

Comparison Study of Hydrogels Properties Synthesized with Micro- and Nano- Size Bacterial Cellulose Particles Extracted from *Nata de coco*

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The effect of different size of bacterial cellulose particles used in the production of hydrogel was investigated. Bacterial cellulose was extracted from *nata de coco*, a local dessert origin from the Philippines. Micro size particle was prepared by conventional grinding of dried sheet of bacterial cellulose whereas cellulose nanoparticle was prepared by acid hydrolysis treatment. Both were then used in hydrogels formulation with acrylic acid (in ratio of 70% of bacterial cellulose dispersion to 30% acrylic acid) and exposed to electron beam irradiation (40 kGy). TEM observation showed nano cellulose particle, having range of dimension of 80 – 160 nm in length and 13 – 22 nm in diameter. It was found that hydrogel with nano cellulose has higher glass transition temperature (39°C) as compared to micro cellulose hydrogel (32°C). SEM observation revealed that swollen nano cellulose hydrogel has smaller and homogenous pores arrangement while micro cellulose has bigger and irregular pores thus affecting their swelling degree.

Key words:

Bacterial cellulose, acrylic acid, electron beam, hydrogel, swelling degree

Introduction

Hydrogels are hydrophilic polymer networks that can absorb and retain large volume of water. However due to chemical or physical crosslink the three dimensional structure remain insoluble.¹ They find application in various field such as drug delivery system, medical devices and tissues scaffold for their good biocompatibility property.^{2–6} Nevertheless, a stimuli sensitive hydrogel that possess sensitivity towards changes in external factor such as temperature, pH, ions, electric field as well as magnetic field attracted widespread interest in waste water treatment and electronic field.^{7–9}

Hydrogels can be produced by reacting one or more polymers, and could be from the combination of synthetic-synthetic or synthetic-natural polymers. Recently, many researches have focus on producing hydrogels from natural polymers due to their biocompatibility and biodegradability with acceptable mechanical strength. Therefore the fabrications of natural based hydrogels have emerged involving the use of natural polymers such as starch, cellulose, chitin, gelatine, hyaluronic acid and much more.

One of the most studied natural polymers is cellulose. Cellulose is the most abundant and renewable biopolymer in nature. It is the main constituent of plant cell walls, fungi, some algae and several bacteria has the ability to produce extra-cellular cellulose as their metabolites. Cellulose is a carbohydrate homopolymer consist of β -D-glucopyranose units joint together by β -1,4-glycosidic linkage.¹⁰ Cellulose fibrils are highly insoluble and inelastic. Their molecular configuration making the tensile strength of cellulose comparable to that of steel and this unique feature provide mechanical support to the tissues which it resides.¹¹

Solvent extraction is an important method to remove the extractable fraction from cellulosic fibres. However this procedure may cause slight damages to the fibre structure and results in a more expose cellulosic surface. Considering this, bacterial cellulose has a lot to offer. The cellulose produced by this organism is of exceptionally high purity and resembles the same feature of that from plants and algae in term of crystalline unit structure and average microfibrillar width.¹² Therefore pure cellulose can be easily obtained from this source without having the usual difficulties associated with hemicellulose and lignin removal process.

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Previously, hydrogel composite synthesized using purified cellulose from *nata de coco* has been investigated. The cellulose used in the formulations was in the form of dried powder in micron size.¹³ The mechanism by which the hydrogel was formed has been proposed and it was also found to be pH and temperature sensitive.^{2,14} The present study was conducted to synthesize hydrogel using different size of cellulose particles. The cellulose nanoparticles were prepared by acid hydrolysis and expected to alter several physicochemical properties of the hydrogel. As the nano cellulose has more surfaces available for reaction, the crosslink density and swelling degree was also expected to be modified.

Materials and Methods

Preparation of micro- cellulose powder

Nata de coco was soaked and washed with distilled water until the pH become neutral. It was then blended in wet blender and dried in conventional oven at 60°C. The dried sheets were later grinded to produce white, fluffy powder.

