Development of thermo-sensitive poly N-isopropyl acrylamide grafted chitosan derivatives

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ABSTRACT

In this study, the thermo-sensitive copolymers were synthesized by graft copolymerization of poly N isopropyl acrylamide (PNIPAm) onto chitosan (Ch) and aminated chitosan (AmCh) derivative. Free radical polymerization using potassium persulphate (KPS) as initiator was used for performing the grafting process. FTIR, TGA and DSC analysis were used to verify the structure of the resulting graft copolymers. The thermal sensitivity of the prepared copolymers was determined by monitoring the phase transition temperature at 550nm using a UV–VIS spectrophotometer. The results show that the transition of the PNIPAm-g-Ch is sharper than that of the PNIPAm-g-AmCh and the tr-ansition, in both cases, became sharper as increase the PNIPAm grating percentage.

INTRODUCTION

Environmental sensitive polymers, also known as "smart polymers", are those kinds of polymers which possess the ability to respond to the surrounding environmental conditions in different physic-chemical manners of changes (Lendlein and Shastri, 2010; Grainger and El-Sayed, 2010; Tanaka, 2011). The smartness of those polymers came from their capability to recover its native state when the environmental changes are removed (Stuart *et al.*, 2010). Such interesting behavior raising new areas of applications ranged from pharmaceutical ones (Moroni *et al.*, 2008; Fleige *et al.*, 2012), through biomedical *(*Thammakhet *et al., 2011,* Herber *et al.,* 2005*,* Brahim *et al.,* 2002*;* Do *et al.,* 2008*;* Kanno *et al.,* 2011*),* food technology

and Kakati, 2013; Fernandez-Saiz *et al.*, 2010), textile industries (Liu *et al.*, 2009; Hu *et al.*, 2007) to biotechnological ones (Galaev and Mattiasson, 2007; Gautam *et al.*, 2012; Wang and Chen, 2007; Jeong and Gutowska, 2002). Environmental changes could be categorized into three categories namely; physical, chemical, and biological ones (Kumar *et al.*, 2007; Biazar and Pourshamsian, 2011). Polymers sensitive to thermal changes of the media, thermo-sensitive polymers, show sharp solubility changes around a specific solution temperature. Beyond this temperature, the polymer turns to precipitate and could be dissolved again by lowering the solution temperature. This temperature is known as Low critical solution temperature (LCST); (Dusek, 1993; Aoki *et al.*, 1994).

The well known pH sensitive of chitosan, in the pH ranged between 6 and 7, contributed in preparation of new smart polymers have sensitivity to more than stimuli where several studies have been performed to prepare pH/thermo-sensitive poly N- isopropyl acrylamide grafted chitosan (Kim *et al*., 2000; Cho *et al*., 2004; Cai *et al*., 2006; Chen *et al*., 2012).

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In this study, new combination of poly N- isopropyl acrylamide with new derivatives of chitosan was carried out. The effect of extra amine groups in chitosan on the temperature sensitivity of the prepared copolymers was studied compared to chitosan itself. Evidences of the modifications were proved using FT-IR, TGA, and DSC.

MATERIALS AND METHODS

Materials

Shrimp shells were collected from domestic wastes. acetic acid (purity 99.8%), p-benzoqunione PBQ (purity 99%), sodium hydroxide pellets (purity 99–100%), N- isopropyl acrylamide (purity 97%), ethanol (purity 99%) and Acetic acid (purity 98%) were purchased from Sigma Aldrich (Germany). Ethylenediamine (purity 99%) was obtained from Alfa Aesar (Germany).

Extraction of chitin from shrimp shells

Chitin extraction was performed according to (Islama *et al.*, (2011). Briefly, demineralization of shells was carried out via shells dispersing in 5% HCl at room temperature at solid: liquid ratio of 1:14 (w/v) for 24 hrs. The shells were quite squashy and then rinsed with water to remove the un-reacted acid and the produced calcium chloride. The obtained shells were then treated with 5% NaOH for neutralization at room temperature for 24 hrs at solid: liquid ratio of 1:12 (w/v). The residues were then collected and washed using tap water followed by distilled water to obtain chitin.

