an increasing in the number of starch macro radicals terminated prior to monomer addition and increased in homopolymer formation or might be due to accumulation of a large number of free-radicals resulting into premature termination of the growing chains and the grafting. (Lkhuoria and Okieimen, 2010; Kaewtatip, K. and V. Tanrattanakul, 2008; 2006).

1.2.3 Effect of reaction temperature

Reaction temperature played an important role on graft copolymerization. As ANOVA result table for %add-on, the reaction temperature factor was significant but not for %GE. From Figure 24, the %GE had a little bit declined with the increasing of reaction temperature while the %add-on reduced significantly. Undoubtedly, every polymerization system has its own optimum reaction temperature. Similar result was reported, the maximum %add-on and %GE in tapioca starch-graft-PDMC copolymerization with KPS was at 40°C. It is well established that the decomposition temperature of KPS is between 40 to 80°C. When increasing the reaction temperature not only leading to more free-radicals, but also increasing their molecular mobility and rate of diffusion of monomer. Thus, grafting reaction was promoted. Decrease in grafting parameter after the optimum temperature was attributed to premature termination of growing chains and grafted chains as well as chain transfer reactions. It should be noted that the present study used very low reaction temperature because the pre-gelatinized tapioca starch was treated before graft copolymerization.

2. Physicochemical characteristic of tapioca starch-graft-PDMC

2.1 FT-IR

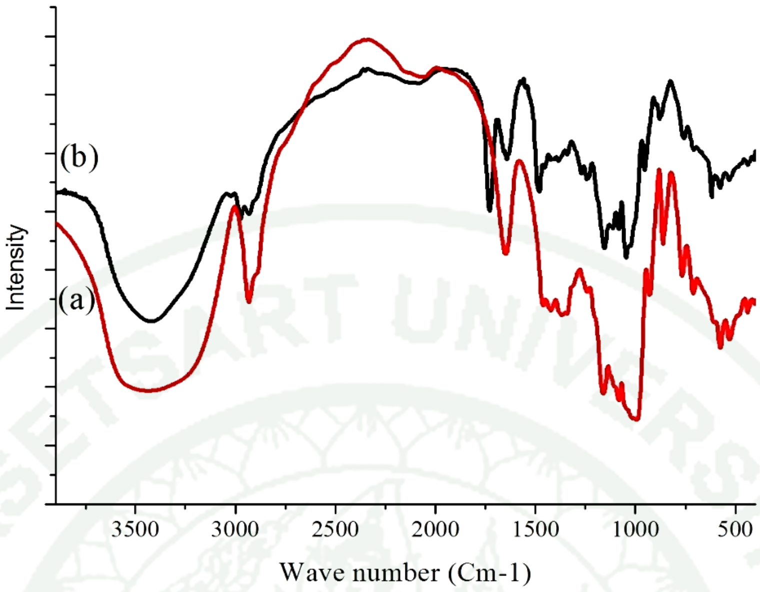


Figure 23 FTIR spectra of (a) tapioca starch-graft-PDMC, and (b) tapioca starch

Figure 23 the infrared spectroscopy of original tapioca starch and the tapioca starch-graft-PDMC. The newly peaks were noticed in the spectrum of the tapioca starch-graft-PDMC which different from tapioca starch, at 1729 cm^{-1}corresponding to the vibrating absorption of carbonyl group in PDCM chains. The other two peaks 1480 and 953 cm^{-1}, could be assigned to methyl group and quaternary ammonium group, respectively. The characteristic peaks of non-modified and tapioca starch-graft-PDMC are demonstrated in Table 20.

Table 20 Characteristic peak of tapioca starch and PDMC (Wang et al., 2013)

Wavelength (cm^{1})	Assignment
Tapioca starch	
3600-3000	O-H stretching
2933 and 2881	C-H stretching of CH_2
1190-950	
PolyDMC	
1729	Carbonyl in PDMC
1480 and 953	Methyl group and quaternary ammonium

2.2 X-ray diffraction (XRD)

The XRD profiles of tapioca starch-graft-PDMC exhibited the disappearance of crystalline peaks, compared with native tapioca starch (Figure 27). The profiles of native tapioca starch was showed four peaks around 2θ = 15°,17°,18° and 23° which presented a characteristic A-type X-ray diffraction pattern. The modified tapioca starch graft copolymer, tapioca starch-graft-PDMC, had a dispersive broad peak and shown no crystal starch peak. Because of decreasing in crystallinity suggested that the introduction of DMC onto starch backbone destroyed the original ordered structure of starch.

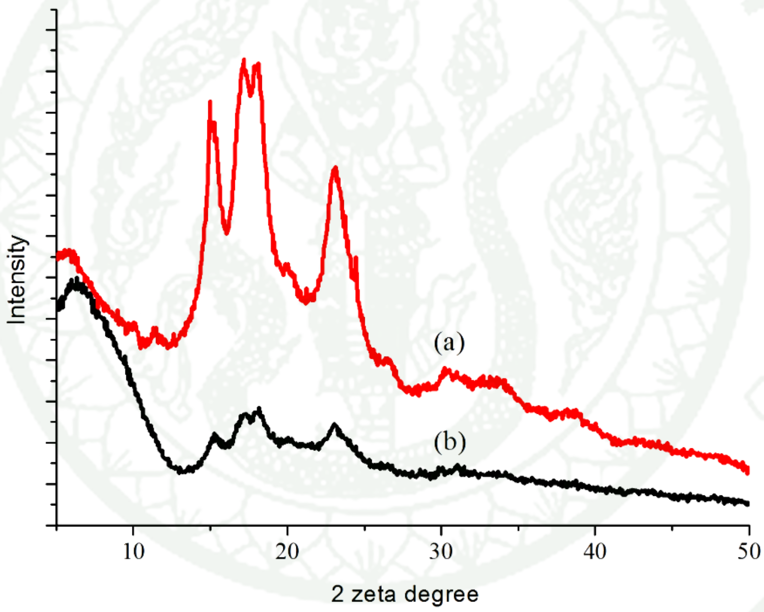


Figure 24 Crystalline peak of (a) tapioca starch and (b) tapioca starch-graft-PDMC

2.3 Elemental analysis (CHN analysis)

The successful of the grafting could also be indicated from the increase of nitrogen (N-content) in the grafted copolymer. Generally, there should no nitrogen element (N-content) in the non-modified tapioca starch. However the present of N-content 0.1577% might come from the N_2 in atmosphere. Table 21 listed the comparison of elemental analysis value. There were 2.71% N-content of the tapioca grafted copolymer (after Soxhlet extraction) which increased from 0.1577% of the non-modified tapioca starch. The completely removal of PDMC homopolymer and DMC monomer by ethanol Soxhlet extraction could be implied from N-content of the tapioca starch grafted copolymer was lower than before Soxhlet extraction. Hence, the Soxhlet extraction by ethanol was effectively to remove PDMC homopolymer and DMC monomer like in the study of Wang et al,.(2013).

Table 21 The presence of N-content before and after soxhlet extraction of tapioca starch-graft-PDMC by ethanol for 24 hr.

Elementals	**%weight**			
	C	**H**	**N**	**O**
Tapioca starch	35.06	7.212	0.1577	57.5703
Before-Soxhlet	34.81	8.588	3.7936	52.8084
Tapioca starch-graft-PDMC (after Soxhlet)	31.54	8.02	2.71	57.73

2.4 Thermal gravimetric analysis (TGA analysis)

TGA analysis was used to investigate thermal stability properties and confirm the graft copolymerization occurrence. As shown in Figure 25 and Table 22, the thermal behavior of tapioca starch-graft-PDMC was different from native tapioca starch. The TGA profile of tapioca starch-graft-PDMC (black line) showed two distinctly quickest weight loss, the first around 268 °C to 297 °C and the later around 452°C to 490°C, respectively. The first weight loss might be due to the decomposition of starch backbone, and the second one might be due to the grafted PDMC chain. There were no water absorb due to the sample were dry in oven before TGA analysis. The comparative of on-set and end-set weight loss temperature of native tapioca starch and tapioca starch-graft-PDMC were summarized in Table 22. It might means that the graft copolymerization significantly changed to enhance the thermal stability of native tapioca starch.

The decomposition temperature of native tapioca starch has been also explained by Wang et al., (2013) and S. Mishra et al., (2011). There were two quickest weight loss of starch, the first at 30-150°C (Wang) and 30-120°C (Mishra) due to the loss of moisture. After that the decomposition of starch would be occurred at 280-480°C (Wang) and 250-330°C (Mishra), respectively. S. Mishra et al., (2011) have been mention about the present of PAM in starch showed the two extra zone of weight loss 340-460°Cand 340-460°C, respectively.

