Synthesis of sulfonated poly-(eugenol divinylbenzene) nanosilver composite and its application as antibacterial compound of cotton fabric

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Abstract. In recent years, nano-sized composite particles have been developed as antibacterial agents. We investigated antimicrobial activities of sulfonated poly-(eugenol divinylbenzene) nanosilver composite coated on cotton fabric. Firstly, sulfonated poly-(eugenol divinylbenzene) was synthesized through a cationic copolymerization between eugenol and divinylbenzene, followed by sulfonation using sulfuric acid. Furthermore, the nanosilver material was synthesized by reducing silver nitrate using sodium citrate solution as a reducing agent. The composite formation has been successfully carried out through the interaction of the polymer and nanosilver. The resulting inhibition zone diameter of copolymers and composites shows moderate inhibitory activity. The data shown by SEM-EDX reveals that the spray coating method is successful in making the composites coated well on cotton fabrics. Coated cotton fabric has hydrophobic properties and exhibits good antibacterial activity against E. coli and S. aureus bacteria.

1. Introduction
The development of chemistry in the field of polymers, both natural and synthetic polymers, is rapidly increasing. Polymer technology is widely used in various fields, one of which is as an antibacterial agent [1,2,3]. Eugenol, majorly contained in cloves, cinnamon, and tulsi leaves, is one of the most widely used natural antibacterial agents [4,5]. Having allyl and benzene groups, eugenol can be further modified into derivative compounds that also have antibacterial properties. Our group has conducted polymerization of eugenol and tested its antibacterial activity. However, the antibacterial activity of the polyeugenol is still classified as a slow response [6].

Polymer modification through crosslink reaction can be approached to produce polymers with better structural properties, reactivity, and selectivity [7,8]. Many researchers studied DVB as a crosslinking agent to provide physical strength to the synthesized material. Copolymerization of DVB can increase molecular weight, thermal resistance, particle size, swelling degree and viscosity of the polymer [9,10,11].

Polymer modification with the addition of ionic or ionizable groups in the form of acids, bases, or salts has the advantage since it can bind with other materials to increase its function as active materials [12,13]. The sulfonate group is known to give a negative charge to the polymer chain so it can bind to metal materials that have good antibacterial properties [14]. Nanosilver particles (AgNPs) have been shown to have good ability as antimicrobials. Moreover, nanosilver can be interacted with polymers to perform high antibacterial properties [15,16].
In this context, we synthesized PEDVB-SO₃H/AgNPs composites as well as carried out as coating material on cotton fabrics. Furthermore, its antibacterial activity is also investigated.

2. Experimental section

2.1. Materials and equipment

The materials used in this study were obtained from Merck, such as eugenol, divinylbenzene, silver nitrate, sodium citrate, chloroform, boron trifluoride etherate, 98% sulfuric acid, methanol, anhydrous sodium sulfate, sodium hydroxide, sodium chloride, phenolphthalein indicator, yeast extract, peptone, nutrient agar, DMSO, amoxicillin, *Escherichia coli* and *Staphylococcus aureus* bacteria.

The equipment used in this research were set of laboratory glasswares, analytical balance, hot plate stirrer, desiccator, mortar and pestle, Ubbelohde viscometer, Laminar Air Flow (LAF), autoclave, petri dish, ose, tweezers, micropipette, disc paper, cotton cloth, airbrush, FTIR (PerkinElmer Spectrum Version 10.4.00), UV-Vis (Shimadzu UV-1280), and Analytical Scanning Electron Microscope (SEM-EDX JEOL JSM-6510LA).

2.2. Synthesis of PEDVB

The synthesis of PEDVB referred to the research of our group [13]. Eugenol (10.64 g, 0.0648 mol), DVB (0.844 g 0.00648 mol), and chloroform (5 ml) were stirred under nitrogen atmosphere at room temperature. Then, boron trifluoride etherate (2.5 mL) was added dropwise. Polymerization was carried out overnight until it formed a gel and then the reaction was quenched with methanol. The product was dissolved with chloroform and neutralized with distilled water. The organic layer was separated and dried with anhydrous Na₂SO₄. The solvent was then evaporated. The isolated solid was washed with methanol and ethanol sequentially. Finally, the desired PEDVB was dried to obtain a brown powder with a yield of 92%. The product was characterized by FTIR. Moreover, its molecular weight was measured using an Ubbelohde U-tube viscometer. The intrinsic viscosity was measured at 25°C in chloroform, and the average molecular weight (Mᵣ) was calculated using the Mark-Houwink-Sakurada equation:

\[ [\eta] = K \times M_\alpha^a \]

where K (a parameter depended on temperature) and \( \alpha \) (a parameter related to molecular weight) were 11×10⁻⁵ mL/g dan 0.725, respectively. Our experiment found that the intrinsic viscosity is 12.8 mL/g to obtain the \( M_r \) of PEDVB of 16.9 kDa. In addition, the melting point of PEDVB is 120-122°C.

