View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by UKM Journal Article Repository

Sains Malaysiana 49(9)(2020): 2251-2260
http://dx.doi.org/10.17576/jsm-2020-4909-22

Synthesis and Characterization of Acylated Low Molecular Weight Chitosan and Acylated Low Molecular Weight Phthaloyl Chitosan

(Sintesis dan Pencirian Kitosan Berjisim Molekul Rendah Terasil dan Kitosan Ftaloil Berjisim Molekul Rendah Terasil)

RAHADIAN PERMADI, VICIT RIZAL EH SUK & MISNI MISRAN*

ABSTRACT

Oral drug delivery is one of the most convenient routes due to its painless administration and high patient compliance. However, oral administration is becoming more difficult to be conducted due to its poor water solubility, poor dissolution rate, and low oral bioavailability in the gastrointestinal tract. Herein, we develop a strategy to produce a chemically modified chitosan using depolymerization and introducing hydrophobic groups onto the chitosan backbone through acylation. By modifying the structure of chitosan, we aim to overcome limitations of drug delivery before and after the oral administration. The successful acylation of protected (using phthalic anhydride) chitosan and unprotected (without phthalic anhydride) chitosan was proved by Fourier transform infrared (FTIR). FTIR was conducted not only to characterize the functional group changes but also to find quantization of degree of acylation (DA) and the degree of substitution (DS) of chitosan before and after acylation. The particle size of chitosan was found ranges from 300-500 nm with zeta potential value shifted from -50 mV to a more positive value as acid anhydrides concentration increased. The Field Emission Scanning Electron Microscopy (FESEM) images showed the low molecular weight of chitosan and acylated chitosan nanoparticle possess non-spherical form with hollow structure. In addition, the size obtained was in accordance with the size measured by particle size. Hydrophobically modified chitosan has been successfully synthesized via acylation on both primary hydroxyl and amine groups on the backbone of chitosan. This chemically modified chitosan can enhance drug solubilization as well as improving biocompatibility and degradability.

Keywords: Acylation; biomaterials; chitosan; oral drug delivery; polymer synthesis

ABSTRAK

Penghantaran ubatan melalui oral merupakan cara paling mudah berikutan tidak menyakitkan dan dipatuhi oleh pesakit. Walau bagaimanapun, kaedah oral menjadi susah untuk dijalankan berikutan keterlarutan air yang rendah, kadar pelarutan yang rendah dan bioketersediaan oral yang rendah pada saluran perut usus. Di sini, kami membangunkan strategi untuk menghasilkan kitosan terubah suai kimia menggunakan kaedah pempolimeran dan memperkenalkan kumpulan hidrofobik pada rantaian kitosan melalui tindakan pengasilan. Dengan mengubah suai struktur kitosan, kajian ini bertujuan untuk mengatasi batasan penghantaran ubatan sebelum dan selepas pengambilan secara oral. Kejayaan proses pengasilan pada kitosan terlindung (menggunakan anhidrida phthalik) dan kitosan tak terlindung (tanpa anhidrida phthalik) telah dibuktikan menggunakan spektroskopi inframerah transformasi Fourier (FTIR). FTIR bukan sahaja digunakan untuk pencirian kumpulan berfungsi tetapi juga untuk mengkuantumkan darjah pengasilan (DA) dan darjah penukargantian (DS) kitosan sebelum dan selepas pengasilan. Purata saiz zarah kitosan adalah antara 300-500 nm dengan nilai keupayaan zeta dianjakkan dari -50 mV ke nilai yang semakin positif selari dengan peningkatan kepekatan asid anhidrida. Mikrograf daripada mikroskop pengimbas elektron medan pancaran (FESEM) menunjukkan nanozarah kitosan berjisim molekul rendah dan kitosan terasil mempunyai bentuk tak sfera dengan struktur berongga. Tambahan lagi, saiz yang diperoleh adalah bertepatan dengan purata zarah saiz yang telah diukur. Kitosan terubah suai hidrofobik telah berjaya disintesis melalui pengasilan pada kumpulan hidroksil primer dan amina pada rantaian kitosan. Kitosan terubah suai kimia ini berupaya untuk meningkatkan pemelarutan ubatan dan juga menambahbaik biokeserasian dan penguraian.

Kata kunci: Biobahan; kitosan; pengasilan; penghantaran ubatan oral; sintesis polimer

