

Full Length Research Paper

X-ray photoelectron spectroscopy and atomic force microscopy studies on crosslinked chitosan thin film

W. F. Yap^{1*}, W. M. M. Yunus¹, Z. A. Talib¹ and N. A. Yusof²

¹Department of Physics, Faculty of Science, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

²Department of Chemistry, Faculty of Science, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

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Crosslinked chitosan solution was synthesized by homogeneous reaction of medium molecular weight chitosan in aqueous acetic acid solution with glutaraldehyde as crosslinking agent. The solution was then deposited on glass cover slip by spin coating to form thin film. The crosslinked chitosan thin film had been studied by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). The XPS data was obtained to evaluate the state of crosslinked chitosan in thin film. The AFM data shows a relatively smooth morphological characteristic.

Key words: Crosslinked chitosan, X-ray photoelectron spectroscopy, atomic force microscopy.

INTRODUCTION

Chitosan is a linear copolymer of glucosamine and N-acetyl glucosamine linked by β -1, 4 glucosidic bonds. Chitosan is derived by alkaline N-deacetylation from chitin. It is a non-toxic, biocompatible and biodegradable natural polymer. This low cost material had been used for a wide range especially in industry such as absorption of metal ions, dyes and protein (Onsoyen and Skagrud, 1990; Covas et al., 1992; Kawamura et al., 1993; Bassi et al., 2000; Dambies et al., 2000; Chu, 2002; Ngah et al., 2002; Qi and Xu, 2004; Guibal, 2004; Merrifield et al., 2004). It also had been used widely in biomedical applications such as wound dressing, bone substitute, tissue engineering and drug-delivery vehicles (VandeVord et al., 2002). Compared to chitin, chitosan is more efficient in absorption capacity due to the presence of a large number of amino groups on chitosan chain. Chitosan is soluble in organic acid, such as acetic acid and formic acid (Shauer et al., 2003). Crosslinking is an important step to improve the chemical stability of chitosan (Bhumkar and Pokharkar, 2006). One crosslinking agent, glutaraldehyde, is an organic compound with the formula $\text{CH}_2(\text{CH}_2\text{CHO})_2$. Glutaraldehyde is frequently used in biochemistry

applications as an amine-reactive homobifunctional crosslinker (Vieira and Beppu, 2006). The possible structure of chitosan crosslinked with glutaraldehyde was shown in Figure 1.

Atomic force microscopy (AFM) is a very high-resolution type of scanning probe microscopy with resolution on the order of fractions of a nanometer that is more than 1000 times better than the optical diffraction limit (Zhao and Xu, 2010). AFM is versatile analytical tool that excels at topographic characterization of surface. Furthermore, it can be used to probe the identity of chemical constituents at a surface and the mechanical properties of the near surface region with high spatial resolution (Chen et al., 1998; Roa et al., 2011).

The surface properties of polymer are important in many applications. They are dependent on the structure and composition of the outermost molecular layers. The thickness of the surface layer involved is typically of the order of a few nanometers. Therefore, understanding surface structure property relationships requires analytical technique that has this degree of surface sensitivity or specificity such as X-ray photoelectron spectroscopy (XPS). XPS can be used to measure the elemental composition, empirical formula, chemical state and electronic state of the elements that exist within a material. By irradiating a beam of X-rays, photoelectrons excite from the material. The energy of the photoelectrons leaving the sample are determined and

*Corresponding author. E-mail: yapwingfen@gmail.com Tel: 60128190393. Fax: 60389454454.

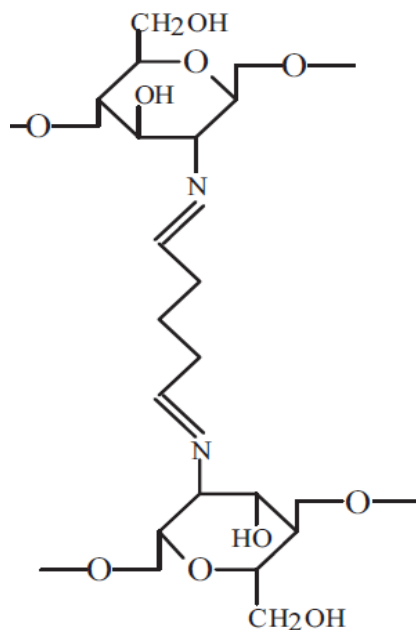


Figure 1. Possible structure of chitosan formed by crosslinking with glutaraldehyde.

