Indonesian Physical Review
Volume 7 Issue 1, January 2024
P-ISSN: 2615-1278, E-ISSN: 2614-7904

Analysis of Hydroxyapatite/Gelatine Composition on the Filament Formation Using Piston Extrusion Method

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Article Info

Article info:
Received: 13-09-2023
Revised: 12-01-2024
Accepted: 15-01-2024

Keywords:
Filaments; Viscosity; Hydroxyapatite; Gelatine; Extrusion Piston

How To Cite:
Y. A. Tandanu, D. J. D. H. Santjojo, and Masruroh, "Analysis of Hydroxyapatite/Gelatine Composition in the Filament Formation Using Piston Extrusion Method", Indonesian Physical Review, vol. 7, no. 1, p 152-165, 2024.

DOI:
https://doi.org/10.29303/ipr.v7i1.273.

Abstract

HA/Gelatine bio composite is the main material in making scaffolds that have the advantage of biocompatibility and high biodegradability. One method of making scaffolds is using piston extrusion 3D printing technology, which is compatible with several types of materials, especially HA/gelatine biocomposites. This study aims to determine the effect of HA/gelatine bio composite composition on the filament formation process which is influenced by the rheological properties of the material. The filament extrusion process is influenced by rheological properties in the form of viscosity. The synthesized HA material was then dissolved with gelatin in the ratio of 1:2, 1:3, 1:4, 1:5, and 1:6 homogeneously. After that, viscosity measurements were made on each variation of HA/gelatine composition with a viscometer. The biocomposite solution that has been mixed homogeneously is then extracted until it comes out of the nozzle. Meanwhile, the viscosity of the HA/gelatine bio composite solution when given piston pressure can be known through the calculation process. The viscosity test results show that there is a change in the viscosity of the solution. This is caused by the shear-thickening phenomenon due to the application of pressure on the fluid. Based on the experimental results, the extrusion results still do not form filaments, which indicates that the rheological properties of the HA/gelatine bio composite solution are still too liquid so other material modifications are needed. The extrusion speed of 0.42 mm/s used in this study is too fast for the HA/gelatin material solution, so it has not formed optimal filaments.

Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is an alternative material that is often used in the health world to treat bone fracture problems. Hydroxyapatite is composed of calcium phosphate material so that it can regenerate bone tissue itself. This is because most mineral fractions in human bones have the same structure as hydroxyapatite. Hydroxyapatite also has other advantages, such as high bioactivity and biocompatibility, and is not corrosive [1]. However, hydroxyapatite has disadvantages, namely low mechanical strength, and biodegradation, so modification is needed to increase the efficacy of good bone regeneration [2]. Hydroxyapatite also cannot be used as a single scaffold material [3]. Therefore, a combination of organic polymer materials is needed to handle this problem. Gelatine is an organic polymer material that is currently being developed. Gelatine has biocompatible and biodegradable properties, so it can resemble an extracellular matrix (ECM) because it has the same functional groups and can create a porous structure [4]. In addition, gelatine can increase the cell differentiation process [5]. The hydroxyapatite and gelatine bio composite is used as the main material in making scaffolds [6].

Scaffolds are significant in tissue engineering because they allow cells to grow and develop into bone tissue. In addition, the scaffold plays a role in stimulating cell adhesion so that bone tissue function can be maintained [7]. Scaffolds are made using several methods, such as freeze-drying [6] and electrospinning [8]. However, both methods have disadvantages, namely difficulties in controlling pore size, shape, and porosity. One method that can overcome this problem is 3D printing. 3D printing is an alternative method for overcoming tissue engineering problems in these scaffolds. With appropriate modifications and techniques, biologically active molecules and ligands can be recognized by cells physically or chemically associated with the surface of the scaffold. This is because 3D printing has a high level of precision and can handle interconnectivity problems in scaffold manufacturing [9].

