CHAPTER IV

RESULTS AND DISCUSSION

4.1 Emulsification properties of PCTS

4.1.1 Characterization of PCTS

Chitosan derivative was synthesized by substitution of phosphate groups onto chitosan main chain by phosphorylation to obtain phosphorylated chitosan. Sodium salt formation was used to improve water solubility of phosphorylated chitosan. Therefore, the obtained sodium phosphorylated chitosan (PCTS) was water soluble. The structure of the obtained PCTS was analyzed by FT-IR spectrometer. Figure 4.1 shows FT-IR spectra of chitosan and PCTS. Chitosan shows characteristic peaks of O-H stretching and N-H stretching at 3434 cm^{-1}, C=O stretching and N-H bending at 1666 and 1604 cm^{-1}, respectively and C-O stretching of pyranose ring at 1072 cm^{-1}. Whereas, PCTS exhibits similar peaks as chitosan including new specific peaks at 823 cm^{-1}, 980 cm^{-1}, and 1263 cm^{-1} which are characteristic peaks of P-O-C, P-OH and P=O stretching, respectively[127]. Furthermore, the absorption peak at 823 cm^{-1}, and 980 cm^{-1} tend to increase with the mole ratios of phosphorous pentoxide to chitosan residue increased.

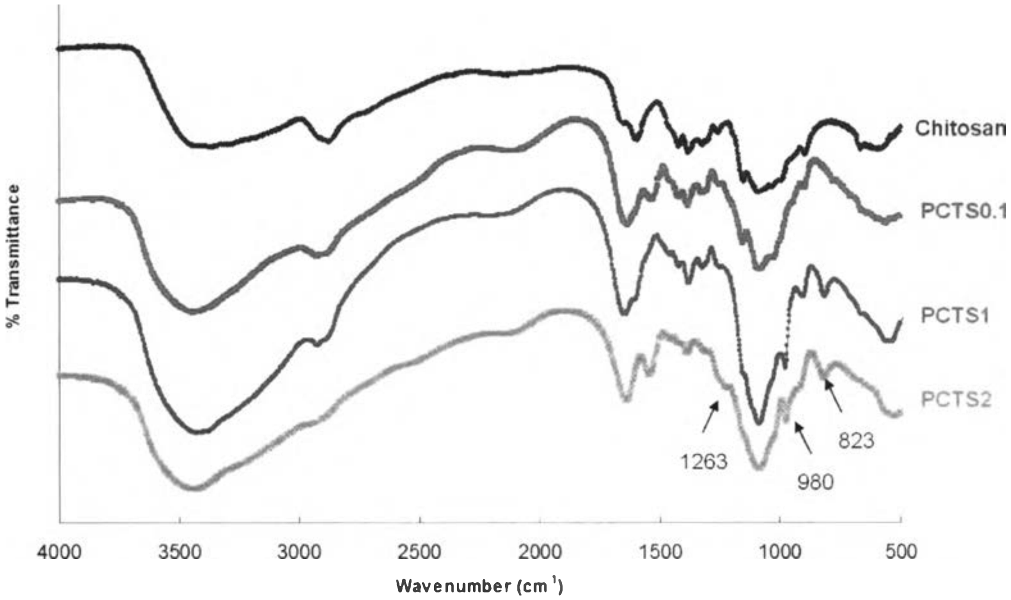


Figure 4.1 FT-IR Spectra of chitosan, PCTS0.1, PCTS1, and PCTS 2.

The molecular weight of PCTS and the degree of phosphate substitution to chitosan residue were shown in Table 4.1. From FT-IR spectra and degree of phosphate substitution indicate that sodium phosphorylated chitosan can be synthesized with higher phosphate groups as increasing mole equivalent of phosphorus pentoxide.

Table 4.1 The characteristic of PCTS at various degree of substitution.

Samples	Degree of phosphate substitution of PCTS [b]	Weight Average Molecular Weight of PCTS (kDa) [c]	Number Average Molecular Weight (kDa)[c]	Polydispersity Index
PCTS[a] 0.1	0.04	58	50	1.16
PCTS[a] 1	0.25	69	65	1.06
PCTS[a] 2	0.62	78	74	1.05

[a] Synthesis from chitosan with degree of deacetylation 85

[b] Determination by a Scanning Electron Microscope, JSM-5800, JEOL equipped with an Energy –Dispersive X-ray spectrometer calculating from Eq.3.1

[c] Determination by gel performance chromatography (Shimadzu class-VP V 6.14, Japan).

4.1.2 Hydrophilic-Lipophilic Balance (HLB) of PCTS

The HLB is described by a number which indicates the relative affinity of emulsifier for the oil and aqueous phase [62]. A molecule with a high HLB number has a high ratio of hydrophilic groups to lipophilic groups, and vice versa. The HLB number of emulsifier shows a useful to indicate its solubility in oil or water phase and can be used to predict the type of emulsion that will be formed by emulsifier. The emulsifier with a low HLB number (3-6) preferably dissolved in oil and stabilized W/O emulsion. The emulsifier with a high HLB number (10-18) preferably dissolved in water and stabilized O/W emulsion [30].

A drop of toluene diameter over PCTS solution was 5.848 ± 0.183 mm, illustrated that HLB value of PCTS around was 19 calibrating from the calibration curve in Figure 4.2. The structure of PCTS contains acetamido groups to the pyranose main chain that support the hydrophobicity. Furthermore, it contains hydrophilic parts of phosphate and amino groups. The degree of deacetylation of initial chitosan was around

85 resulting in possessing more amino groups than acetamido groups cause the high number of HLB or high balance of strength of the hydrophilic to lipophilic groups.

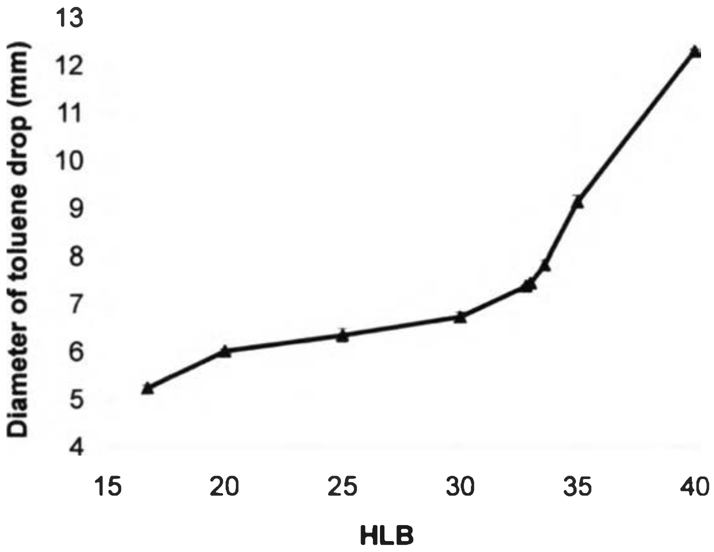


Figure 4.2 Calibration curve between HLB values and diameter of the toluene drops over mixed emulsifier solutions at 25 °C.

4.1.3 Critical Micelle Concentration (CMC) of PCTS

A Critical Micelle Concentration (CMC) is well known as the lowest concentration of emulsifier that promotes micelles formation. Furthermore, CMC value is important for systems which emulsifiers' effect is primary consideration [36]. For example, low concentrations of emulsifier are desirable in personal care product in order to reduce irritation [36]. In order to investigate the lowest concentration for PCTS to form micelle, CMC was determined by measuring surface tension of PCTS solution as a function of PCTS concentrations.

Equilibrium values of surface tension of PCTS at 25°C are shown in Figure 4.3. The CMC of PCTS was determined as the concentration at the inflection point of the surface tension curve which was 0.13 %w/v. Therefore, PCTS has potential to from micelles over CMC.

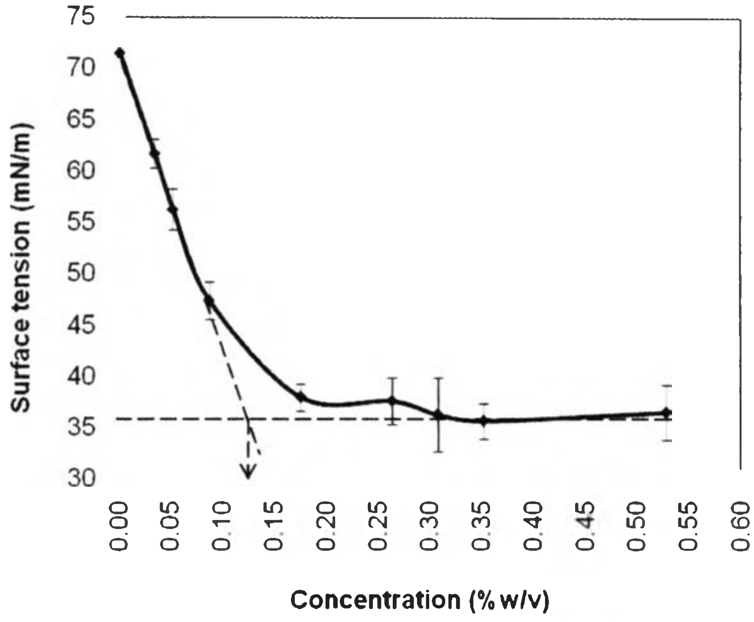


Figure 4.3 Surface tension of PCTS0.1 aqueous solutions related to its concentrations determined at 25°C. CMC was read at intersection point.

4.1.4 Preliminary emulsion type evaluation of PCTS

In order to preliminary evaluate emulsion type, the aqueous solution to oil ratios were varied to 2:8, 4:6, 5:5, 6:4 and 8:2. The concentration of PCTS0.1 was fixed at 1%w/v which higher than the CMC of PCTS0.1. The preferable emulsion type was evaluated from emulsion index, surface mean diameter and drop test.

Figure 4.4 shows relationship between emulsion index, surface mean diameter (d_{32}) of the system using PCTS0.1 as emulsifier and water to oil ratios. The emulsion index is founded to be dependent on the PCTS aqueous solution to oil ratios. The emulsion index increased with increasing volume ratio of PCTS aqueous solution in the system. This result indicates that PCTS would rather suitable emulsified in the system which the volume of aqueous phase was higher than that of oil phase. It should be noted that oil was significantly remained in higher volume as increasing volume ratios of oil phase. According to HLB number, because of high HLB number indicating that

PCTS has higher hydrophilic moiety than hydrophobic moiety. Therefore, PCTS would rather promote the formation of oil-in-water emulsions (O/W emulsions). This HLB number can confirm type of the produced emulsion as O/W emulsion. The HLB number was considered as a factor directly influenced to the emulsion type. This result was also corresponded to a previous report [30]. The emulsifier with a low HLB number (3-6) preferably dissolved in oil and stabilized W/O emulsion. The emulsifier with a high HLB number (10-18) preferably dissolved in water and stabilized O/W emulsion [30].

Furthermore, the drop test of as prepared emulsion in distilled water or mineral oil was observed to preliminary investigate the continuous phase. The results showed that all as prepared emulsions using various concentrations of PCTS, a drop of emulsion dispersed in distilled water, while it aggregated in mineral oil. Therefore, it is indicated that the continuous phase was an aqueous phase. This phenomenon also supported that PCTS was suitable functioned as emulsifier to prepared O/W emulsion.

To find optimum water to oil ratio for preparing emulsion, the droplet size and droplet size distribution of emulsion was also considered. The d_{32}, the mean surface diameter of droplet, decreased with increasing water to oil ratios. The d_{32} reached a constant when the ratios of water to oil became between 6:4 and 9:1.

The droplet size distribution of emulsion is one of the most important characteristic of emulsion because it is one factor to control dominant mechanism of instability such as flocculation, Ostwald ripening, droplet coaleasence [95,128]. The droplet size distribution also influenced to viscosity of the emulsion [129]. An emulsion with same mean droplet size may exhibit different behavior due to differences in their droplet size distribution [95]. Some research reported that the emulsion within the same range of mean droplet size having a bimodal distribution exhibited lower magnitude of viscosity than the emulsion having a unimodal distribution [130].

Figure 4.5 shows droplet size distribution of emulsion at various PCTS0.1 aqueous solutions to oil volume ratios in emulsion. The result showed that the emulsions prepared at the ratios of water to oil of 2:8, 4:6, 5:5 showed bimodal droplet size distribution , whereas the emulsions prepared at the ratios of water to oil of 6:4, 8:2, 9:1 showed unimodal with narrow droplet size distribution. The emulsion had bimodal droplet size distribution, it tended to occur Ostwald ripening after storage emulsion for a period of time. Ostwald ripening is the main mechanism for nano-emulsion destabilization [61,79]. Ostwald ripening occurs from the different pressure of internal phase within the small and big droplets containing in the emulsion. As a result, the internal phase tends to diffuse from small droplets into bigger one leading to a progressive displacement of the droplet size distribution up to higher average sizes [105]. The emulsion having unimodal size distribution, in contrast, can be predicted to have stability.

From the above results, the PCTS aqueous solution to oil ratio of 8:2 and 9:1 showed the smallest droplet size, unimodal droplet size distribution and possessed emulsion index around 1. But the water to oil ratio of 8:2 could contain higher volume of oil phase than 9:1. This result showed that water to oil ratio of 8:2 was the most effective condition to prepare emulsion using PCTS0.1 as emulsifier. This was because of no remained oil at the surface of as prepared emulsion including the smallest droplet size. Therefore, this proportion was selected to prepare the emulsion for further evaluating emulsifying efficiency of PCTS0.1 and emulsion stability.

