Modification of Chitosan for Preparation of Poly(*N*-isopropylacrylamide/ O-nitrochitosan) Interpenetrating Polymer Network

(Pengubahsuaian Kitosan untuk Penyediaan Polimer Rangkaian Saling Menembus Poli(*N*-isopropilakrilamida/O-nitrokitosan))

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ABSTRACT

An interpenetrating polymer network (IPN), N-isopropylacrylamide/O-nitrochitosan (NONK) was developed in the presence of ammonium persulfate using solution polymerization technique. O-nitrochitosan (ONK) was synthesized from chitosan, before it was further reacted with N-isopropylacrylamide. A new vibration of the C-N bond formed between the NH from ONK and the CH₂ from NIPAAm was detected at the peak 1154 and 1171 cm⁻¹ using attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy. In the polymerization, the primary amine became a secondary amine and the peaks of the amide I (C=O peak) and amide II (N–H) were broadened. The structure of ONK was confirmed using nuclear magnetic resonance (NMR). At 10°C, the result from swelling test was found that IPN poly(NONK82) had the highest swelling percentage, 1171%, compared to poly (NIPAAm) at 467%. Micrograph from scanning electron microscopy (SEM) indicated the pore size of IPN NONK increased with an increasing ratio of ONK.

Keywords: N-isopropylacrylamide(NIPAAm); nuclear magnetic resonance(NMR); O-nitrochitosan (ONK)

ABSTRAK

Polimer rangkaian saling menembus (IPN) N-isopropilakrilamida/O-nitrokitosan (NONK) telah dibangunkan dengan kehadiran amonium persulfat dengan menggunakan teknik pempolimeran larutan. O-nitrokitosan disintesis daripada kitosan terlebih dahulu sebelum ditindak balaskan dengan N-isopropilakrilamida. Satu getaran baru ikatan C-N terbentuk di antara NH dari ONK dan CH₂ dari NIPAAm pada puncak 1154 dan 1171 cm⁻¹ diperhatikan dengan menggunakan spektroskopi (FTIR-ATR). Kumpulan amina primer menjadi amina sekunder dan puncak amida I (puncak C=O) dan amida II (N–H) semakin melebar akibat pempolimeran. Struktur ONK ditentukan melalui nuklear magnetik resonans (NMR). Pada suhu 10°C, hasil ujian pembengkakan mendapati IPN poli(NONK82) mempunyai peratus pembengkakan yang paling tinggi iaitu 1171% berbanding poli (NIPAAm) iaitu 467%. Mikrograf mikroskopi elektron imbasan (SEM) menunjukkan saiz liang IPN NONK meningkat dengan peningkatan nisbah ONK dalam IPN.

Kata kunci: N-isopropilakrilamida (NIPAAm); nuklear magnetik resonans(NMR); O-nitrokitosan (ONK)

INTRODUCTION

Chitosan, a polysaccharide which composes of β-(1-4)-linked D-glucosamine units, has been derived from chitin through deacetylation process (Kumar 2000). Chitosan and its derivatives are used in various fields: Biomedical (Prabaharan & Jayakumar 2009), pharmaceutical (Baldrick 2010) and food industrial (Dutta et al. 2009).

Currently, considerable attention is given to smart hydrogels that can undergo a reversible and discontinuous volume phase change in response to environmental stimuli, such as changes in pH and temperature (Don & Chen 2005; Verestiuc et al. 2004). Among these hydrogels, poly (NIPAAm) is the most widely studied because it is temperature and pH responsive, which are crucial to the human body. Normally, this polymer is synthesized in a form of gel or beads. It is used for drug delivery, to separate the bioactive molecules and acts as a catalyst (Guo & Gao 2007). This polymer is unique because it has a low critical solution temperature (LCST) with approximately 30 - 32°C.

In order to obtain other unique properties for applications such as drug delivery and biosensors, NIPAAm has been introduced into biocompatible and biodegradable chitosan. The advantage of chitosan over other polysaccharides (cellulose, starch and galactomannans) is that its chemical structure can undergo accepts specific modifications at C-2 position (Rinaudo 2006).