One type of 3D printing often used is piston extrusion 3D printing. Piston extrusion 3D printing uses an extrusion system with a press as a piston to expel the solution from the nozzle to form a filament. The advantages of this type are that it is compatible with various materials and has a high resolution and level of precision [10]. The factor that influences the success of the filament formation process in piston extrusion is the material ratio [11], machine 3D printing parameters [12], and environmental influences [13]. This article focuses on determining the effect of hydroxyapatite and gelatine composition on the filament formation process. Based on research from Syifa et al. [14], a solution consisting of 3 grams of gelatine dissolved in 10 mL of distilled water succeeded in optimally forming filaments. Meanwhile, the solution consisting of 2 grams and 4 grams of gelatine dissolved in 10 mL of distilled water did not form optimal filaments. A high viscosity will cause the extrusion to clot at the nozzle. Meanwhile, a too low viscosity will result in extrusion results that are too runny.

Therefore, further research is needed to add hydroxyapatite material to the composition of the gelatine solution so that it can be utilized in scaffold development. This study aims to determine the effect of adding gelatine by 2, 3, 4, 5, and 6 mixed with 1 gram of hydroxyapatite on the filament formation. The suitable bio composite composition is hoped to be found as an optimal and repeatable filament formation parameter.

Theory and Calculation

Viscosity is a rheological property that measures how resistant a substance is to movement or flow. Viscosity plays a significant role in the process of filament formation. If the viscosity of the solution is too low, then filaments cannot be formed from 3D printing because the solution is too liquid. However, if the viscosity is too high, it will be difficult for the solution to exit the nozzle so that filaments cannot form [15].

Based on the flow behavior and viscosity, viscosity properties can be divided into two types, namely Newtonian viscosity, and non-Newtonian viscosity [16]. Newtonian viscosity is a fluid property where the viscosity remains constant or does not depend on the applied pressure or shear speed. The solution will flow similarly and will not experience changes in flow behavior at high or low pressure [17]. Meanwhile, non-Newtonian viscosity is the property of a fluid with a viscosity value that changes with pressure or shear speed. In non-Newtonian viscosity, Newton's law of viscosity does not apply. Therefore, the stress curve is not linear [18]. Viscosity will change along with shear rate or stress changes [19]. Research conducted by Trachtenberg et al. [20] revealed that changes in viscosity values were caused by the shift rate, as shown in the following equation.

$$\eta_{app} = \frac{\tau_W}{\dot{\gamma}} \tag{1}$$

Based on the equation above, it shows that the viscosity after applying pressure (η_{app}) is influenced by shear stress(τ_W) and shear rate ($\dot{\gamma}$). Shear stress occurs in a fluid caused by two forces in opposite directions and parallel to the plane of the fluid. In other words, shear stress occurs due to parallel forces in the cross-section of the fluid [21]. The following equation can be used to determine the shear stress's magnitude.

$$\tau_W = \frac{d}{2}\frac{\Delta P}{2L} \tag{2}$$

Where d is the nozzle diameter (mm), ΔP is the pressure drop value, and L is the nozzle length (mm). Pressure drop significantly influences the print results from 3D printing extrusion pistons. When pressure is applied to a solution, friction will occur between the syringe wall and the solution, resulting in resistance between the syringe wall and the solution [22].

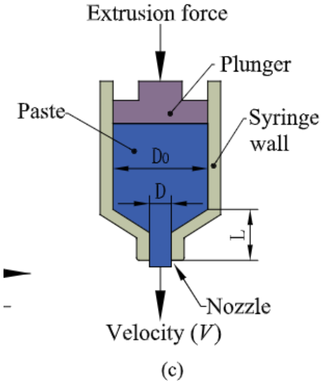

Figure 1 Extrusion Model with Plunger [22]

Based on the image above, it shows that the extrusion force generates the pressure. Factors such as nozzle size, material type, and viscosity influence the extrusion force. The plunger's pressure will increase as the nozzle size becomes smaller [23]. The pressure drop value can be calculated using the following Hagen-Poiseuille equation [24].

$$\Delta P = \frac{128\eta L Q}{\pi d^4} \tag{3}$$

η is the initial viscosity value measured using a viscometer, and Q is the solution flow rate. The following equation can be used to find out the Q value.