Introduction

Among the multiple drug delivery routes, the oral administration has gained much attention due to some advantages such as high patient compliance, ease of administration, and cost-effective process (Araujo et al. 2017; Banerjee et al. 2016; Choi et al. 2014, 2013; Hu & Luo 2018). Even though the oral route is the most convenient and most desirable delivery method to administer active pharmaceutical ingredients, there are not many commercial drugs available on the market that can survive from the harsh environment in the gastrointestinal (GI) tract (Homayun et al. 2019). After the oral administration, drugs need to pass through some barriers before arriving in the systemic circulation thereby reducing oral bioavailability (Werle et al. 2009). Moreover, viscous mucosal layer within GI tract hinder the drug attachment and following administration to the systemic circulation. In order to address this problem, mucoadhesion is used as one of the effective strategies to prolong drug residence time in GI tract (Ensign et al. 2012). For the past decades, chitosan has been investigated as one of the promising drug delivery carriers due to its excellent physical and biological properties. Chitosan offers some advantages as an oral delivery drug carrier such as it is safe, biodegradable, and it shows excellent mucoadhesive properties (Balata et al. 2018; Khajuria et al. 2018; Ren et al. 2018; Takayama et al. 1990; Yu et al. 2018). Despite the fact of the advantages chitosan possess, the usage of chitosan in the industry has been greatly limited by its poor solubility in water as it is only soluble in acidic aqueous medium. Various types of chemically modified chitosan have been studied in order to address this limitation. Modification to improve the properties and add additional functionality to chitosan is usually conducted within two reactive functional groups, one is amino group at the 2-carbon position and the others are two hydroxyls at the 3 and 6 carbon position (Lee et al. 2012). The presence of free electron pair at amine group make chitosan a reactive compound which leading to the formation of new functionality by chemical modifications.

Acylation is one of chemical modification methods which can increase the organic solubility of chitosan. Acylation is achieved by altering the functional groups in chitosan and replace it with acyl group. Most of the acylation process occurred at amine group as it is more reactive than hydroxyl group, but it is also possible to conjugate the acyl group at hydroxyl groups via protection of amine group. To the best of our knowledge, so far there is no report on studies of acylation of chitosan on hydroxyl group and amino group at the same time. We wish to report a new synthesis method using acyl anhydride to attach hydrophobic moieties to the chitosan backbone resulting in the formation of chitosan self-aggregates with the hydrophobic core in the aqueous environment.

Materials and Methods

Materials

Chitosan (with molecular weight of 100,000-150,000 kDa) was obtained from Acros Organics (USA). Phthalic anhydride, hexanoic anhydrides (C6), *N,N*-dimethylformamide (DMF), acetic acid glacial, methanol, ethanol, and acetone were purchased from Merck. Sodium nitrite ($NaNO_2$) was obtained from Spectrum. Sodium hydroxide (NaOH) was obtained from Fluka (Switzerland).

Preparation of low molecular weight chitosan (LMWC)

The preparation of low molecular weight chitosan was conducted as previous method reported by Tan and Misran (2013) with slight modification. First, chitosan solution (1% w/v) was prepared by adding chitosan (MW_{avg} = 150 kDa) in acetic acid 1% and mixed thoroughly. Then, an appropriate amount of 0.1 M $NaNO_2$ was added in a dropwise manner into chitosan solution and it was stirred under magnetic stirring for 1 h. After that, the pH was adjusted to an alkaline pH of 8-9 in order to precipitate the undissolved chitosan. Next, the undissolved chitosan was filtered, and the filtrate was neutralized to pH7 followed by precipitation using acetone. The precipitate was collected by using a centrifugation at 6500 rpm for 5 min at 25 °C and dried overnight using a vacuum oven (Tan & Misran 2013).

Preparation of *N*-phthaloyl chitosan (LMWC-PA)

The preparation of *N*-phthaloyl chitosan was conducted as previous method reported by Kurita et al. (2002). To a solution of 6 g of phthalic anhydride in 100 mL of *N,N*-dimethylformamide (DMF) containing 5% (v/v) water was added 3 g of low molecular weight chitosan, and the reaction mixture was heated at 120 °C with stirring under N_2 atmosphere. After 5 h of reaction, the mixture was cooled to room temperature and poured into ice water. The precipitate was collected on a filter, washed with methanol, and dried to give the final product (Kurita et al. 2002).

Preparation of low molecular weight acylated chitosan (LCha)

Acylation of low molecular weight chitosan was carried out using a reported method by Tiew and Misran (2017). Briefly, 1% (w/v) of low molecular weight chitosan was dissolved in a mixed solvent of 1% acetic acid:methanol at a ratio of 1:1. The mixed solution was then neutralized before the dropwise addition of acid anhydrides.

Afterwards, the solution was left overnight and neutralized before it was precipitated with large quantity of acetone. The solution was then centrifuged at 5000 rpm for 2 min at 25 °C. The collected precipitate was washed excess methanol and dried overnight under vacuum (Tiew & Misran 2017).

CHARACTERIZATION

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The FTIR analysis was conducted using Perkin Elmer FTIR-Spectrum 400 spectrometer for analysis and identification of absorption bands associated with the vibration of functional groups present in the molecule. The spectrometer was run at a frequency range of 450-4000 cm^{-1} with a resolution of 4 cm^{-1}. Triplicate measurement was made, and the mean average was used. The degree of acylation (DA) was then calculated by using an equation described in previous report (Kasaai 2008) as shown herewith:

$$DA = \left(\frac{A_{1655}}{A_{3450}}\right) x\ 100/1.33$$

where A_{1655} is the absorbance of the amide I band at 1655 cm^{-1} and A_{3450} is the absorbance of the hydroxyl band at 3450 cm^{-1}. The factor '1.33' denoted the value of the ratio of A_{1655}/A_{3450} for fully N-acetylated chitosan. The degree of substitution (DS) was determined by calculating the degree of acylation (DA) difference between LMWC and LCha.