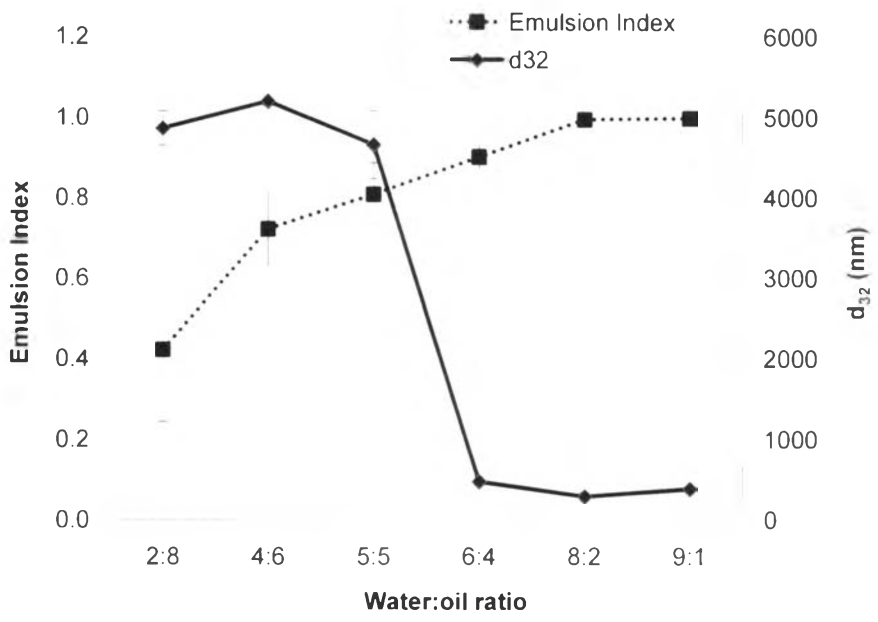


Figure 4.4 Emulsion index and surface mean diameter (d_{32}) at various PCTS0.1 aqueous solutions to oil volume ratios. Concentration of PCTS0.1 was fixed at 1%w/v for all emulsions.

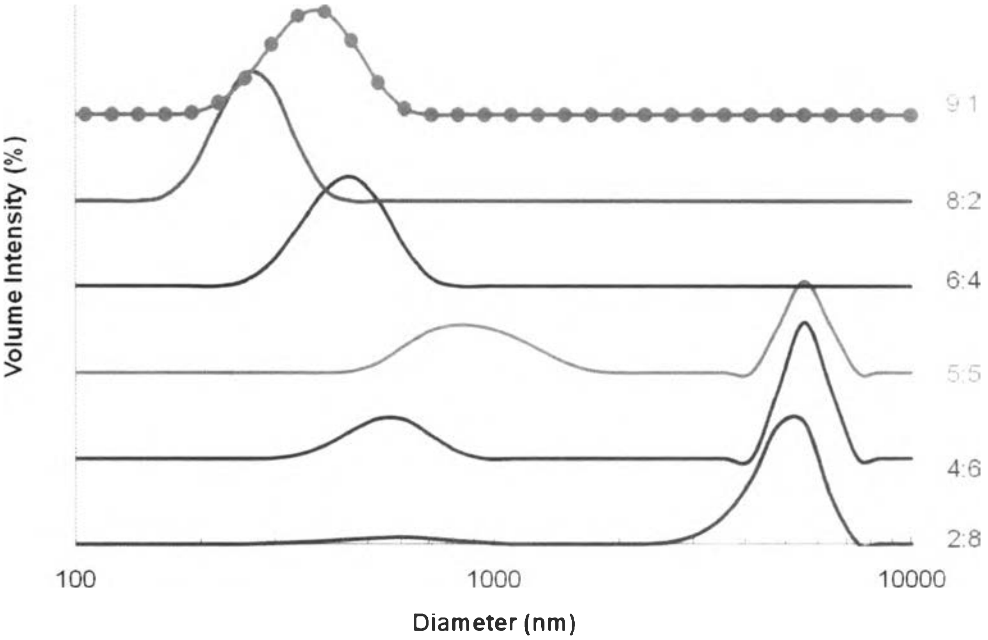


Figure 4.5 Droplet size distribution of emulsion at various PCTS0.1 aqueous solutions to oil volume ratios. Concentration of PCTS0.1 was fixed at 1%w/v for all emulsions.

4.1.5 Emulsifying efficiency and emulsion stability

4.1.5.1 Emulsifying efficiency

The droplet size of fresh prepared emulsion at room temperature was shown in Figure 4.6. The droplet size decreased as increasing PCTS concentrations until reach constant at PCTS concentration of 1%w/v. Obviously, the droplet sizes of as prepared emulsions below CMC (0.063%w/v) was large, whilst that of as prepared emulsions above CMC was smaller and constant. It is implied that there were enough emulsifiers presented to form a strong layer at the interface of oil droplets and to stabilize the emulsion resulting in constant emulsion droplet size.

This result could be confirmed under confocal scanning laser microscopy (CLSM) by visualization of the interfacial PCTS distribution between oil and water phase. PCTS was labeled with fluoresceine isothiocyanate and visualized the fluorescent emission through the microscope.

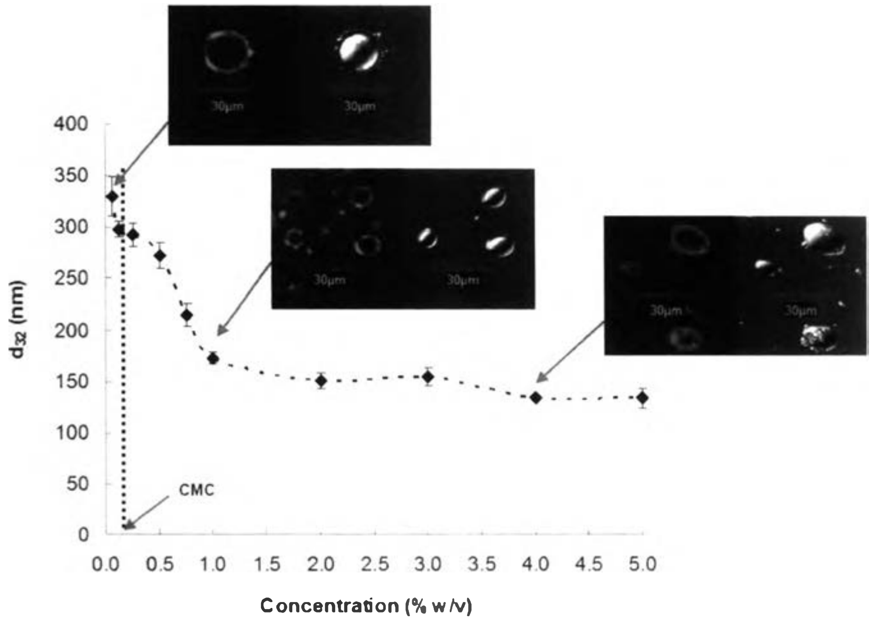


Figure 4.6 Surface mean diameter (d_{32}) of emulsion droplets prepared from various PCTS concentrations at fixed water to oil ratio of 8:2, measuring at room temperature. Insert picture showed CLSM micrograph of emulsion droplet at magnification of 40x, fluorescence light image (left), Nomarski image (right).

The CLSM micrograph showed that emulsion can form at PCTS concentration below CMC (0.063%w/v), but PCTS does not clearly covered all over oil droplet. This may be influenced to emulsion stability. Whilst at concentrations of PCTS above CMC, PCTS rearranged in high density covering around oil droplets to stabilize micelle and produce small droplets.

4.1.5.2 Emulsion stability

4.1.5.2.1 Storage appearance alteration

The storage appearance of emulsions for 1, 7 and 90 days was presented in Figure 4.7. Almost all fresh emulsions prepared from various concentrations of PCTS showed homogeneous white colloidal solution, except only the emulsion prepared by using the concentration of PCTS below CMC (0.13%w/v). They provided the gradient creaming, an opaque white gradient on the top of emulsion

After 7 days, the emulsion prepared from the PCTS concentrations around CMC in the range of 0.06-0.25% w/v and much above CMC ($\geq$ 2%w/v), showed gradient creaming at upper phase and in a clear lower serum occurred at bottom phase. While, the emulsion contained 0.5-1.0 %w/v of PCTS produced only gradient creaming at upper phase. These phenomenons also were occurred after storage emulsion after 90 days but significantly increased for serum and creaming.

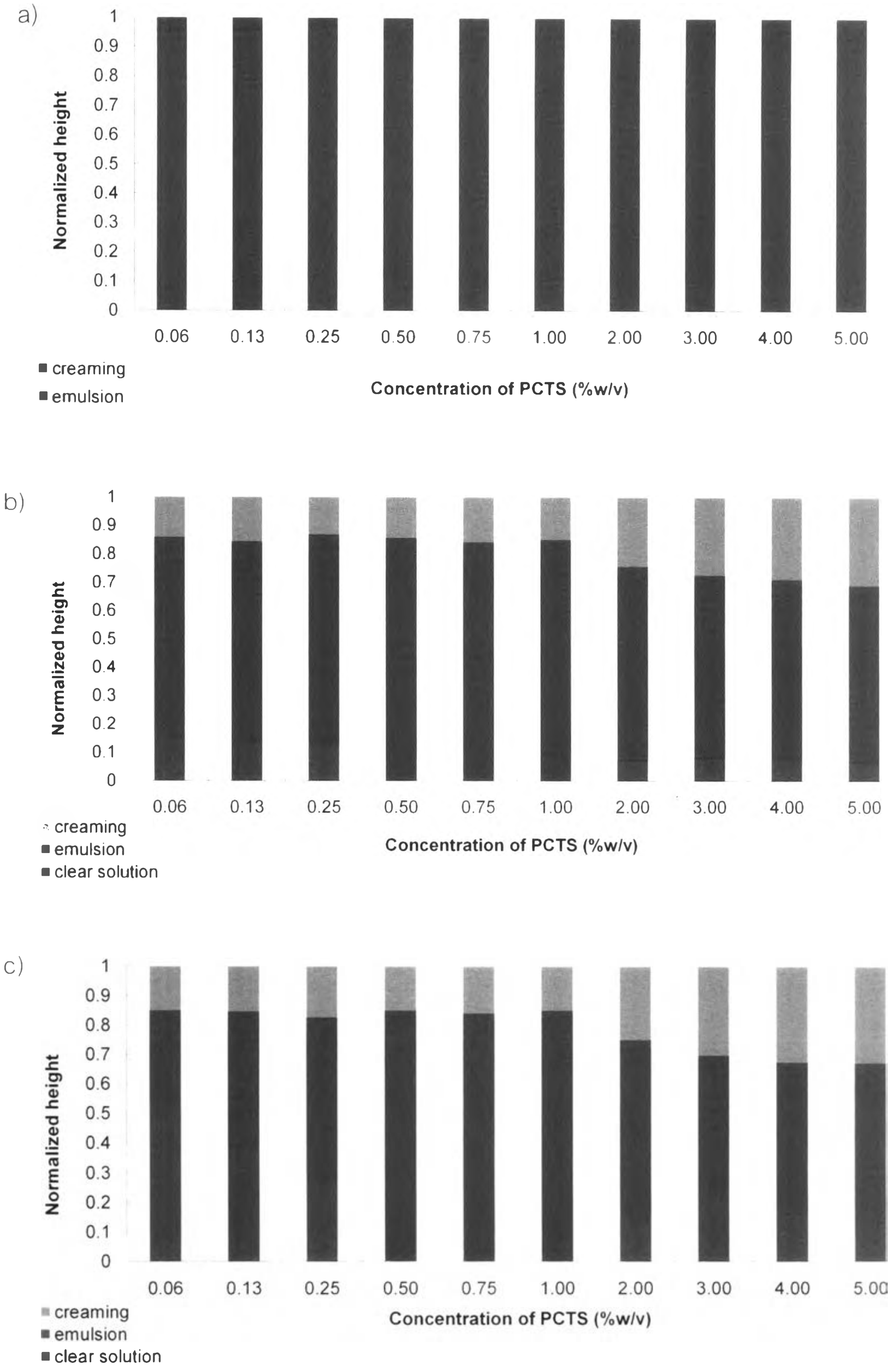


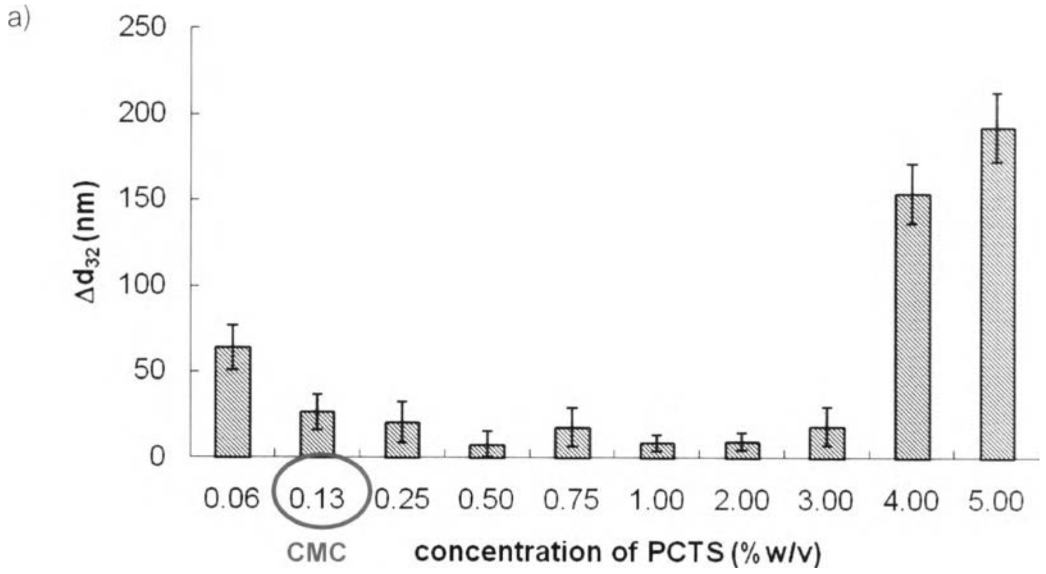
Figure 4.7 Schematic drawing represented appearance of a) fresh prepared emulsion, and that of emulsions after b) short term storage (7 days), c) long term storage (90 days).

4.1.5.2.2 Droplet size alteration

The alteration of droplet size after storage at room temperature for short term (7 days) and long term (90 days) comparing with fresh prepared emulsions was analyzed in order to study the stability of as prepared emulsions. Figure 4.8 showed droplet size alteration from that of the fresh prepared emulsions using various concentrations of PCTS.