Preparation of nano- cellulose powder

5 g of the fluffy white powder that was prepared earlier was reacted with sulphuric acid (65% by weight, 200 ml) at 45°C for 45 minutes. The reaction was quenched by fivefold dilution. The mixture was then concentrated and rinsed by centrifugation (10 000 rpm for 10 minutes). The nano cellulose was re-suspended and neutralized by dialysis against distilled water.¹⁵ The suspension was then freeze dried to produce nano cellulose powder.

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was conducted using a Philips CM30 microscope to investigate the morphology of nanoscale cellulose. A droplet of a diluted suspension was deposited on a Cu grid covered with a thin carbon film. To enhance the contrast, the cellulose were negatively stained with 2 wt% uranyl acetate solution (an organometallic complex) in deionized water for 1 min and then dried at room temperature.

Hydrogel formulation

Hydrogel formulation was prepared by mixing 1% of cellulose dispersion in distilled water with acrylic acid. In this case the ratio used was 7:3 (cellulose dispersion: acrylic acid). In this case, two formulations were prepared, the first one was using micro- cellulose (Hydrogel A) and the second one was using the nano-cellulose (Hydrogel B). The

formulations were stirred with magnetic stirrer for 1/2 hours to ensure proper mixing and later on heated at 40°C in water bath for 3 hours to eliminate bubbles entrapped. The formulations were then poured into plastic mould with dimension of 12x12 cm and place onto the conveyer that will bring the tray pass under the electron beam window. In each pass, 5 kGy of electron beam will be delivered until a total dose of 40 kGy was achieved. This process was done in the presence of air. Under the effect of ionizing irradiation polymerization of polyacrylic acid and grafting of polyacrylic acid onto cellulose backbone took place and cause the formulation to solidify, forming the hydrogel. In this case the thickness of the hydrogel is 3mm.

FTIR analysis

Infrared spectra of the hydrogels were recorded using FTIR Spectra 2000 (Perkin Elmer) at room temperature. The hydrogels were cut into pieces with dimension of 1cm x 1cm and 2mm thickness and dried in convectional oven until a constant dried weight was achieved. The samples were then analyzed by directly placing the dried hydrogels on the top plate of Diamond attenuated total reflectance (DATR) and analyzed over the range of 500 – 4000 cm⁻¹.

Swelling degree

Hydrogels were extracted in distilled water at room temperature for 7 days. The extraction process was done to remove any sol that maybe present in the hydrogels. A known weight of dried extracted hydrogels (G_d) was then immersed in distilled water to evaluate the swelling degree of hydrogels for 48 hours at room temperature (25°C). Swollen samples were weighed (G_s) at every hour where excess water was pad dried. The percent of swelling was calculated by using the following equation:

$$\text{Swelling (\%)} = \frac{G_s - G_d}{G_d} \times 100$$

DSC analysis

Differential scanning calorimetry (DSC) analysis was performed using Metler Toledo STAR DSC. Five milligrams (5 mg) of dried grinded hydrogels were measured, placed and sealed in aluminium sample pans. The test was then conducted under nitrogen flow (20 ml/min) and heated from -50°C to 150°C (heating rate 5°C/min).

Scanning electron microscopy (SEM)

Morphological study was done onto dried hydrogels and swollen (48 hours) hydrogels. Samples were observed using SEM, Philips XL-30. Hydrogels samples were sputter coated with gold and images were taken under 500X magnification.

Results and discussion

TEM analysis

TEM observation of nanoparticles cellulose from *nata de coco* is shown in Figure 1. *Nata de coco* cellulose nanoparticles ranged in length from 80 – 160 nm, with an average value around 120 nm. The diameter was in the range 13–22 nm. These results demonstrate the efficiency of the conditions used for the acid hydrolysis treatment of *nata de coco* and confirm that the aqueous suspension contained individual nanoparticles. The nanoparticles provide more surface area for acrylic acid grafting as the number of celluloses ‘islets’ has been increased, being the cellulose amount the same (see

Figure 2). The increased surface area would also expect to increase the swelling degree of hydrogels as the fraction of cellulose islets (proportional to crosslink density) was decreased.