$$Q = \frac{V}{\Delta t} = \pi \left(\frac{d}{2}\right)^2 F \tag{4}$$

F is the total system speed in the form of extrusion and flow speeds. The extrusion speed is known to be 0.42 mm/s. Extrusion speed is the speed required by the lead screw to push the plunger so that it can rise and fall using a stepper motor. Meanwhile, flow speed is required for the solution to come out of the nozzle and form a filament. Based on research conducted by Geng et al. [25], increasing the filament width significantly influences the extrusion speed of 0-30 mm/minute. However, it tends to stabilize at the next extrusion speed. Apart from that, the filament width remains stable at a certain speed, so it is essential to know the printing speed limit that can be used. To determine the flow velocity, it can be determined using the following equation [25].

$$\frac{v_x}{v_a} = \left(\frac{D}{d}\right)^2 \tag{5}$$

Meanwhile, shear rate is a change in velocity that occurs in a fluid layer that passes through another layer at a certain distance [26]. The following equation can be used to find the shear rate value.

$$\dot{\gamma} = \frac{4Q}{\pi\left(\frac{d}{2}\right)^3} \tag{6}$$

Experimental Method

Preparation of Hydroxyapatite and Gelatin Bio composite Solution

Hydroxyapatite powder was previously synthesized via the coprecipitation method by Nawafi et al. [6]. The study has formed hydroxyapatite characteristics at 2θ peaks of 25.88^0, 31.72^0, 32.19^0, and 32.84^0 and successfully formed a Ca/P ratio of 1.69. Then, 1 gram was weighed and dissolved into 10 mL of distilled water. Gelatin powder made from cowhide (Nurra Gemilang, Malang) with mass variations of 2, 3, 4, 5, and 6 grams was dissolved into 10 mL of distilled water with a magnetic stirrer at 40^0C with a speed of 400 rpm. Then, the two solutions were mixed at the same temperature and speed for 1 hour.

Viscosity Testing Before Extrusion

The viscosity of the hydroxyapatite and gelatin bio composite solution with a predetermined composition ratio was then measured using a Brookfield viscometer. The viscometer spindle is selected as RV-4 type and then installed into the viscometer. Then, the rotation speed of the viscometer is set to be constant, namely 50 rpm.

Extrusion of Bio composite Solutions and Calculation of Viscosity Changes When Pressure is Applied

The solution is measured with a measuring tube of 1.5 mL and then put into a syringe tube. The extrusion system is arranged as in Figure 2. The extrusion speed and direction can be adjusted constantly at 0.42 mm/s with the Arduino IDE. Next, the plunger will apply constant pressure to the solution until it comes out of the nozzle. The viscosity of the bio composite solution when pressure is applied is then calculated using equation (1-5).

