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Cite as: AIP Conference Proceedings **2663**, 020010 (2022); https://doi.org/10.1063/5.0108828
Published Online: 20 September 2022

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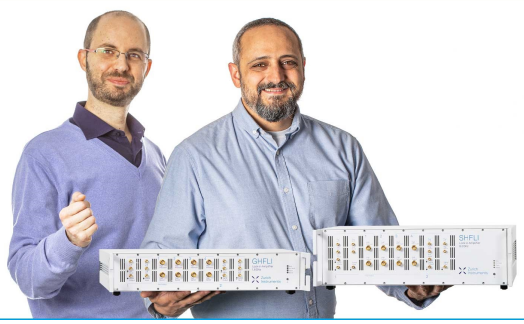

Synthesis and Characterization of Cryogel Apple Pectin-Chitosan As Physical Barrier Intraperitoneal Antiadhesion Postsurgical

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Abstract. Peritoneal Adhesion achieve high rates of general surgery and gynecological surgery.Adhesion can cause intestinal obstruction, slow healing process after surgery, abdominal pain and even death Cryogel aple pectin-chitosan is a new study that uses pectin from apples, chitosan and reacts with methods cryotopic gelation. This study will prove the effect of variations in chitosan concentration (0.6%, 0.7%, 0.8% and 0.9%) and identify the optimal concentration of chitosan through Fourier Transform Infrared (FTIR) test, tensile test and degradation test. The FTIR test results show that there is a vibration of protonated amine group (NH^{3+}) around the wave number of 1600 cm^{-1} and there is polyelectrolyte complex bonding. The optimal composition of intraperitoneal antiadhesi membrane is at 0.9% chitosan concentration with Ultimate Tensile Strength of 1.241 MPa, and the degradation rate of 87.9% on the 7th day. Based on the results of tensile test and degradation test, the higher concentration of chitosan, the lower Ultimate Tensile Strength and the faster degradation rate. Optimal composition is found in cryogel sample with 90% chitosan concentration.

Keywords: *intraperitoneal adhesion; cryogel, apple pectin; chitosan.*

INTRODUCTION

Abdominal surgery often cause the formation of adhesions. Surgical processes that can causeadhesion include cholecystectomy, gasterctomy, appendectomy, hysterectomy, colectomy, resection and vascular abdominal surgery [1]. Furthermore, the use of gloves during surgery can also lead to adhesion [2]. Adhesion occurs due to adhesions of two surfaces of the peritoneum that are injured after surgery. The percentage of intraperitoneal adhesions is 67-93% in general surgery and 97% in gynecological surgery [3]. In addition, adhesion canincrease the cost of care for patients [4]. In this regard, good anti-adhesion material is needed [5]. The requirements for the material used for antiadhesion is must have nontoxic, nonimmunogenic properties, can cover and attach to the wound area and hold on between 5-7 days. In addition, other properties that mustbe possessed by antiadhesion agents are biodegradable, and are easy to apply [6].There are several adhesion prevention agents that have been studied and developed such as anti-inflammatory drugs, anticoagulants, but the results are still not satisfying. There is also liquid intraperitoneal antiadhesion agents such as hyaluronic acid / carboxymethylcellulosa (Seprafilm) solutions. Seprafilm can reduce the incidence and level of postoperative adhesion. Unfortunately, not only the cost is expensive, but also in some cases it is thought to cause an increase in anastomotic leakage (incorporation of healthy intestines after the diseased intestine is cut) in intestinal splicing surgery [7]. Several attempts have been made to resolve the lack of antiadhesi agents, one of which is using cryogel that made from apple pectin and chitosan. Pectin is an anionic polysaccharide which mustbe added chitosan as a cation to form polyelectrolytes when making gels and films [8]. While the properties of chitosan can inhibit microbial activity, antitumor, hemostatic activity and can accelerate the process of wound healing. In addition,the characteristic of

The 3rd International Conference on Physical Instrumentation and Advanced Materials (ICPIAM) 2021
AIP Conf. Proc. 2663, 020010-1–020010-5; https://doi.org/10.1063/5.0108828
Published by AIP Publishing. 978-0-7354-4393-8/$30.00

chitosan is biodegradable and biocompatible [9]. The antibacterial properties of chitosan can fightbacteria Staphylococcus aureus and Streptococcus pyogenes which have been known to be one of the main causes of postoperative wound infection [10].

In a previous study by Konovalova et al (2017) [11] given chitosan concetration 0.7%. The results showed good antiadhesion activity, but the degradation time was around 4 days. This is not in accordance with the requirements of a good antiadhesion agent which must hold on up to 5-7 days in intraperitoneal [6]. The addition of chitosan affects the rate of degradation due to the presence of amine groups found in chitosan [11]. Based on that result , this study will focus on the synthesis and characterization of apple pectin-chitosan antiadhesion agent with variations concentrations of chitosan 0.6%, 0.7%, 0.8% and 0.9% to improve the degradation rates of cryogel.