For both short term (Figure 4.8a) and long term storage (Figure 4.8b), it is evident that the significant change in droplet size dependent on PCTS concentrations. For short term storage, below CMC (0.13%w/v) and much above CMC (above 3%w/v), the emulsions were quite unstable. Larger emulsion droplets were rapidly formed at very high concentrations. At CMC or above CMC (less than 3%w/v), the emulsions were obviously stable owing to stability in droplet size alteration. For long term storage showed similar evidence on droplet size alteration to short term storage, but exhibiting obviously observed in droplet size alteration. However, the emulsion was unstable at CMC. It was implied that over CMC there was enough emulsifiers presented to form a strong layer at the interface of oil droplets and stabilized the emulsion resulting in constant emulsion droplet.

Above CMC to 3% w/v, the droplet size slightly changed. It was implied that it possessed optimum thickness of interfacial layer to generate protective membrane for emulsion droplet. Above 3%w/v, the droplet size of emulsion increased again.



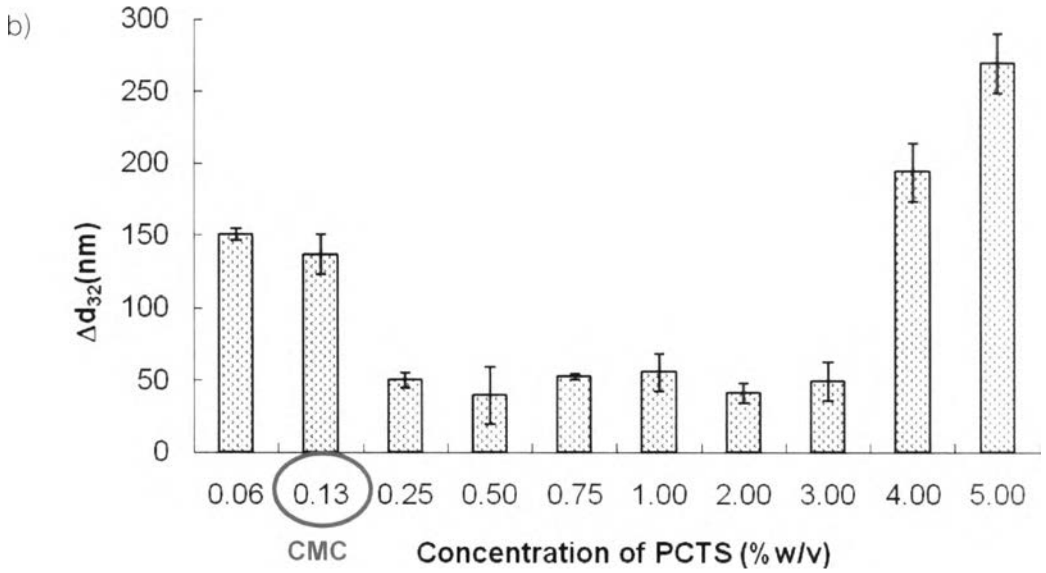


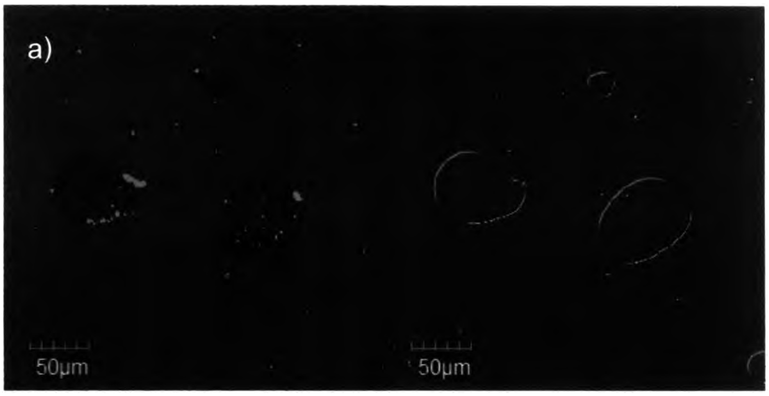
Figure 4.8 Droplet size differences of emulsions after a) short term storage (7 days) and b) long term storage (90 days) from that of fresh prepared emulsions.

4.1.6 Physical mechanism of unstable emulsions

4.1.6.1 Visualization of the PCTS distribution on the emulsion droplet

The emulsion using PCTS concentration below CMC and much above CMC after storage for a given periods of time, they tend to increase in droplet size (Figure 4.8) in a consequence of phase separation (Figure 4.7) into creaming (white cream at upper phase) and syneresis (clear serum at lower phase). An unstable emulsion mostly shows two phenomena depending on the comparative density of dispersed phase to continuous phase, which are (i) creaming at upper phase and syneresis clear serum at lower phase and (ii) sedimentation at lower phase and syneresis clear serum at upper phase. Therefore, it is our interests to investigate the physical mechanism suit to explain those phenomena. Physical mechanism of an unstable emulsion can be classified into two main categories which are reversible process:- flocculation and irreversible process:-coalescence and Ostwald ripening. In this research, the physical mechanism of the unstable emulsion form by using PCTS0.1 concentrations of 0.063%w/v and 4%w/v was preliminary investigated by visualization of the PCTS distribution on the emulsion droplets via confocal scanning laser microscope.

Figure 4.9 shows CLSM micrograph of emulsion droplet E85/0.1 (using PCTS 0.1 as emulsifier) after storage for 7 days at PCTS concentrations of 0.063%w/v and 4%w/v. The emulsion prepared by using PCTS concentration of 0.063%w/v showed big droplets (Figure 4.9a), whereas that prepared by using PCTS concentration of 4%w/v showed individual small droplets clump together containing of polymer bridging at the center (Figure 4.9b). Therefore, this can be explained that an unstable mechanism of emulsion prepared by using PCTS concentration of 0.063%w/v might be coalescence, whereas that prepared by using PCTS concentration of 4%w/v might be flocculation. Coalescence can occur when droplets close with each other due to Brownian motion and van der Waals attraction [Sing, A.J.F., Gracia, A., Lachaise, J., Brochette, P., 1999]. Furthermore, thin film of emulsifier distorts and the disperse phase in droplet is drained leading to rupture of separating film. Then, two or more liquid droplets fuse together to form a single larger droplet [104]. Flocculation is generally a reversible aggregation of droplets, leads to separation in a gravity field, i.e., creaming. The droplets, however, retain their integrity [131].



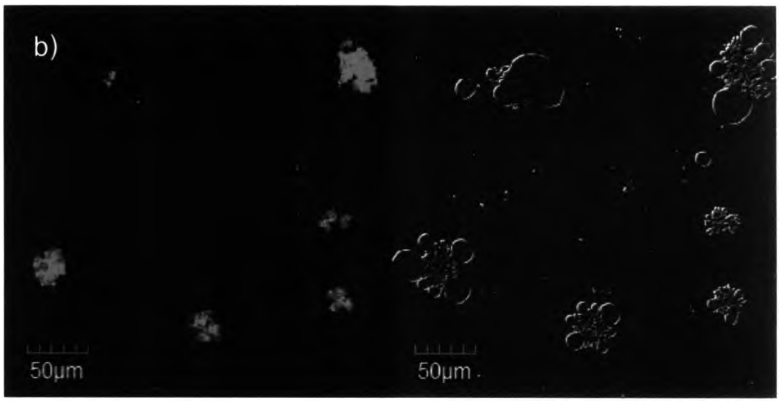


Figure 4.9 CLSM micrograph of emulsion droplet E85/0.1 (after 7 days) using PCTS0.1 at a) 0.063%w/v, b) 4%w/v, respectively as emulsifier a) fluorescence light image, b) Nomarski image.

4.1.7 Physical reversibility of the unstable emulsion under pH variation

The physical mechanism of an unstable emulsion after storage for the emulsion prepared by using PCTS concentration of 0.063%w/v and 4%w/v were further investigated in detail to support the above data. For the limited data, the physical reversibility of emulsion prepared by using PCTS concentration of 4%w/v will be explained in this dissertation. However, the physical reversibility of emulsion prepared by using PCTS concentration of 0.063%w/v should be studied further. The reversibility of the emulsion prepared from PCTS concentration of 4%w/v was investigated under pH variation after storage the emulsion for 7 days. Surface charge and surface mean diameter were measured to explain the mechanism.

4.1.7.1 ζ-Potential of emulsion droplets

Figure 4.10 shows ζ-Potential of emulsion droplets as function of pH. It showed that the zeta potential of droplet decreased with increasing pH of the environment. At lower pH, the amino groups on PCTS structure were protonated and changed to NH_3^+, so the surface of droplets showed positive charges. When increased pH of system, the phosphate groups on PCTS structure were deprotonated and changed to PO_3^{2-} resulting in negative charges at the surface. The negative potential was strong or weak depending on the amount of phosphate substitution. PCTS0.1 had phosphate substitution only 0.04 units per chitosan residue. Therefore, the emulsion prepared from PCTS 0.1 showed quite low negative charges at the surface. Interestingly, the emulsion droplets aggregated at pH around 5 which was an isoelectric point of PCTS0.1.

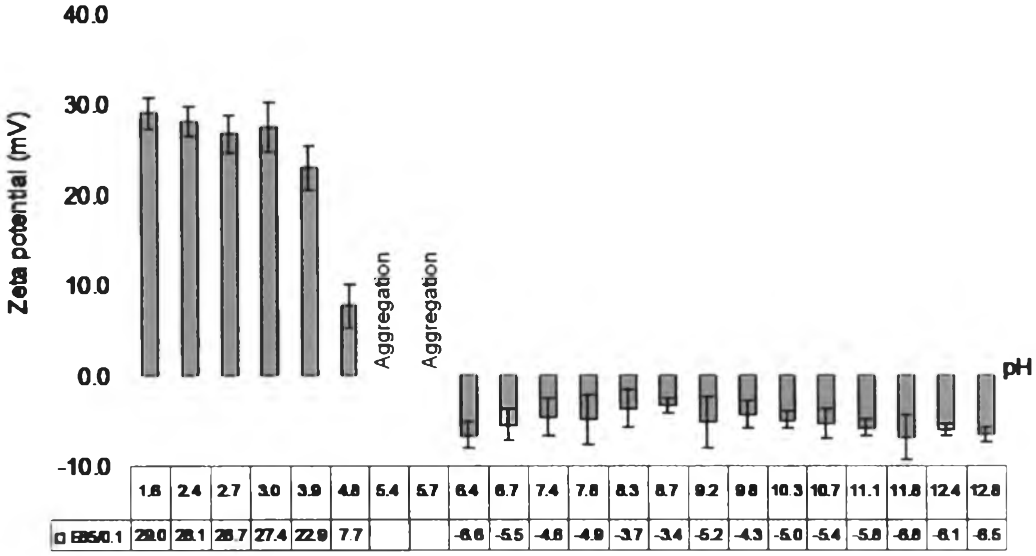


Figure 4.10 ζ-Potential of emulsion droplets as function of pH. The 4%w/v PCTS0.1 as emulsifier.

4.1.7.2 The droplet size of emulsion at various pH

Since the significant increase in droplet size after storage at ambient temperature for 7 and 90 days was first observed in emulsion using PCTS concentration of 4%w/v. Therefore, to verify assumption about flocculation of emulsion when using PCTS0.1 at concentration much above CMC, the emulsion prepared from PCTS0.1 concentration of 4%w/v was chosen to investigate the physical mechanism of an unstable emulsion.

Generally, many methods were utilized to verify flocculation of emulsion, for example, (i) investigation the viscosity of emulsion or the shear stress under applied various shear rates [102-103] The low droplet-droplet interaction in emulsions and non-flocculation, are supposed to be responsible for the Newtonian behaviors of the fluids. In contrast, the shear-thinning behavior of emulsions could be attributed to the formation of clusters or flocculated of droplet [102-103], (ii) investigation the flocs of droplet using microscope [132]. The optical image shows droplets clump together.

Owing to PCTS was an amphoteric molecule consisting both positive and negative charges in the same molecule. In this research, flocculation of emulsion was verified at the first time through investigating changing in emulsion droplet size under various environmental pH. The emulsion droplets clumped together in a loose arrangement and formed big particles was defined as flocculation, so that, the emulsion droplets surrounding with amphoteric PCTS expected to change their droplets size under changing pH of the continuous phase .

The surface mean diameter (d_{32}) of emulsion droplets using 4%w/v PCTS0.1 as emulsifier was plotted against various pH in Figure 4.11. As mentioned above, PCTS0.1 mainly exhibited positive charges at lower pH. The results showed that the surface mean diameter decreased with decreasing pH of system. It could be implied that at lower pH the amino groups on PCTS structure were protonated to be NH_3^+ and induce electrostatic repulsion of the positive charges between the droplets in concomitant with separating into small particles, as a result of small surface mean diameter. When pH of system increased, the phosphate group on PCTS structure was deprotonated to be PO_3^{2-}. As mentioned above that at pH over than 6, PCTS0.1 showed very low negative surface charges that may not enough to repulse each droplet, so each droplets clump together, resulting in large surface mean diameter.

This phenomenon showed that the clump of emulsion droplets using 4%w/v PCTS as emulsifier could separate into individual droplet at lower pH, so means the physical mechanism of this emulsion was flocculation, not coaleacence.