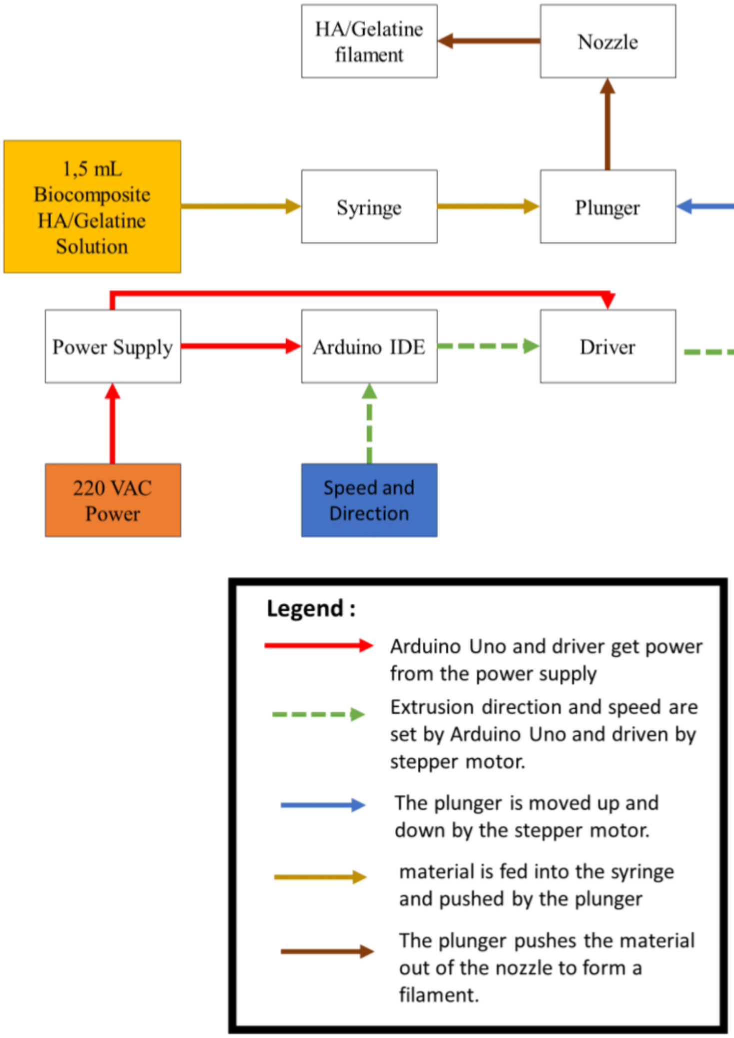

Figure 2 Design Extrusion System

Result and Discussion

Rheological Properties

Several factors influence extrusion molding results. One of the most critical factors that significantly influence the printing results is the rheological properties of the HA/gelatin bio composite solution. This rheological property is a reference in 3D printing because it concerns the deformation and flow of materials or fluids [27]. The rheological property that is generally measured is viscosity. Viscosity here can be interpreted as the resistance of a material to a flow, or in other words, it can also be interpreted as the force required to maintain a constant flow rate in a fluid [28]. This research uses measurements via a viscometer and calculations to determine the viscosity value. Viscosity measurement aims to determine the viscosity value before piston pressure is applied. Meanwhile, viscosity when pressure is applied can be obtained through a calculation process. The results of viscosity measurements before being subjected to pressure can be shown in the following graph.