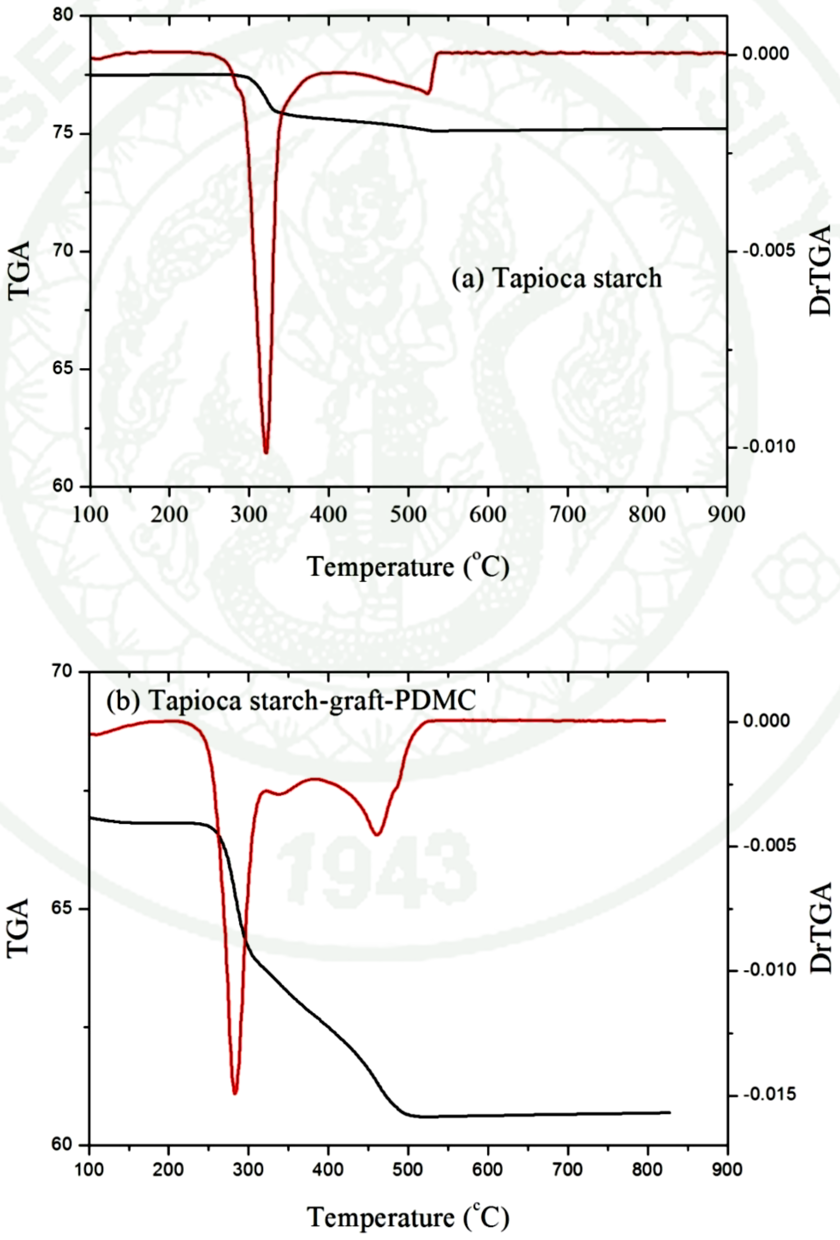


Figure 25 TGA and DrTGA of (a) tapioca starch, and (b) tapioca starch-graft-PDMC

Table 22 Comparison of onset and end set temperature between tapioca starch and tapioca starch-graft-PDMC

Temperature (°C)	**Tapioca starch**	**Tapioca starch-graft-PDMC**	
	1st	**1st**	**2nd**
On set	295.47	267.71	452.59
End set	321.41	296.98	489.95

2.5 Solubility

The introducing of cationic DMC polymer chain onto starch backbone improved the hydrophilicity of starch. Because of a quaternary ammonium salt in grafted DMC polymer chain could be dissolved in waster readily. Along of the original inter-molecular and intra-molecular hydrogen bonding was destroyed in the grafting process, the graft copolymer could be dissolved in acidic, neutral and alkaline solution as shown by naked eyes experimental results in Table 23.

Table 23 Solubility of tapioca starch and tapioca starch-graft-PDMC in acid, neutral and basic conditions

Sample	**Acidic (**HCl,0.1M**)**	**Neutral** (H_2O)	**Basic** (NaOH,0.1M)
Tapioca starch	Not soluble	Not soluble	Not soluble
Tapioca starch-graft-PDMC	soluble	soluble	soluble

2.6 Zeta potential

Potential-pH profiles are very useful to describe the charge of particle suspension and predict the flocculation mechanism. As can be seen in Figure 30, native tapioca starch showed negatively surface charged at any pH conditions whereas tapioca starch grafted copolymer was positively charged along pH study ranges. The zeta potential value was increased with the increasing of pH values. Between pH 4-8 showed the highest values and then trend to decrease after pH around10-12. However, the potential value of tapioca starch grafted copolymer was still positively at any pH conditions. From the appearance of positive zeta potential value, it might be implied that there were PDMC in the flocculants chains.

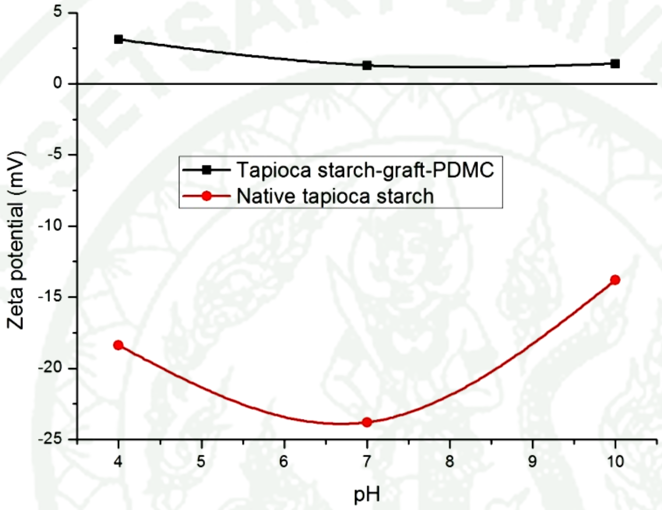


Figure 26 Variation of zeta potential-pH profile of tapioca starch-graft-PDMC (st-g-PDMC) and tapioca starch (non-modified).

2.7 Scanning electron microscopy (SEM) analysis

It was evident form SEM micrographs of starch in Figure 27, the morphology of non-modified tapioca starch changed in from of transition from granular to lace like structure. It could be implied that the morphology of starch was lost after grafting and transformed into lacy morphology. The loss of starch granular happened before grafting copolymerization because starch was gelatinized at 65°C (gelatinization temperature of tapioca starch between 62-75°C. The pre-gelatinized tapioca starch might enhance the probability of DMC monomer movement via water media to graft with starch backbone both of amylose and amylopectin.