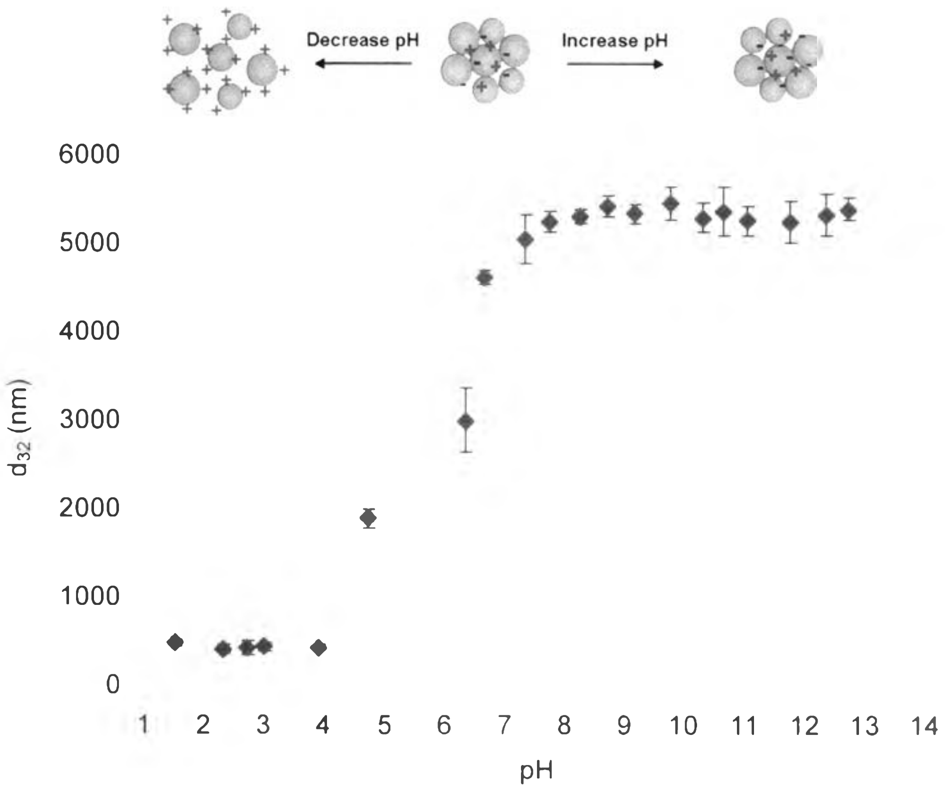


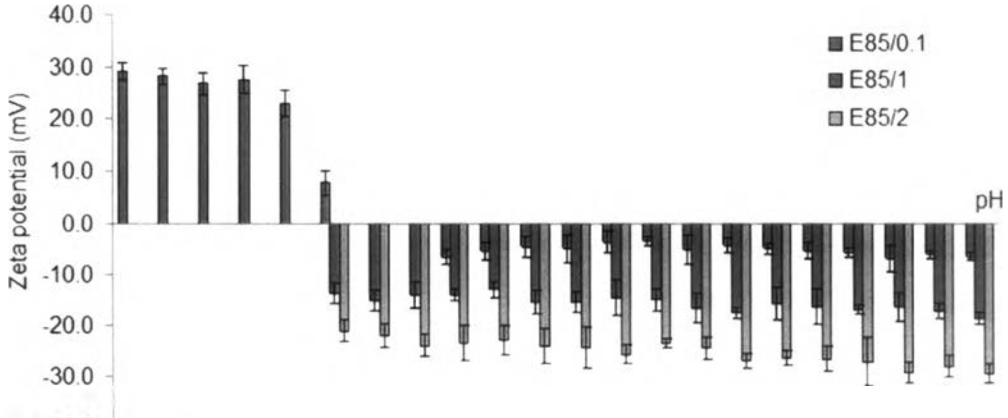
Figure 4.11 Surface mean diameter of emulsion droplets as function of pH. Emulsions were prepared from PCTS0.1 concentration of 4%w/v.

4.1.8 Effect of ionic levels of PCTS on the physical mechanism of unstable emulsion

Ionic levels were also considered as a factor influenced to physical mechanism of unstable emulsions. Therefore, the physical mechanism of unstable emulsions formed from various ionic levels of PCTS was also explained in terms of surface charges and surface mean diameter under variation of pH.

4.1.8.1 ζ-Potential of emulsion droplets

The zeta potential of emulsion prepared by using 4%w/v PCTS0.1, PCTS1, and PCTS2, respectively as emulsifiers plotted as function of pH was shown in Figure 4.12.



	1.6	2.4	2.7	3.0	3.9	4.8	5.4	5.7	6.4	6.7	7.4	7.8	8.3	8.7	9.2	9.8	10.3	10.7	11.1	11.8	12.4	12.8
■ E85/0.1	29.0	28.1	26.7	27.4	22.9	7.7	Aggregation		-6.6	-5.5	-4.6	-4.9	-3.7	-3.4	-5.2	-4.3	-5.0	-5.4	-5.8	-6.8	-6.1	-6.5
■ E85/1	Aggregation					-13.9	-15.3	-14.2	-14.2	-13.1	-15.6	-15.6	-14.8	-15.1	-16.7	-17.6	-15.8	-16.4	-17.0	-16.5	-17.2	-18.7
■ E85/2						-21.1	-22.0	-23.9	-23.5	-22.8	-24.1	-24.3	-25.6	-23.4	-24.4	-26.7	-26.3	-26.4	-27.0	-29.1	-27.8	-29.2

Figure 4.12 ζ-Potential of emulsion droplets as function of pH. PCTS0.1, PCTS1, and PCTS2 at concentration of 4%w/v were used as emulsifiers for E85/0.1, E85/1, and E 85/2. respectively.

The zeta potential of emulsion using PCTS 0.1 as emulsifier (E85/0.1) started with quite high positive surface charges of around +30 in the pH range of 1-3. The surface charges decreased as increasing pH until reached the isoelectric point aroud pH 5.6 and kept constantly low negative charges in the pH range of 6-13. This result can be explained follow this, at lower pH, the amino groups on PCTS structure were protonated to be NH_3^+, so the surface charge of droplet showed positive charges. When pH of system increased, the small amount of phosphate group on PCTS structure was deprotonated to be PO_3^{2-}. However, NH_2 was not protonated at pH over than 8. The negative potential was strong or weak depending on the amount of phosphate substitution. For PCTS0.1 had phosphate substitution only 0.04 per chitosan residue. Therefore, the emulsion prepared from PCTS 0.1 showed quite low negative charges at the surface.

The emulsion using PCTS 1 showed tends of zeta potential as same as that using PCTS2. The zeta potential of emulsion using PCTS 1 as emulsifier decreases from -14 mV to -19 mV and that of emulsion using PCTS 2 as emulsifier decreases from -20 mV to -30 mV when pH in system increased from 5.0 to 12.8. It may be because phosphate groups on PCTS structure were additionally deprotonated. The decreasing in zeta potential at higher pH can be more significantly observed in emulsion using PCTS 2 as emulsifier than PCTS 1 as emulsifier because PCTS 2 contained more phosphate groups than PCTS 1 around 2.5 times.

4.1.8.2 The droplet size of emulsion at various pH

To evaluate the effect of ionic levels of PCTS on the physical mechanism of unstable emulsion, so PCTS was synthesizes at various degree of substitution (DS) follow this PCTS 0.1, PCTS 1, PCTS 2 (The characteristics of these PCTS were shown in Table 3.1) to use as emulsifiers at concentration of 4%w/v.

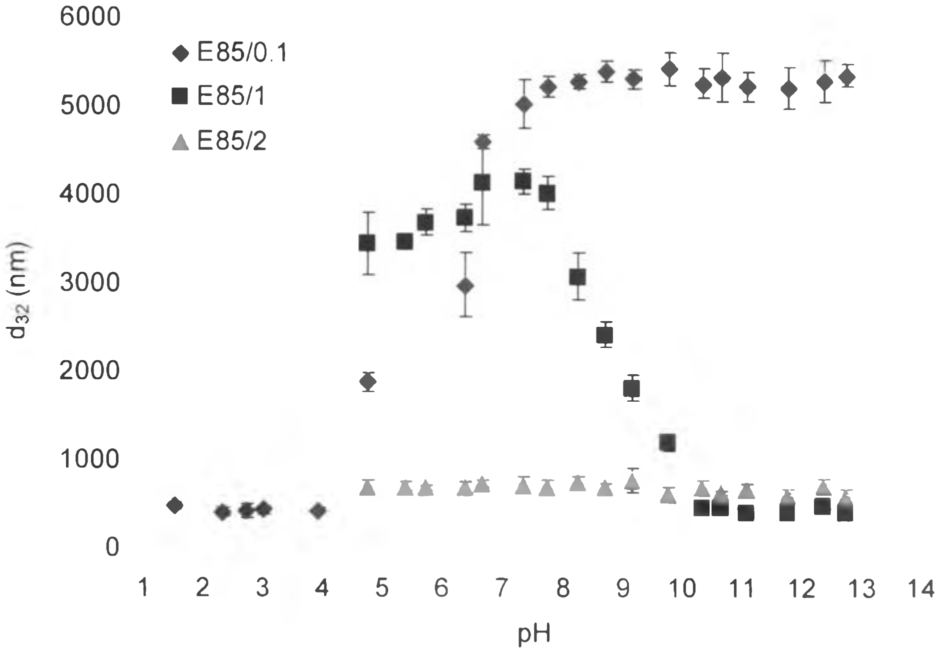


Figure 4.13 Surface mean diameter of emulsion droplets as function of pH. E85/0.1, E85/1 and E85/2 defined as the emulsion prepared by using PCTS0.1, PCTS1, and PCTS2 as emulsifier, respectively , with PCTS concentration of 4%w/v.

Surface mean diameter of emulsion droplets plotted as function of pH was shown in Figure 4.13. The result of these PCTS showed different feature for each PCTS. The emulsion using PCTS 0.1 as emulsifier displayed increase in droplet size when the pH of system increased from pH around 1.5 to 12.8. It showed small droplet size during pH range of 1.5-4, increased in droplet size abruptly during pH range of 4-7 and kept constantly in large droplet size as increasing pH more than 7. The emulsion using PCTS 1 as emulsifier exhibited constant of droplet size at pH in range of 5.0-8.0 and dramatically decrease at pH in range of 8.0-10.25, then it possessed constant value of droplet size. Meanwhile, the emulsion using PCTS 2 as emulsifier showed constant value of droplet size even increase pH from 4.8 to 12.8.

These phenomena can be described as follows: first, the emulsion using PCTS 0.1 as emulsifier, at lower pH the large amount of amino groups on PCTS structure were protonated to be NH_3^+ and induced electrostatic repulsion of each droplet resulting in small surface mean diameter. When increasing pH of system, the phosphate groups on PCTS structure were deprotonated to be PO_3^-. As mentioned above that at pH over than 6, PCTS0.1 showed very low negative surface charges that may not enough to repulse each droplet, so each droplet clump together, resulting in large surface mean diameter.

Second, the emulsion using PCTS 1 as emulsifier, owing to it has low negative potential. Therefore, in pH range of 5.0-8.0, during this pH range some of the amino groups can be protonated to be NH_3^+. Therefore, the deprotonated PO_3^- can induce electrostatic attraction with protonated NH_3^+ and clump each droplet together, resulting in large surface mean diameter. Then, droplet size of emulsion using PCTS 1 as emulsifier showed dramatically decrease at pH in range of 8.0-10.25 because the phosphate groups were deprotonated to PO_3^- to contain negative charged enough to repulse adjacent droplet resulting in decreased in droplet size. The E85/1 maintained constant in droplet sizes unless pH in system increased over 10.25. The phosphate groups of PCTS 1 had been deprotonated totally to be PO_3^- since pH 10.25, resulting in constant value of zeta potential as mentioned above, in a consequence of constant emulsion droplets size. This implied that the constant droplets size over pH 10.25 was a mean individual emulsion droplets size.

In fact, the emulsion droplets prepared from PCTS2 should show similar behavior with emulsion droplets prepared from PCTS1. Interestingly, the emulsion droplets prepared from PCTS2 showed constant small droplets size in a wide range of pH. This may be due to PCTS2 has strong negative potential enough to repulse into individual droplets.

Schematic drawing of droplets of emulsion E85/0.1, E85/1, and E85/2 prepared by using 4% w/v PCTS 0.1, 1, and 2, respectively as emulsifier was showned in Figure 4.14. This scheme was described about the physical mechanism in mean droplet size when increasing or decreasing the environmental pH. All emulsions were prepared in distrilled water at pH 5.6. After changing environmental pH, the physical mechanism can be explained as follows. E85/0.1 droplets dominated in positive surface charges, they replused into individual dropltes when decresing pH and agglomerated into large particles because of charge neutralization. E85/1 droplets dominated in weak negative surface charges, they agglomerated at pH 5.6 due to electrostatic interaction between NH_3^+ and PO_3^{2-}, and aggregated at pH below 5.6 because of charge neutralization, but replulsed into individual droplets when increasing pH. Meanwhile, E85/2 droplets dominated in strong negative surface charges, they maintained their droplet size in a wide range of pH owing to their strong negative potential. They also aggregated at pH below 5.6 as same as E85/1.

This scheme showed that the clump of emulsion droplets using 4%w/v PCTS as emulsifier were reversible into individual droplet at lower pH for E85/0.1 and at higher pH for E85/1. E85/2 maintained individual droplet over wide pH range. Furthermore, PCTS used as emulsifier should possessed excess negative charges to stabilize emulsion, in other words, PCTS used as emulsifier in this system should possessed degree of phosphate substitution above or equal 0.65 (PCTS2).

From this limitation of data, it can be concluded that the physical mechanism of unstable emulsion was flocculation for the emulsion preparing by using PCTS with DS lower than 0.65. When using PCTS with DS over than or equal to 0.65, the emulsion did not represent an unstable mechanism.

