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Effects of Tricalcium Phosphate Addition as A Filler on The Properties of Chitosan Based Adhesive

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Abstract. Interest in medical bioadhesives, such as wound closing and tissue repair, has increased in recent decades because of its advantages. Chitosan has been investigated in several studies and can become a bioadhesive. In this study, fillers and photoinitiators were added to the chitosan based bioadhesive, and the mechanical properties of the bioadhesive were analyzed. The fillers were added into bioadhesive at concentrations of 0.25, 0.5, and 1 %w/v. The photoinitiator was added into bioadhesive at 0, 0.05, 0.1, and 0.2%w/v concentrations. The results of SEM, FTIR, and DSC were analyzed. The analysis results show that the filler concentration of 1% w/v has mechanical properties near optimal, where the viscosity is 62.54 cP, solid content 12.1%, and tensile strength is 34 kPa. The SEM results show that adding filler will increase the homogeneity and quality of the bioadhesive. The FTIR results show that the bioadhesive has amine and alcohol groups with and without filler in the adhesive. Adding a photoinitiator to the bioadhesive, which was analyzed using DSC, showed that it would slightly speed up the adhesive reaction time and increase the material's melting point. The increase in filler concentration will also increase the viscosity and solids content of the bioadhesive. The best adhesive combination is the TCP filler concentration 1%w/v and photoinitiator BPO 0.05%w/v. In the future, bioadhesives in medical treatment can be a potential. This research will be a benchmark for applying bioadhesives in medical treatment.

Keywords: Benzoyl Peroxide, Chitosan, Bioadhesive, Filler, Photo Initiator, Tricalcium Phosphate

INTRODUCTION

The adhesive is one of the materials often encountered in everyday life and can be used to permanently glue two or more objects through an adhesive binding process (Ebnesajjad, 2008). Bioadhesive is frequently used in the medical field, especially in surgery, to glue tissue or wounds (Li *et al.*, 2020). The advantages of using bioadhesive compared to the thread are that it is easier to use, is strong and flexible, does not interfere with the wound healing process, and reduces the risks of swelling and trauma for the patients (Vakalopoulos *et al.*, 2013). The bioadhesive must have high biocompatibility, low toxicity, and be biodegradable (Kuznetsova *et al.*, 2020). Bioadhesive is also used to deliver drugs to tissue, also known as a drug delivery system. A drug delivery system delivers drugs to certain tissues in humans or animals (Tiwari *et al.*, 2012). One

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of this field's most popular bioadhesives is cyanoacrylate or super glue. However, this product is toxic in high concentrations and has a biocompatibility problem, so this product is restricted to several uses only (Cohen *et al.*, 2014).

Chitosan ($C_{56}H_{103}N_9O_{39}$, MW: 1526.45) is a cationic polysaccharide compound from deacetylated chitin and can be used as wound hydrogel. Chitin is usually found in invertebrate animals. The chitosan structure is a straight-chain polymer composed of D-glucosamine and N-acetyl-D-glucosamine, shorter than the chitin structure (de Alvarenga, 2011). Nowadays, chitosan has the potential to be used in biotechnology and the medical field because the characteristics of chitosan are low toxicity, antimicrobial, and biodegradable, and it depends on the deacetylation degree of chitin. Chitosan is more widely used than chitin due to its higher water solubility.

Much of the present scientific literature states that chitosan has potential as a bioadhesive and is still under investigation. It is proposed that chitosan provides amine functional groups, and in bioadhesive, it is a positive sign because it could induce blood coagulation. This effect will be beneficial for patients with clotting factor deficiency. Some research has stated that the surface tension of chitosan decreases with increasing concentration, so it is easier to spread many types of materials. Other researchers stated that the viscosity of chitosan increases when the concentration of chitosan increases in that fluid, and the viscosity will decrease if the temperature increases (Saha *et al.*, 2020). A few researchers focused on the characteristics of chitosan itself and its potential for medical applications, such as tissue repair and wound healing. Limited studies have been concerned with the effect of adding filler to chitosan to strengthen its shear strength and initiator to increase its curing time.

This research aims to study the effect of adding a tricalcium phosphate filler to chitosan based bioadhesive on its mechanical properties, such as shear strength, viscosity, and solid content. Filler is a material that was added to the bioadhesive to increase its mechanical properties. Tricalcium phosphate is usually used in medical and pharmacy applications as a bioactive filler for bone. Another objective is to study the effect of peroxide initiator on chitosan based bioadhesive curing time. So, the main objective of this study is to analyze the potential chitosan based bioadhesive when the filler tricalcium phosphate and benzoyl peroxide were added.