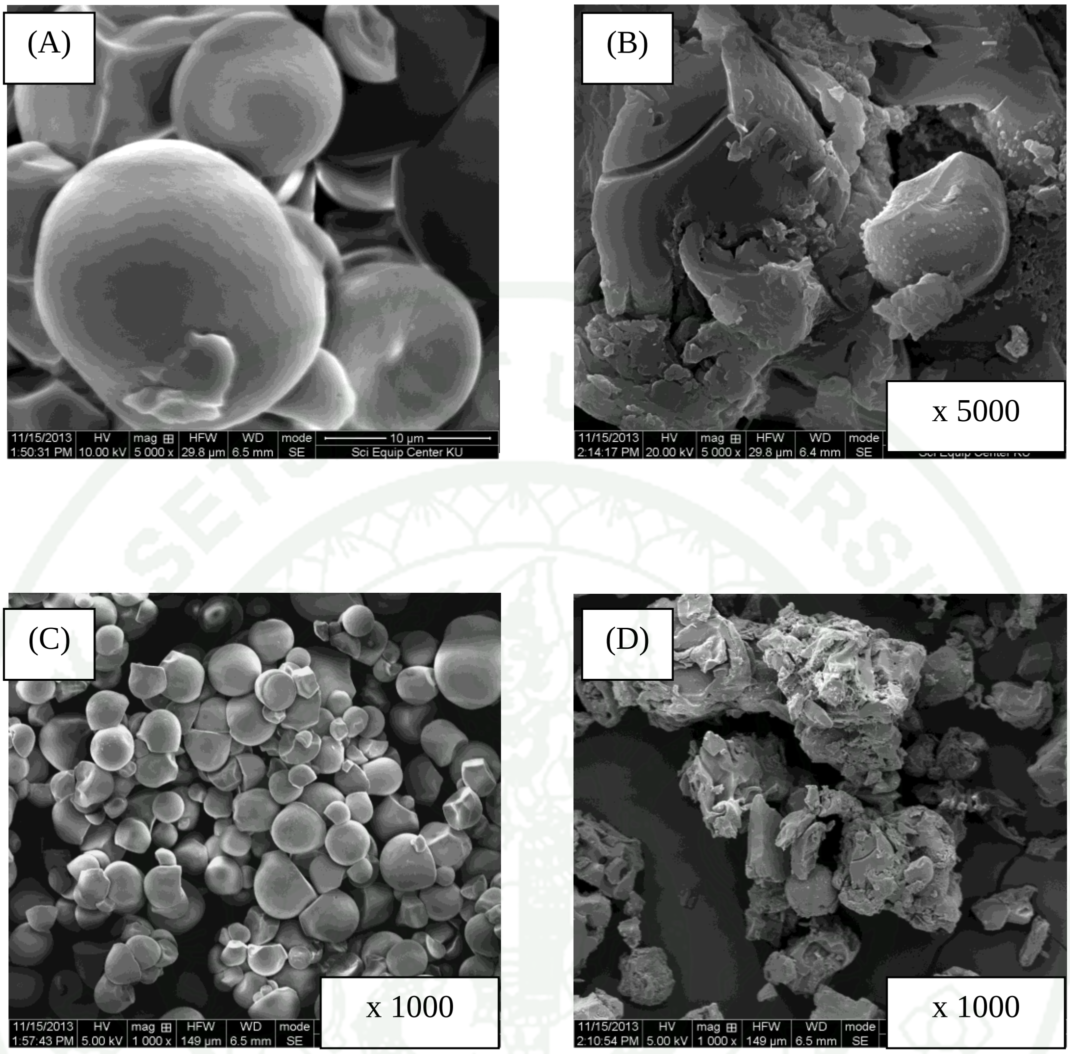


Figure 27 SEM image of (A) and (C) native tapioca starch at x5000, (B) and (D) tapioca starch grafted x1000, respectively

3. Flocculation ability of tapioca starch-graft-PDMC copolymer

It is well known that in kaolin suspension flocculation, pH plays a role important factor to control the efficiency of turbidity reduction. Kaolin suspension flocculation only happens within a specific pH range using different flocculants types and dosages. In this study, kaolin suspension 0.25%wt was prepared as synthetic wastewater treatment to study the flocculation ability of tapioca starch-graft-PDMC. The effects of pH and flocculation dosage were studied. The flocculation ability of tapioca starch-graft-PDMC in various pH conditions pH4, pH7, and pH10 were represented to acidic, neutral and alkali conditions, respectively. The flocculation ability was explained by %Transmittance from UV-vis spectroscopy at λ = 630 nm. (*(Wang, L.-J. et al., 2009)*. The zeta potential-pH profiles of supernatant solution after kaolin flocculating and kaolin suspension were shown in Figure 31 and Figure 32, respectively.

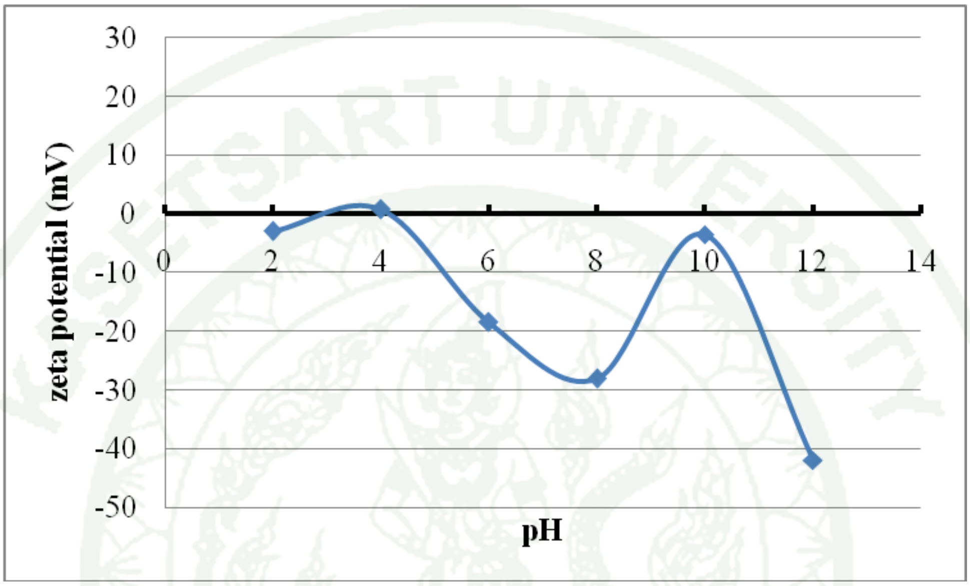


Figure 28 zeta potential-pH profile of kaolin suspension 025% w/v

The zeta potential-pH profile of kaolin suspension (Fig. 28) showed mostly negatively, except pH 3-4 which showed nearly zero. The close or equal to zero of zeta values often happen only when pH is around pH-isoelectric point (pH_{pzc}). At this pH point, the suspension surface repulsion force becomes weaker, unstable, and begin to agglomerate. It was claimed that at pH isoelectric point (pH_{pzc}), it might be easy to destabilize the particle and lead to good flocculation. Consequently, the pH_{pzc} of kaolin suspension in this study might be around pH 3 to 4. When pH of solution below pH_{pzc} point, the surface charge of kaolin suspension would show positively while in pH higher pH_{pzc} point the surface charge would show negatively, respectively.

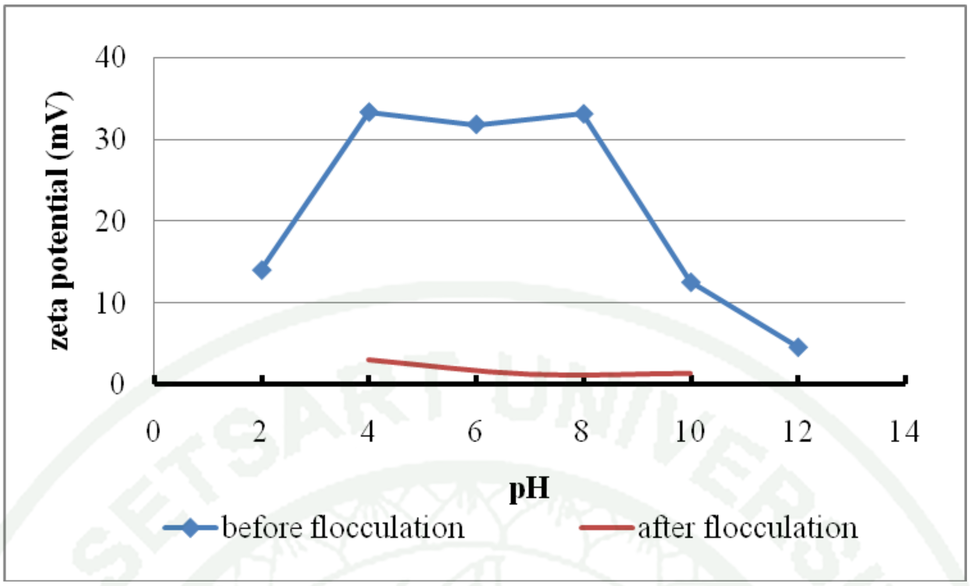


Figure 29 The zeta potential-pH profile of before and after kaolin flocculation by tapioca starch-graft-PDMC

The zeta potential-pH profile of supernatant before and after flocculation was demonstrated in Figure 29. It could be seen that after flocculation by tapioca starch-graft-PDMC, the zeta potential value was nearly close to zero in all representative pH conditions. It might be emphasized that this flocculants might be used as high performance flocculants. The clearness compared to before flocculation process of synthetic wastewater after flocculation process was showed in Figure 30.