Deacetylation of chitin

According to (Rigby, 1936) method, preparation of chitosan is a simple deacetylation process of chitin in an alkaline medium. Removal of acetyl groups from the chitin was achieved by using 50% NaOH solution with solid: liquid ratio of 1:50 (w/v) at 100-120°C for 12 hrs. The resulted chitosan was washed with bi-distilled water to remove excess of NaOH.

Preparation of aminated chitosan (AmCh)

Aminated chitosan was prepared through three steps according to our previous work (Mohy Eldin *et al.*, 2012). In the first step, 4 g of chitin were dispersed in 50 ml of a known concentration of p- benzoquinone (PBQ) at selected pH and temperature and were stirred for 6 hrs. The PBQ-conjugated chitin was separated and washed with distilled water to remove excess unreacted PBQ. In the second step, PBQ-conjugated chitin was dispersed in 50 ml of a known concentration of EDA at definite temperature and was stirred for 6 hrs.

The aminated modified chitin was separated and washed with distilled water to remove unreacted EDA. In the last step, aminated modified chitin was deacetylated through treatment with 50% aqueous solution of NaOH at 120–150 ^{o}C for 6 hrs. The obtained aminated chitosan was separated and washed with distilled water to remove the excess of NaOH.

Preparation of the PNIPAm grafted Chitosan derivatives

The PNIPAm-g-Ch and PNIPAm-g-AmCh copolymers were prepared as the following; 0.2 g of Ch/AmCh was dissolved in 20 ml acetic acid solution (2%) and 20 ml of the NIPAm monomer solution with desired concentration was added and then mixed well. The mixture was stirred at room temperature for 30 min under a nitrogen atmosphere to get a uniform solution. Then, KPS was added and the temperature was elevated to 60°C for initiating the polymerization reaction. The polymerization was allowed to process for 6 hrs. The resulting product was precipitated in 200 ml of ethanol and the precipitate was filtered and washed with distilled water and ethanol several times. The product was filtered and dried in a vacuum oven at 60°C overnight. The preparation conditions were listed in Table1.

Table 1: Grafting polymerization condition of NIPAm on Ch /Amch at 60 ^{o}C, for 6 hours using 0.2 (mM) of KPS.

Sample code	Ch/Amch (mM)	NIPAm (mM)	Polymer: Monomer ratio
Ch-PNIA 0	1.24	0	-----
Ch-PNIA 1	1.24	6.2	1:5
Ch-PNIA 2	1.24	12.4	1:10
Ch-PNIA 3	1.24	18.6	1:15
Ch-PNIA 4	1.24	24.8	1:20
Ch-PNIA 5	1.24	31	1:25
AmCh -PNIA 0	1.24	0	-----
AmCh -PNIA 1	1.24	6.2	1:5
AmCh -PNIA 2	1.24	12.4	1:10
AmCh -PNIA 3	1.24	18.6	1:15
AmCh -PNIA 4	1.24	24.8	1:20
AmCh -PNIA 5	1.24	31	1:25

CHARACTERIZATION

Infrared spectroscopic analysis (FT-IR)

The structure of the graft copolymers samples were investigated using Fourier Transform Infrared Spectrophotometer (Shimadzu FT-IR - 8400 S, Japan) connected to a PC and analysis the data by IR Solution software, Version 1.21.

Thermo-gravimetric analysis (TGA)

TGA of the graft copolymers samples was carried out using thermo-gravimetric analyzer instrument (Shimadzu TGA-50, Japan) in the temperature range from 20 °C to 600 °C under nitrogen at a flow rate of 20 ml/min and at a heating rate of 10 °C/min.

Differential scanning calorimetric (DSC)

DSC Analysis of the graft copolymers samples was conducted using differential scanning calorimetric analyzer (Shimadzu DSC-60A, Japan).

Determination of the phase transition temperature

The thermal behavior of the graft copolymers samples was observed at 550 nm using a UV–VIS spectrophotometer (Chung *et al.*, 1999). To examine the reversible thermo-response properties, optical transmittance was measured using a controlled temperature circular program from 20 to 40°C. LCST of the

polymer solutions were defined as the temperature at which the optical transmittance of the solution reduced to 50% of its original value.