The combination and conjugation of copolymers have the advantage of creating copolymers with improved properties (Alli & Hazer 2008). For example, reaction of an AB-crosslinked graft copolymer based on maleilated chitosan with NIPAAm was carried out through a two-step reaction (Don & Chen 2005). Carboxymethyl chitosan-g-NIPAAm has improved its thermal and mechanical properties and water content (Zhang et al. 2009). In addition, poly (NIPAAm)/carboxymethyl chitosan (poly (NIPAAm)/CMCS) membrane has been synthesized as described in the literature by Chen et al. (2007) with no change in the LCST shift when the CMCS is introduced into

NIPAAm. The DSC and optical transmittance results indicate that the hybrid hydrogel with low chitosan composition exhibits an obvious phase transition.

In the present paper, O-nitrochitosan (ONK) was derivatized from chitosan and used for preparation of IPN (NIPAAm)/ONK. The effects of chitosan modification will be discussed in the aspects of synthesis and characterizations of IPN (NIPAAm)/ONK.

MATERIALS AND METHODS

Chitosan (Fluka Biochemica) was used as a raw material for the ONK synthesis. Both NIPAAm (Aldrich) and ammonium persulfate were stored at room temperature until used. Acetic acid (Systerm) and ethanol (Systerm) were used as solvents. Deionized water was used as a solvent during the polymerization procedure. All chemicals were used without further purification.

SYNTHESIS OF O-NITROCHITOSAN

The O-nitrochitosan (ONK) was prepared by adding several quantities of chitosan to 100 mL of an isopropanol solution and stirring with a glass rod. Sodium hydroxide (50% v/v) was introduced dropwise over the first 10 min. The solution was stirred using a magnetic stirrer. After 1 h, the fume nitric acid was added dropwise while stirring. The solution was then submerged into water bath at 55°C for a few minutes. The solution was filtered and precipitated in methanol. 1% acetic acid was added until the solution achieved at pH 7.0. ONK was purified via precipitation and washed with ethanol three times (Zeng et al. 2005). The product was filtered and dried in an oven for one day at 60°C.

PREPARATION OF POLY (N-ISOPROPYLACRYLAMIDE-O-NITROCHITOSAN) (NONK)

The NONK was prepared via solution polymerization of aqueous precursor solutions containing NIPAAm, ONK and ammonium persulfate (APS). Three different polymeric membranes with various compositions that according to weight percentage were prepared-100:0 NIPAAm, 90:10 NIPAAm: ONK labeled as poly(NONK91) and 80:20 NIPAAm: ONK labelled as poly(NONK82). In a round-bottomed flask equipped with a magnetic stirrer, an individual mixture containing various quantities of NIPAAm and ONK were mixed with 1.6% w/w of APS initiator. The mixture was then dissolved in 5 mL of deionized water and purged with nitrogen gas for 10 min. Then, NONK was refluxed continuously for 7 h at 70°C. Petroleum ether was used to wash the product.

CHARACTERIZATION METHODS

Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy was carried out using a Spectrum 400 FTIR/FT-NIR spectrophotometer (Universal ATR). The spectra were collected in the range of 4000 to 650 cm^{-1} over 64 scans with a resolution of 4 cm^{-1}. The measurements were made at room temperature on a diamond using a variable-angle ATR unit at a nominal incident angle of 458°. The optical alignment of the unit was set up to achieve a maximum throughput of the infrared beam to the detector. Samples, in powder form, were taken randomly from the flat films. The curve-fitting simulations of the ATR-FTIR spectra were performed using the standard Origin 6.0 software package.

^{1}H and ^{13}C NMR spectra were obtained from Bruker AVANCE III 600 MHz spectrometer. The ONK sample (5 mg) was diluted in 3 mL of CH$_3$COOH/D$_2$O (50:1, v/v) and stirred for a week prior to the analysis. All analysis were performed at ambient temperature, 27°C, using 16 scan pulse accumulation and an LB parameter of 0.30 Hz. The spectral width was 20 ppm with 65 K data points (Guinesi & Cavalheiro 2006).