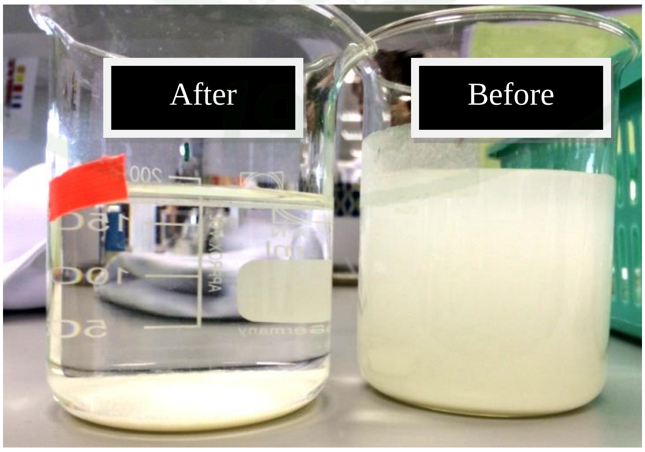


Figure 30 Before and after flocculating of 0.25% w/v kaolin suspension by tapioca starch-graft-PDMC copolymer

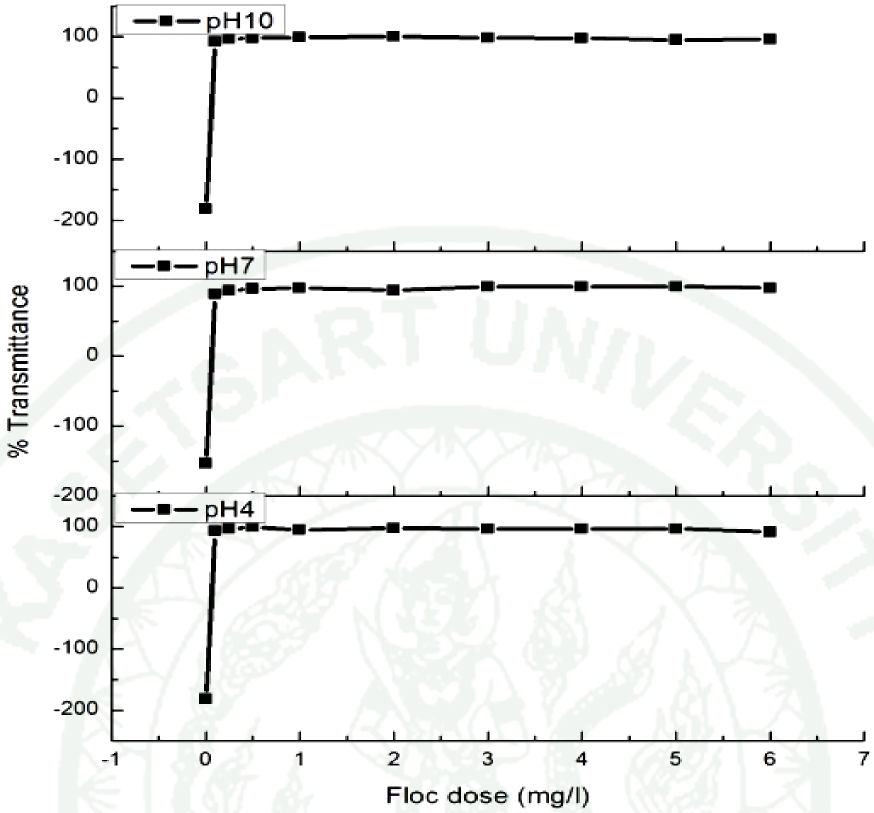


Figure 31 Effect of tapioca starch-graft-PDMC flocculent dosage in various pH conditions to flocculation efficiency

Kaolin suspension is hardly to remove without adding some chemical agent to destabilize and allow them agglomerate to easily set down in sedimentation process. The pH is well-known parameter in kaolin suspension flocculation and acts as an important role to control the efficiency of turbidity reduction. Figure 31 showed the effect of pH (4, 7 and 10) on flocculation ability at different dosages. It should be noted that native starch showed no flocculation ability under all tested conditions. The grafted starch showed high performance under acidic, neutral and alkaline conditions. Apparently, the decrease in turbidity of the kaolin suspension was observed even at a low dosage of grafted starch as 0.1 mg/L. The flocculation rate increased rapidly as soon as applying the grafted starch and reached 100% transmittance with increasing the dosage. It could be implied that the minimum dosage to remove kaolin suspension 0.25 wt% was only 0.1 mg/L. Schwarz and co-worker [S. Bratskaya, S. Schwarz, T. Liebert, T. Heinze, Starch derivatives with high degree of functionalization: 10

.Flocculation of kaolin dispersion, Colloids Surf.A 254 (2005) 75-80.] investigated the flocculation properties of water-soluble cationic starch derivatives have suggested that the flocculation of kaolin dispersion is a combination of the bridging and charge patch process. Therefore, the cationic property of quaternary ammonium group in the grafted copolymer chains may neutralize the negatively charged of kaolin suspension and the high molecular weight of PDMC could also enhance bridging effect.

4. Comparative flocculation performance between tapioca starch-graft-PDMC to non-modified tapioca and commercial flocculants (PDADMAC)

The study of flocculation ability of tapioca-starch-graft-PDMC to remove turbidity compare to PDADMAC and native tapioca starch was carried out by jar test in 0.25%w/v kaolin suspension flocculation at pH 4, 7 and 10, respectively. The flocculant dosage that used in this study was in the range of 0.25 to 6 mg/l.

Figure 32 described the turbidity reduction efficiency express in %Transmittance of supernatant from kaolin flocculation by tapioca starch-graft-PDMC, PDADMAC, and native tapioca starch as flocculants were affected by their flocculants dosage. Almost 100% turbidity reduction efficiency could be achieved by adding tapioca starch-graft-PDMC around 0.8 mg/l. For commercial flocculants PDADMAC also showed high turbidity reduction efficiency at all pH conditions due to its low molecular weight and high charge density. The native tapioca starch showed no turbidity reduction efficiency at all pH conditions. Moreover, the overdosing of flocculation by PDADMAC occurred after 0.8 mg/l flocculant dosage was added while the turbidity reduction efficiency of tapioca starch-graft-PDMC flocculants performed high efficiency (Chowdhury *et. al.*, 2010; Ayguna and Yilmazb, 2010).

From this result, it could be confirmed that only less than 1.0 mg/l of tapioca starch-graft-PDMC used as high performance flocculants at all pH condition. In the opposite of highly charged density PDADMAC flocculant, the insufficient might be the limitation of usage.