AVERAGE MW DETERMINATION

The average molecular weight (MW) of chitosan was determined using Static Laser Scattering (SLS) method using a Malvern Nano ZS Zetasizer Zen 3600 (Malvern Instruments Ltd, UK) (Wu et al. 1995).

CONTACT ANGLE MEASUREMENTS

Contact angles of wettability were measured using a Krüss goniometer contact angle (Krüss GmbH, Germany). A glass slide (1 cm^2) was put on a movable sample stage and levelled horizontally; then, a drop of about 3 µL of samples was placed on the surface of the glass slide using microsyringe. Triplicate measurement was made, and the mean average will be used.

PARTICLE SIZE AND ZETA POTENTIAL

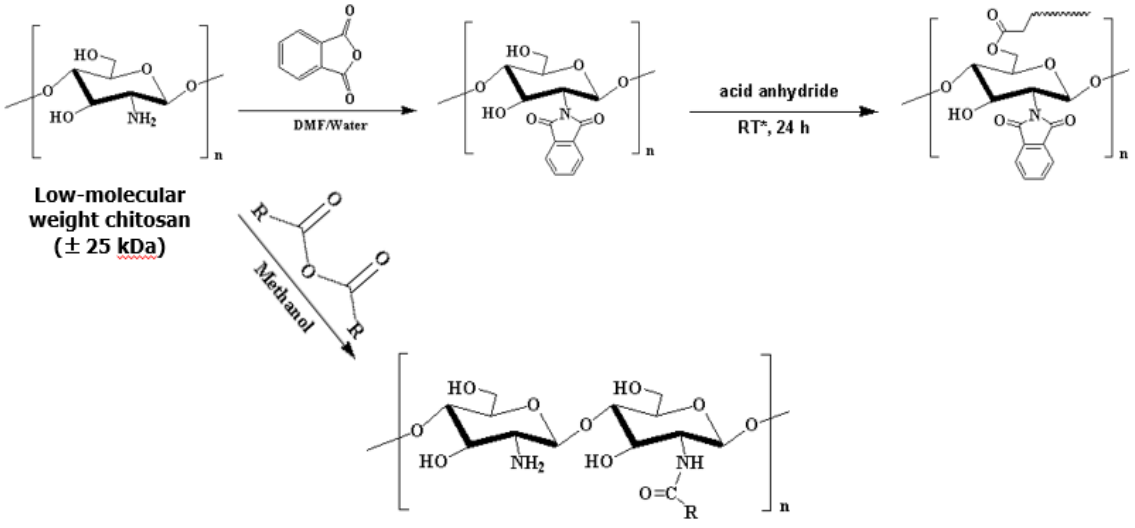
The measurement of mean particle size and zeta potential of *N*-acylated chitosan and *O*-acylated chitosan were measured using Malvern Nano ZS Zetasizer Zen 3600 (Malvern Instruments Ltd, UK). The sample was dissolved in 1% acetic acid and carefully transferred into gold plated U-shaped capillary cell (Bondar et al. 2012; Klodzinska et al. 2010). The samples were investigated at backscattered angle of 173° and using scattering laser light 633 nm at 25 °C. The mean particle size and zeta potential were obtained from three measurements.

FIELD-EMISSION SCANNING ELECTRON MICROSCOPY

The surface of low molecular weight chitosan and acylated chitosan were studied using FESEM (Model SU8220 Hitachi). Pieces of samples were taken and mounted in copper stubs. Samples were observed using an accelerating voltage of 5 kV and magnification at 10,000 and 20,000×.

RESULTS AND DISCUSSION

The present study describes acylation of low molecular weight chitosan (LMWC) using acyl anhydride to introduce hydrophobic moieties for use as a matrix for drug delivery, as shown in Scheme 1.



SCHEME 1. Synthesis of acylated chitosan

Acylation process was conducted after oxidative depolymerization of chitosan using sodium nitrite. By having degradation process, chitosan can be converted to its LMWC which present good water solubility. The LMWC nanoparticle and acylated chitosan (LCha) structure was examined by Fourier-transform infrared (FTIR) spectroscopy as depicted in Figure 1.