FTIR analysis

The IR spectra of bacterial cellulose, acrylic acid, Hydrogel A and Hydrogel B are shown in Figure 3. The bacterial cellulose IR spectrum has shoulders between 985 to 1000 cm^{-1} representative of C-O stretching. The peaks at 1163 cm^{-1} and 2926 cm^{-1} are attributed to the C-O-C of ether linkage [1,4- β -D-glucoside] of cellulose and C-H stretching respectively while the prominent peak at 3440 cm^{-1} correspond to O-H stretching of intermolecular hydrogen bonding.¹⁶ Acrylic acid spectra showed distinctive peaks at 1600 cm^{-1} correspond to C=C stretching. The prominent peak at around 1700 cm^{-1} is assigned to C=O stretching and broad peak at 3040–2340 cm^{-1} refers to O-H stretching.¹⁷

The analysis also shows that all peaks present in both hydrogels spectra are comparable, suggesting that the formation of hydrogels involved the

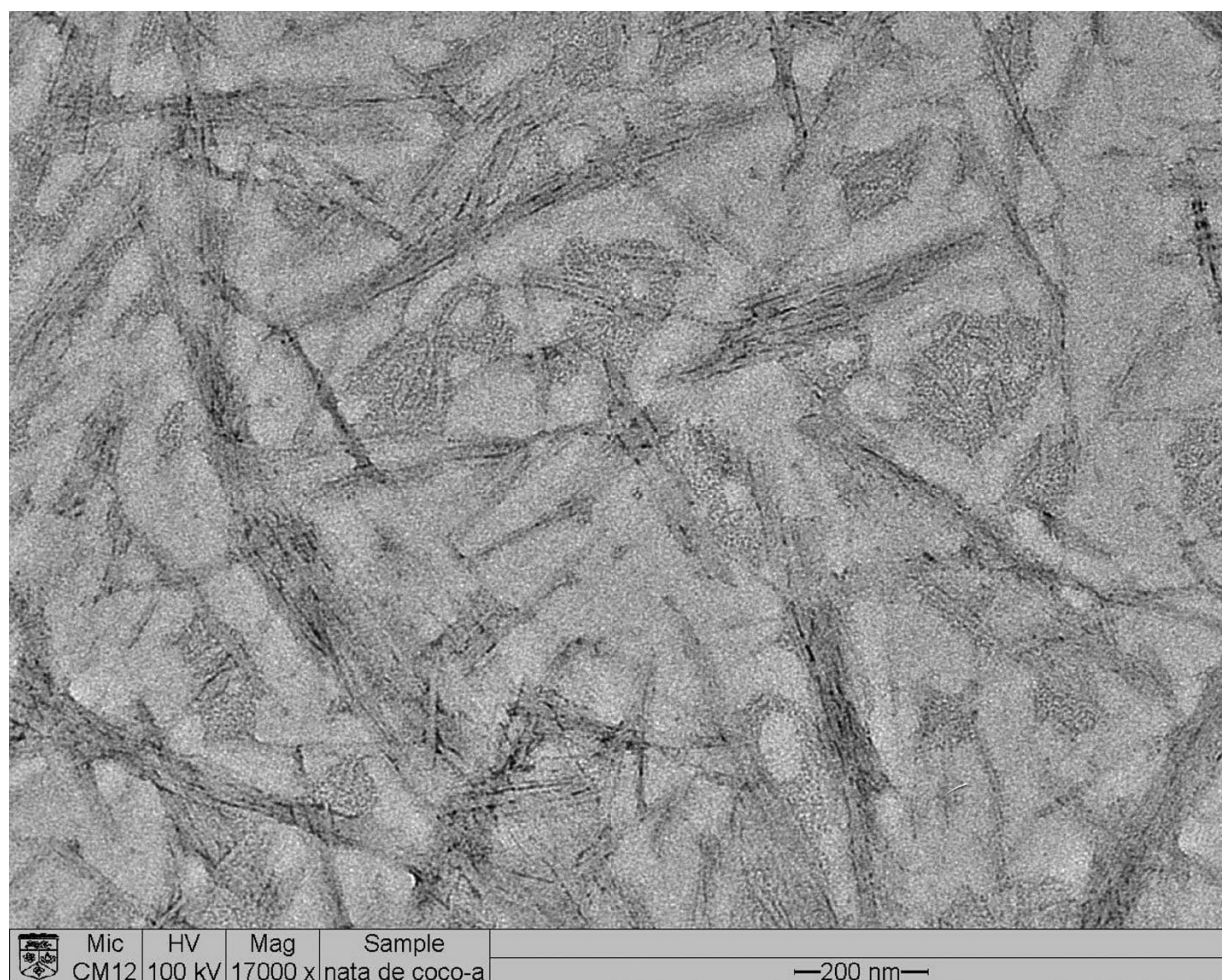


Fig. 1 – Transmission electron micrograph of cellulose nanocrystals extracted from *nata de coco*

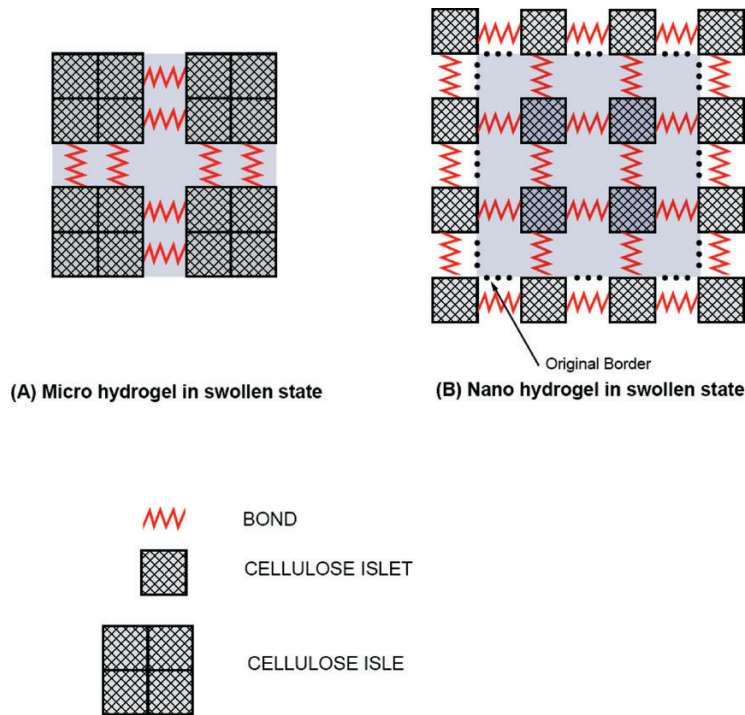


Fig. 2 – Illustration of crosslink points in hydrogel with micro cellulose (a) and nano cellulose (b)