this gives a spectrum with a series of photoelectron peaks. The binding energy of the peaks are characteristic of each element. The peak areas can be used to determine the composition of the materials surface. The shape of each peak and the binding energy can be slightly altered by the chemical state of the emitting atom. Hence XPS can provide chemical bonding information as well (Yahya and Afor, 2004; Arof et al., 1998; Dambies et al., 2001; Nascente, 2005).

MATERIALS AND METHODS

Medium molecular weight (MMW) chitosan was purchased from Sigma Aldrich (St. Louis, MO, USA). The molecular weight of MMW chitosan is 190,000-310,000 and the degree of deacetylation is 75 to 85% as noted by the manufacturer. Acetic acid (assay $\geq 99.7\%$) and glutaraldehyde (Grade 1, 25% in H_2O) were also purchased from Sigma Aldrich (St. Louis, MO, USA). The substrate, glass cover slips 24×24 mm with thickness 0.13 to 0.16 mm were purchased from Menzel-Glaser.

Preparation of chitosan solution and chitosan thin film

To prepare chitosan solution, 0.40 g of chitosan was weighed and dissolved in 50 ml 1% acetic acid. The solution was stirred until all the chitosan was dissolved in acetic acid. Then 0.05 g of glutaraldehyde was added to the solution to cross-link chitosan. The resulting solution was stirred thoroughly. Spin coating technique was used to produce a thin layer of crosslinked chitosan film on the substrate. Approximately 0.55 ml of the solution was placed on the glass cover slip covering the majority of the surface.

The glass cover slip were spun at 6000 rev./min for 30 s using Spin Coating System, P-6708D.

X-ray photoelectron spectroscopy (XPS)

The XPS study in this work was carried out using XPS XSAM-HS (Kratos, UK) with a focused monochromatic $Mg K\alpha$ X-ray source. The x-ray source was run at a power of 120 W, that is operating voltage of 12 kV and operating current of 10 mA. The pressure inside the vacuum chamber was 5×10^{-8} torr. The substrate was mounted onto a standard stainless steel sample holder. The XPS scan was recorded in the energy range between 10 and 1100 eV. The pass energy and step size energy was 160 and 1 eV per step respectively.

X-ray photoelectron (XP) spectra was recorded in the constant pass energy mode and expressed as intensity on the y-axis versus binding energy on x-axis. The spectra was fitted using Gaussian-Lorentzian curve fitting program for each peak with a linear background in order to determine the binding energy of the various element core levels.

Atomic force microscopy (AFM)

The sample was imaged with an AFM (Q-scope 250, Quesant Instrument Corporation, Agoura Hills, CA) in tapping mode. The scan resolution of 600 was chosen for data acquisition at a scan speed of 2 Hz at ambient conditions. The sample was measured in air after drying in room temperature.

RESULTS AND DISCUSSION

Chemical compositions of crosslinked chitosan thin film were characterized using XPS and the XPS spectra were shown in Figure 2. The spectra confirmed the presence of carbon, oxygen and nitrogen. The theoretical and experimental C/N ratio was 8.5 and 8.8 respectively, where as for C/O ratio was 1.7 and 1.2 respectively. The theoretical C/N and C/O for crosslinked chitosan was calculated by assuming two chitosan monomers for one glutaraldehyde molecule. There was a little relative error due to the precision limit of the experimental technique. However, the numbers were in very good agreement with the expected results. Variations can possibly caused by side-reactions and polymerization of crosslinkers that are known in the literature (Simionescu et al., 1991; Vieira and Beppu, 2006).