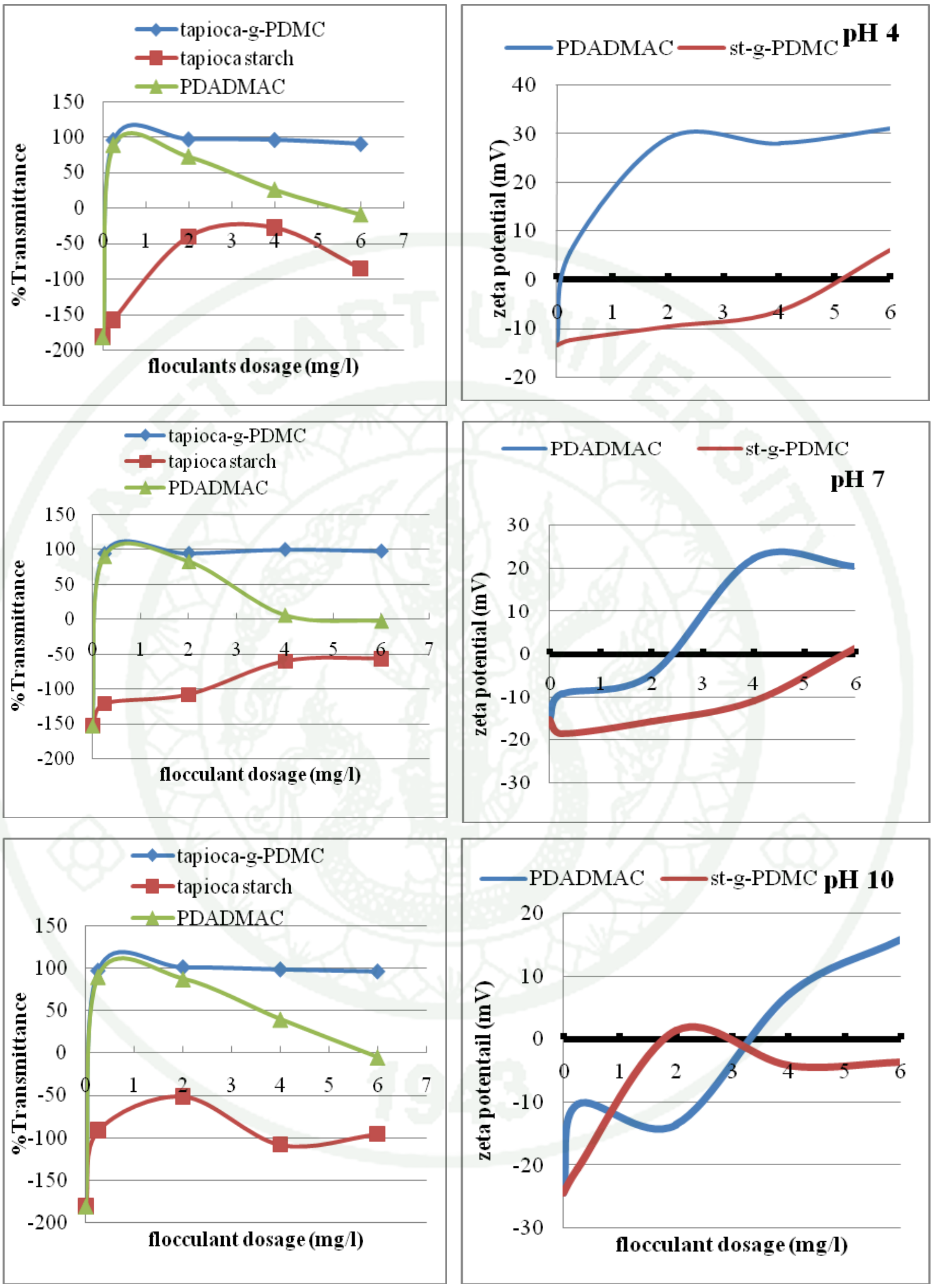


Figure 32 Comparative flocculation ability of Tapioca starch-graft-PDMC to tapioca starch (controlled) and commercial flocculent (PDADMAC)

5. Dual functional of tapioca starch-graft-PDMC copolymer on turbidity reduction and hexavalent chromium Cr(VI) and Congo Red dye (CR)removal efficiency

The dual functional of tapioca starch-graft-PDMC copolymer on turbidity reduction and hexavalent chromium Cr(VI) and Congo red dye (CR) removal efficiency were verified by using two systems. The first one was the system contained kaolin suspension 0.25% wt and hexavalent chromium 1 mg/l. The second was the system contained 0.25%wt of kaolin suspension and 5 mg/l of Congo red dye. The effect of flocculant dosage, pH of solution, and kaolin suspension to Cr(VI) removal efficiency was studied. %Transmittance value expressed how good of turbidity reduction efficiency same as the previous section.

5.1 Kaolin suspension and hexavalent chromium flocculation

The calibration curve of Hexavalent chromium (Cr(VI)) was prepared before Jar test and showed in Figure 33. The linear regression equation of calibration curve y = 0.931x with R^2 = 0.975, respectively was obtained to determine the concentration of hexavalent chromium in the solution. Jar test was used to study the tapioca starch-graft-PDMC flocculation ability. The effect of pH and flocculant dosage resulted below

.

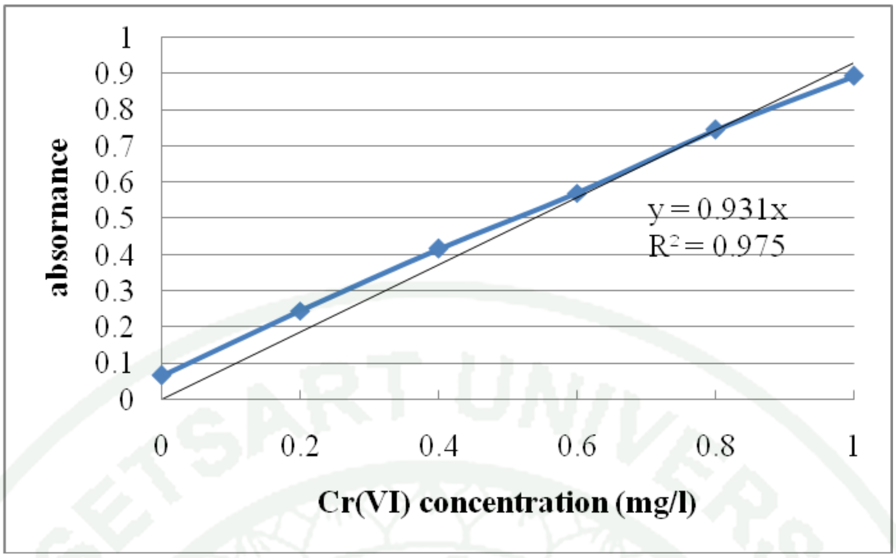


Figure 33 Calibration curve of hexavalent chromium

(A) Effect of pH to Cr(VI) removal and turbidity reduction efficiency

Flocculation experiments were carried out in the pH ranging from 2 to 10, and kept all other parameters constant (Chromium conc. = 1 mg/l, flocculants dosage = 0.25. mg/l, stirring patterns including rapidly speed 200 rpm 2 min, slowly speed 60 rpm 15 min, then settling 5 min). The supernatant was drawn and separated to 2 parts, one to form DPC-complex for tracing the residuals chromium (VI) ions and other one was used for turbidity reduction efficiency which measured by UV-spectroscopy. The effect of pH to %Cr(VI) removal and turbidity reduction efficiency were showed below in Figure 34 (a).

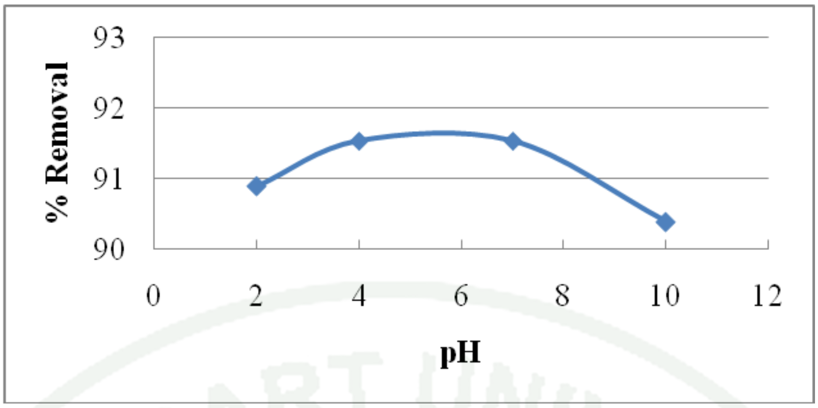


Figure 34 The percentage of Cr(VI) removal against pH

The result showed the Cr(VI) removal efficiency increased with increasing of pH ranging from 2.5 to 3.5. The maximum Cr(VI) removal efficiency by tapioca starch-graft-PDMC flocculants nearly closed to 92 %. Thereafter, there was a decline in alkalis condition of the aqueous solution. The Cr(VI) removal efficiency was largely related to the type and ionic state of functional groups presented on the flocculants and chromium speciation in solution.

Chromium (VI) normally exist in the anionic form, as $Cr_2O_7^{2-}$, $HCrO_4^-$ or CrO_4^{2-} forms, depending on the pH and concentration in the solution. At pH values below 1, the predominant species in chromic acid (H_2CrO_4) with the dissociation constant of K_1=4.1 and K_2 = $1.2x10^{-6}$ respectively, in acidic media with pH values of 2-4, Cr(VI) exists mostly in the form of dichromate ($Cr_2O_7^{2-}$) ions. At pH between 4 and 6, $Cr_2O_7^{2-}$ and $HCrO_4^-$ ion exist in equilibrium; under alkali conditions (pH 8), it exists predominantly as chromate anion (CrO_4^{2-}). (Jain, M., V. K. Garg and K. Kadirvelu, 2010). Consequently, the $Cr_2O_7^{2}$ removal efficiency showed the highest value in acid condition due to electrostatic mechanism between negatively charged of chromium species and strongly positively charged of tapioca starch-graft-PDMC copolymer flocculants. However, the percentage of Cr(VI) removal showed highly efficiency remove up to 90% over pH conditions in this study due to the highlight properties of tapioca starch-graft-PDMC in pH-independent and their strongly positively charged quaternary ammonium salt in PDMC chain (Wei *et al*., 2008).