MATERIAL AND METHODS

Acetylation Of Chitosan

Chitosan was carried out in a water-alcohol medium with a ratio 1: 1.7 v / v. 1gram of chitosan was dissolved in 1% acetic acid-methyl alcohol and stir for 0.5hours. 2.5 mL acetylating agent 6.4 mM was added into 1 gram of chitosan. Thereaction takes 5 minutes at a temperature of 22°C. Then chitosan is dialyzed andfreeze dry [12].

Making Pectin Solution

Apple pectin powder (1 gram) is weighed on a digital balance, then inserted littleby little into a glass beaker. Add the solvent until the volume reaches 100 ml. This solution is then stirred until it is homogeneous [11].

Mixing Chitosan and Pectin

Chitosan solution 0.6%, 0.7%, 0.8% and 0.9% was poured into 0.25% acetic acid and added with Calcium Chloride ($CaCl_2$) 0.12 M. Then, 1 ml of chitosan- calcium solution was frozen at temperature -18°C for 1 hour, coat with solutionof apple pectin and leave it for 8 hours at room temperature [6]. The gel formedis freeze dried and then sterilized using UV-irradiation for 1 hour [11].

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is an infrared spectroscopic method that uses an infrared beam that has different light frequencies and measures how much beam of light can be absorbed by the sample [13]. FTIR steps are cryogel membrane is placed between two KBr plates. Analysis of sample functional groups was carried out by comparing the transmittance bands formed in the infrared spectrum with correlation tables andusing a comparative spectrum [6].

Tensile Test

Tensile tests are carried out to complete the information on the strength of a material and supporting data for material specifications [14]. Tensile test steps are, first the membrane is cut, then the thickness of the membrane is measured with a digital micrometer and membrane is associated with the test equipment. After that, the load is loaded (Newton) and the membrane is pulled at a certain speed until it breaks. When cryogel breaks, the weight of the pulling load is recorded and put into the equation to get the Ultimate Tensile Strength value.

$$UTS = Fmax / A_0 \quad (1)$$

$$e = (l_i - l_0) / l_0 \quad (2)$$

Degradation Test

Cryogel sample degradation test is carried out by simulating the sample under physiological conditions which serves to observe quantitatively the weight of the sample lost before and after immersion in a Phospate Body Simulation (PBS) solution. Cryogel samples were incubated at 37 oC for 1, 3, 5, and 7 days. A 1.5 cm membrane is weighed as a dry weight (Wo). Then the cryogel sample was immersed in 3 mL PBS (pH = 7.4). After soaking, the sample is removed from the medium, washed with distilled water. After that, sample is frozen and freeze dried. Then weighed as wet weight (Wt) [11]. The rate of degradation is expressed as a percentage of weight loss at each time interval calculated.

RESULTS AND DISCUSSIONS

Fourier Transform Infrared Spectroscopy (FTIR) Test Result

In the FTIR spectrum of apple pectin can be seen in the wave number 1739.79 cm^{-1} and 1625.99 cm^{-1} each has a C = O stretching vibration of methyl esterified carboxylic groups and COO- asymmetric stretching vibration which is a characteristic group of pectin. The use of apple pectin with a low esterification level due to good ability gelling when the presence of Ca^{2+} ions [16]. Based on the FTIR test, in all cryogel samples there were absorption of chitosan and pectin wave numbers and the presence of NH_2 protonation to NH^{3+} was evidenced by the absorption at 1600 cm^{-1} that overlapping each other.

In the spectrum of chitosan that has been carried out acetylation, it can be seen in the wave number 3463 cm^{-1} that there is an overlapping of OH stretching vibration and NH stretching. At wave number 1654 cm^{-1} there is a C = O vibration of amide groups are also commonly known as amide I ribbon. The addition of an acetyl group on the process of the emergence of acetylation visible absorption at wave number 1081 cm^{-1} which is -CO stretching vibration and the absorption at wave number 2922 cm^{-1} which is a CH stretching vibration. From the spectrum results of the two main ingredients above, it will be compared with the results of the sample spectrum of cyogel apple pectin- chitosan. From the results of the spectrum of cryogel samples in various chitosan concentrations there is an overlapping between the vibrations of OH groups and NH stretching groups in the wave number around 3700-3000 cm^{-1}. At wave numbers 1726-1739 cm^{-1} shows the vibration of carbonyl (C = O) groups from pectin. Overlapping occurs again in wave numbers around 1600 cm^{-1} in all cryogel samples. It is because, at wave numbers around 1600 cm^{-1} there is a vibration of Amide I from chitosan, COO- stretching from apple pectin and asymmetric bending from NH^{3+}. The presence of absorption asymmetric bending from NH^{3+} indicates the amine group of chitosan has been protonated to NH^{3+} [17]. There is a shift in the absorption groups of cryogel sample. This is because there are polyelectrolyte complex bonds between chitosan and apple pectin [17].