The peak deconvolution of the high resolution spectra provided the chemical composition of the thin film of the surface. The resolved C 1s spectrum regarding crosslinked chitosan thin film revealed three peaks as shown in Figure 3. The C 1s peak at 285.0 eV was mainly assigned to the contaminated carbon or C-C chemical binding. The peak at 286.6 eV was assigned to C-O, C-N or C-O-C and the peak at 288.2 eV to C=O or O-C-O chemical bindings (Briggs, 1990; Beamson, 1992; Ratner and Caster, 1997). This result had a good agreement with the theoretical structural binding of C binding in crosslinked chitosan.

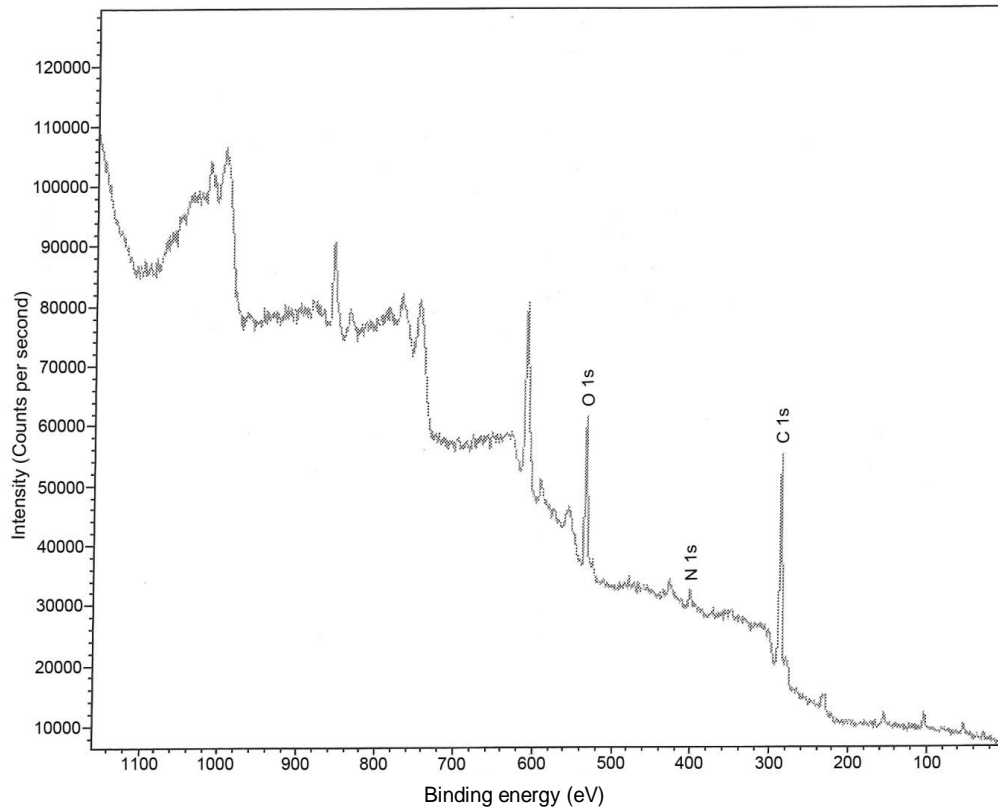


Figure 2. XPS spectra of chitosan crosslinked with glutaraldehyde.