RESULTS AND DISCUSSION

Characterization of the PNIPAm grafted Chitosan derivatives

In this study, the effect of the extra amine groups of AmCh (Kenawy *et al*, 2015) on the thermo-sensitivity of the grafted chitosan with different amounts of poly N- isopropyl acrylamide (PNIPA) was studied. Series of the grafted copolymers were prepared using different molar ratios of Ch or AmCh and PNIPAm. The grafting process of N-Isopropyl acrylamide at 60°C temperature, which is more than its LCST (32°C), has been oriented the polymerization process to produce short chains rather than long chain graft polymers. Therefore, the obtained copolymers were precipitated at this temperature. The structures of the obtained grafted polymers were proved by comparing FT-IR, TGA and DSC analysis with others reported in previous literatures.

FT-IR analysis

The FT-IR spectrums of the PNIPAm-g-Ch and the PNIPAm-g-AmCh were investigated as shown in figures 1 and 2 respectively. The results exhibit that Ch and AmCh have a broad band around 3440 cm^{-1} corresponding to -OH and -NH stretching as well as, bands in between 2835-2950 cm^{-1} in a combination of C-H stretching of the methyl and methylene groups. In addition, the stretching vibration of C=O and NH-C=O was observed at 1620 cm^{-1}. Bands at 1066-1069 cm^{-1} corresponding to C-O-H stretching were detected. A new band at 1642 cm-^{1} was generated which can be attributed to the –C=N vibrations characteristic of the imines (Colthup *et al*., 1990) while they are not observed in the Chitosan spectrum.
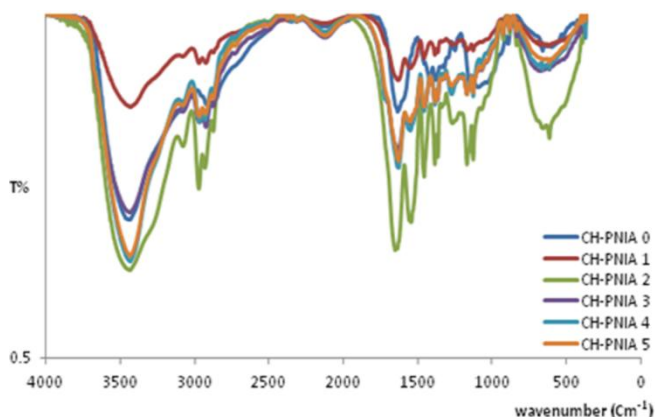

Fig. 1: FT-IR of chitosan and PNIPAm-g-Ch.

The grafting of the PNIPAm onto Ch and AmCh show clearly change in the chart and the characteristic absorption bands of amide I and II were observed at 1668 cm^{-1}and 1560 cm^{-1}. Peaks at 1392 cm^{-1}and 1371 cm^{-1} are related to the deformation of the two methyl groups in the isopropyl acrylamide (Gui and Jin,

2014). Besides, N-H stretching was observed at 3470 cm^{-1}. The peaks at 2979 cm^{-1}and 1465 cm^{-1} were corresponding to the asymmetric stretching vibration and asymmetric deformation of the methyl groups, respectively (Liang *et al*., 2000).
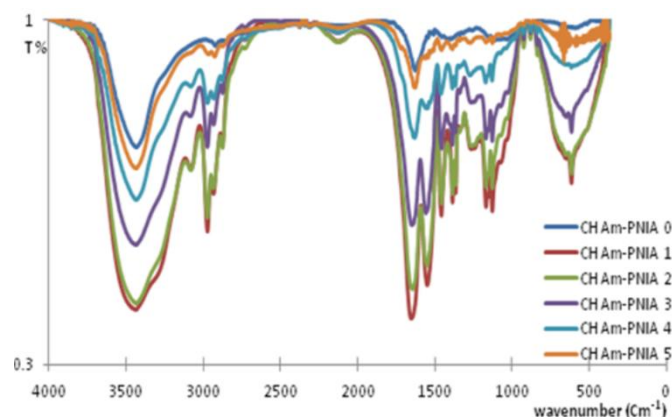

Fig. 2: FT-IR of aminated chitosan and PNIPAm-g-AmCh.