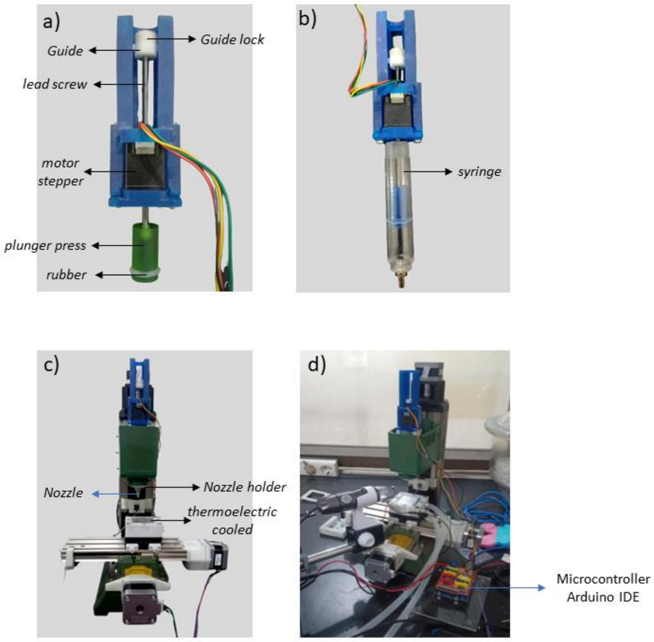

Figure 3 System Extrusion Piston Method

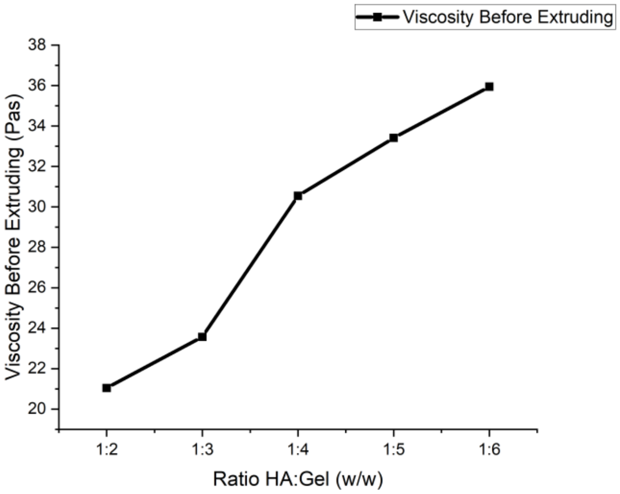

Figure 4 Viscosity Before Extruding

The viscosity value of the HA/gelatine bio composite solution increases when various gelatine concentrations are added. This is due to physicochemical properties and hydrogen bonds. Gelatine is a polysaccharide synthesized from collagen from animals. These physicochemical properties are influenced by several factors, namely temperature, pH, concentration, and time [29]. Gelatine has excellent thickening properties, so it will form a dense network when it is at ambient temperature. Therefore, the addition of gelatine will increase the viscosity of the solution. The more particles that influence this in the solution, the greater the particle density must be accommodated. Based on research conducted by Cutini et al. [30], hydrogen bonds exist between the PO_4^{3-} group in hydroxyapatite and the NH_2 group in collagen material. This is supported by research conducted by Siswanto et al.

[31], where hydrogen bonds are formed between hydrogen atoms in the OH- group in gelatine and oxygen atoms in hydroxyapatite. This interaction allows gelatine molecules and hydroxyapatite particles in solution to bond with each other, thus forming a strong and stable matrix. Therefore, hydrogen bonds will affect the viscosity of the solution.

Meanwhile, to find the viscosity value of the HA/gelatine bio composite solution when pressure is applied, one can use equation (1-5). Based on equation (6), the fluid flow and nozzle diameter influence the shear rate. Based on equation (4) calculations, the fluid discharge value is 18.62 mm^3/s. This is because the extrusion speed used in this study is only one variation of 0.42 mm/s with a flow speed of 94.2 mm/s. So, the shear rate of the fluid also has the same value, namely 2.96 s^{-1}. Meanwhile, to find the shear stress value, the pressure drop value must be known first using the Hagen-Poiseuille equation. This equation explains fluid flow through a cylindrical tube involving piston extrusion in 3D printing. Equation (3) shows several factors influencing the pressure drop value in piston extrusion 3D printing: solution viscosity, flow rate, nozzle diameter, and nozzle length. However, the Hagen-Poiseuille equation only applies to laminar solution conditions and the absence of turbulence [31]. This laminar flow occurs when fluid particles move in regular and parallel layers [32]. In laminar flow, fluid particles near the syringe walls move more slowly, while fluid particles in the middle of the flow move faster. Piston extrusion 3D printing requires a fluid classified as laminar flow with no turbulence effects so that the filament can exit the nozzle [33]. Other research conducted by Dee et al. [34] used calcium phosphate as a ceramic material to manufacture scaffolds, including laminar flow fluid. Based on equation (3), a pressure drop graph can be obtained for each gelatine hydroxyapatite composition ratio variation as follows.

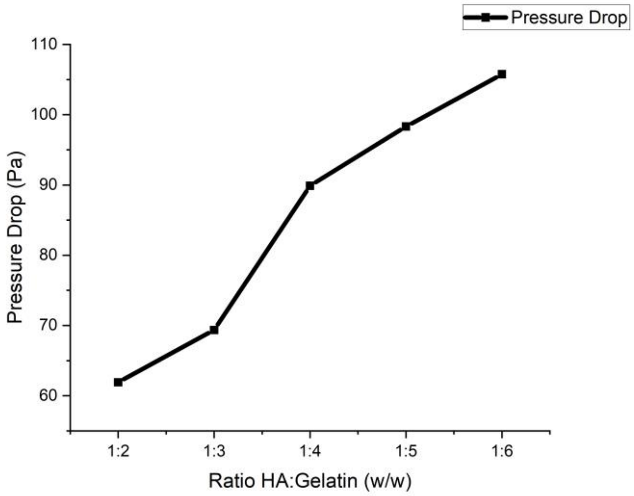

Figure 5 Pressure Drop for Each Composition Comparison of HA: Gelatin

The graph above shows that as the concentration of gelatine increases, the pressure required by the piston to push the hydroxyapatite gelatine bio composite solution also increases. The viscosity of the hydroxyapatite gelatine bio composite solution influences this. Higher viscosity can increase flow resistance, so more pressure is needed to push the solution. The pressure drop value for each gelatine hydroxyapatite composition is then used to calculate the shear stress value. The following is a graph of the shear stress for each variation of Gelatine Hydroxyapatite composition.