The dried NIPAAm, poly(NONK91) and poly(NONK82) hydrogels were immersed in deionized water at 4, 10 and 60°C in a closed container. The weight changes of the samples were recorded after 24 h and the percentage of water contents were then calculated using the following equation:

$$Swelling\ ratio = \frac{weight\ of\ swelled\ sample - weight\ of\ the\ dried\ sample}{weight\ of\ the\ dried\ sample}$$

$$\times\ 100\%.$$

At chosen times, the samples were taken out and carefully wiped with filter paper to remove any excess water prior to weighing (Han et al. 2009).

A scanning electron microscopy (Carl Zeiss EVO MA10-SEM) was used to analyze the sample morphology. The samples (ONK, poly(NONK91) and poly(NONK82)) were prepared by freeze-drying under an argon gas flow and coated with a conductive layer of sputtered gold (Han et al. 2009).

RESULTS AND DISCUSSION

IPN hydrogels of poly (NIPAAm)/ONK were prepared via free radical polymerization in the presence of ONK. Before the polymerization step was complete, the ONK was synthesized from chitosan. Figure 1 shows the reaction from chitosan to ONK. The product refers to ONK, a form of yellow powder with a darker compared to pure chitosan. The synthesis was carried out an alkaline sodium hydroxide medium. Under these conditions, the alkoholate anion was more reactive and O-substitution occurs (Lang 1995). While in acidic medium, nitrogen group (N-) will be activated. Figure 2 shows the reaction between the ONK and NIPAAm.

Figure 3 provides the ATR/FTIR spectra for (a) chitosan, (b) ONK (c) poly(NONK82) (d) poly(NONK91) and (e) poly (NIPAAm). The amide groups of pure chitosan generally exhibit absorption bands at 1650 cm^{-1} (amide I,
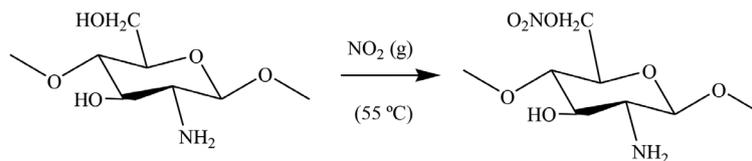
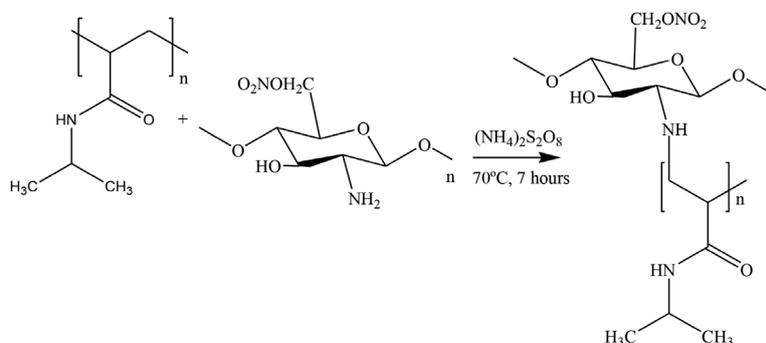FIGURE 1. Synthesis scheme of O-nitrochitosan
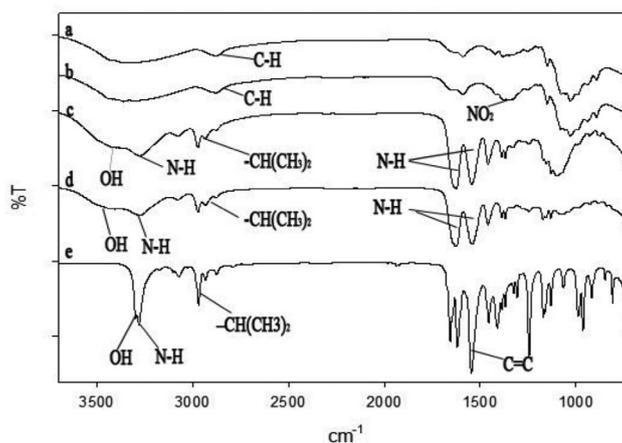