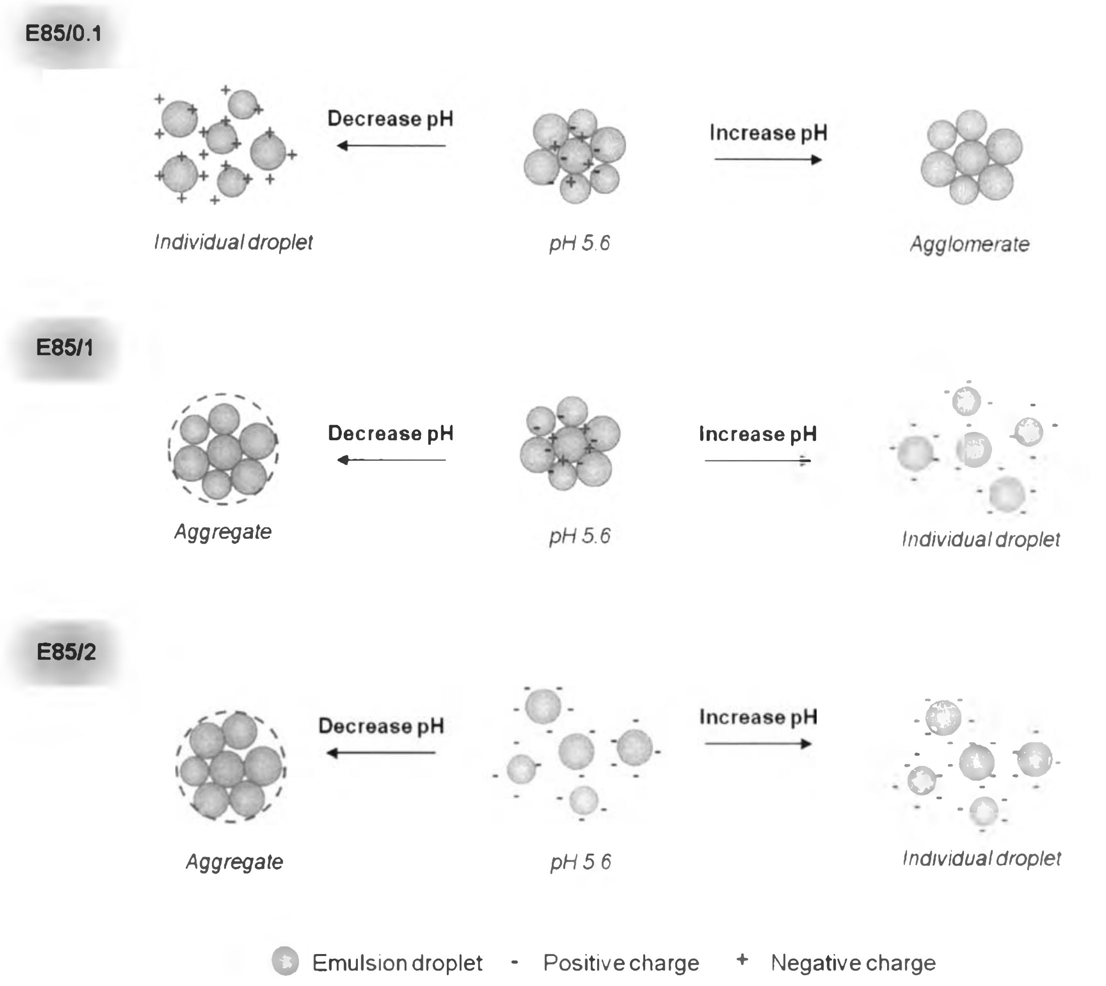


Figure 4.14 Schematic drawing represented the physical mechanism of emulsion E85/0.1, E85/1, and E85/2 using 4% w/v PCTS 0.1, 1, and 2, respectively as emulsifiers which ocuured after increased or decreased pH of system.

4.2 Effect of systematic conditions (pH, ionic strength, and temperature) on emulsion stabilization

Because PCTS is amphoteric containing both amino groups from chitosan and phosphate substituted groups, a change of some systematic conditions such as pH may be possessed effect on stability of emulsion using PCTS as emulsifier. Furthermore, emulsion using PCTS as emulsifier exhibited ionic characteristic in distilled water, so we hypothesized that ionic strength may also effect on stability of emulsion.

Moreover, PCTS also showed amphiphilic property. Therefore, the environmental temperature might effect to its hydrophilicity. To proof our hypothesis, in this part, the stability of emulsion in various systematic conditions viz. pH, ionic strength, and temperature was studied.

The PCTS used in this part only varied degree of phosphate substitution on PCTS around 0.04, 0.25, and 0.62 which was called PCTS0.1, PCTS1, and PCTS2, respectively. It should be noted that the number followed PCTS was denoted equivalent mole of P_2O_5 feeding in the phosphorylation reaction with chitosan. Furthermore, The PCTSs possessed weight average molecular weight and number average molecular weight in the range of 58-78 kDa and 50-74 kDa, respectively (Table 4.1). The emulsion prepared by using PCTS0.1, PCTS1, and PCTS2 as emulsifiers were named E85/0.1, E85/1 and E85/2, respectively.

4.2.1 Effect of pH on stability of emulsion using PCTS at various degree of substitution as emulsifiers

4.2.1.1 Surface charge of emulsion at various pH

The surface charge of 'dilute emulsion' with various pH (1-13) at fix concentration of 1%w/v PCTS was illustrated in Figure 4.15. The surface charges for all samples of emulsion droplet gradually changed from positive to negative charges when pH of the system increased.

E85/0.1, which was prepared by using PCTS0.1, showed positive surface charges in pH range of 1-7. Surface charges of E85/0.1 was gradually changed from positive to be neutral around pH 8 (isoelectric point), then constant negative charge at pH 9-13 due to less degree of phosphate substitution on chitosan structure. Since PCTS is amphoteric and shows an isoelectric point (pI), the pH value at which PCTS is neutral. Therefore, the emulsion prepared by using PCTS as emulsifier also exhibits amphoteric property.

E85/1 and E85/2, which were prepared by using PCTS1 and PCTS 2, respectively, shifted their isoelectric point to be lower at pH 5.5 and 3.9, respectively. At given pH, surface charges of emulsion droplets dominated to be positive or negative depending on degree of phosphate substitution. The higher the degree of phosphate substitution, the lower the isoelctric point was. The emulsion droplets exhibited positive surface charges below the isoelectric point, and exhibited negative surface charges above the isoelectric point. Negative charges of surface droplets occurred from the presence of deprotonated phosphate groups along its side chain in condition over isoelectric point.

It should be noted that the emulsions using PCTS 1 and 2 as emulsifiers aggregated at low pH (1-3). It is possibly because phosphate groups were neutralized at low pH [33].

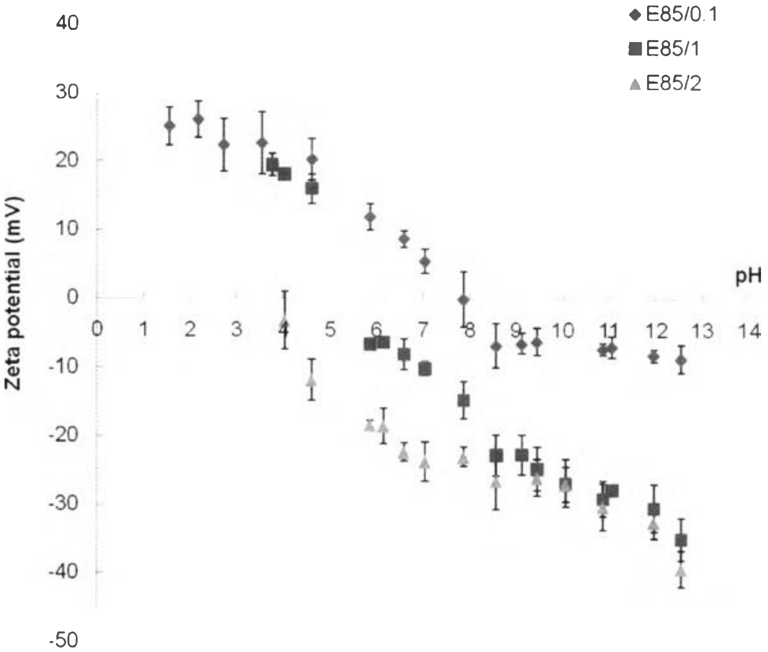


Figure 4.15 Surface charge of emulsion droplets as function of pH. Emulsions were prepared from PCTS0.1, PCTS1, and PCTS2 at concentration of 1%w/v and were definded as a) E85/0.1, b) E85/1, and E85/2, respectively.

4.2.1.2 Droplet size and droplet size distribution of emulsion at various pH

As mentioned in Table 4.1, PCTS was synthesized from chitosan with degree of deacetylation of 85 and substituted with phosphate groups with various degree of phosphate substitution as 0.04, 0.25 and 0.62, so named as PCTS0.1, PCTS1 and PCTS2, respectively. It should be noted that the number following the PCTS was denoted equivalent mole of P_2O_5 feeding in the phosphorylation reaction with chitosan. On the other word, PCTS0.1, PCTS1 and PCTS2 had percent of phosphate to amino groups of 4:85, 25:85 and 62:85, respectively.

Figure 4.16 showed surface mean diameter under pH variation of the emulsion prepared by using PCTS0.1, PCTS1 and PCTS2 which were named as E85/0.1, E85/1 and E85/2, respectively. The results showed that E85/0.1 changed the droplet size in the opposite manner with E85/1 and E85/2 when pH in system was changed.

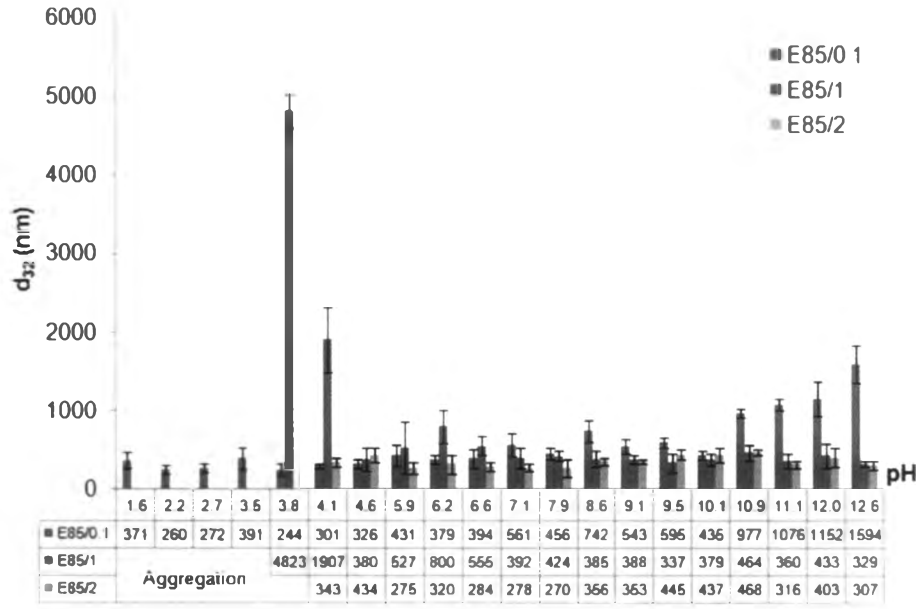
E85/0.1, containing percent of amino groups more than phosphate groups, showed small droplet size in range of 200-500 nm and kept constant even increased the environmental pH during pH range below the isoelectric point (pH 1-7). It was because at pH below isoelectric point, the amino groups were protonated, resulting in electrostatic repulsion between adjacent droplets into individuals droplets. Therefore, the droplets size was constant without considering the strength of positive surface charges. Furthermore, at pH above the isoelectric point, it was found that droplet size increased with increasing environmental pH. The phosphate groups should be deprotonated to be PO_3^{2-} . While NH_2 was not protonated at pH over than 8. The negative potential was strong or weak depending on degree of phosphate substitution. For PCTS0.1 had phosphate substitution only 0.04 per chitosan residue. Therefore, the emulsion prepared from PCTS 0.1 showed quite low negative charges at the surface that may not enough to repulse adjacent droplets and induced the agglomeration of the droplets.

On the other hand, E85/1 and E85/2 which had higher percent of phosphate groups than E85/0.1 (percent of amino groups was fixed at 85) showed more negative surface charges in wide range of pH. Even percent of amino groups was higher than that of phosphate groups, the total surface charges still be negative. It may be because the different in ionization ability between phosphate groups and amino groups. From the above reason, E85/1, E85/2 showed the different phenomena of change in droplets size to E85/0.1. Both E85/1 and E85/2 showed quite constant in droplet size over a wide pH range from pH 4.6 to pH 12.6 without considering the strength of negative charge at surface area. E85/1 and E85/2 aggregated below their isoelectric points, thus, the droplet size was not possible to be measured. This indicated that the environmental pH did not effect to the stability for the emulsion using anionic PCTS with degree of phosphate substitution more than 0.25. From above phenomenon can be concluded that amino group in E85/0.1 and phosphate group in E85/1 and E85/2 played an important role to repulse adjacent droplets through electrostatic repulsion and to stabilize the emulsion without considering the strength of surface charge (zeta potential). From zeta potential and droplet size data, it should be noted that the emulsion should had surface charges over than +5 for E85/0.1 and lower than -10 for E85/1 and E85/2. The strength of charge did not influence to size of individual emulsion droplets.

Furthermore, not only droplet size of emulsion was considered for the stability of emulsion but also droplet size distribution. The droplet size distribution plays a key on emulsion properties such as texture, long-term stability, and optical appearance [133]. Monodisperse emulsions and a narrow size distribution lead to emulsion stability and facilitate control of their properties [134].

Figure 4.17 shows droplet size distribution of E85/0.1, E85/1, and E85/2, respectively at various environmental pH. The droplet size distribution of E85/0.1 showed unimodal and had size in range of 350-500 nm at pH below isoelectric point, which composed in term nano-emulsions (20-500 nm) [69] and gradually increased to 600-2000 nm at pH above isoelectric point.

The droplet size distribution of E85/1 and E85/2 also showed unimodal droplet size distribution (droplet size 200-600 nm) over wide pH range (over their isoelectric points). Except only E85/1 still showed bimodal droplet size distribution below its isoelectric point, indicating the aggregation stating point.