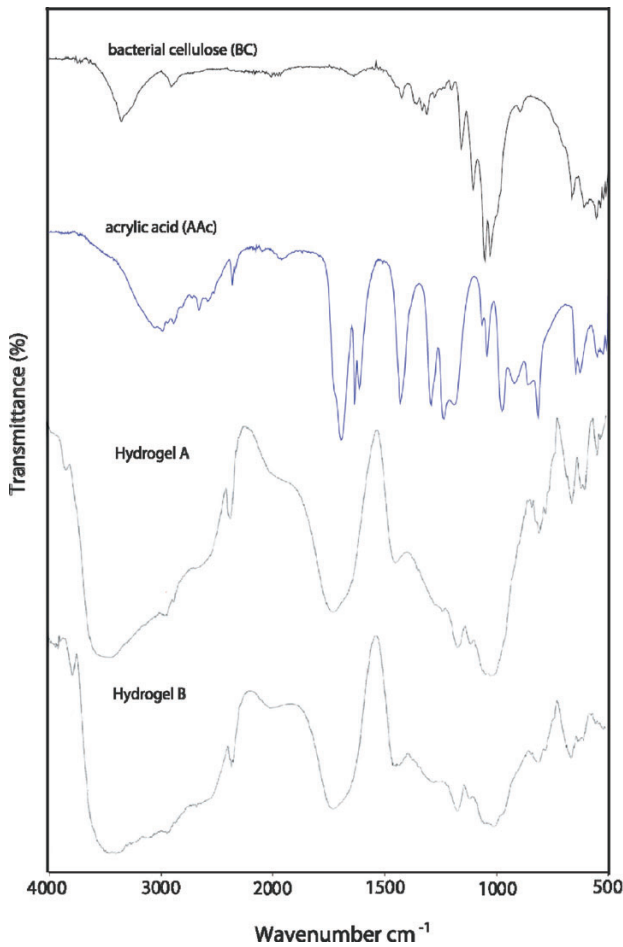


Fig. 3 – FTIR spectra of bacterial cellulose, acrylic acid, Hydrogel A and Hydrogel B

same interaction between cellulose fibres and AAC polymer chains. The reaction mechanism by which the hydrogel was formed has been earlier proposed by Halib et al. (2009).¹⁴ In this case, the acrylic acid was grafted onto cellulose backbone. Since cellulose used in Hydrogel B was in nano size, more surfaces were available for reaction.

Swelling degree

Swelling degree of hydrogels was obtained with distilled water at room temperature and hydrogels were let to swell until equilibrium state for 48 hours. It was found that Hydrogel B with nano cellulose had a swelling degree 18% higher as compared to Hydrogel A with micro cellulose (Figure 4). This can

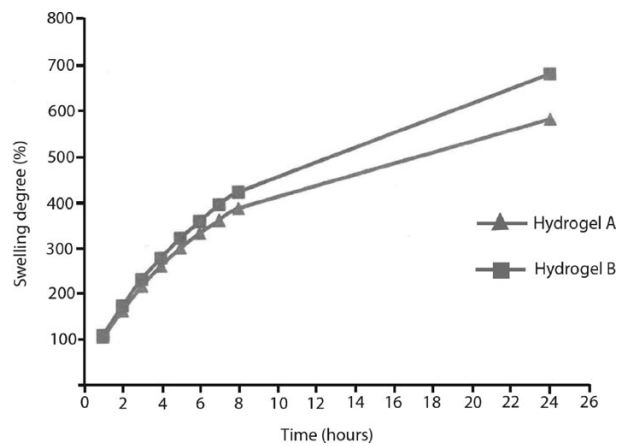


Fig. 4 – Comparison of swelling degree on Hydrogel A and Hydrogel B

be explained according to the interpretation given in Figure 2. The finding also demonstrated that the same amount of cellulose would actually produced hydrogel with different performance as the physical properties of the fibres being modified. Furthermore this modification would contribute in the cost effective production of the hydrogel.

DSC analysis

DSC thermogram of Hydrogel A and Hydrogel B is shown in Figure 5. It was found that Hydrogel B has higher glass transition temperature (T_g) (39°C) as compared to Hydrogel A (32°C). This can be probably due to the more intense interpolymer chains interaction occurring in Hydrogel B (see Figure 2). Indeed, theoretically, the presence of extensive hydrogen bonding between PAAc and cellulose hydroxyl group creates stronger interaction between polymer and it should enhance the thermal stability of the hydrogels.¹⁸