TGA analysis

TGA analysis of the Ch, the AmCh, the PNIPAm-g-Ch and the PNIPAm-g-AmCh were carried; Figures 3 and 4. The illustrated data of the thermal degradation under Nitrogen atmosphere show a depression from ambient temperature to about 150°C which may be attributed to the elevation of the moisture content in the polymers (Pawlak and Mucha, 2003; Zawadzki and Kaczmarek, 2010).
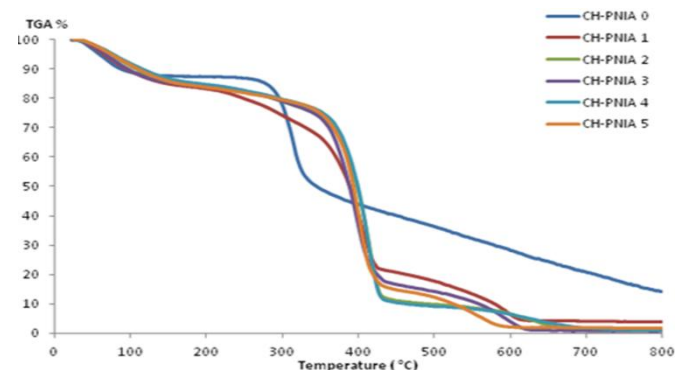

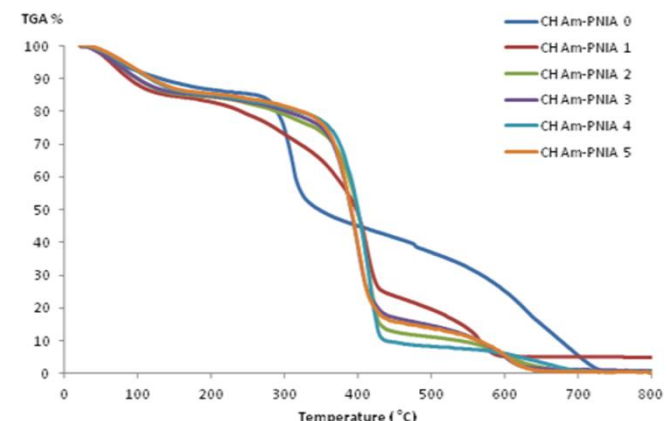**Fig. 3**: TGA of chitosan and PNIPAm-g-Ch.

Fig. 4: TGA of aminated chitosan and PNIPAm-g-AmCh.

The most important depressions of each sample are summarized in Table 2. The second depression for chitosan and aminated chitosan, was appeared between 270 to 320 ^{o}C and they lost about 32.5 and 31.54% from their weights, respectively. The observed behaviors may be attributed to oxidative decomposition of the Chitosan backbone. In this stage, the depression was resulted from decomposition of the amine groups in pyranose ring to form new crosslinked fragments (Pawlak and Mucha, 2003). On the other hand, the highly grafted copolymers show more thermal stability where the mean depression was shifted to start from about 345 to 450°C due to PNIPAm backbone degradation (Wang *et al*. 2013; Mu and Fang, 2008).

The lower grafted polymers, PNIPAm-g-Ch (1&2) and the PNIPAm-g-AmCh (1&2) show two degradation stages which were caused by the degradation of the chitosan and the grafted PNIPAm chains, respectively (Don and Chen, 2005).

DSC analysis

DSC analysis of the Ch, the AmCh, the PNIPAm-g-Ch and the PNIPAm-g-AmCh was conducted; Figures 5 & 6. The first endothermic peak for all the samples which was started from 50 to 120°C can be explained by the loss of the moisture contents. Polysaccharides usually have a strong affinity for water while in solid state; these macromolecules haven't unstable structures which can be easily hydrated (Kacurakova *et al*., 1998) Figure 5.

The second thermal event of the Ch and the AmCh may be related to the decomposition of the glucose amine (GlcN) units with correspondence exothermic peak at 295°C (Guinesi and Cavalheiro, 2006). The second peak was disappeared in the highly grafted polymers which resulted from increase in their thermal stability (Wang *et al*., 2013; Mu and Fang, 2008); Figure 6.