	1.6	2.2	2.7	3.6	3.8	4.1	4.6	5.9	6.2	6.6	7.1	7.9	8.6	9.1	9.5	10.1	10.9	11.1	12.0	12.6
■ E85/0.1	371	260	272	391	244	301	326	431	379	394	561	456	742	543	595	436	977	1076	1152	1594
■ E85/1	Aggregation				4823	1907	380	527	800	555	392	424	385	388	337	379	464	360	433	329
■ E85/2						343	434	275	320	284	278	270	356	353	445	437	468	316	403	307

Figure 4.16 Surface mean diameter of emulsion droplets under pH variation. Emulsions were prepared from PCTS0.1, PCTS1, and PCTS2 at concentration of 1%w/v and were definded as a) E85/0.1, b) E85/1, and c) E85/2, respectively.

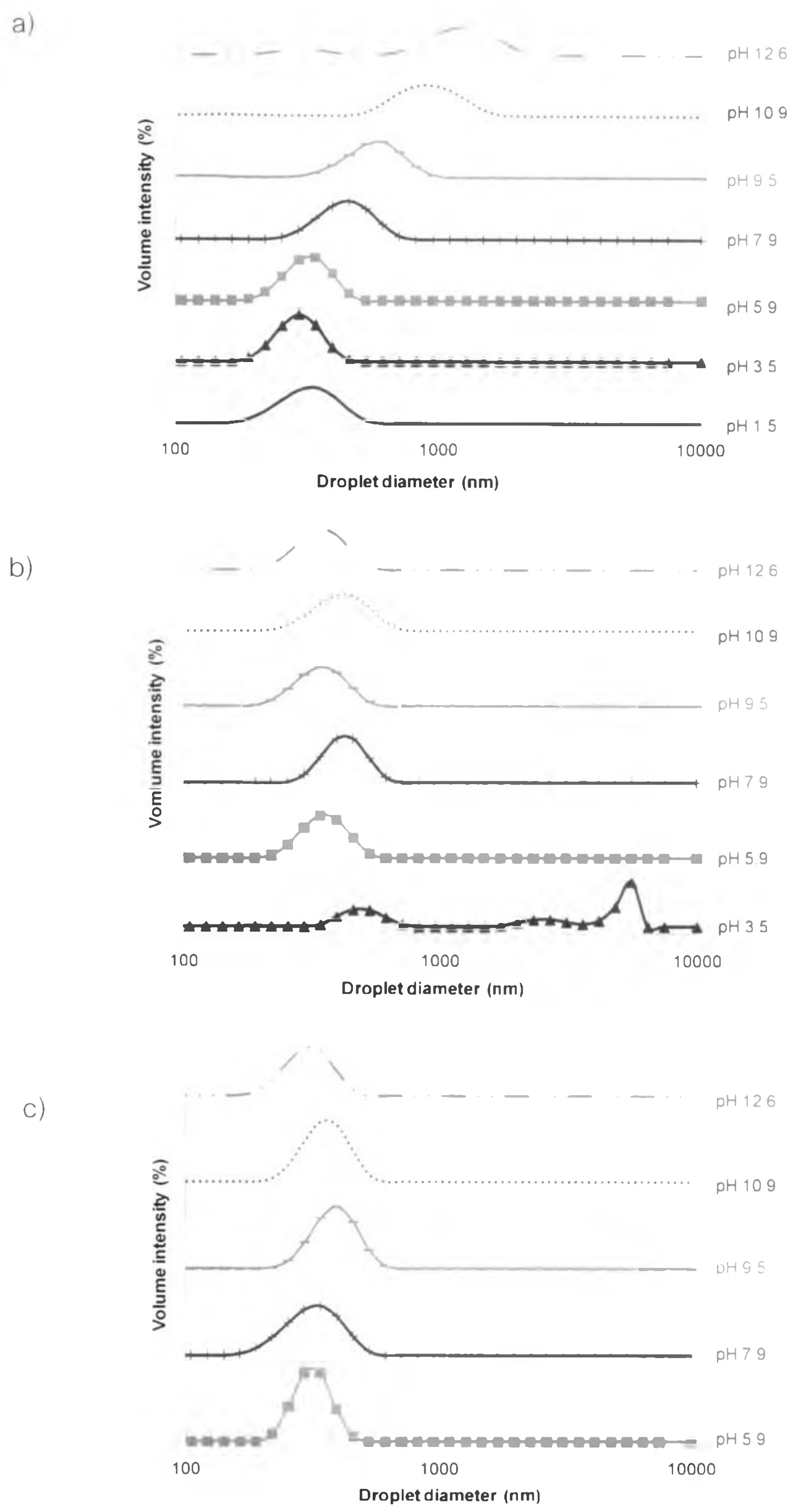


Figure 4.17 Droplet size distribution of emulsion under pH variation. Emulsions were prepared from PCTS0.1, PCTS1, and PCTS2 at concentration of 1%w/v and were definded as a) E85/0.1, b) E85/1, and c) E85/2, respectively.

Schematic drawing of droplets for emulsion E85/0.1, E85/1, and E85/2 prepared by using 1% w/v PCTS 0.1, 1, and 2, respectively as emulsifiers was showned in Figure 4.18. This scheme was described about the physical mechanism in mean droplet size under pH variation.

All emulsions were prepared in distrilled water at pH 5.6. After changing environmental pH, the physical mechanism can be explained as follows. E85/0.1 droplets dominated in positive surface charges, they replused into individual dropltes when decresing pH. When pH of system increased, they clumped together at isoelectric point (pH8) due to zero net charge and agglomerated into large particles at pH over 8 because of less of charge to stabilize droplets .

E85/1 droplets dominated in weak negative surface charges, when decreased pH, they clump together at isoelectric point (pH 5.3) due to zero net charge, and aggreate at pH less than isoelectric point due to charge neutralization. However, they replused into individual dropltes when pH above isoelectric point.

Meanwhile, E85/2 droplets dominated in strong negative surface charges, they maintained their droplet size over wide pH range owing to their strong negative potential. E85/2 exhibited isoelectric point at pH 3.9. They aggregated at pH below isoelectric point due to charge neutralization and no steric repulsion.

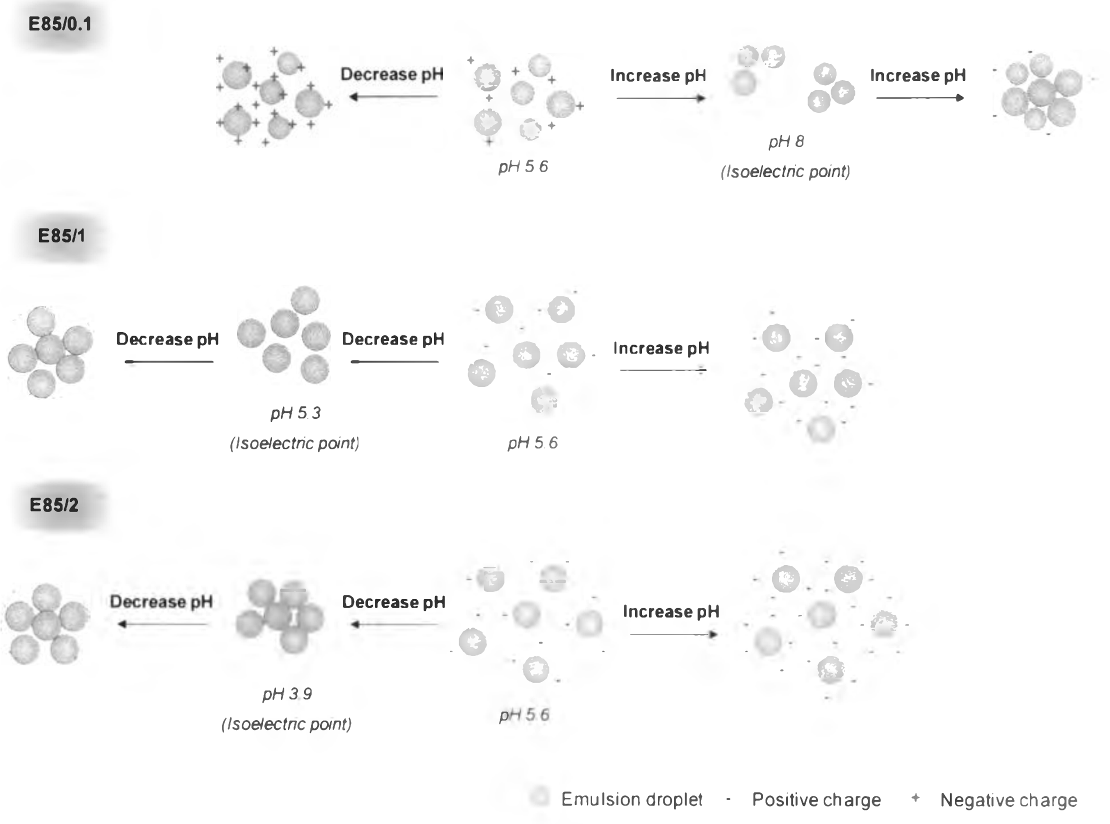


Figure 4.18 Schematic drawing represented the physical mechanism of emulsion E85/0.1, E85/1, and E85/2 using 1% w/v PCTS 0.1, 1, and 2, respectively as emulsifiers which occured under pH variation.

4.2.2 Effect of ionic strength on stability of emulsion using PCTS at various degree of substitution as emulsifiers

4.2.2.1 Surface charge of emulsion at various ionic strengths

Figure 4.19 shows surface charge of emulsion droplets as a function of NaCl concentrations. The zeta potential of E85/0.1 droplets gradually decreased with increasing salt concentrations owing to salt complex between NH_3^+ and Cl^-. Whilst, the zeta potential of E85/1 and E85/2 decreased with increasing salt concentrations until reaching the concentration of 0.8 mM, indicating that ionic strength promoted ionization of phosphate groups. Above this concentration, the zeta potential of E85/1 and E85/2 increased with increasing salt concentrations. From this result, it should be noted that Na+ or Cl- may neutralize the emulsion surface charges in a consequence of reducing the electrostatic repulsion of the individual droplets, so called electrostatic screening effect , [31,116]: counter-ions in the aqueous phase shielded the charges on the emulsion droplet surfaces [103]. However, the zeta potential of E85/2 droplets in NaCl 500 and 1000 mM were not available due to their aggregation and precipitation.

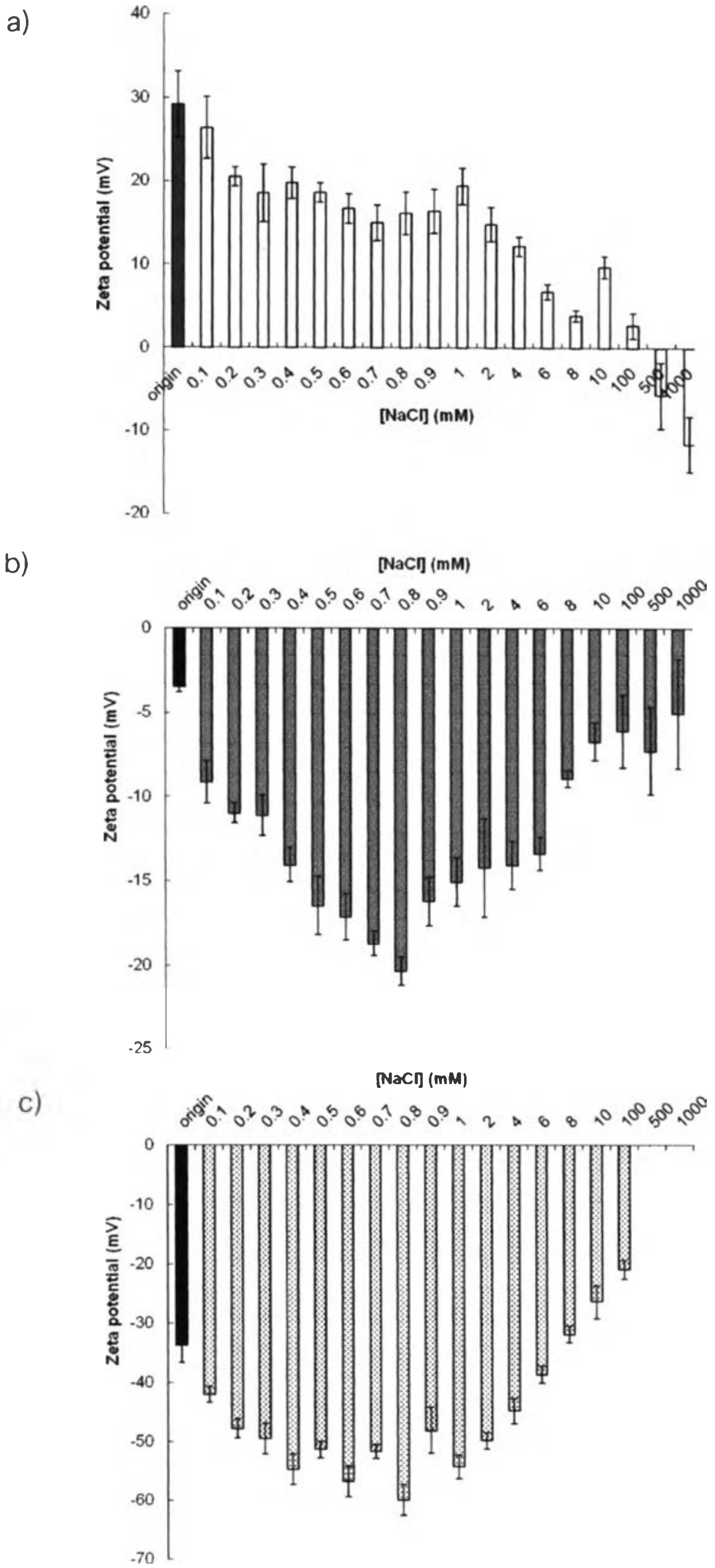


Figure 4.19 Surface charge of emulsion a) E85/0.1, b) E85/1, and c) E85/2, using PCTS as emulsifier at concentration 1%w/v under various ionic strengths.

4.2.2.2 Droplet size and droplet size distribution of emulsion at various ionic strengths

The droplet size of E85/0.1, E85/1, and E85/2 in various ionic strengths was shown in Figure 4.20. The results showed that all emulsions were constant in droplet size around 350-500 nm with NaCl concentrations below 1 mM. E85/0.1 started to increase in size at NaCl concentration of 2mM and increase abruptly at NaCl concentration of 6mM. Whereas, E85/1 and E85/2 increased their size abruptly when concentration of NaCl above 1mM.