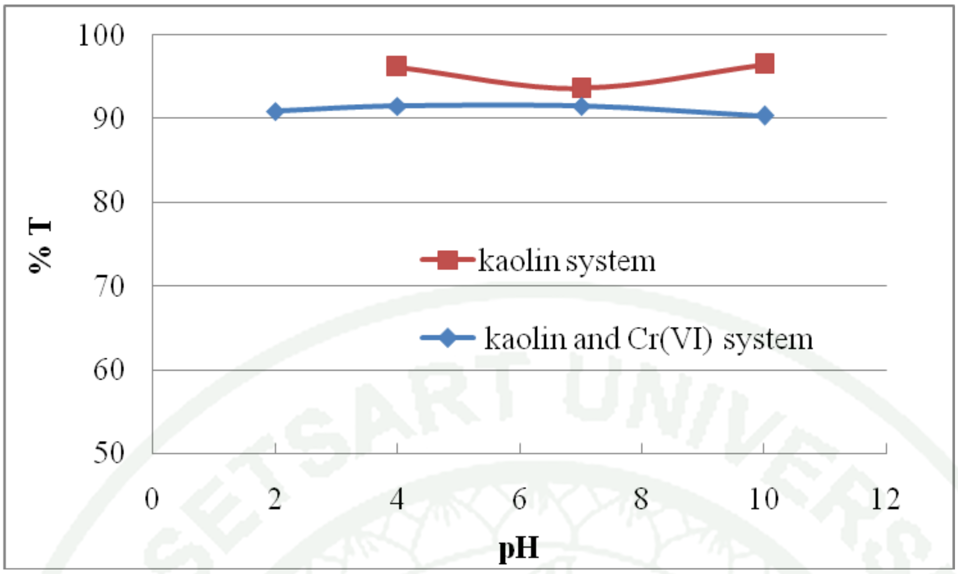


Figure 35 Effect of pH to turbidity reduction efficiency when used in single and dual System

In Figure34 (b), the both lines of kaolin system with and without Cr(VI) ions showed the same trend that high turbidity reduction were achieved more than 80% at all pH conditions. The kaolin system showed higher turbidity reduction more than synthetic wastewater kaolin suspension with Cr(VI) system. It might be due to the competition between Cr(VI) ions species and kaolin suspension to tapioca starch-graft-PDMC copolymer.

Effect of flocculants dosage to Cr(VI) removal and turbidity reduction efficiency

The Cr(VI) removal efficiency increased immediately after added 0.25 mg/l of tapioca starch-graft-PDMC copolymer and then slightly decreased then its equilibrium were achieved. It could be implied that the more active sites for adsorption of Cr (VI) ions species were provided when added more flocculent dosage (Atia, 2006)

The high flocculent dosage was influence directly to the turbidity reduction efficiency only in acid condition.

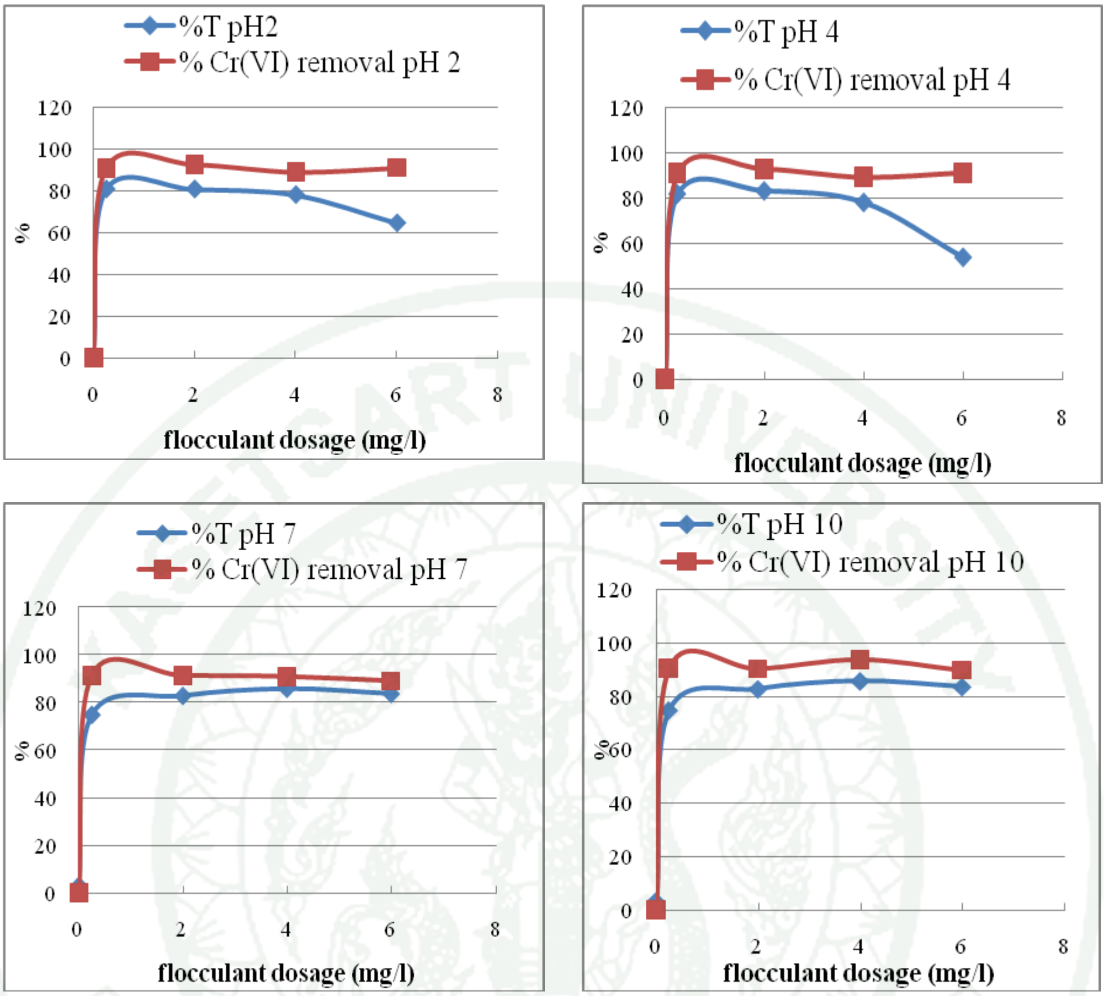


Figure 36 Effect of tapioca starch-graft-PDMC dosage in dual functional flocculation to %Transmittance and Cr(VI) removal at pH 2, 4, 7, and 10.

(C) Effect of kaolin suspension to the percentage of Cr(VI) removal

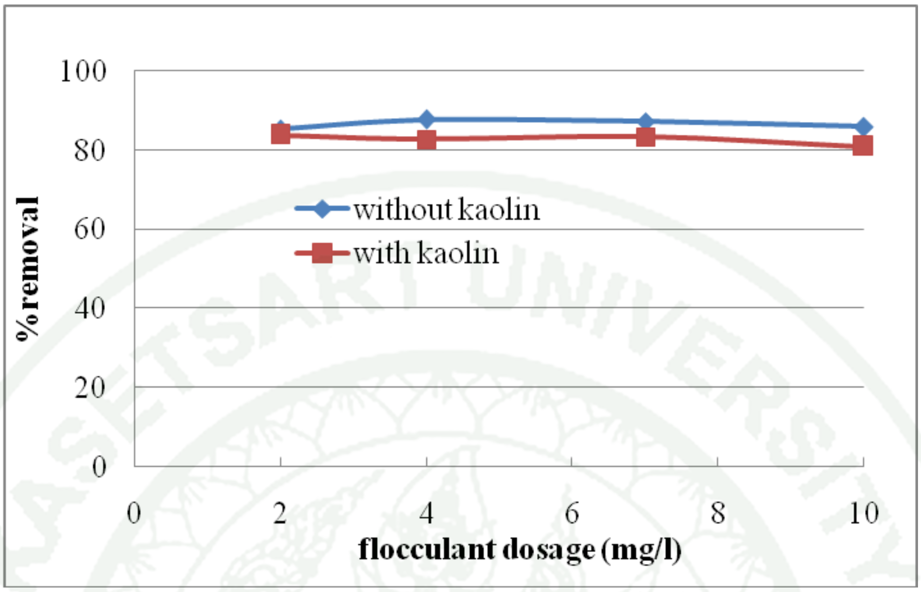


Figure 37 Effect of kaolin suspension 0.25% w/v to the percentage of Cr(VI) removal by st-g-PDMC flocculants

The influence of kaolin suspension to the Cr(VI) removal against pH from 2 to 10. The percentage of Cr(VI) removal of without kaolin system shows higher than system with kaolin. In Figure 36, when added kaolin suspension at pH 2 Cr(VI) removal decreased from 85.23% to 83.88% , at pH 4 decreased from 87.73% to 82.78%, at pH 7 decreased from 87.19% to 83.34%, and at pH 10 decreased from 85.86% to 80.98% , respectively.

The decreasing of Cr(VI) removal when flocculated in kaolin suspension due to the competition between negatively kaolin surface charged and chromate anion at pH < 3.5 and Cr(VI) anion might be blind by kaolin suspended solid. The isoelectric point of kaolin suspension was around 3.3 to 3.5. Hence, pH < 3.5 , kaolin have a negatively surface charged and pH > 3.5, kaolin suspension became positively surfaced charged and then higher pH the OH^- also increased, so at pH from 7-10 the competition of kaolin and OH^- might be predominated influent. At the same time, the st-g-PDMC had to neutralize negatively surface charged of kaolin also.