MATERIALS AND METHOD

Materials

Chitosan (CAS 9012-76-4, technical grade), carrageenan (CAS 9000-07-1, technical grade), p-Toluenesulfonic acid (CAS 104-15-4, technical grade), ethanol (70%, CAS 64-17-5, technical grade), aquadest (CAS 7732-18-5), polyethylene glycol 4000 (CAS 25322-68-3, technical grade), and citric acid (CAS 77-92-9, technical grade) were purchased from CV General Labora. Tricalcium Phosphate (CAS 7758-87-4, technical grade), Benzoyl Peroxide (CAS 94-36-0, technical grade), phosphate buffered saline (CAS 7758-11-4, technical grade), and potassium sulfate (K_2SO_4, CAS 7778-80-5, technical grade) were purchased from Alfa Kimia. All materials were used without further purification. For tensile strength, the materials used were porcine skin, 2.5 cm x 2.5 cm wood block, and cyanoacrylate.

Instrument

Instruments used in this study were Brookfield Viscometer DV-E to measure the viscosity of the bioadhesive, universal testing machine Lloyd LR 5K to measure the tensile strength, SEM JSM-6510LA, FT-IR Spectrometer Thermo Scientific Nicolet iS10, and DSC-60 Plus Shimadzu for thermal properties analysis.

Methods

a. Carrageenan Crosslinked Preparation
   Carrageenan was weighed 2 grams and dissolved in 100 mL of distilled water at 50°C. The solution was heated and stirred using a hot plate magnetic stirrer up to 80°C.17.4 grams of potassium sulfate (K_2SO_4) weighed and dissolved in 100 mL of distilled water. The 0.1 N K_2SO_4 solution was added to the carrageenan solution after reaching 80°C and heated for 30 minutes at a fixed temperature. Ethanol with a temperature of 8℃ was added to the solution after 30 minutes of heating to precipitate carrageenan and form crosslinked carrageenan (CRG CL).
b. Bioadhesive synthesis
   Cross-linked carrageenan (CRG CL) was weighed 2 grams and dissolved in 100 mL aquadest at 50°C. The CRG CL solution was heated and stirred using a hot plate magnetic stirrer until the temperature reaches 80°C. Citric acid was weighed as much as 3.2 grams, and p-Toluenesulfonic acid catalyst was weighed as much as 1 gram. The citric acid and p-Toluenesulfonic acid catalyst were then put into the CRG CL solution, which was being heated. Chitosan (CS) was weighed 2 grams and then put inside the mixture after the temperature reached 80°C. Heating was carried out for 1 hour at a temperature of 80°C.
c. Filler adding
   Bioadhesive that had been heated for 1 hour at 80°C has been added with filler, namely tricalcium phosphate, with various concentrations of 0.125, 0.25, and 0.5% w/v. After added, the adhesive and filler were stirred for 30-45 minutes with a hot plate magnetic stirrer at 70°C until 50% of the solution remained from the initial solution.
d. Viscosity testing
   The adhesive that had been finished and added with variations in the ratio of filler was aged for 2 days. The test was carried out using Brookfield Viscometer DV-E. The Brookfield Viscosimeter was turned on, and a specified-sized spindle was mounted on the tool. The spindle specification was the s-02 type spindle with a rotating speed of 50-60rpm. This measurement was carried out on each experimental sample.
e. Tensile strength testing
   The sample was prepared according to ASTM F2258 – 05 (ASTM Standard). The tensile strength testing was conducted using the universal testing machine Lloyd LR 5K and carried out on each experimental sample.
f. Solid content testing
   Empty petri dishes were washed and dried in an oven at 105℃ for 10 minutes and then cooled in a desiccator for 15 minutes. The empty petri dish was weighed using a digital analytical balance. The adhesive that had been made was weighed as much as 3 grams, placed on a petri dish, and then baked in the oven for 2 hours at 105°C. A petri dish containing the adhesive was put into the desiccator to cool for 15 minutes. The petri dish and adhesive were cooled and then weighed using a digital analytical balance.

g. Scanning Electron Microscopy and Fourier Transform Infrared testing
   This testing was conducted with SEM JSM-6510LA for SEM analysis and FT-IR Spectrometer Thermo Scientific Nicolet iS10 for FTIR analysis.
h. Bioadhesive synthesis with photoinitiator
   Bioadhesive was polymerized, and a filler was added, as in section c. Benzoyl peroxide (BPO) photoinitiator prepared by dissolving in PEG and aquadest with a ratio of 30:70. BPO solution was made with a concentration of 0.05, 0.1, and 0.2% w/v. BPO solution that had been made was added into the remaining 50% liquid bioadhesive as much as 5 ml and stirred until room temperature. The bioadhesive and photoinitiator that had been made were tested for their thermal properties by using Differential Scanning Calorimetry.