Table 2: Thermo-gravimetric analysis of the thermo-grafted Chitosan derivatives.

| Sample code	1st Depression Ambient- 150 ^{o}C (%)	2nd Depression		3rd Depression	4th Depression	T_{50}	Residue at 800 ^{o}C
Ch-PNIA 0	12.3	270 - 330 ^{o}C 32.5 %		330 - > 800 ^{o}C 39.19 %	-----	345.19	14.23 %
Ch-PNIA 1	15.06	150 - 212 ^{o}C 2.28 %	212-347 ^{o}C 15.24 %	347- 423 ^{o}C 44.38 %	423 - 615 ^{o}C 18.39 %	388.26	3.85 %
Ch-PNIA 2	14.07	150 - 215 ^{o}C 1.87 %	215-351 ^{o}C 10.35 %	351- 432 ^{o}C 61.13 %	432 - 670 ^{o}C 11.07 %	397.98	0.94 %
Ch-PNIA 3	14.44	150 - 343 ^{o}C 10.86 %		343 - 430 ^{o}C 55.96 %	430 - 620 ^{o}C 17.33 %	388.57	0.90 %
Ch-PNIA 4	13.35	150 - 351 ^{o}C 11.15 %		351- 433 ^{o}C 59.13 %	433 - 695 ^{o}C 14.59 %	399.5	1.067 %
Ch-PNIA 5	14.33	150 - 345 ^{o}C 10.12 %		345 - 430 ^{o}C 59.16 %	430 - 589 ^{o}C 13.93 %	393.24	1.71 %
Am Ch -PNIA 0	11.15	270 - 330 ^{o}C 31.54 %		330 – 724 ^{o}C 51.53 %	--------	347.53	0.38 %
Am Ch -PNIA 1	15.31	150 - 426 ^{o}C 58.03 %			426 - 590 ^{o}C 21.17 %	397.29	0.47 %
Am Ch -PNIA 2	14.47	150 - 228 ^{o}C 1.85 %	228-354 10.58 %	354 - 435 ^{o}C 59.18 %	435 - 590 ^{o}C 12.61 %	398.82	0.74 %
Am Ch -PNIA 3	14.21	150 - 349 ^{o}C 10.56 %		349 - 428 ^{o}C 55.56 %	428 - 626 ^{o}C 17.85 %	390.05	0.728 %
Am Ch -PNIA 4	13.26	150 - 358 ^{o}C 10.78 %		358 - 434 ^{o}C 65.52 %	434 - 700 ^{o}C 9.66 %	399.98	0.568 %
Am Ch -PNIA 5	13.12	150 - 358 ^{o}C 8.68 %		339 - 435 ^{o}C 61.19 %	435 - 638 ^{o}C 16.07 %	390.2	0.18 %
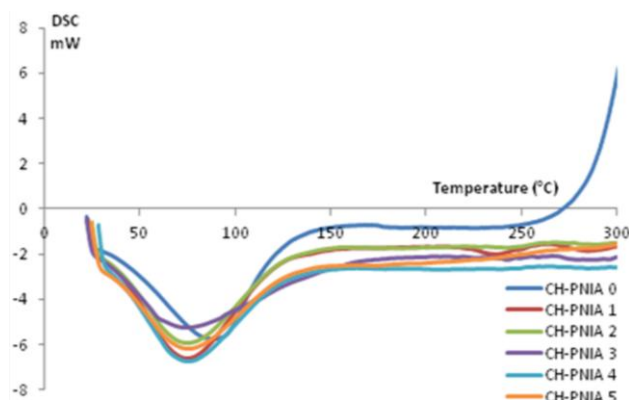
Fig. 5: DSC of chitosan and PNIPAm-g-Ch.
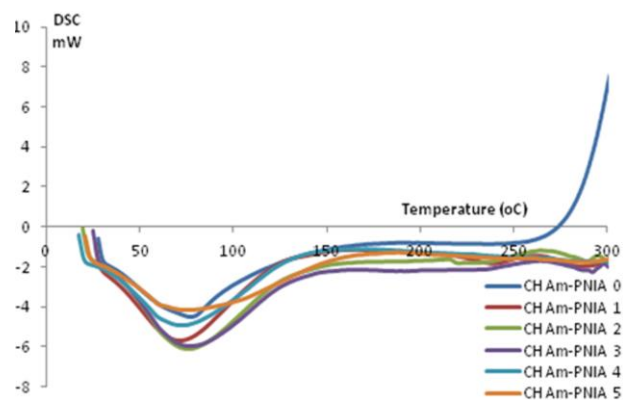
Fig. 6: DSC of aminated chitosan and PNIPAm-g-AmCh.