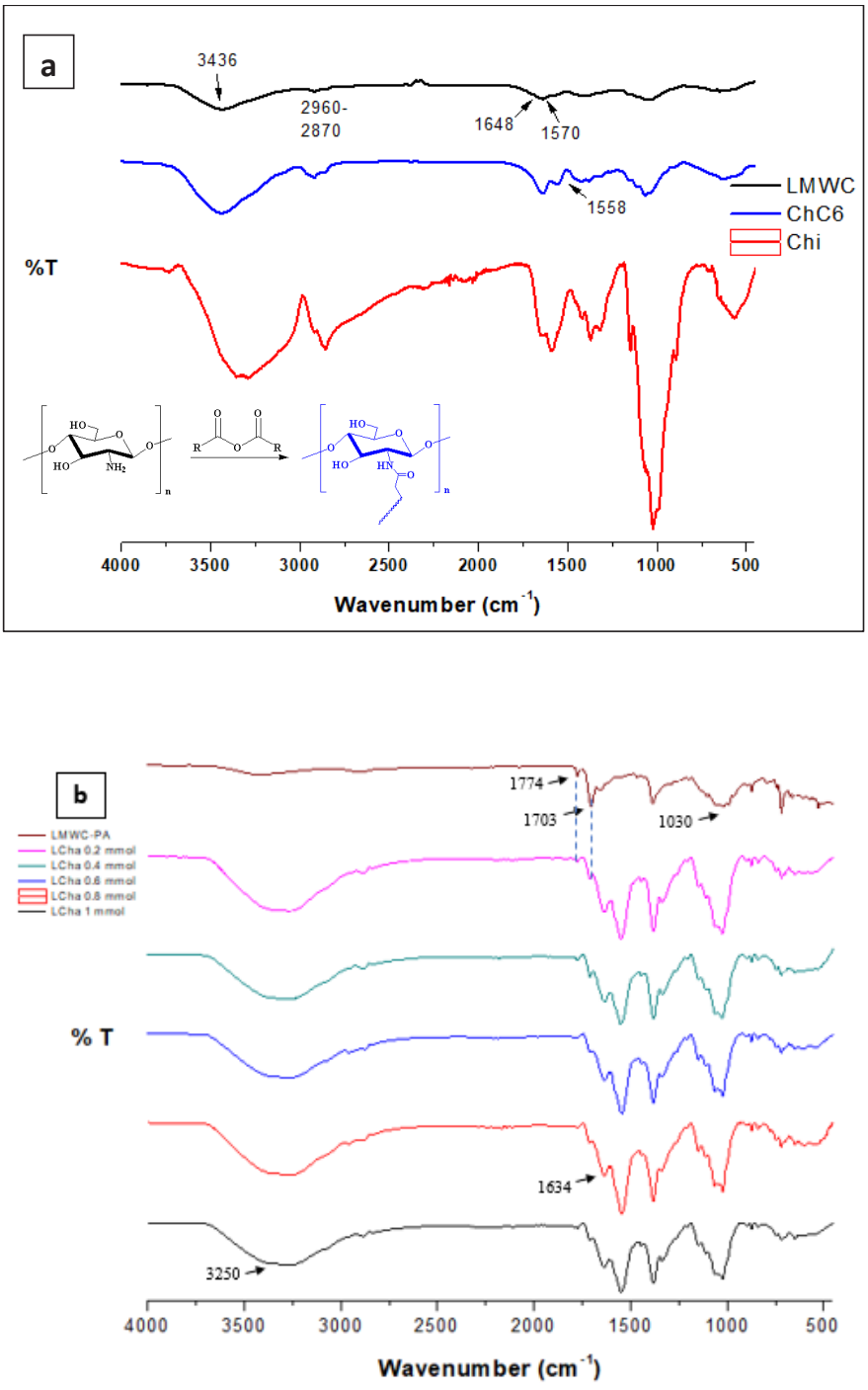


FIGURE 1. FTIR spectra of (a) chitosan (chi), LMWC, and LCha (ChC_6); (b) LMWC-PA and LCha (0.2-1 mmol addition of hexanoic anhydride)

As observed in Figure 1(a), there is no significant change in the spectra of low molecular weight chitosan (LMWC in black line) and native chitosan before depolymerization (Chi in red line). This was proved by a peak at 3436 cm^{-1} which attributed to OH vibration, a peak at 1570 cm^{-1} corresponding to NH bending of primary amine and a peak at 1648 cm^{-1} from carbonyl stretching of secondary amide. All these peaks attributed to the major functional group of chitosan, indicating there is no structural change after depolymerization of chitosan. After acylation on LMWC (ChC6 in blue line), a new peak appeared at 1558 cm^{-1} attributed to NH bending of secondary amide which is confirmed that the acylation has been successfully done on amine group of chitosan.

Acylation on hydroxyl group has also been successfully conducted via protection of amino group using phthalic anhydride as shown in Figure 1(b). After the protection on amino group, there was some new peaks at 1703 and 1774 cm^{-1} corresponding to carbonyl stretch imide of phthalimide group confirming that the protection of amino group chitosan has been made. Next, the acylation on hydroxyl group was examined by observing a new peak at 1634 cm^{-1} from carbonyl stretch of acyl group and some peaks which is remained the same at 1703 and 1774 cm^{-1} for phthalimide and 3250 cm^{-1} for the remaining hydroxyl group of chitosan.

The molecular weight (MW) of the sample was determined using Static Light Scattering (SLS) which measure the intensity of the scattered light to obtain the average molecular weight of a macromolecule as described in Rayleigh equation (Priyadarshini et al. 2013). According to the equation, the scattering light intensity at a given angle depends on several factors, some of them include molecular size and molecular weight of the sample. The larger the molecules in molecular weight, the more light will be scattered by them. The average MW of LMWC was found around 23 kDa. The degree of substitution (DS) was determined by calculating the degree of acylation (DA) difference between LMWC-PA and LCha as shown in Table 1 and was found around 7-8%. The DA indicates the number of hydrophobic moieties (acyl groups) that is successfully conjugated to the chitosan, while DS designates the amount of hydroxyl group that is being replaced with acyl groups.