RESULTS AND DISCUSSION

Viscosity Analysis and Bioadhesive Solid Content of Chitosan and Carrageenan with TCP filler

Viscosity is a parameter of the thickness of a fluid. The thicker the fluid, the higher the viscosity. In this study, a Brookfield Viscosimeter with a spindle size of 02 and a speed of 60 rpm was used to measure the viscosity of bioadhesive with the filler concentrations of 0%, 0.25%, 0.5%, and 1% w/v. Viscosity data on research is shown in Figure 1.

The initial viscosity of adhesive with the concentration of 0% w/v filler is about 32 cP, and the highest viscosity is reached when the filler concentration is 1% w/v. It can be seen that the x-axis is the concentration of filler in the bioadhesive, and the y-axis is the viscosity of the bioadhesive. It can be seen that the higher the filler concentration, the more viscous the bioadhesive becomes. The relationship between adhesive viscosity and filler concentration is close to linear, with the coefficient of determination 0.9413. Besides filler concentration, another factor that causes an increase in viscosity is the solids content in the adhesive. An oven with a temperature of 105℃ was used in this experiment to evaporate the liquid in the bioadhesive for 2 hours. The results of solids content in the bioadhesive with each filler concentration is shown in Figure 2.

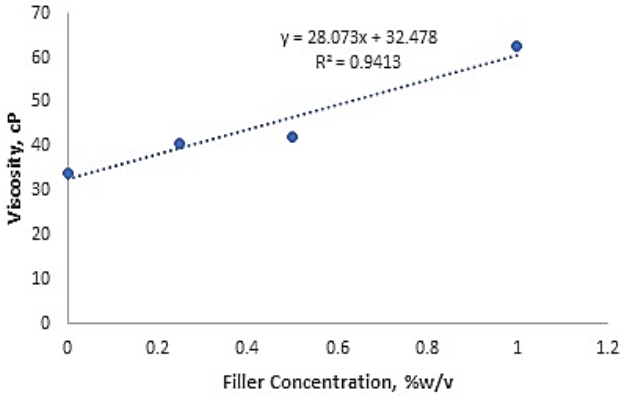

Fig. 1. Viscosity of adhesive for each filler concentration

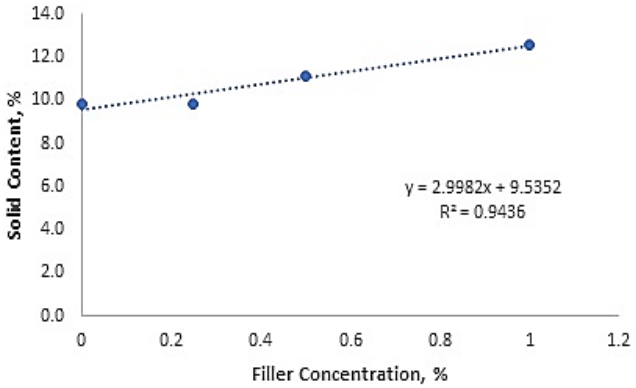

Fig. 2. Solid content of adhesive for each filler concentration

Solid content is the amount of solids contained in a solution. It can be seen that the higher the filler concentration, the more solids it contains because the greater the

mass of solids used. The relationship between filler concentration and solid content in bioadhesive shows an almost linear relationship with a coefficient of determination of 0.9436. The more solid content, the more viscous the bio-adhesive will be and the stronger the adhesion.

The mechanical properties, which are viscosity and solid content, are one of the properties that determine the quality of adhesive. The higher the viscosity of the adhesive, the higher the solid content. It will increase the strength of the adhesive, which will be explained in the next section.

Bioadhesive Tensile Strength Analysis of Chitosan and Carrageenan with TCP Filler

The tensile strength test was carried out using a universal testing machine with a sample of the dermis of pig skin. Tensile strength is the maximum tension of the adhesive in holding the two surfaces coated by the adhesive when pulled apart. It is one of the factors that determine the quality of a bioadhesive. In this study, the tensile strength of the adhesive was affected by the filler concentration, where the higher the filler concentration, the tensile strength increased because the filler could add tensile strength by filling in the empty spaces in the adhesive. The relationship between adhesive tensile strength and filler concentration can be seen in Figure 3.