One possible explanation for the agglomeration of emulsion droplets, the increasing in salt concentrations may cause to compress the electric double layer in a consequence of reducing the electrostatic repulsive force and in strength of charge potential lead to agglomeration of emulsion droplets which was according to the explaination of J.N Israelachvili [31] who reviewed about emulsion in high salt concentration.

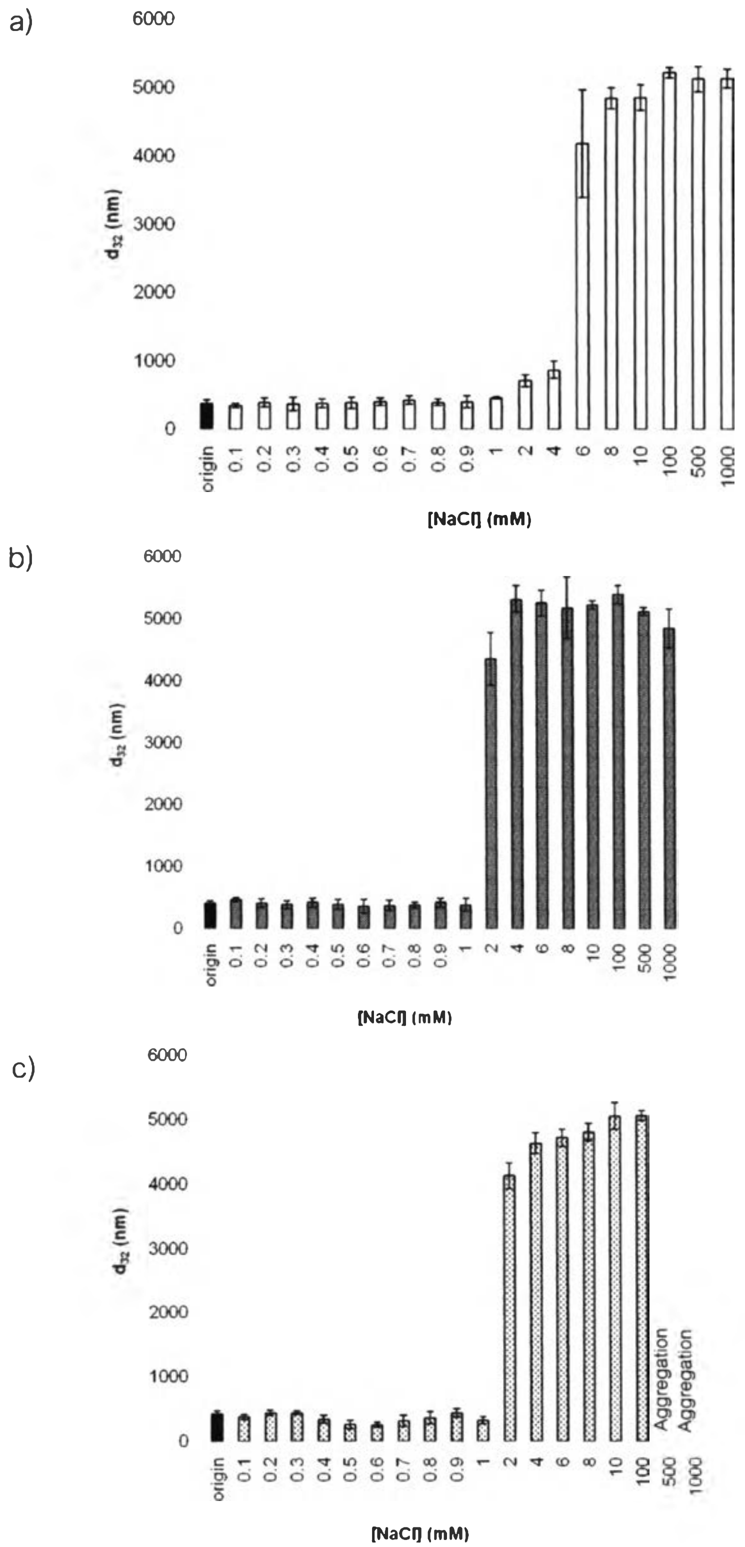


Figure 4.20 Surface mean diameter of emulsion a) E85/0.1, b) E85/1, and c) E85/2, using PCTS as emulsifier at concentration of 1%w/v under various ionic strengths.

The droplet size distributions are given in Figure 4.21. It showed that the droplet size distribution depend on concentration of NaCl, the droplet size distribution was unimodal when NaCl concentration below 6 mM for E85/0.1 and below 2 mM for both E85/1 and E85/2. A bimodal was observed that one large peak centered at large droplet size with a small additional peak centered at small droplet size. Likewise, the large droplet size occurred from agglomeration of the droplets. Furthermore, in case of the formation of large peak of droplet size distribution centered at large droplet size was the main reason for increasing the total surface mean diameter.

It is known that strong electrolytes produced O/W unstable emulsion because of the remarkable decrease in the thickness of the electrical double layer of the oil droplet [135].

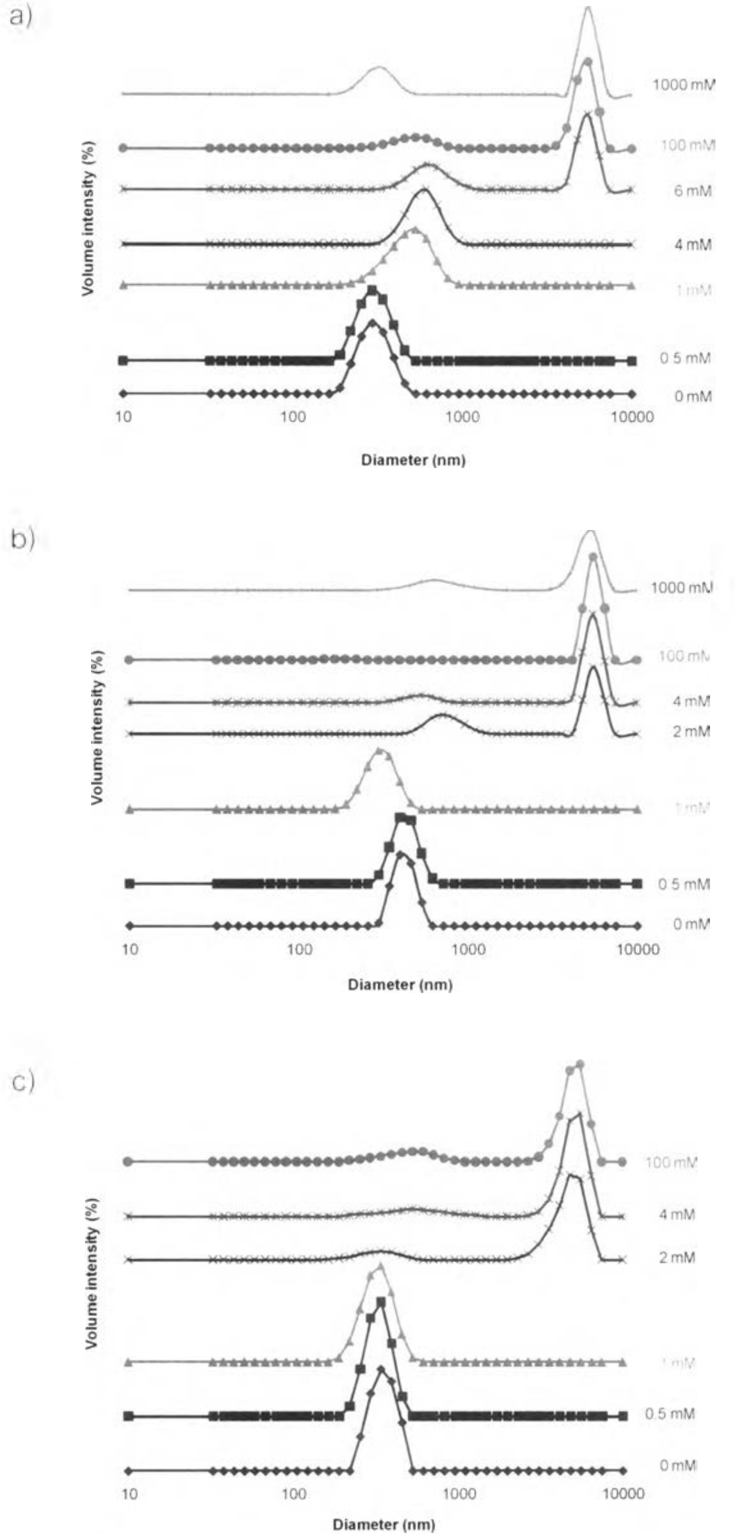


Figure 4.21 Droplet size distribution of emulsion a) E85/0.1, b) E85/1, and c) E85/2, using PCTS0.1, PCTS1, and PCTS2, respectively as emulsifier at concentration of1%w/v under various NaCl concentrations.

Schematic drawing of droplets for emulsion E85/0.1, E85/1, and E85/2 prepared by using 1% w/v PCTS 0.1, 1, and 2, respectively as emulsifiers was showned in Figure 4.22. This scheme was described about the physical mechanism in mean droplet size when increasing electrolyte concentrations. E85/0.1 was dominant in positive charges, whilst E85/1 and E85/2 were dominant in negative charges without electrolyte. With increasing electrolyte concentrations in the range of 0.1-4mM, the declining in positive surface charges of E85/0.1 may be due to salt complex between NH_3^- and Cl^-. Meanwhile, with increasing electrolyte concentrations in the range of 0.1-1mM, the rising in negative surface charges of E85/1 and E85/2 indicated that ionic strength promoted ionization of phosphate groups. The droplet size for those E85/0.1, E85/1 and E85/2, in contrast, maintained constant without considering the surface charges. With increasing electrolyte concentrations more than or equal 6 mM for E85/0.1 and 2 mM for E85/1, E85/2, may cause to compress the electric double layer in a consequence of reducing the electrostatic repulsive force and in strength of charge potential lead to agglomeration of emulsion droplets with bimodal size distribution.

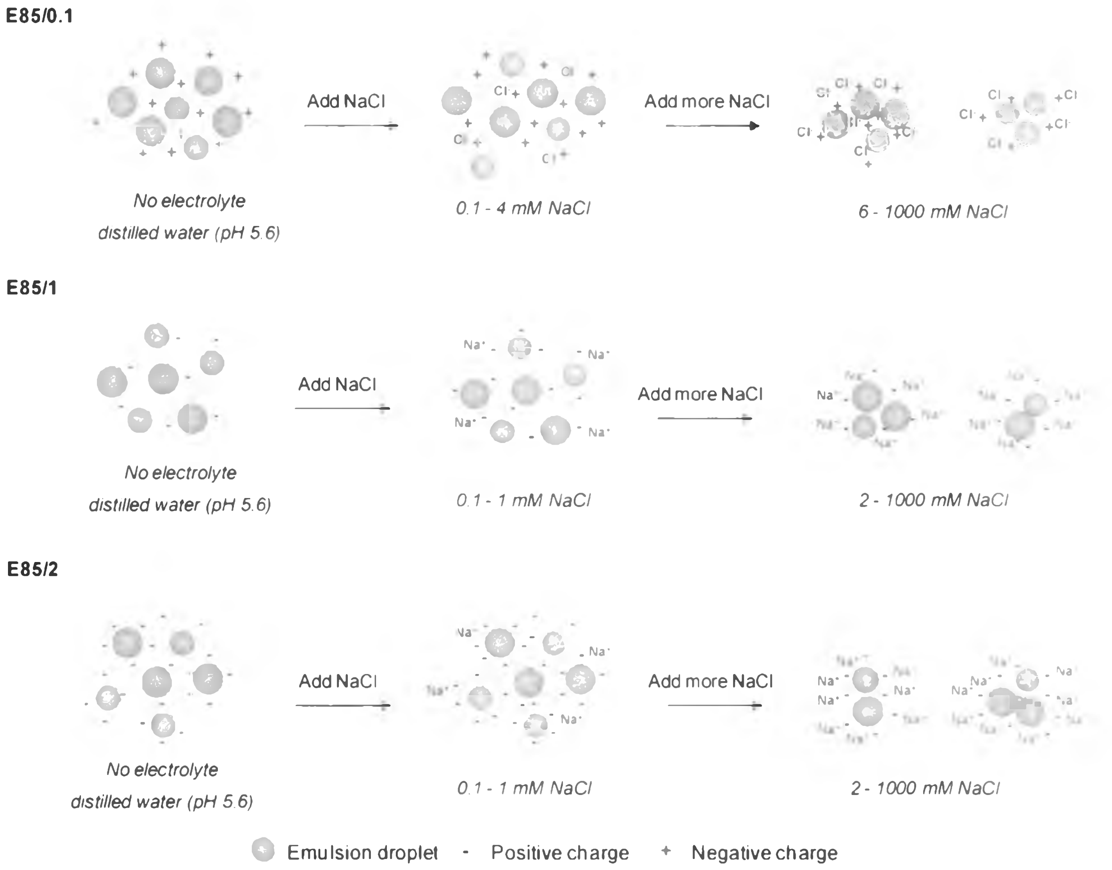


Figure 4.22 Schematic drawing represented the physical mechanism of emulsion E85/0.1, E85/1, and E85/2 using 1% w/v PCTS 0.1, 1, and 2, respectively as emulsifiers which occured after incrasing electrolyte concentrations.

4.2.3 Effect of temperature on stability of emulsion using PCTS at various ionic levels as emulsifiers

To determined effect of temperature on the stability of emulsion using PCTS at various ionic levels as emulsifiers, emulsions system were varied at five temperature points (30°C, 40°C, 50°C,60°C, and 70°C) and their surface charge, surface mean diameter of droplets, and droplet size distribution were determined along the temperature axis.