FIGURE 2. Reaction of IPN poly(NIPAAm)/ONK

FIGURE 3. FT-IR spectra of (a) chitosan and (b) ONK (c) poly
(NONK82), (d) poly(NONK91) and (e) PNIPAAm

C=O stretching) and 1560 cm⁻¹ (amide II, N-H bending) (Don & Chen 2005, Guo & Gao 2007) and 1350 cm⁻¹ (amide III, stretching vibration of C-N) (Samantha et al. 2014). After synthesis of the ONK, the primary amine is converted to a secondary amine, which can be observed in the broadening of the amide I (C=O) and amide II (N-H) bands. Stretching of the secondary N-H appears at 1625 and 1542 cm⁻¹ (Alli & Hazer 2008; Caykara et al. 2006; Don & Chen 2005). Unlike chitosan, the ONK exhibits new bands at 1645 and 1348 cm⁻¹ confirming the attachment nitro groups on chitosan by replacing the hydrogen atoms. The ATR/FTIR spectra of the ONK, poly(NONK82) and poly(NONK91) also exhibit new bands between the C-N and N-H bands that arise from ONK and CH₂ in the NIPAAm at 1154 and 1171 cm⁻¹, respectively, in the spectra in (b) and (c). In NIPAAm monomer, C=C

band at 1650 cm⁻¹ is no longer exist in the interpenetrating polymer network, poly (NIPAAm)/ONK. ONK exhibits one broad band at approximately 3400 cm⁻¹ representing a stretching band of C-H and O-H, while poly(NONK82) and poly(NONK91) exhibit several bands; ONK has aromatic C–H while C–H band in poly(NONK82) and poly(NONK91) at 1458 cm⁻¹ represents methyl group and the band at 1376 cm⁻¹ represents methylene group (Guo & Gao 2007). Isopropyl group in NIPAAm can be observed at 2900 cm⁻¹ (Don & Chen 2005; Zhang et al. 2009).

The confirmation of ONK structure was determined via NMR spectroscopy. The ¹³C-NMR spectrum for ONK shows 6 signals attributed to 6 carbon atoms in ONK (Table 1). The total number of carbons of ONK is the same as in pure chitosan because no carbon atoms were added or changed. Figures 4 and 5 show the ¹³C-NMR and ¹H-NMR

TABLE 1. Chemical shifts for proton in (D_2O) and carbon nuclei in O-nitrochitosan determined by ^{1}H NMR or ^{13}C NMR spectroscopy