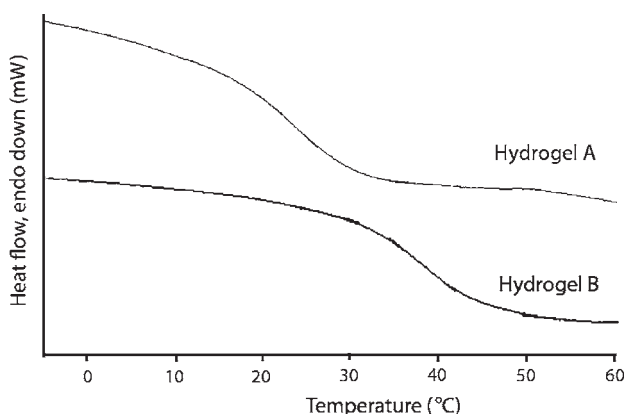


Fig. 5 – DSC thermogram of Hydrogel A and Hydrogel B

SEM analysis

SEM micrograph for dried and swollen hydrogels are shown in Figure 6(a & b) and Figure 7(a & b). It was observed in dry state that hydrogel A and Hydrogel B has smooth surface area with the absence of pores. This is because in dry state, the pores were collapsed and the surface appeared as non-porous structure; in contrast, in swollen state, the pores were open up. As observed, Hydrogel A has bigger and irregular pores whereas Hydrogel B has smaller and more uniform pores. The distribution of pores in Hydrogel B also appeared more even and homogenous throughout the surface area. This obviously affected the swelling degree of both hydrogels. The homogenous distribution and uniform pores of Hydrogel B enables water molecules to be absorbed in every direction with faster response time. Thus, in agreement with the result in Figure 4, Hydrogel B has increased swelling degree. This would suggest that the use of nano cellulose would enhance and im-

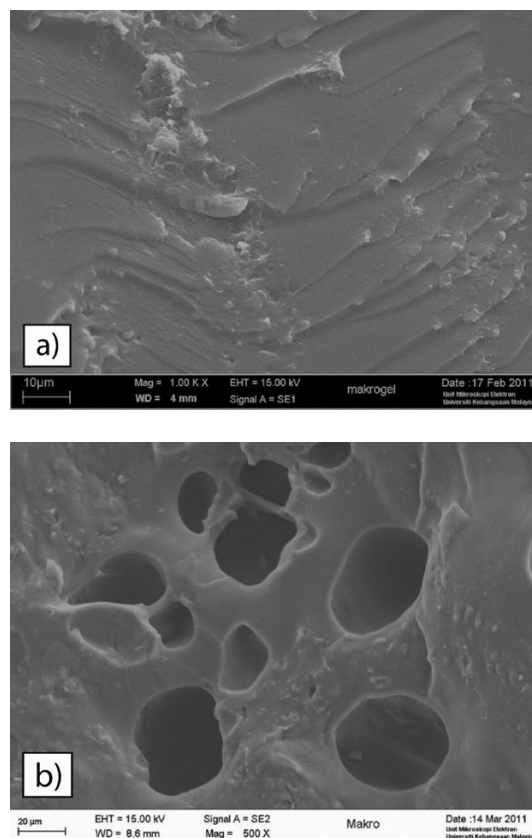


Fig. 6 – a) SEM micrograph of dried Hydrogel A; b) SEM micrograph of swollen, freeze dried Hydrogel A