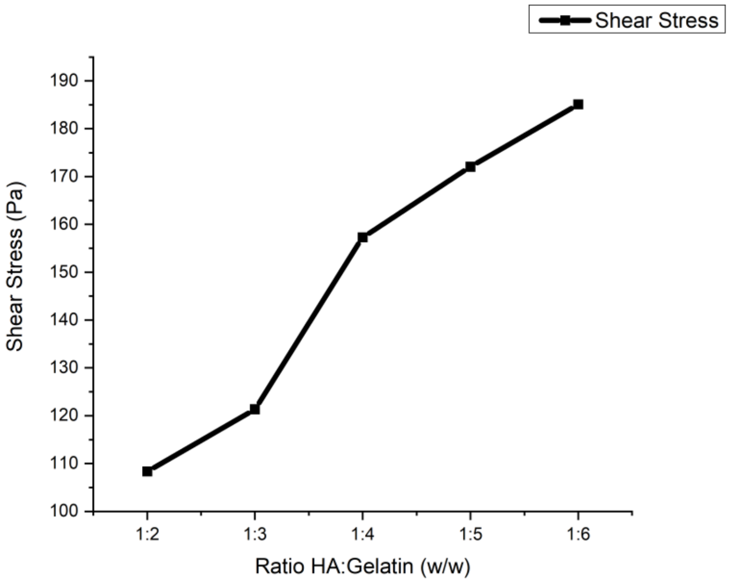

Figure 6 Shear Stress in Each Composition HA/Gelatine

Based on Figure 3, it shows that the shear stress increases as the gelatine concentration increases. This indicates that the higher the viscosity of the gelatine hydroxyapatite bio composite solution, the more shear force per unit area is needed to push the solution out of the nozzle. The viscosity when pressure is applied can be known after knowing the shear stress and shear rate values. The following results were obtained based on the calculation results.

Table 1 Viscosity Bio composite Hydroxyapatite During Extruding

Ratio HA: Gelatine (w/w)	Viscosity During Extruding (mPas)
1:2	36.53
1:3	40.91
1:4	53.02
1:5	58
1:6	62.39

Then, the viscosity of the hydroxyapatite gelatine bio composite solution, when compared with that before pressure is applied, can be shown as follows.

Based on this, it shows that the increase in viscosity of the hydroxyapatite gelatine bio composite solution when pressure is applied is caused by the shear thickening effect. When materials are stressed, these particles interact and can form locked or interlocked structures. This structure causes an increase in the stiffness and density of the material [39]. This is also supported by research conducted by Wang et al. [40], the interaction between amino hydroxyapatite and gelatine forms a structure that is interlocked and interacts chemically through hydrogen bonds.