| Proton/Carbon position	δ[1]H (ppm) multiplisiti (*J* Hz)			δ^{13}C (ppm)		
	ONK 2	Zhang et al. 2003	Kasaai 2010	ONK 2	Zhang et al. 2003	Kasaai 2010
1	4.65 (s)	4.76	4.62-4.85	97.52	97.5	102.7-105.7
2	3.01 (*t, J* = 8.7)	3.09	3.18-3.24	55.66	55.6	55.2-57.6
3	3.55, 3.60, 3.72 - 3.78 (*m*)	3.43-3.81	3.52.3.65 3.74-4.34	69.99	70.0	73.1-75.7
4	3.55, 3.60, 3.72 - 3.78 (*m*)	3.43-3.81	3.52.3.65 3.74-4.34	76.19	76.5	80.9-85.7
5	3.55, 3.60, 3.72 - 3.78 (*m*)	3.43-3.81	3.52.3.65 3.74-4.34	74.66	75.0	73.1-75.7
6	3.55, 3.60, 3.72 - 3.78 (*m*)	3.43-3.81	3.52.3.65 3.74-4.34	59.78	60.0	59.6-60.8
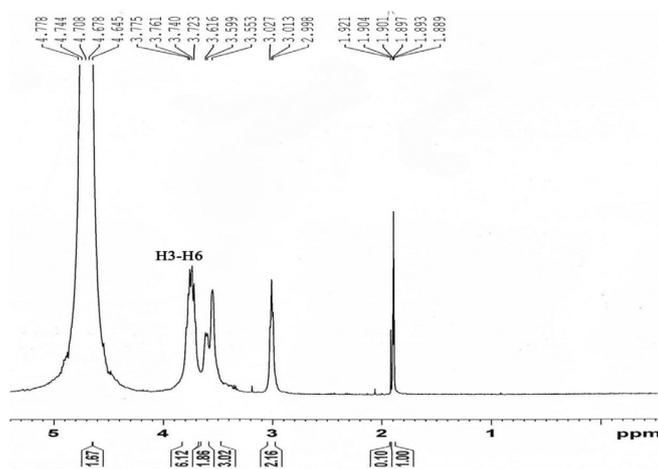
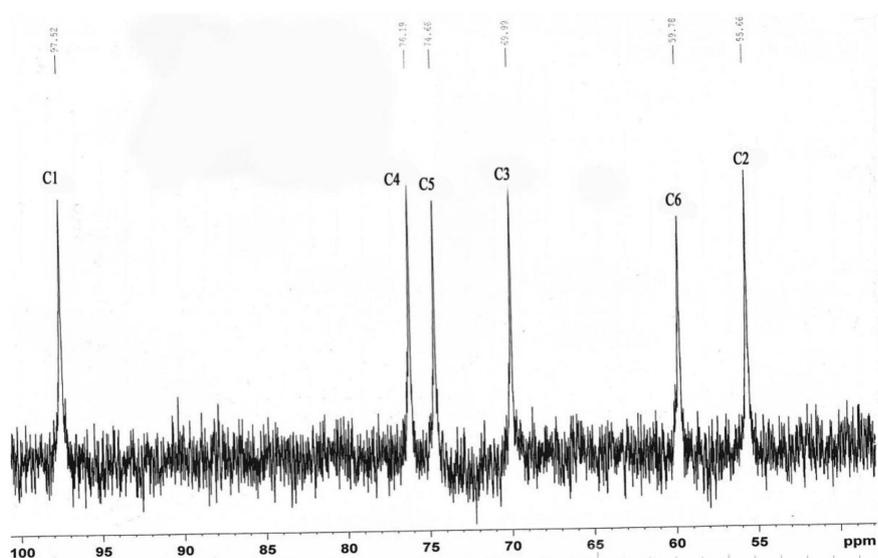FIGURE 4. H[1] Nuclear magnetic resonance (NMR) of ONK

FIGURE 5. C[13] Nuclear magnetic resonance (NMR) of ONK

spectra of ONK. Proton signal at d_H 3.01 is referred to H2 while proton of H3 to H6 is referred at d_H 3.55, 3.60 and 3.72-3.78. NMR spectrum showed the presence of six carbon signal which is assigned to six carbon atom on ONK at d_C 55.66 (C3), 59.78 (C6), 69.99 (C3), 74.66 (C5), 76.19 (C4) and 97.52 (C1).