TABLE 1. Degree of acylation (DA) and degree of substitution of LMWC-PA and LCha

Sample	DA (%)	DS (%)
LMWC-PA	82.473	-
LCha 0.2	74.921	7.552
LCha 0.4	73.798	8.675
LCha 0.6	74.150	8.323
LCha 0.8	74.746	7.727
LCha 1	73.958	8.515

TABLE 2. Contact angle of pure chitosan and LCha

Sample	θ_1	θ_2	θ_3	$\theta_{Average}$
Chitosan	18°	22°	16°	18.7°
Lcha 0.2	28°	32°	35°	31.7°
Lcha 0.4	30°	29°	23°	27.3°
Lcha 0.6	38°	42°	40°	40°
Lcha 0.8	48°	47°	50°	48.3°
Lcha 1.0	40°	42°	42°	41.3°

The contact angle of chitosan and LCha has been conducted as shown in Table 2. There was a difference between pure chitosan and LCha as chitosan show smaller angle compared to the acylated chitosan. Normally, contact angle less than 90° attributes to hydrophilicity of surface, while if it is more than 90° represents hydrophobic surface (Yuan & Lee 2013). Even though all samples showed contact angle less than 90°, the acylated chitosan possessed higher contact angle. This is because the pure chitosan has more hydrophilic groups such as hydroxyl groups and amino groups and it is in agreement with the previous report (He et al. 2019). Thus, we can justify that the hydrophobic moieties have been successfully conjugated to the acylated chitosan.

The particle size and zeta potential of LMWC and acylated chitosan were carried out using zetasizer. As shown in Figure 2(a), the mean particle size of LMWC ranges from 200-300 nm with polydispersity index (PDI) around 0.5. After the amino group of chitosan being protected, the mean particle size increased as the addition of phthalimide group. The increment of size might because of the steric hindrance of phthalimide group which is causing the particles to become larger compared to LMWC particles. The acylation process causes the mean particle size of acylated chitosan (LCha) to decrease as the amount of mole feed increasing. This result is in lined with the investigation conducted by Lee et al. (1998) and Mekhail et al. (2012). As the DS of chitosan increased, the higher amount of hydrophobic alkyl group was substituted to the backbone of chitosan resulting a stronger hydrophobic interaction between polymer chains and as a result more compact and smaller particle size formed.

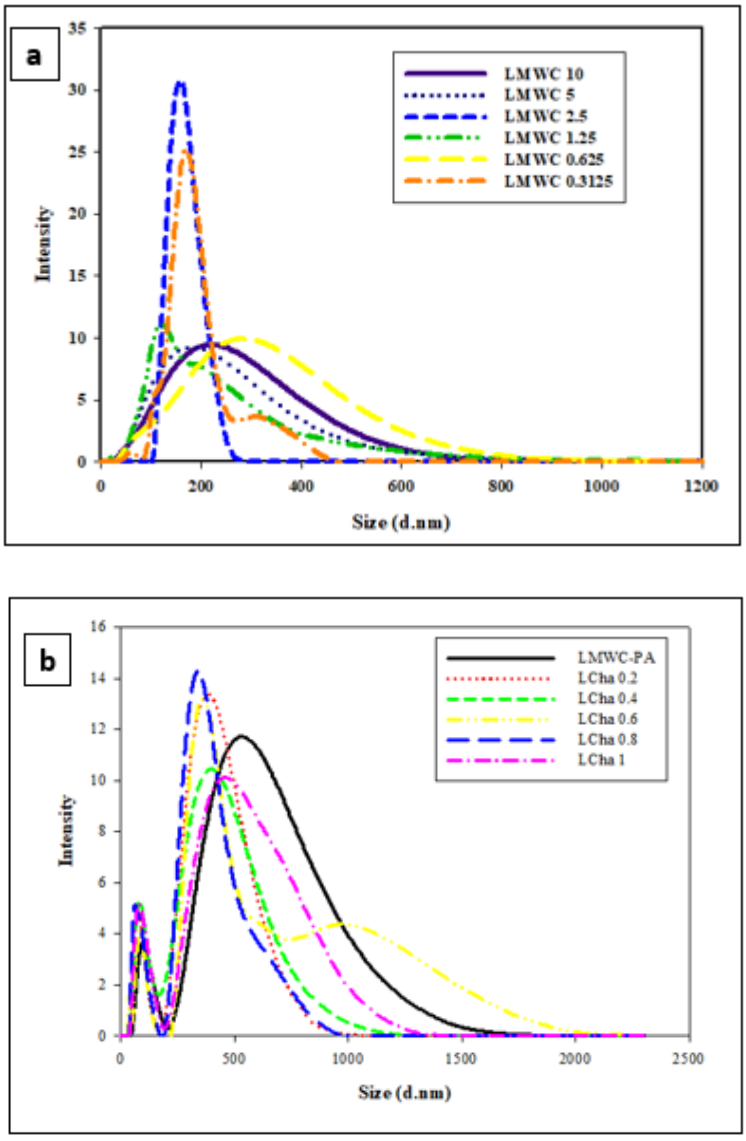


FIGURE 2. Particle size of (a) LMWC (b) LMWC-PA and LCha

The modification of hydroxyl group of chitosan can also be observed by the change of zeta potential as depicted in Figure 3. The ceaseless addition of acyl anhydrides shifted zeta potential to a more positive value. The zeta potential in 0.2 dropped because the DS is higher compared to LMWC-PA causing the cationic charge of amino groups being reduced to the higher amount. In addition, the zeta potential change in 0.8 and 1 mole was higher than the 0.2-0.6 mole of acyl anhydrides added. This might be because the positive charge amount of the unprotected amino group from 0.8 and 1 mole was higher compared to the alkyl group that reduced charges on the 0.2-0.6 mole ratio.