Using the tapioca starch-graft-PDMC as a dual functional flocculent not only remove kaolin suspension but also Cr(VI) could be removed. It is stated clearly in Figure 37, the turbidity reduction efficiency was still high more than 80%. Moreover, the Cr(VI) removal could be highly also, the percentage of Cr(VI) removal almost 100% after optimum tapioca starch-graft-PDMC of 0.25 mg/l.

5.2 Kaolin suspension and Congo red dye flocculation

The calibration curve of Congo red was prepared before Jar test and showed in Figure 37. The linear regression y = 0.047x, R^2 = 0.972 using to determine the concentration of Congo red dye in solution was obtained. Jar test was used to study the tapioca starch-graft-PDMC flocculation ability. The effect of pH and flocculant dosage resulted below

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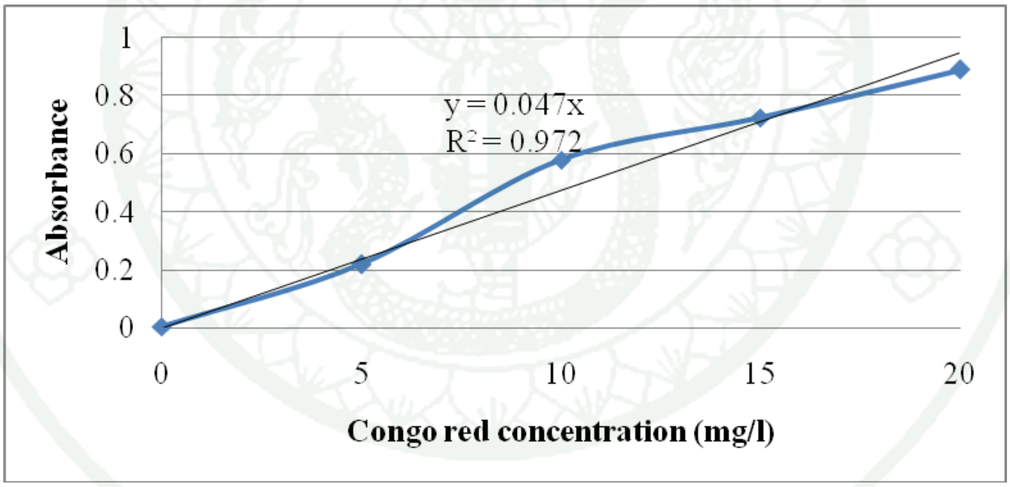

Figure 38 The calibration curve of Congo red

(A) Effect of pH on the turbidity reduction and Congo red dye removal efficiency

The performance of color removal by chemical flocculation depends on the characteristic of both dyes and flocculants and pH condition. Congo red dye is reactive dye and high solubility. The charge of dye depends in large extent on pH of the dye solution. Hence, the pH is very important for selection of flocculants type.

Congo red dye exists as the dark blue anionic form in acidic solution and red color cationic form in alkali solution, but different red color in neutral condition.

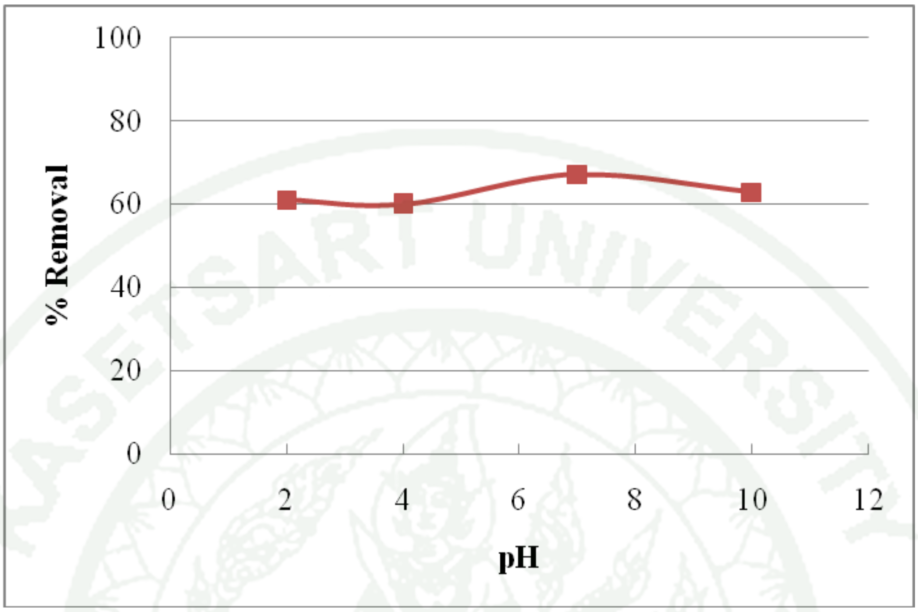

Figure 39 The effected of pH to Congo red dye (CR) removal efficiency of tapioca starch-graft-PDMC

In Figure 38 pH 2, the CR removal efficiency was around 60 %, and then at pH 10, the CR removal increased to almost 70%. The removal efficiency slightly increased with the increasing of pH value because of the charge neutralization at the surface of the adsorbents. It could be safely assumed that by increasing the pH of the solution preference on the negative center (SO_3^-) of the dye for the active sites of the adsorbent increase, which in turn facilitates the adsorption process which agree with the studied of A. Mittal et.al, 2009. In Figure 43, while the pH was increase, the turbidity reduction efficiency still showed high value up to 80%. However, the reduction on turbidity reduction efficiency could be seen in pH 4 due to the completion between the negatively charged of kaolin suspension and Congo red dye anions.

(B)Effect of flocculants dosage to turbidity reduction and Congo red removal efficiency

A batch jar test study was performed to ascertain the effect of variation of amount of flocculant dosage on Congo red dye removal efficiency. Tapioca starch-graft-PDMC flocculants dosages were taken in separated beaker in 200 ml of 0.25%w/v kaolin suspension having Congo red dye solution of 5.0 mg/l concentration at pH 2, 4, 7, and 10.