The hydrogel swelling behaviour has been studied with the temperature range between 10 and 60°C. The effect of temperature on the degree of swelling is shown in Figure 6. The swelling percentage of poly(NIPAAm) decreases slightly with the increasing temperature. Generally, the degree swelling hydrogels is affected by the repulsive and attractive specific intermolecular interactions such as Van der Waals forces, hydrophobic interactions, hydrogen bonding and electrostatic interactions. The degree of swelling poly (NIPAAm) depend on the hydrophilic (CONH-) and hydrophobic (–CH(CH$_3$)$_2$) groups. At the LCST, the hydrophilic group (CONH–) becomes dominant through the formation of H-bonds between water and –OH, –NHCO and –NH$_2$ groups in the hydrogel (Guo & Gao 2007). As the temperature increases, poly(NIPAAm) undergoes deswelling and most of the absorbed water is removed. This result could be due to the breaking collapse of poly (NIPAAm) segments with the increasing temperature. The hydration of the gel is suppressed by interaction of the intermolecular, polymer-water hydrogen bonding with intramolecular hydrogen bonding involving poly (NIPAAm) alone (Verestiuc et al. 2004). As ONK was introduced into poly (NIPAAm), these hydrogels poly(NONK91) and poly(NONK82)) exhibited a greater swelling capacity than the poly (NIPAAm). For example, poly(NONK82) facilitates water uptake by approximately 1171%, possibly due to additional hydrogen bonding interactions between chitosan and NIPAAm in the interpenetrating polymer networks. In addition, two competitive phenomena appear to determine the behavior of the gel; as the temperature rises, the hydration capacity of chitosan increases, whereas that of poly (NIPAAm) decreases as the segments collapse because of the temperature-controlled conformational transition (Verestiuc et al. 2004). The water content of poly(NONK82) also drops sharply when the temperature exceeded 25°C. The same pattern is observed in the water the crosslinked chitosan-g-poly (NIPAAm) content when the temperature is greater than 30°C (Don & Chen 2005).

SEM results provide information regarding the morphological changes and miscibility of poly (NIPAAm)/ONK. The SEM images of the ONK, poly(NONK82) and poly(NONK91) surfaces are presented in Figure 7 at varying 1000× and 10,000× magnifications. According to Nagahama et al. (2009), the surface morphology of a chitosan membrane is smooth with no large pores on the surface. This smooth morphology is due to the presence of chitosan hydrogels in the membrane. In this study, ONK exhibits a rough texture with no pores, unlike poly(NONK82) and poly(NONK91), which have pores and smoother textures. Furthermore, poly(NONK82) has the more heterogeneous morphology than poly(NONK91) with some holes caused by the dispersion of ONK particles in the poly (NIPAAm) networks. This result is due to the increasing of ONK content from 10 to 20 wt. % in NIPAAm. Therefore, the morphology of the poly(NONK82) exhibits a dense and heterogeneous surface. This observation proves the effectiveness of the intermolecular penetration between both ONK and poly (NIPAAm). The poly(NONK91) has a smoother texture and is dense with few pores compared to poly(NONK82). As reported by Don and Chen (2005), A-B crosslinked graft copolymers based on a maleilated chitosan (MA-CS) and NIPAAm are smooth and dense. From these micrographs, we can conclude that both components, ONK and NIPAAm, have a good miscibility in polymer networks.

CONCLUSION

In this study, interpenetrating polymer network of poly (N-isopropylacrylamide/O-nitrochitosan) was successfully
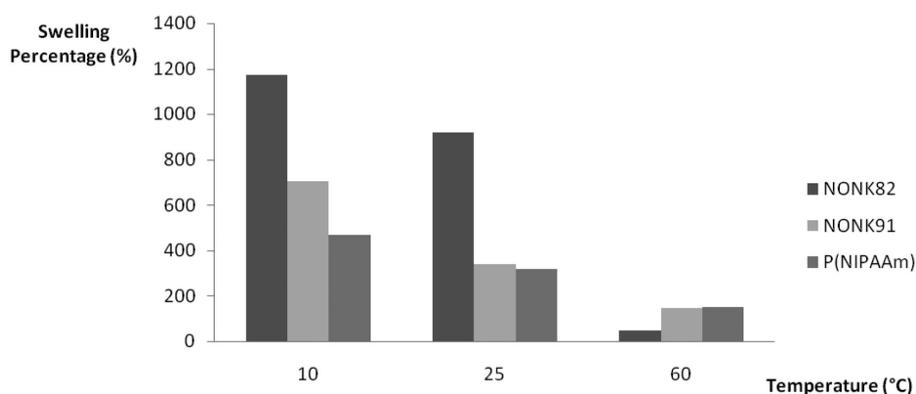