It can be seen that the relationship between adhesive tensile strength and filler concentration is linearly related to the coefficient of determination of 0.9819. However, from the data obtained, the optimum filler concentration cannot be determined to provide maximum tensile strength. The more filler is added, the denser the adhesive will become because the solids content increases and will lose its tensile strength. From the data obtained, the best filler concentration is 1% w/v, and the value is better than 0% w/v filler. This result proves that the results obtained from this study are quite good and close to the reference results. The comparative study with another research is shown in Table I. However, it should be noted that the possibility of tensile strength can still increase if the filler concentration is more than 1% w/v. The optimum concentration needs to be studied further.

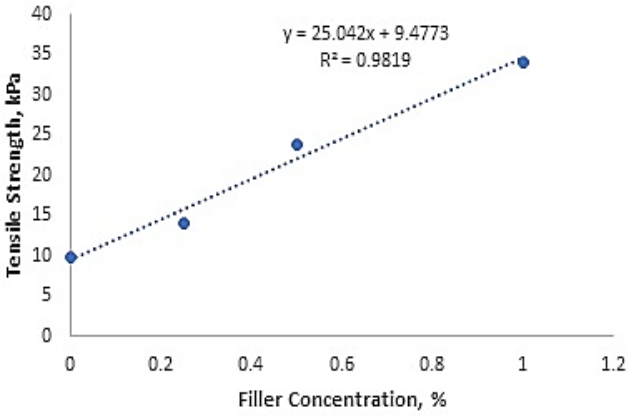

Fig. 3. Tensile strength of adhesive for each filler concentration

Table 1 shows a comparative study of tensile strength of materials. It can be seen from the table that the tensile strength for bioadhesive is around 15 – 50 kPa, making the bioadhesive from TCP filler the potential to be applied for medical treatment. The tensile strength of the adhesive is one of the important parameters for bioadhesive. Higher tensile strength makes the bioadhesive bonds stronger in the body and can be used as an alternative for thread in medical applications. The adhesive can be used to close the wound in medical applications, and the tensile strength of the adhesive is the parameter that determines the quality of adhesive that can be used on the wound.

Table 1. Comparative study for tensile strength

	Material	Research	Tensile Strength
1	Gelatin-Alginate based Bioadhesive using TCP Filler	Cohen *et al.* (2014)	15 kPa
2	Chitosan-Polyethylene Glycol based Bioadhesive	Du *et al.* (2021)	20 – 50 kPa
3	Biocomposite Material from Poly(Lactic Acid) with TCP Filler	Ferri *et al.* (2016)	56 MPa for 10%w/t
4	β-TCP for Adhesive Dentin Bonding	Alrefeai *et al.* (2021)	30.38 MPa
5	Calcium Phosphate Cements	Luo *et al.* (2016)	3 ± 0.6 MPa
6	Chitosan Film Bioadhesive with Lactic Acid Solvents	Khan *et al.* (2000)	59.87 ± 2.21 MPa
7	Chitosan Film Bioadhesive with Lactic Acid Solvents	Khan *et al.* (2000)	67.11 ± 1.27 MPa
8	Chitosan-Alginate Hydrogel	Rudiyardjo & Wijayanto (2017)	9.01 ± 0.65 MPa
9	Chitosan-Carageenan		34 kPa

Bioadhesive SEM Analysis of Chitosan and Carrageenan with TCP Filler

Figure 4 shows SEM analysis of material with or without filler addition. It can be seen that with the same magnification ratio, the filler concentration of 1%w/v is more homogeneous than the filler concentration of 0%w/v. This causes the tensile strength at 1% w/v filler concentration to be stronger because adding filler can affect the material's mechanical properties. Also, the filler will fill the space in the adhesive without reacting with the adhesive (Ebnesajjad, 2015). In a more homogeneous adhesive, the possibility of air filling the space will be smaller. If air is in the adhesive, the adhesive power will decrease, and the adhesive quality will decrease. Thus, the filler will improve the bioadhesive properties, such as viscosity and tensile strength.