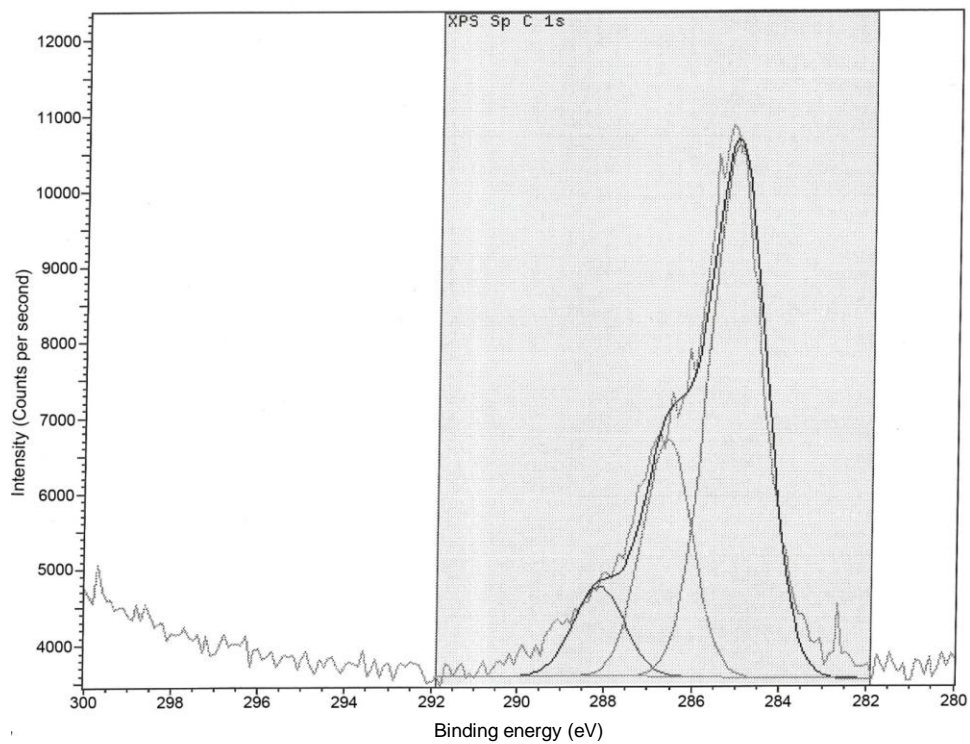


Figure 3. Narrow scan of C 1s peak.

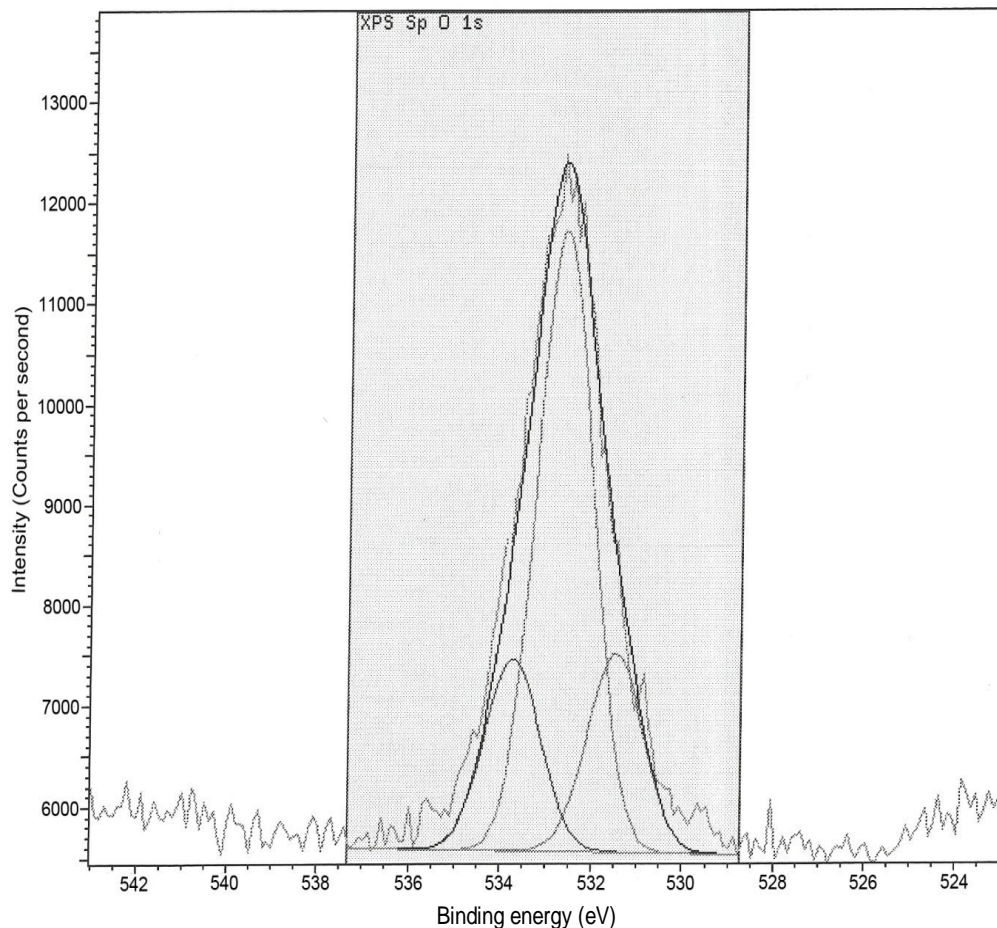


Figure 4. Narrow scan of O 1s peak.