FIGURE 6. Swelling test of poly(NIPAAM) , poly(NONK82) and poly(NONK91) 10, 25 and 60°C in aqueous solution
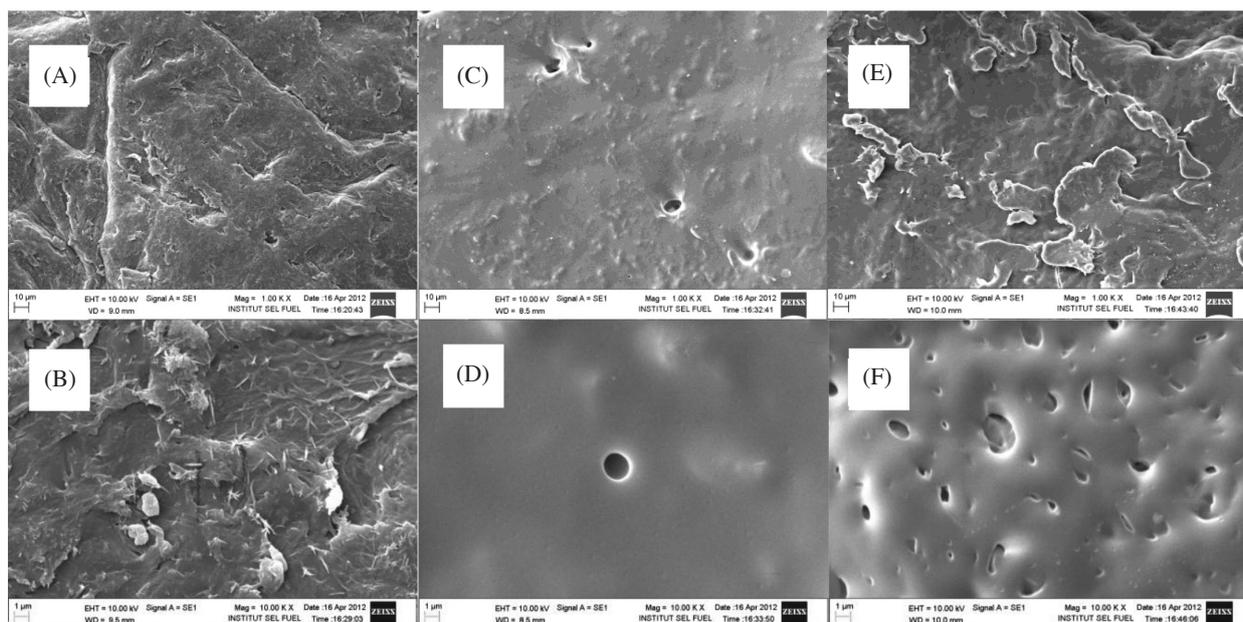
FIGURE 7. SEM micrographs for ONK surface (A, B), poly(NONK91) (C, D) and poly(NONK82)

synthesized using solution polymerization technique. The new chitosan derivative, ONK has been confirmed by ¹H and ¹³C NMR analysis. This chitosan derivative improved the properties of poly(NIPAAm) in swelling behaviour compared to poly(NIPAAm) hydrogel. This new IPN of poly (N-isopropylacrylamide/O-nitrochitosan) hydrogels exhibited thermoresponsive characteristic over that of the poly (NIPAAm) hydrogel. The ONK and NIPAAm also show good miscibility as presented in SEM.

REFERENCES

Alli, A. & Hazer, B. 2008. Poly (N-isopropylacrylamide) thermoresponsive cross-linked conjugates containing polymeric soybean oil and/or polypropylene glycol. *European Polymer Journal* 44(6): 1701-1713.

Baldrick, P. 2010. The safety of chitosan as a pharmaceutical excipient. *Regulatory Toxicology and Pharmacology* 56(3): 290-299.

Caykara, T., Kiper, S. & Demirel, G. 2006. Thermosensitive poly(N-isopropylacrylamide-co-acrylamide) hydrogels: Synthesis, swelling and interaction with ionic surfactants. *European Polymer Journal* 42(2): 348-355.