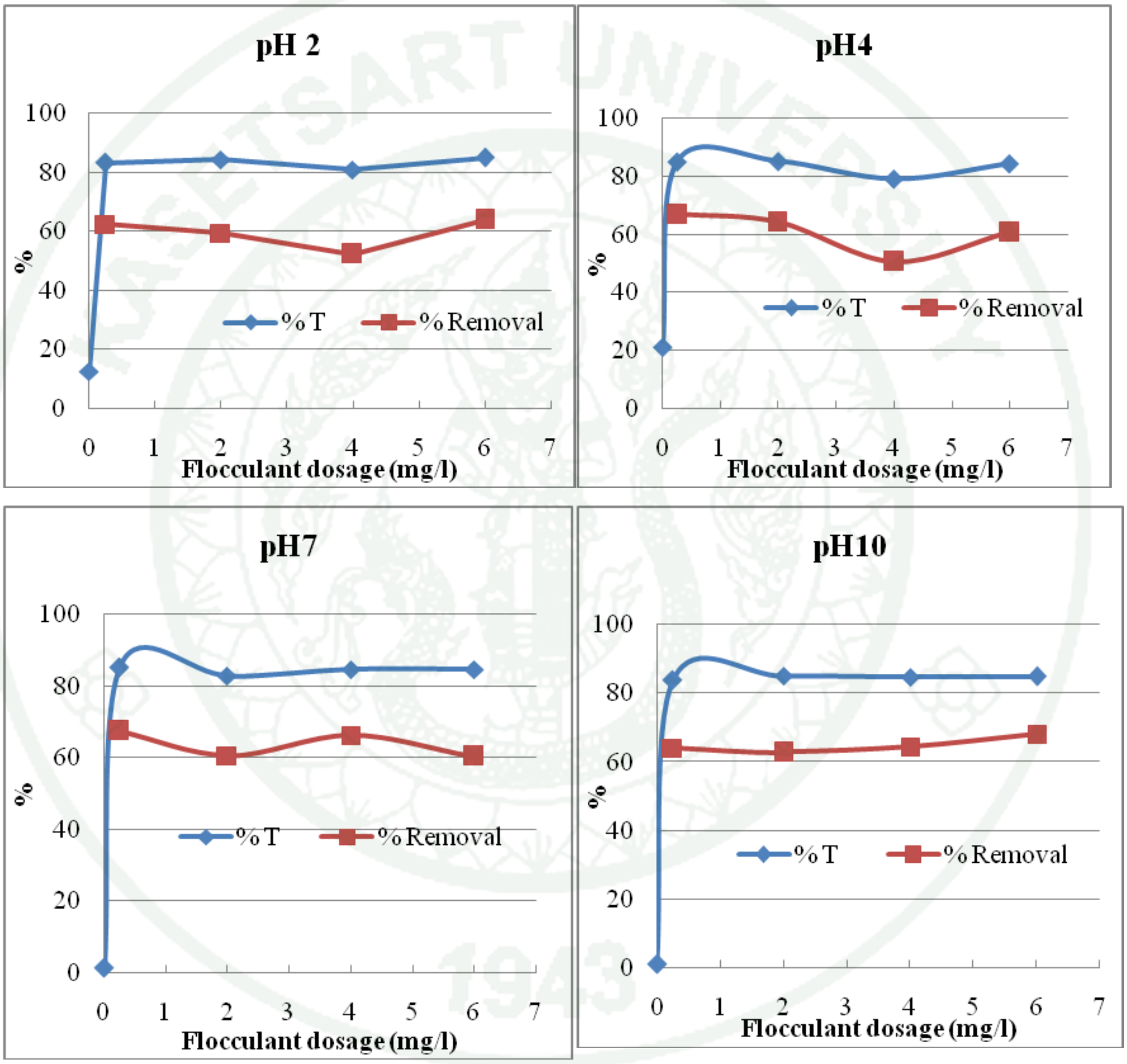

Figure 40 Tapioca starch-graft-PDMC dual function in removal of kaolin and CR

As shown in Figure 39, the percentage of Congo red removal were increased up to 60% when 0.25 mg/l of flocculants was added and decreased after 4.0 mg/l for all pH conditions. The increasing of Congo red removal efficiency could be explained by the increasing of the flocculants active sites due to flocculant dosage. However, the amount of flocculants did not bring any considerable change in the turbidity reduction and dye removal efficiency. Thus using at least 0.5 mg/l could be attributed to remove up to 70% of 5.0 mg/l of Congo red dye solution while still highly turbidity reduction up to 80%. The Insufficient dosage or overdosing would result in the poor flocculation performance. Therefore, it was significant to determine the optimum dosage in order to minimize the cost and sludge formation and then obtain the optimum performance in treatment (Patel et al., 2010; Kuar et al., 2013).

(C) Effect of kaolin suspension to Congo red dye removal efficiency

To study the effect of kaolin suspension to Congo red dye removal efficiency, two synthetic wastewater systems were conduced. First, the system kaolin suspension and Congo red dye system. Second one was only 5.0 mg/l Congo red dye in the system to use as controlled experiment.

The Congo red dye removal efficiency in two systems was resulted in Figure 41. At pH 2, the dye removal efficiency in kaolin suspension and Congo red dye solution performed higher value than the controlled system. Given that the pH isoelectric point of kaolin suspension around pH 3.5, an acidic condition pH 2 was necessary to provide positive charged surface facilitating increasing the dye removal via complexation. In addition, the high positively in any pH range of tapioca starch-graft-PDMC and anionic forms of Congo red dye were attracted by electrostatic attraction.

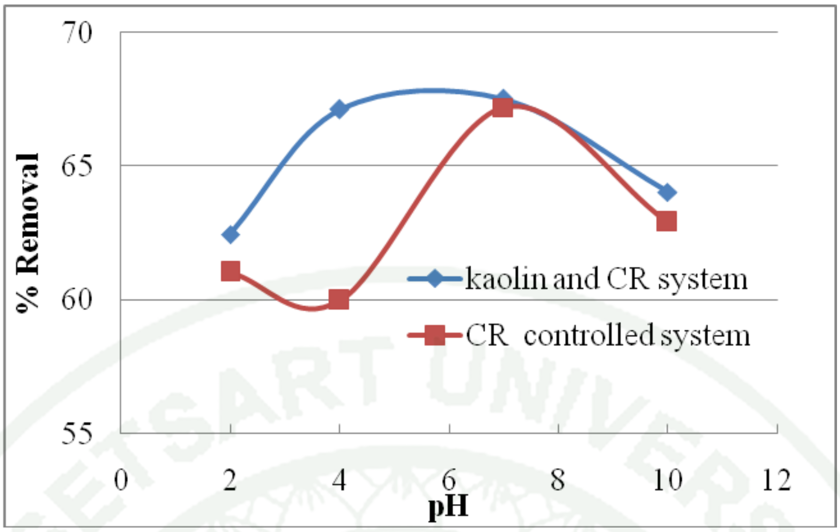

Figure 41 The effect of kaolin suspension on the Congo red dye removal efficiency after added 0.25 mg/l of tapioca starch-graft-PDMC copolymer

The highest different dye removal efficiency between kaolin suspension system and kaolin suspension with Congo red dye solution in acidic condition was distinctly seen in Figure 40. It showed high up to 65% from 60 % at pH 4. Because the reactivity of kaolin suspension forms from pH 4 was negatively. The complex between kaolin suspension and positively Congo red dye might form and be a major diving force of dye removal efficiency in acidic condition. After pH 7, the dye removals of both systems were significantly reduced because of the electrostatic repulsion between positive surfaces charged of tapioca starch-graft-PDMC and Congo red dye cationic forms. So, it was clearly that Congo red dye removal efficiency significantly affected by the presence of kaolin suspension (Wang and Wang, 2006).

CONCLUSION AND RECOMMENDATION

Conclusion

The design of experiment (Box-Behken design) could be usefully applied to optimize graft copolymerization conditions. Starch content, concentration of KPS initiator and reaction temperature was expressed that they were significance variables response to the %add-on and %GE. The estimated model of %add-on and %GE were determined by applying analysis of variance (ANOVA) and their contour and surface plots were also displayed.

A novel environmentally friendly and cost-effective flocculants for wastewater treatment process was developed by introducing well-known highly effective flocculant PDADMAC in tapioca starch backbone by free-radical graft copolymerization. The optimum conditions which obtain the highest of %add-on and %GE were 2.5g of tapioca starch, 0.1195 M of KPS concentration, and reaction temperature of 40°C, respectively.

The physicochemical characteristic of tapioca starch grafted copolymer was investigated by FTIR, TGA, SEM, X-ray diffraction, zeta potential and CHN elemental analysis to confirm the existing of PDMC grafted chains.

The flocculation ability of tapioca starch-graft-PDMC was studied in 0.25%w/v kaolin suspension. The high performance was obtained when using at least 0.25 mg/l flocculent dosage at all pH condition due to the strongly positively charged of quaternary ammonium chloride group in flocculants chains. The tapioca starch-graft-PDMC was also high flocculation ability equal to commercial flocculants PDADMAC while native tapioca starch showed no flocculation efficiency in kaolin suspension.

Due to highly positively charged, not only remove turbidity but also remove anion substance including hexavalent chromium and Congo red dye at low concentration. The up to 70 % Removal of Cr(VI) at pH 2 and 80% Removal of Congo red dye at pH 7 was achieved when use only 0.25 mg/l of flocculent dosage.

After the dual functional flocculation ability study of Cr(VI) and CR, we could consider that the tapioca starch-graft-PDMC have still highly flocculation ability in kaolin suspension while could be remove anionic substance in the range of low concentration.

Recommendation

In pilot plant, these novel tapioca starch-graft-PDMC flocculants might be applied to reduce turbidity both of low and high at the ground and surface water treatment plant, respectively.

For ground water treatment plant, It could use only these flocculants to remove the turbidity less than 10 NTU without adding inorganic coagulant before. The turbidity in surface water treatment plant always change suddenly especially after raining, these flocculants could use to reduce high turbidity and suspended solid particles leading to reduce the cost of chemical consumption. Moreover, they could reduce the fouling effect resulting to reduce cost of membrane and equipments maintenances. Because of the strongly cationic surface charged in wide range of pH, tapioca starch-graft-PDMC might act as an absorbent to remove some kinds of anion in solution such as PO_4^{3}, NO_3^-, F^- or CN^-, etc,

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CURRICULUM VITAE

NAME : Miss Manida Kaewphetsuwan

BIRTH DATE : October 19, 1989

BIRTH PLACE : Uttaradit, Thailand

EDUCATION :

YEAR	INSTITUTE	DEGREE/DIPLOMA
2012	Silpakorn Univ.	B.Eng (Petrochemical and polymeric materials)

POSITIVE/TITLE : Postgraduated student

WORKPLACE : Faculty of Engineering, Kasetsart University

SCHOLARSHIP/AWARDS :

2012 TAIST-Tokyo Tech Scholarship (Master’s degree)

2014 an Oral Presentation in the International Conference on Pure and Applied Chemistry 2014 (PACCON 2014)