For O 1s spectrum, the results of the fit assumptions were summarized in Figure 4. Three peaks were identified from the spectrum. The peak at 531.5 eV was assigned to C=O chemical bindings in *N*-acetylated-glucosamine units. The peaks at 532.7 eV was assigned to C–O or O–H or bound water, while the peak at 533.7 eV was assigned to O–C–O chemical binding (Beamson, 1992).

The resolved N 1s spectrum of crosslinked chitosan thin film was shown in Figure 5. Two peaks were identified. The peak at 399.6 eV was assigned to C–N or NH₂ chemical bindings, while the peak at 400.9 eV was assigned to amino groups in the ammonium form (NH₃⁺) (Lindberg et al., 1983). Same as C binding, the results also proved the experimental assigned O and N bindings had a good agreement with the theoretical bindings.

Figure 6 shows the surface morphology of the crosslinked chitosan thin film over an area of 15 x 15 μm by atomic force microscopy. A relatively smooth morphological characteristic with granular structure was observed in Figure 6. This morphology is mostly related

to the physical property of the outmost chitosan component. The high intrinsic chain stiffness (Brugnerotto et al., 2001) and high molecular weight for the chitosan molecules affect its diffusion ability during coating process.

Conclusions

The thin film of chitosan crosslinked with glutaraldehyde had been studied by XPS and AFM. The XPS spectra obtained for this crosslinked chitosan thin film confirmed the presence of carbon, oxygen and nitrogen. The experimental ratio of C/O and C/N showed a very good agreement with the expected results. The C, O and N spectra was fitted using Gaussian Lorentzian curve fitting program to determine the binding energy for each peak. The assignments for main spectral bands for C, O and N based on their binding energies also showed a good agreement with the possible structure of chitosan formed by crosslinking with glutaraldehyde. The AFM measurement confirmed the successful formation of the