Chen, J., Sun, J., Yang, L., Zhang, Q., Zhu, H., Wu, H., Hoffman, A.S. & Kaetsu, I. 2007. Preparation and characterization of a novel IPN hydrogel memberane of poly(N -isopropylacrylamide)/carboxymethyl chitosan (PNIPAAM/CMCS). *Radiation Physics and Chemistry* 76(8-9): 1425-1429.

Don, T.M. & Chen, H.R. 2005. Synthesis and characterization of AB-crosslinked graft copolymers based on maleilated chitosan and N-isopropylacrylamide. *Carbohydrate Polymers* 61(3): 334-347.

Dutta, P.K., Tripathi, S., Mehrotra, G.K. & Dutta, J. 2009. Perspectives for chitosan based antimicrobial films in food applications. *Food Chemistry* 114(4): 1173-1182.

Guinesi, L.S. & Cavalheiro, E.T.G. 2006. The use of DSC curves to determine the acetylation degree of chitin/chitosan samples. *Thermochimica Acta* 444(2): 128-133.

Guo, B.L. & Gao, Q.Y. 2007. Preparation and properties of a pH/temperature-responsive carboxymethylchitosan/poly(N-isopropylacrylamide) semi-IPN hydrogel for oral delivery of drugs. *Carbohydrate Research* 342(16): 2416-2422.

Han, J., Wang, K., Yang, D. & Nie, J. 2009. Photopolymerization of methacrylated chitosan/PNIPAAm hybrid dual-sensitive hydrogels as carrier for drug delivery. *International Journal of Biological Macromolecules* 44(3): 229-235.

Kasaai, M.R. 2010. Determination of the degree of N-acetylation for chitin and chitosan by various NMR spectroscopy techniques: A review. *Carbohydrate Polymers* 79: 801-810.

Kumar, M.N.V.R. 2000. A review of chitin and chitosan applications. *Reactive Functional Polymer* 46(1): 1-27.

Lang, G. 1995. Chitosan derivative- preparation and potential uses. In *Chitin and Chitosan: The Versatile Environmentally Friendly Modern Materials*, edited by Zakaria, M., Muda, W.M.W. & Abdullah, M.P. Bangi: Penerbit Universiti Kebangsaan Malaysia. pp. 109-118.

Nagahama, H., Maeda, H., Kashiki, T., Jayakumar, R., Furuike, T. & Tamura, H. 2009. Preparation and characterization of novel chitosan/gelatin membranes using chitosan hydrogel. *Carbohydrate Polymers* 76(2): 255-260.

Prabaharan, M. & Jayakumar, R. 2009. Chitosan-*graft*-β-cyclodextrin scaffolds with controlled drug release capability for tissue engineering applications. *International Journal of Biological Macromolecules* 44(4): 320-325.

Rinaudo, M. 2006. Chitin and chitosan: Properties and applications. *Progress in Polymer Science* 31(7): 603-632.

Samanta, H.S. & Ray, S.K. 2014. Controlled release of tinidazole and theophylline from chitosan based composite hydrogels. *Carbohydrate Polymers* 106: 109-120.

Verestiuc, L., Ivanov, C., Barbu, E. & Tsibouklis, J. 2004. Dual-stimuli responsive hydrogels based on poly(*N*-isopropylacrylamide)/chitosan semi-interpenetrating networks. *International Journal of Pharmaceutics* 269(1): 185-194.

Zeng, M., Zhang, L. & Kennedy, J.F. 2005. Intermolecular interaction and properties of cross-linked materials from poly(ester-urethane) and nitrochitosan. *Carbohydrate Polymers* 60(3): 399-409.

Zhang, C., Ping, Q., Zhang, H. & Shen, J. 2003. Synthesis and characterization of water-soluble O-succinyl-chitosan. *European Polymer Journal* 39: 1629-1634.

Zhang, H.F., Zhong, H., Zhang, L.L., Chen, S.B., Zhao, Y.J. & Zhu, Y.L. 2009. Synthesis and characterization of thermosensitive graft copolymer of *N*-isopropylacrylamide with biodegradable carboxymethylchitosan. *Carbohydrate Polymers* 77(4): 785-790.

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