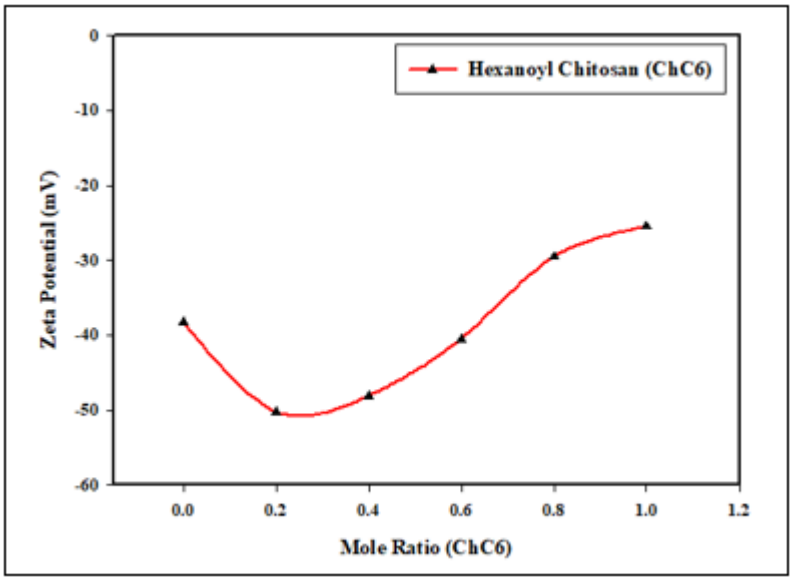
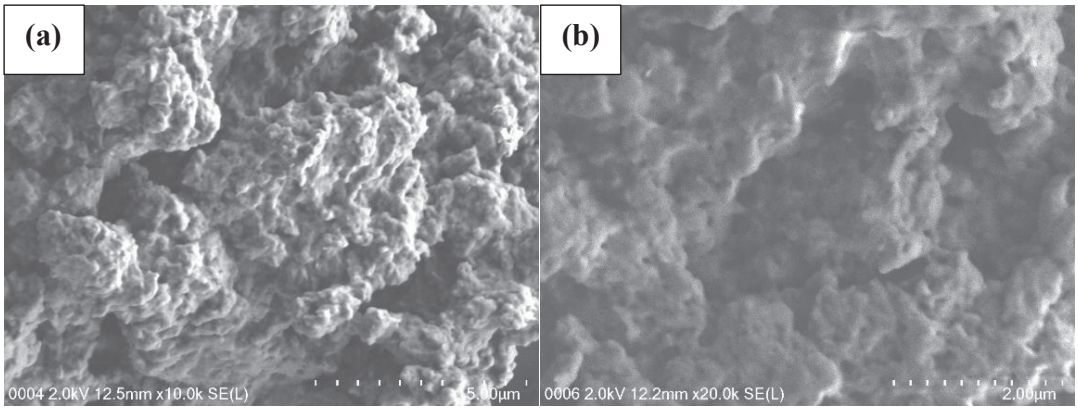


FIGURE 3. Zeta potential of LMWC-PA and LCha

Morphological studies were investigated using FESEM instrument. As portrayed in Figure 4, FESEM images of the LMWC surface and modified acylated chitosan exhibited vivid differences between them as they possess non spherical form with hollow structure.