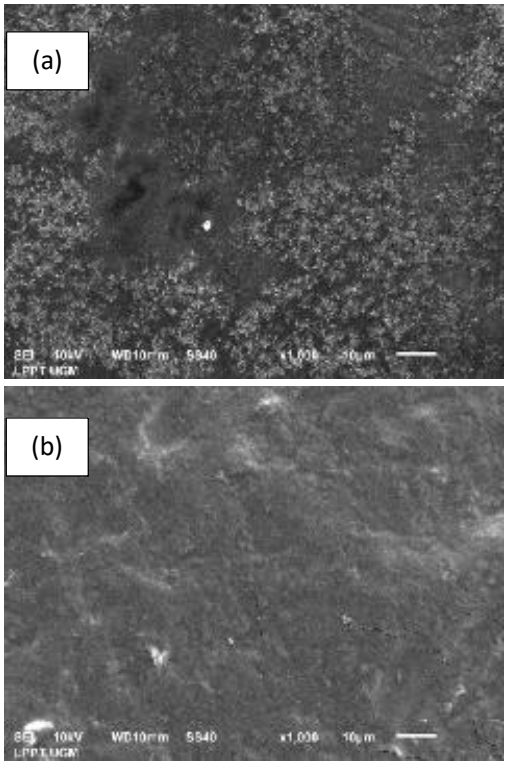

Fig. 4. SEM analysis result for filler concentration of (a) 0%w/v (b) 1%w/v

Bioadhesive FTIR Analysis of Chitosan and Carrageenan with TCP Filler

Figure 5 shows the FTIR spectra of material with or without filler addition. It can be seen from the FTIR analysis that there are 2 peaks at a filler concentration of 0%w/v and 3 peaks at a filler concentration of 0.5%w/v with a not too large height difference. This proves that the composition of the two types

of bioadhesive has similar groups and compositions. However, the two variations show only slight differences. Both variations have peaks at 3341.59 (0%w/v) and 3318.04 (0.5%w/v), indicating aliphatic primary amines in the form of N-H stretching secondary amines. The other peaks are at wavelengths of 1635.16 (0%w/v) and 1630.40 (0.5%w/v), which show the N-H deformations of primary amines. At 0.5%w/v, there is a peak at 1414.33, which indicates O-H bending alcohol.

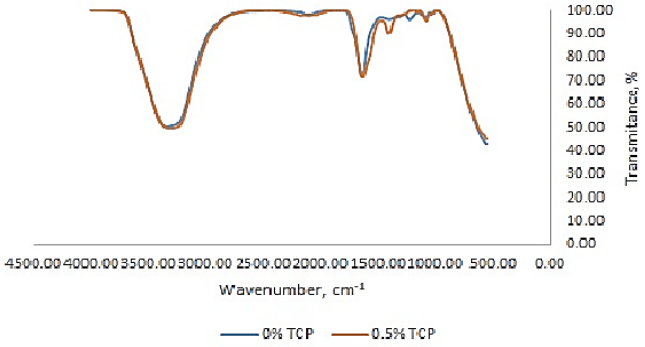

Fig. 5. FTIR analysis result

From the FTIR characterization, it can be concluded that the major bases of the bioadhesive are amines and bending alcohol. The chitosan has N-H and O-H bonds, while carrageenan has O-H bonds. The TCP filler has calcium and phosphate but is not shown in the FTIR graphic. This means that the filler didn't react with the bioadhesive; it is the same as the theory that the TCP only acts as a filler to increase the mechanical properties of the adhesive.

Bioadhesive Thermal Properties Analysis of Chitosan and Carrageenan with TCP Filler and Benzoyl Peroxide (BPO) Photoinitiator

The melting point of adhesive can be a parameter that determines if the adhesive is fit for the body. The average temperature of the human body is 36°C; if the adhesive melting point is below that, the adhesive will be too dilute for the body. Figure 6 shows the DSC curve of adhesive. The blue line shows the DSC vs. timeline. The peaks that show the melting point are 26.94°C at a photoinitiator concentration of 0%w/v and 27.34°C at a photoinitiator concentration of 0.05%w/v. This proves that the increase in melting point is due to an increase in the concentration of the photoinitiator, but the increase is quite small due to the addition of benzoyl peroxide (BPO), which is not too much. If the addition of a photoinitiator is excessive, it will cause the photoinitiator not to be able to dissolve in the adhesive mixture. In addition, the reaction time due to the addition of the photoinitiator concentration will decrease; this means that the compound will undergo a reaction faster, but the change is not too significant. The resulting reaction is exothermic; heat is required for a larger reaction at a smaller BPO concentration. The other parameter is the curing time of the adhesive. The faster the curing time for adhesive, the better the quality of the adhesive will be because the adhesive will dry up faster. The use of other photoinitiators need to be studied.