Thermo-sensitive behaviors

The phase transition behavior of the PNIPAm-g-Ch and the PNIPAm-g-AmCh in water was investigated by measuring the optical transmittance at 550 nm in the temperature range of 20 - 40ºC (figure 7 and 8). It was clear that there was no phase transition observed for both the Ch and the AmCh at the investigated temperature range.

For the grafted copolymers, the transparency has maintained its constant value at low temperatures and then starts to drop until zero when it reach to 32ºC (ie; LCST of PNIPAm). It was clear that the optical transmission depends on the grafting degree where the transition became sharper with increasing the grafting percent. This behavior is similar to the behavior PNIPAm surface grafting (Zhang *et al.*, 2005; Mu and Fang, 2008).

Several mechanisms described the LCST of the PNIPAm in the literatures (Diez-Pena, 2002; Tanaka, 1979; Aoki *et al.*, 1994) which the precipitation of the PNIPAm above the LCST is due to the disruption of the hydrogen bonding with the water molecules and increasing of the hydrophobic interactions among the isopropyl groups.
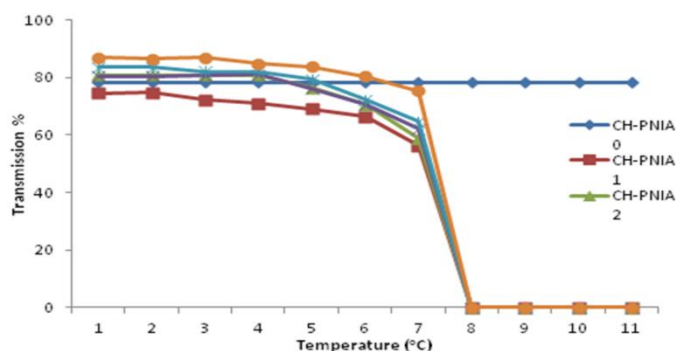

Fig. 7: Transparency of chitosan and PNIPAm-g-Ch in aqueous solution at 550 nm over the temperature between 20 ºC and 40 ºC.
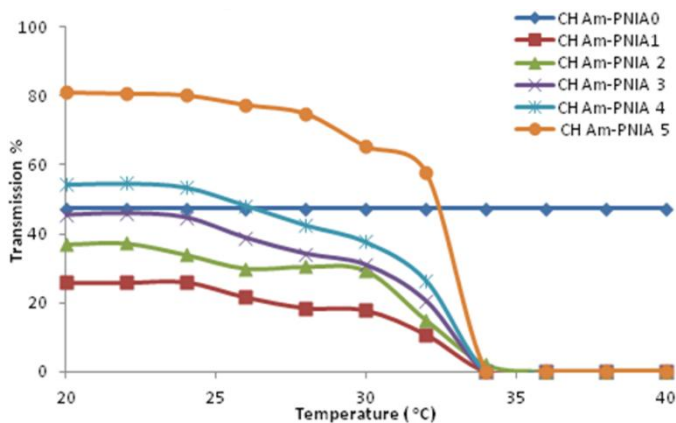

Fig. 8: Transparency of Aminated chitosan and PNIPAm-g-AmCh in aqueous solution at 550 nm over the temperature between 20 ºC and 40 ºC.

CONCLUSION

The PNIPAm-g-Ch and the PNIPAm-g-AmCh were prepared and characterized using FT-IR, TGA and DSC analysis. Increasing the grafting ratio, produced novel copolymers with sharp phase transferee at LCST. On the other hands, increasing the

amine groups of the aminated chitosan affect its thermo sensitivity which acts as an important feature for its application in the medical and the pharmaceutical fields.

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