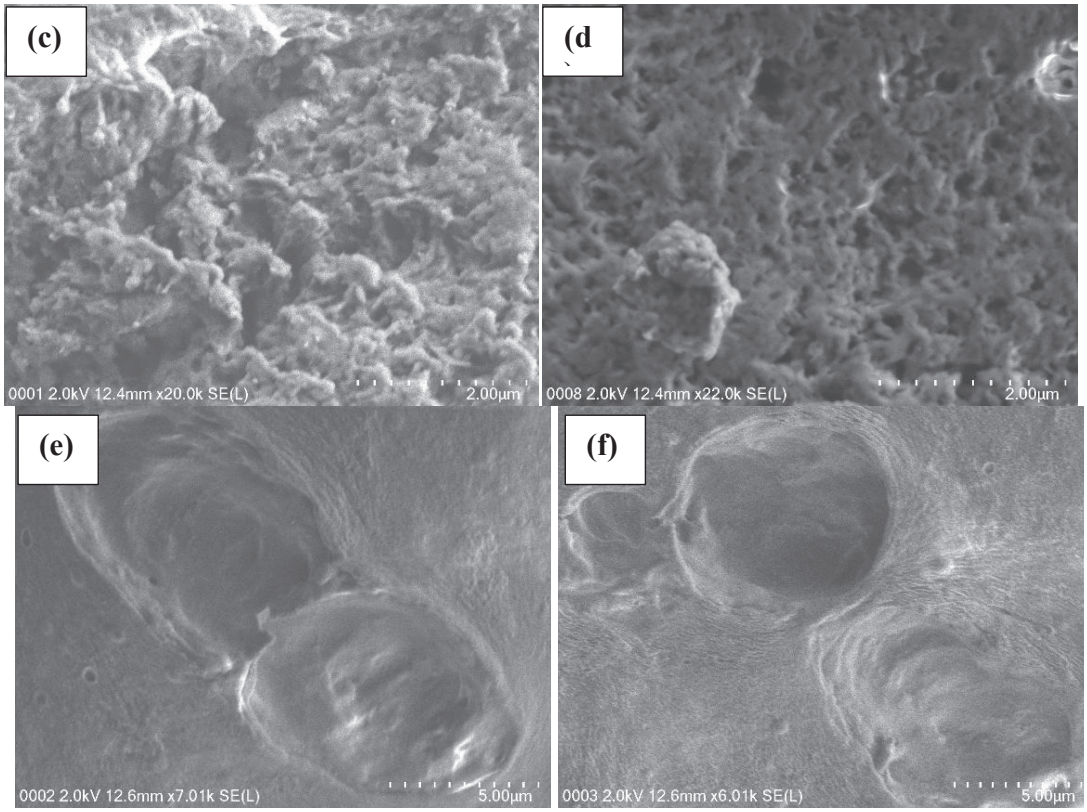


FIGURE 4. FESEM images of LMWC and modified acylated chitosan. The upper image: LMWC (scale bar used 2 and 5 µm) with 10,000 times magnified (a) and 20,000 times magnified (b); the middle image: LCha (scale bar used 2 µm) with 20,000 times magnified (c) and 22,000 times magnified (d); the lower image: LCha-PA (scale bar used 2 and 5 µm) with 7,000 times magnified (e) and 6,000 times magnified (f)

LMWC has net-like structures and multiple pores, this result is in lined with the previous report (Mai et al. 2012), whereas LCha-PA does not have any pores on the surfaces. This is because chitosan presents in a gel form owing to its functional groups which forming a network-like structure. Meanwhile, after the acylation, the structure becomes flat as the network structure is hindered by the acylation. LMWC has larger and various pores shape and its diameter roughly around 500 nm - 1 µm. On the other hand, LCha has smaller and regularly arranged pores and its diameter approximately around 200-600 nm. LMWC-PA has a flat surface with a crater-like structure on the middle of the surface.

Conclusion

Hydrophobically modified chitosan has been successfully synthesized via acylation process on the hydroxyl and amino group of chitosan using acyl anhydrides. Acylation was done after depolymerization of chitosan to its low molecular weight chitosan. The structural changes of chitosan were observed using FTIR instrument and it was found there was no change in depolymerization process of chitosan. The structural changes occurred only after the acylation was done on hydroxyl and amino group of chitosan. The particle size was increased after the acylation process from 200 to 500 nm with a zeta potential shifted to a more positive value. The morphological study performed by FESEM indicated that there is a clear change on the surface of LMWC and synthesized acylated chitosan.

ACKNOWLEDGEMENTS

The authors would like to express our gratitude to Shameer Hisham for his assistance and fruitful discussion.

We would also like to express our gratitude to the financial support given from University of Malaya Research (GPF065B-2018), (PR002-2018A) and (FP075-2018A).

REFERENCES

Araujo, F., das Neves, J., Martins, J.P., Granja, P.L., Santos, H.A. & Sarmento, B. 2017. Functionalized materials for multistage platforms in the oral delivery of biopharmaceuticals. *Progress in Materials Science* 89: 306-344.

Balata, G.F., Abdelhady, M.I.S., Mahmoud, G.M., Matar, M.A. & Abd El-Latif, A.N. 2018. Formulation of saudi propolis into biodegradable chitosan chips for vital pulpotomy. *Current Drug Delivery* 15(1): 97-109.

Banerjee, A., Qi, J.P., Gogoi, R., Wong, J. & Mitragotri, S. 2016. Role of nanoparticle size, shape and surface chemistry in oral drug delivery. *Journal of Controlled Release* 238: 176-185.

Bondar, O.V., Saifullina, D.V., Shakhmaeva, I.I., Mavlyutova, I.I. & Abdullin, T.I. 2012. Monitoring of the zeta potential of human cells upon reduction in their viability and interaction with polymers. *Acta Naturae* 4(1): 78-81.

Choi, H.J., Kim, M.C., Kang, S.M. & Montemagno, C.D. 2014. The osmotic stress response of split influenza vaccine particles in an acidic environment. *Archives of Pharmacal Research* 37(12): 1607-1616.

Choi, H.J., Ebersbacher, C.F., Kim, M.C., Kang, S.M. & Montemagno, C.D. 2013. A mechanistic study on the destabilization of whole inactivated influenza virus vaccine in gastric environment. *PloS ONE* 8(6): e66316.

Ensign, L.M., Cone, R. & Hanes, J. 2012. Oral drug delivery with polymeric nanoparticles: The gastrointestinal mucus barriers. *Advanced Drug Delivery Reviews* 64(6): 557-570.