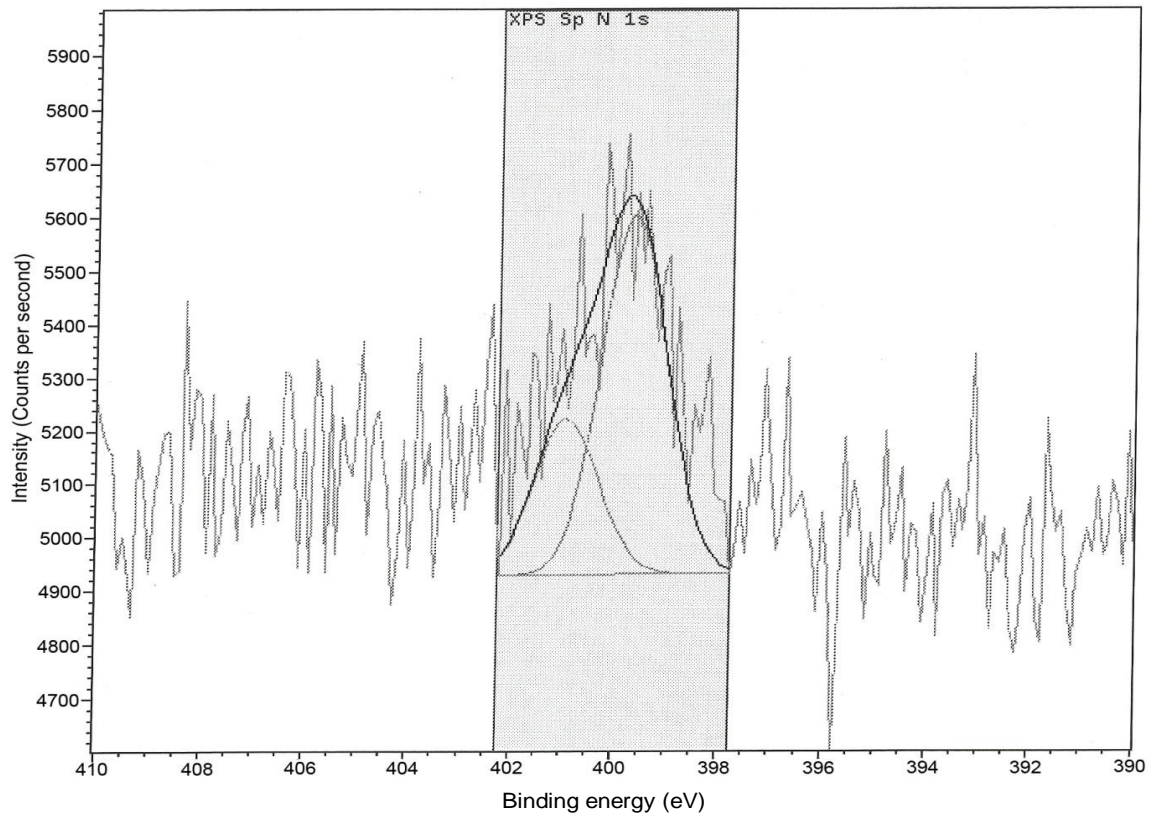


Figure 5. Narrow scan of N 1s peak.

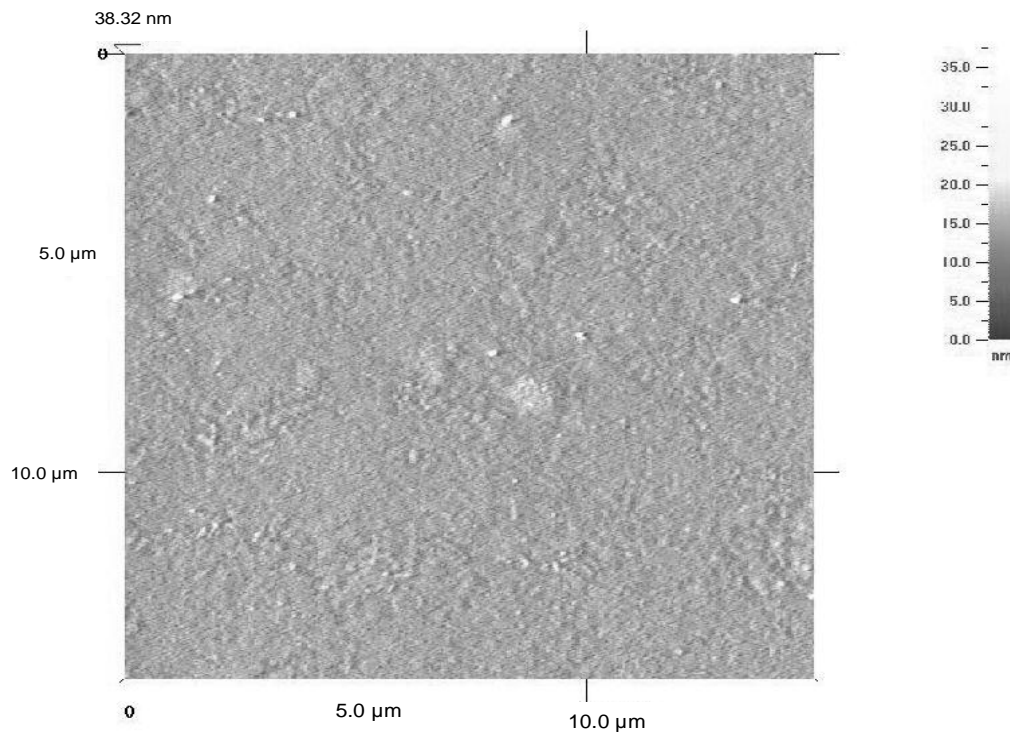


Figure 6. Topography AFM images of crosslinked chitosan thin film. Film morphology was observed in air after drying.

rosslinked chitosan thin film on the substrate.

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