TABLE 1. Fourier Transform Infrared Spectroscopy (FTIR) Test

Groups	Wave Numbe r (cm^{-1})					
	A	B	C	D	Pectin	Kitosan
O-H StretchN-H	3408.22	3428	3405	3394.72	3383.14	3463
Bending	1627.92	1630	1634	1627.92	-	1654
C-H Bending	1436.97	1440	1440	1438.9	-	1420
C=O Esterified	1735.93	1739	1732	1726.29	1739.79	-
C-H Stretching	2937.59	2920	-	2949.16	2937.59	2922
COO- Stretching	1012.63-1147.65	1009- 1243	1009- 1277	1010.7-1246.02	1020.34-1228.66	1027-1260
COO- Stretching	1627.92	1630	1634	1627.92	1625.99	-

Tensile Strength Test

From data tensile strength (Figure 1) is known that the increase in sample's length is very small, which is around 0.15-0.25 cm. This indicates that cryogel samples are not flexible. The degree of deacetylation (DD) of chitosan in each sample is not fulfilled the standard. The acetylation group is very high and NH_2 groups are low. In the material with higher concentration of chitosan, the bonds are weaker because more acetyl groups can block NH^{3+} and COO^- bonds. This could be influence the value of Ultimate Tensile Strength to become lower. At relatively low DD, according to Chatelet *et al.* who reported an increase in brittleness as the DD increased [18]. Wenling *et al*. is stated that at higher DD, the mechanical performance is showed the inverse relationship between chitosan swelling and mechanical strength, as swelling increases, the mechanical strength decreases [19].

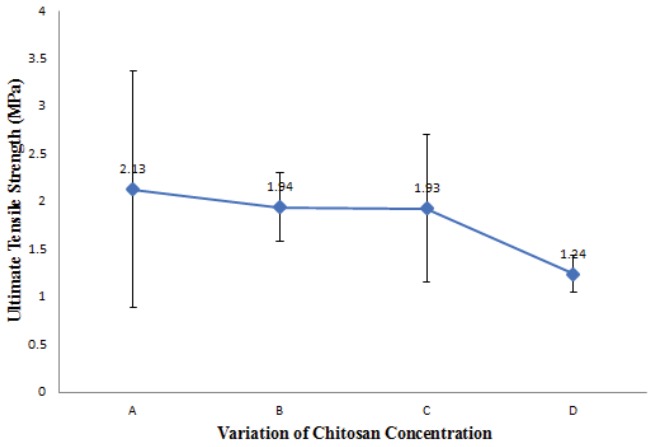

FIGURE 1. Tensile Test Result

Degradation Test

In this study, the degradation percentage of sample A was 83.4%, sample B was84.6%, while for samples C and D were 87.2% and 87.9% respectively on 7th day. From these results showed in Figure 2, the increasing concentration of chitosan given the faster degradation rate. This is because the addition of chitosan with a low deacetylation degree can increase the degradation rate [20]. Chitosan with low deacetylation degree contains more acetyl groups than amino groups. It causes fewer amino groups of chitosan that can bind to carboxyl groups pectin so that the bond becomes weaker and the rate of degradation becomes faster [12].

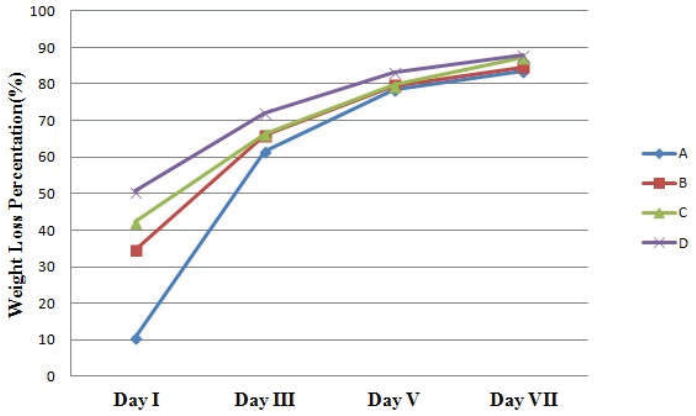

FIGURE 2. Degradation Test Result

CONCLUSION

Based on the results of tensile test and degradation test, the higher concentrationof chitosan, the lower Ultimate Tensile Strength and the faster degradation rate. Optimal composition is found in cryogel sample with 90% chitosanconcentration.

ACKNOWLEDGMENTS

The authors would like to thank Institute of Tropical Disease UniversitasAirlangga for the fasilitation and technical support

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