He, Y., Miao, J., Chen, S., Zhang, R., Zhang, L., Tang, H. & Yang, H. 2019. Preparation and characterization of a novel positively charged composite hollow fiber nanofiltration membrane based on chitosan lactate. *RSC Advances* 9(8): 4361-4369.

Homayun, B., Lin, X.T. & Choi, H.J. 2019. Challenges and recent progress in oral drug delivery systems for biopharmaceuticals. *Pharmaceutics* 11(3): 129.

Hu, Q.B. & Luo, Y.C. 2018. Recent advances of polysaccharide-based nanoparticles for oral insulin delivery. *International Journal of Biological Macromolecules* 120(Part A): 775-782.

Kasaai, M.R. 2008. A review of several reported procedures to determine the degree of N-acetylation for chitin and chitosan using infrared spectroscopy. *Carbohydrate Polymers* 71(4): 497-508.

Khajuria, D.K., Patil, O.N., Karasik, D. & Razdan, R. 2018. Development and evaluation of novel biodegradable chitosan based metformin intrapocket dental film for the management of periodontitis and alveolar bone loss in a rat model. *Archives of Oral Biology* 85: 120-129.

Klodzinska, E., Szumski, M., Dziubakiewicz, E., Hrynkiewicz, K., Skwarek, E., Janusz, W. & Buszewski, B. 2010. Effect of zeta potential value on bacterial behavior during electrophoretic separation. *Electrophoresis* 31(9): 1590-1596.

Kurita, K., Ikeda, H., Yoshida, Y., Shimojoh, M. & Harata, M. 2002. Chemoselective protection of the amino groups of chitosan by controlled phthaloylation: Facile preparation of a precursor useful for chemical modifications. *Biomacromolecules* 3(1): 1-4.

Lee, D., Quan, Z.S., Lu, C., Jeong, J.A., Song, C., Song, M.S. & Chai, K.Y. 2012. Preparation and physical properties of chitosan benzoic acid derivatives using a phosphoryl mixed anhydride system. *Molecules* 17(2): 2231-2239.

Lee, K.Y., Jo, W.H., Kwon, I.C., Kim, Y.H. & Jeong, S.Y. 1998. Structural determination and interior polarity of self-aggregates prepared from deoxycholic acid-modified chitosan in water. *Macromolecules* 31(2): 378-383.

Mai, T.T.T., Ha, P.T., Pham, H.N., Le, T.T.H., Pham, H.L., Phan, T.B.H. & Nguyen, X.P. 2012. Chitosan and O-carboxymethyl chitosan modified Fe_3O_4 for hyperthermic treatment. *Advances in Natural Sciences: Nanoscience and Nanotechnology* 3(1): 015006.

Mekhail, G.M., Kamel, A.O., Awad, G.A.S. & Mortada, N.D. 2012. Anticancer effect of atorvastatin nanostructured polymeric micelles based on stearyl-grafted chitosan. *International Journal of Biological Macromolecules* 51(4): 351-363.

Priyadarshini, N, Sampath, M., Kumar, S. & Kamachi Mudali, U. 2013. Particle size variation and prediction of molecular weight of Bi (III) dydrolyzed polymer using light scattering technique. *ISRN Inorganic Chemistry* 2013: Article ID. 194120.

Ren, X., Chen, C., Hou, Y., Huang, M., Li, Y.B., Wang, D.Q. & Zhang, L. 2018. Biodegradable chitosan-based composites with dual functions acting as the bone scaffold and the inflammation inhibitor in the treatment of bone defects. *International Journal of Polymeric Materials and Polymeric Biomaterials* 67(12): 703-710.

Takayama, K., Hirata, M., Machida, Y., Masada, T., Sannan, T. & Nagai, T. 1990. Effect of interpolymer complex-formation on bioadhesive property and drug release phenomenon of compressed tablet consisting of chitosan and sodium hyaluronate. *Chemical & Pharmaceutical Bulletin* 38(7): 1993-1997.

Tan, H.W. & Misran, M. 2013. Polysaccharide-anchored fatty acid liposome. *International Journal of Pharmaceutics* 441(1-2): 414-423.

Tiew, S.X. & Misran, M. 2017. Encapsulation of salicylic acid in acylated low molecular weight chitosan for sustained release topical application. *Journal of Applied Polymer Science* 134(36): 44849.

Werle, M., Takeuchi, H. & Bernkop-Schnurch, A. 2009. Modified chitosans for oral drug delivery. *Journal of Pharmaceutical Sciences* 98(5): 1643-1656.

Wu, C., Zhou, S.Q. & Wang, W. 1995. A dynamic laser light-scattering study of chitosan in aqueous-solution. *Biopolymers* 35(4): 385-392.

Yu, Z., Li, B.Q., Chu, J.Y. & Zhang, P.F. 2018. Silica *in situ* enhanced PVA/chitosan biodegradable films for food packages. *Carbohydrate Polymers* 184(2018): 214-220.

Yuan, Y. & Lee, T.R. 2013. Surface science techniques. *Springer Series in Surface Sciences* 51(1): 3-34.

Department of Chemistry
Faculty of Science
Universiti Malaya
50603 Kuala Lumpur, Federal Territory
Malaysia

*Corresponding author; email: misni@um.edu.my

Received: 15 October 2019
Accepted: 8 May 2020