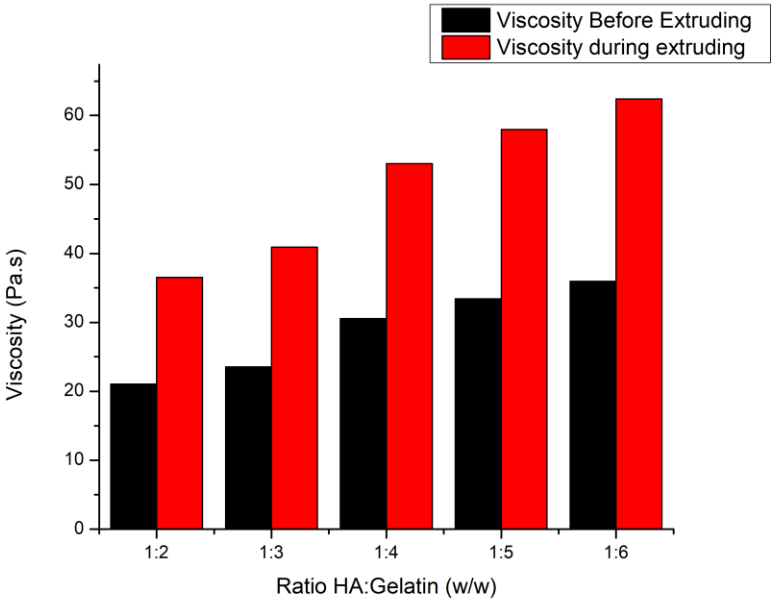

Figure 7 Viscosity of Solution Before and during Extrusion

Extrusion Result

The extrusion results obtained from the research are shown in Figure 8. This indicates that the HA/gelatine composition ratio's viscosity is still unsuitable to form the expected filaments. The analysis results show that several factors can influence viscosity, namely extrusion speed and material composition. An extrusion speed that is too fast means that the material needs more time to thicken when it comes out of the nozzle, so the extrusion results will be too runny [41]. This study used an extrusion speed of 0.42 mm/s, so it was necessary to adjust the extrusion speed again. This extrusion speed will affect the flow rate and pressure the plunger provides. The faster the extrusion speed, the greater the pressure exerted by the plunger to push the HA/gelatine bio composite solution out through the nozzle.

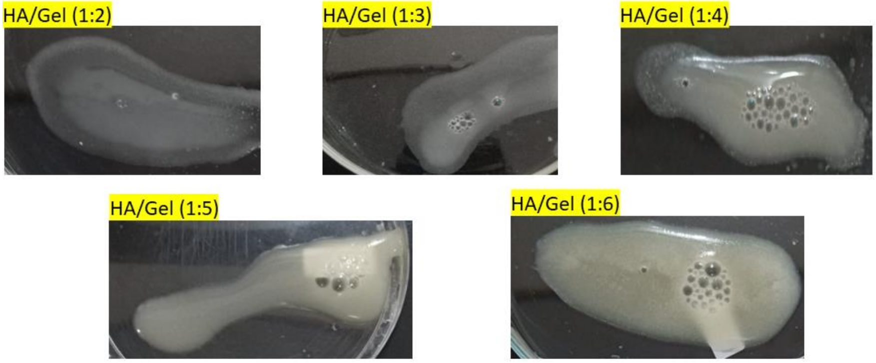

Figure 8 Extrusion Result

Apart from that, inappropriate material composition will also affect the viscosity value. In this study, no cross-linking agent was used in the HA/gelatine bio composite mixture. Cross-linking agents play a role in connecting molecular chains in bio composite materials so that later they can form strong cross-links and increase the strength and stiffness of the material

([42], [43]). Adding a cross-linking agent to the HA/gelatine bio composite solution also reduces the tendency for melting or deformation when passing through the nozzle [44]. This is supported by research conducted by Glukhova et al [45] where the administration of a cross-linking agent increased the mechanical strength and reduced the viscosity of the material after being printed using 3D printing. The use of cross-linking agents can also increase dimensional stability so that it maintains the desired shape and size after the printing process is complete [46].

Conclusion

The composition of the hydroxyapatite gelatin bio composite significantly influences the success of filament formation. This is because the composition of the material affects its viscosity value. Based on the test results, it was found that the higher the concentration of the gelatin mixture, the higher the viscosity value. This viscosity will later influence the 3D printing extrusion piston parameters such as pressure drop, shear stress, shear rate, and fluid flow. The viscosity after being subjected to pressure can be determined by obtaining several parameter values through a calculation process. Based on the calculation results, it shows that there is an increase in viscosity when pressure is applied. This indicates that the hydroxyapatite gelatin bio composite solution is classified as non-Newtonian. The extrusion results show that the gelatin hydroxyapatite compositions 1:2, 1:3, 1:4, 1:5, and 1:6 have not formed the expected filaments. The unsuitable viscosity causes this, so modification of the material composition is required. Apart from that, the extrusion speed of 0.42 mm/s also indicates that it is unsuitable for gelatin hydroxyapatite solutions. Therefore, it is hoped that future research can adjust other extrusion speeds so that 3D printing parameters can be found using the suitable extrusion method.

Acknowledgment

The authors show their appreciation and gratitude to the Faculty of Mathematic and Natural Sciences at Brawijaya University for its financial support. This work funded by Grant No. 4161.3/UN10.F09/PN/2023

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