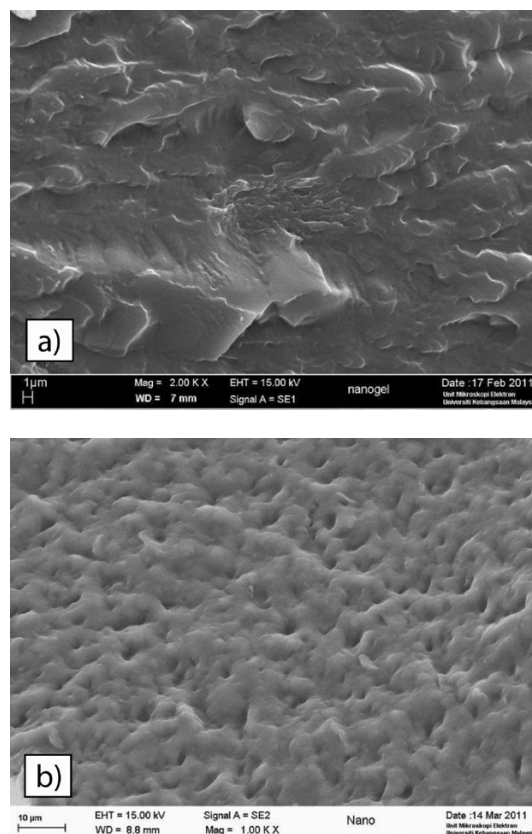


Fig. 7 – a) SEM micrograph of dried Hydrogel B; b) SEM micrograph of swollen, freeze dried Hydrogel B

prove the formation of pores within the hydrogel matrices thus improving its swelling properties.

Conclusion

The modification of cellulose fibre used in the hydrogel formulation would affect its performance. With the increase of surface on the nano cellulose fibre, more interaction can be formed. This in turn will increase the thermal stability as more inter-polymer interactions were present. The formation and distribution of pores and the swelling capacity of hydrogels were also improved with the use of nano cellulose. The above results suggest that hydrogel with improved performance can be produced as the physical properties of the fibres can be modified.

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References

1. Lin, C.C & Metters, A.T., *Adv. Drug. Deliver. Rev.* **58** (2006) 1379
2. Halib, N., Mohd Amin, M.C.I., Ahmad, I., *J. Appl. Polym. Sci.* **116** (2010) 2920
3. Safrany, A., *Nucl. Instrum. Meth. B.* **131** (1997) 376
4. Schmaljohann, D., *e-Polymers.* **21** (2005) 1
5. Wang, B., Liu, M., Chen, Z., Liang, R., Ding, S., Chen, S., Jin, S., *Int. J. Pharm.* **331** (2007) 19
6. Wang, M., Xu, L., Hu, H., Zhai, M., Peng, J., Nho, Y., Li, J., Wei, G., *Nucl. Instrum. Meth. B.* **265** (2007) 385
7. Clough, R.L., *Nucl. Instrum. Meth. B.* **185** (2001) 8
8. Yetimoğlu, E.K., Kahraman, M.V., Ercan, Ö., Akdemir, Z.S. & Apohan, N.K., *React. Funct. Polym.* **67** (2007) 451
9. Pekel, N. & Güven, O., *Polym. Bull.* **51** (2004) 307
10. Qin, C., Soykeabkaew, N., Xiuyuan, N. & Peijs, T., *Carbohydr. Polym.* **71** (2008) 458
11. Yamanaka, S., Watanabe, K., Kitamura, N., Iguchi, M., Mitsuhashi, S., Nishi, Y. & Uryu, M., *J. Mater. Sci.* **24** (1989) 3141
12. Ross, P., Mayer, R. & Benziman, M., *Microbiol. Rev.* **55** (1991) 35
13. Halib, N. 2011. Development and characterization of bacterial cellulose/acrylic acid hydrogels as a system for drug delivery. Phd thesis. The National University of Malaysia.
14. Halib, N., Mohd Amin, M.C.I., Ahmad, Hashim, Z.M., Jamal, N., *Sains. Malays.* **38** (2009) 785
15. Rusli, R. & Eichhorn, S. *Appl. Phys. Lett.* **93** (2008) 033111-1
16. Marchessault, R.H. & Sundararajan, P.R. 1983. Cellulose. In Aspinall, G.O. (editor). *The polysaccharides Volume 2*, page 11–56. New York: Academic Press.
17. Pavia, D.L., Lampman, G.M. & Kriz, G.S. 2001. Infrared Spectroscopy. In *Introduction to Spectroscopy*. 3rd Edition, page 13–101. New York: Thomson Learning, Inc.
18. Lau, C. & Mi, Y., *Polymer.* **43** (2002) 823