4.2.3.1 Surface charge of emulsion at various temperatures

Surface charge of the emulsion was seemed to be depended on temperature. Figure 4.20 shows the surface charge of emulsion as function of temperatures. The zeta potential of E85/0.1 emulsion droplets was gradually decreased in positive range from +30 mV to +15 mV and that of E85/1 and E85/2 emulsion droplets was gradually changed in negative range from -1 mV to -6 mV and from -48 mV to -18 mV, respectively, when increasing the temperature from 30°C -70°C.

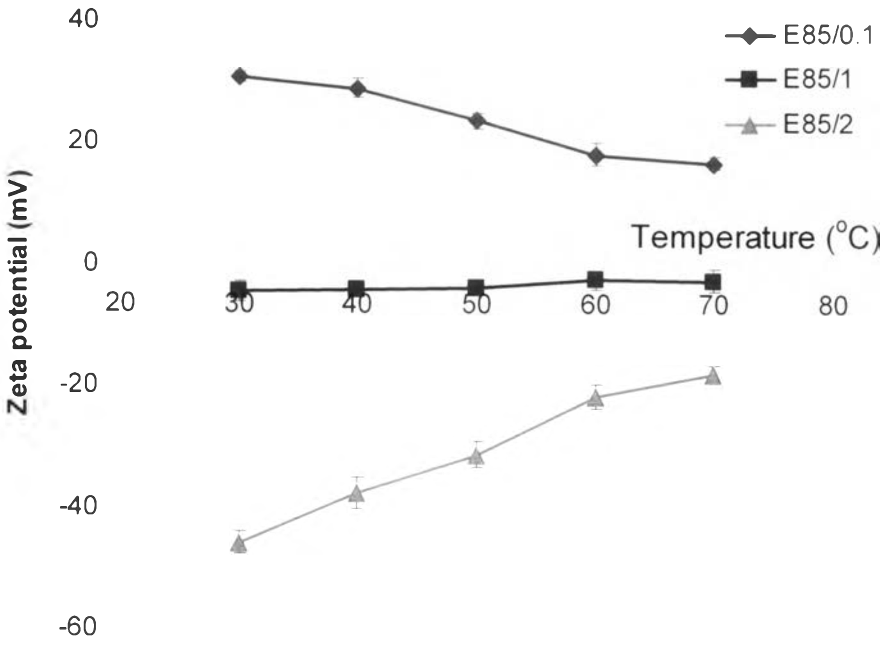


Figure 4.23 surface charge of emulsion droplets under temperature variation. Emulsions were prepared from PCTS0.1, PCTS1, and PCTS2 at concentration of 1%w/v and were definded as E85/0.1, E85/1, and E85/2, respectively.

4.2.3.2 Droplet size and droplet size distribution of emulsion at various temperatures

The surface mean diameter of E85/0.1, E85/1, and E85/2 which used PCTS 0.1, PCTS 1, and PCTS 2 as emulsifiers, respectively under various temperatures were showed in Figure 4.24.

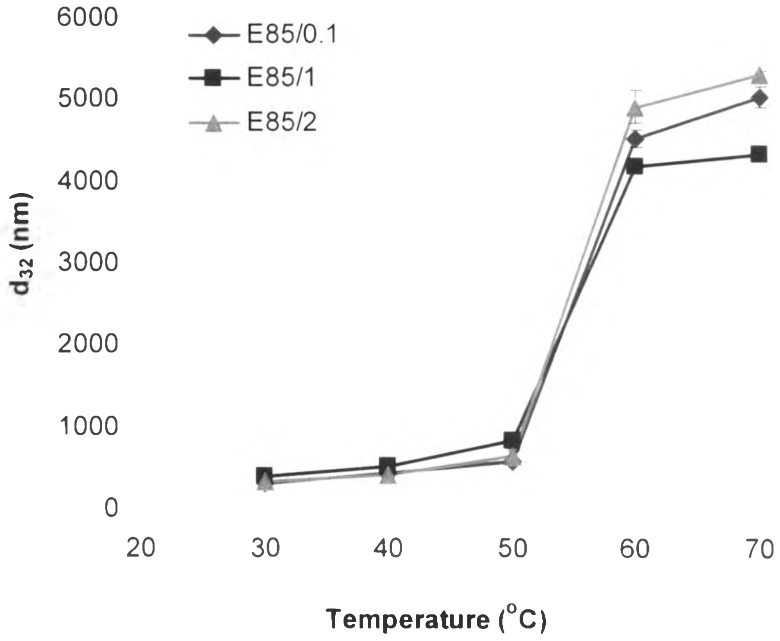


Figure 4.24 Surface mean diameter of emulsion droplets under temperature variation. Emulsions were prepared from PCTS0.1, PCTS1, and PCTS2 at concentration of 1%w/v and were definded as E85/0.1, E85/1, and E85/2, respectively.

The surface mean diameter of E85/0.1, E85/1, and E85/2 exhibited similar trend that the surface mean diameter increased from 300-800 nm at temperature range of 30-50°C. Then, the surface mean diameter dramatically increased during 50-60°C from 300-800 nm to 4000-5000 nm and showed almost constant value after that at 70°C.

It could be assumed that the PCTS chain was rearrangement when increase temperature and clearly observed at temperature in range of 50-60°C. Below 50°C, the hydrophilic surfaces of PCTS chains interact with water, so the molecule is elongated. At temperature in range of 50-60°C and above 60°C, the water molecules around main chain become dehydrate, so the PCTS chains changed into a soluble global with more hydrophobic surfaces.

From the zeta potential (Figure 4.19) and surface mean diameter (Figure 4.20) data indicated that the PCTS molecule rearranged its structure at temperature above 50°C. The change of zeta potential when increasing temperature may influence from increasing in droplet size resulting in decreasing the surface area in a consequence of lowering the strength of charge potential.

The droplet size distribution of E85/0.1, E85/1, and E85/2 under various temperatures was illustrated in Figure 4.25. It showed that the droplet size distribution depend on temperature, the droplet sizes distribution was unimodal when temperature below 50°C in all emulsions. A bimodal distribution with one large peak centered at large droplet size with a small additional peak centered at small droplet size was observed at temperature above 50°C in all emulsions. Therefore, the formation of large peak of droplet size distribution centered at large droplet size was the main reason for increasing the total surface mean diameter.

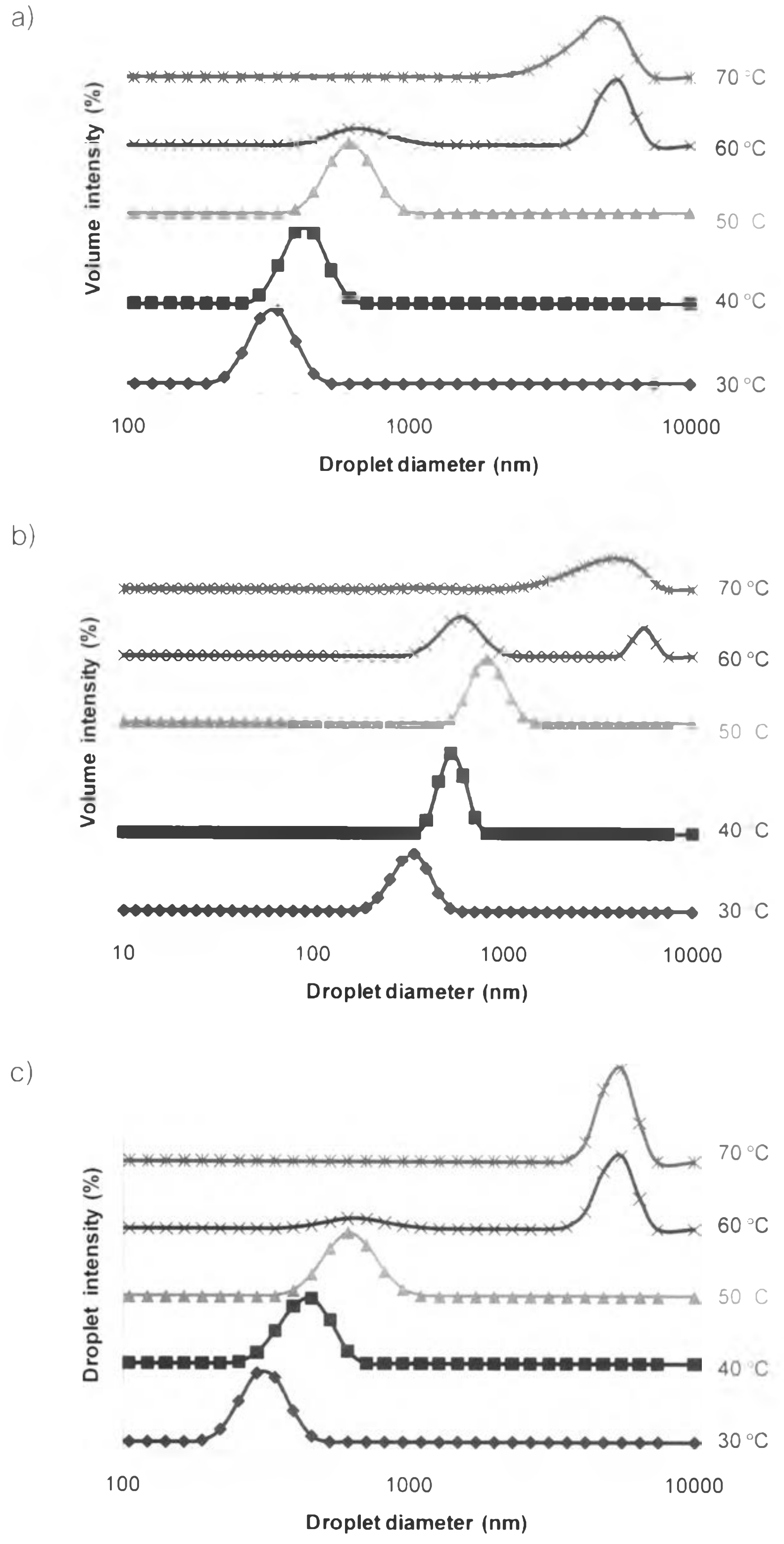


Figure 4.25 Droplet size distribution of of emulsion under pH variation. Emulsions were prepared from PCTS0.1, PCTS1, and PCTS2 at concentration of 1%w/v and were definded as a) E85/0.1, b) E85/1, and c) E85/2, respectively.

Schematic drawing of droplets for emulsion E85/0.1, E85/1, and E85/2 prepared by using 1% w/v PCTS 0.1, 1, and 2, respectively as emulsifiers was showned in Figure 4.26. This scheme was described about the physical mechanism in mean droplet size under temperature variation. The surface mean diameter of E85/0.1, E85/1, and E85/2 exhibited similar trend to increase as temperature increase, and increased abruptly around 50°C. This may be due to the water molecules around main chain become dehydrate, so the PCTS chains changed into a soluble global with more hydrophobic surfaces. Therefore, the individual droplets became closer with hydrophobic interaction, resulting in agglomeration. Surface charge of the emulsion E85/0.1, E85/1, and E85/2 was seemed to be depended on temperature. In fact, the larger in droplet size influenced to less surface area. Therefore, the mean surface charges of the larger size droplets trended to exhibit weaker positive or negative surface charges.

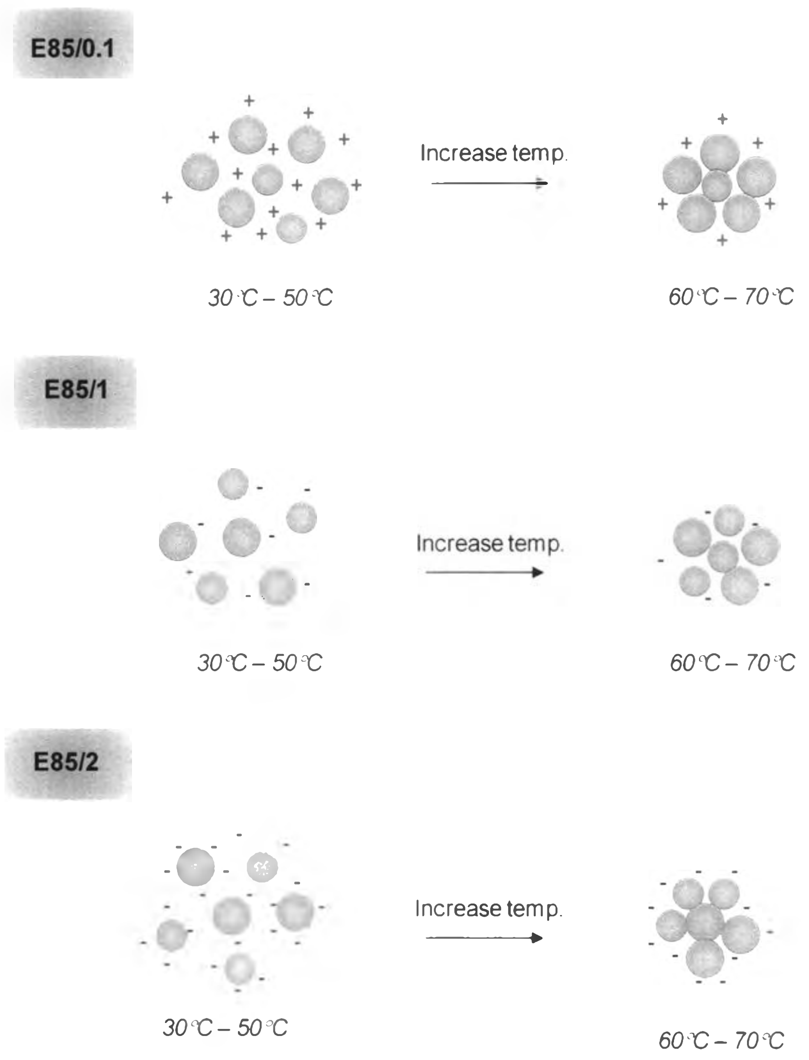


Figure 4.26 Schematic drawing represented the physical mechanism of emulsion E85/0.1, E85/1, and E85/2 using 1% w/v PCTS 0.1, 1, and 2, respectively as emulsifiers which occured under temperature variation.