CONCLUSIONS

From this study, it can be concluded that adding filler will affect the mechanical properties of chitosan based bioadhesive. The higher the concentration of filler added, the better the mechanical properties, especially the tensile strength of the bioadhesive. It is the important parameter that determines the adhesive's quality. The highest tensile strength is reached when the added filler concentration is 1%w/v with the value of 34 kPa. The addition of photoinitiator

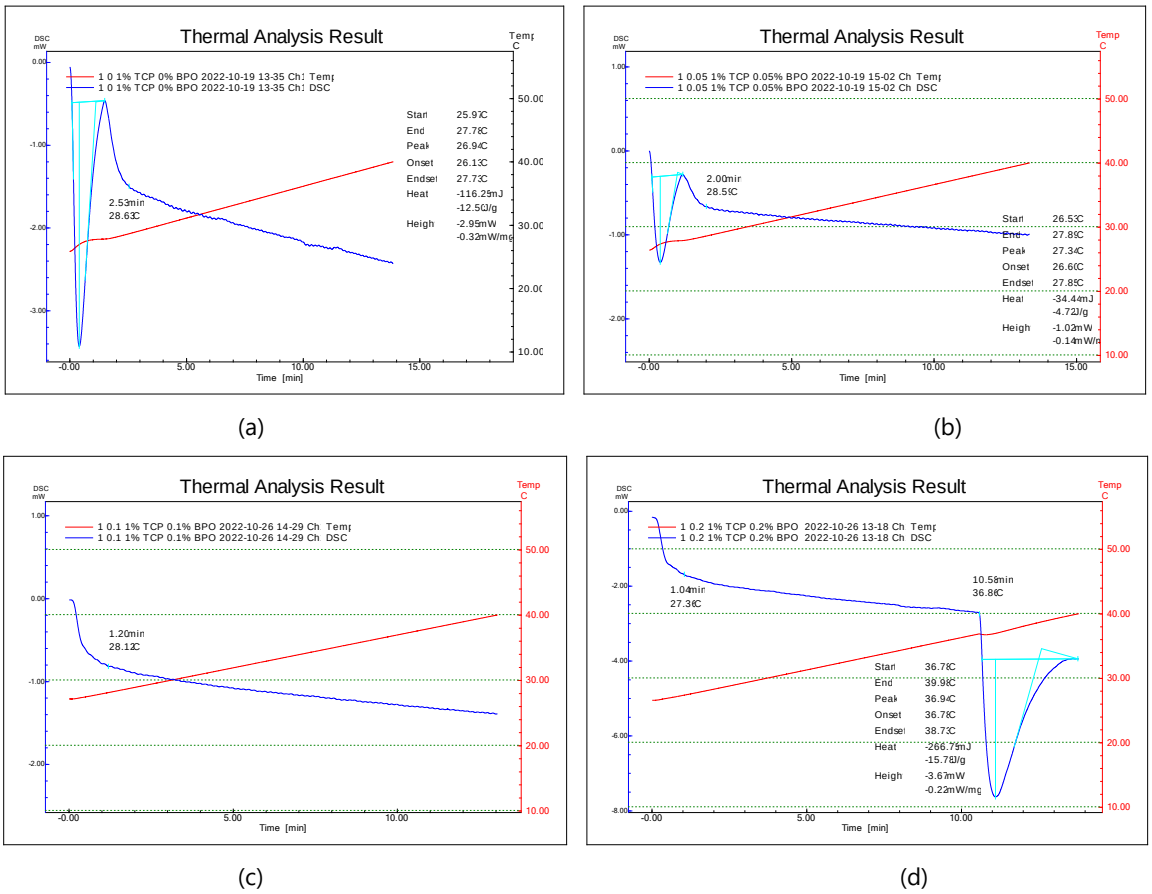

Fig. 6. DSC analysis result for initiator concentration of (a) 0%w/v (b) 0.05%w/v (c) 0.1%w/v (d) 0.2%w/v

to bioadhesive does not significantly affect the mechanical properties of the adhesive, but the best concentration of BPO is 0.05%w/v with a melting point reaching 27.34℃. However, the optimum concentration of the filler must be found by increasing the concentration until it reaches its peak point so that the bioadhesive can work better, and the toxicity of the bioadhesive is needed to measure how safe the bioadhesive is for medical treatment. This research can be further developed so that the use of chitosan based bioadhesive with TCP filler can be applied in medical treatment and improve the potential of bioadhesive. This research can also be a benchmark for another study about bioadhesives and their benefits for medical applications or as an alternative for wound treatment.

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