2.3. Synthesis of sulfonated PEDVB

The synthesis of sulfonated PEDVB refers to [16] with slight modification. PEDVB (5 g) in chloroform (50 mL) was stirred under nitrogen atmosphere, and then H₂SO₄ (2 mL) was added dropwise. The mixture was then heated at 60°C for 2 hours. The mixture obtained was evaporated and washed with distilled water to a neutral pH, then dried in an oven at 100 °C to obtain a black powder with a yield of 88%. The molecular weight of sulfonated PEDVB was obtained using the Ubbelohde viscometer to find the intrinsic viscosity of 14.8 mL/g to obtain the \( M_r \) of sulfonated PEDVB of 20.8 kDa. The product was characterized by FTIR. The degree of sulfonation was measured by the titrimetric method. Sulfonated PEDVB (0.1 g) was immersed in NaCl (10 mL 0.1 M) for 48 hours. It was then filtered, and the obtained filtrate was titrated with NaOH (0.02 M) using PP indicator. The degree of sulfonation (DS) can be calculated using following equation:

\[ DS = \frac{\text{Volume}\text{NaOH} \times M_p}{\text{sample weight}} \]
2.4. Antibacterial activity test using the disc diffusion method
The test was taken to determine the minimum inhibitory concentration (MIC) of PEDVB-SO$_3$H. A total of 30 µL of bacterial suspension fulfilled the turbidity of the standard solution of 0.5 McFarland concentration (turbidity standard of bacterial suspension at $\lambda = 600$ nm with an absorbance of 0.08-0.132) was then spread on solid media. 10 µL of each copolymer solution with various concentrations, positive control (amoxicillin 30 ppm), and negative control (DMSO) was dropped on disc paper. Afterward, the disc paper was placed on the test media. The incubation was carried out for 24 hours by observing the zone of inhibition (ZOI) diameter after 12 and 24 hours. The clear visible zone was measured to obtain the MIC of PEDVB-SO$_3$H.

2.5. Ex-situ synthesis of sulfonated poly-(eugenol divinylbenzene)/nanosilver (PEDVB-SO$_3$H/AgNPs) composite
A total of 50 mL of AgNO$_3$ 0.0015 M (1 mmol) was heated at 90 °C. Then 5 mL of Na$_2$C$_6$H$_5$O; 1% (2.6 mmol) was added dropwise under continuous stirring. When the color of the solution turned yellowish, the stirring was kept for another 2 hours. The obtained solution was analyzed using a UV-Vis spectrophotometer to examine the wavelength in nm for AgNPs. The resulted solution of AgNPs (0.5 mL) was added to the PEDVB-SO$_3$H solution (1 mL, using a concentration based on the MIC value obtained in step 2.4.) under vigorous stirring for 1 hour.

2.6. Composite coating on cotton fabric
For removing the impurities from the surface, the cotton fabric with a size of 1 cm × 1 cm was washed subsequently with distilled water, non-ionic detergent (0.5g/100 mL distilled water), and alcohol (70%) at RT for 20 minutes and dried at 60 °C for 30 minutes. The distilled water, DMSO, amoxicillin (30 ppm), solution of PEDVB-SO$_3$H copolymers (4 and 30 ppm), suspension of AgNPs, suspension of PEDVB-SO$_3$H/AgNPs composite were homogenized under ultrasonication for 30 min. After that, 0.05 mL of each solution was spray-coated with a constant distance from the cotton fabric. Spray coating was carried out 3 times. After that, the coated fabric was oven-dried at 60 °C for 5 min, followed by curing at 150 °C for 1 min. The coated cotton fabrics were stored in a dry place for further characterization against bacterial species.

2.7. Antibacterial activity test of coated cotton fabric using turbidimetric method
A total of 100 µL of the bacterial suspension was put into a 100 mL sterile erlenmeyer containing 10 mL of liquid media. Then each of blank cotton fabric and cotton fabric coated with each of positive control, negative control, PEDVB-SO$_3$H, AgNPs, and PEDVB-SO$_3$H/AgNPs composite were put into the test media and shaked. After that, the changes in the value of bacterial inhibition (absorbance) at the 12th hour were analyzed with a UV-Vis spectrophotometer at $\lambda = 600$ nm.

2.8. Hydrophilicity test of coated cotton fabric
The cotton fabrics coated with PEDVB-SO$_3$H (4 ppm and 30 ppm) and the PEDVB-SO$_3$H/AgNPs composite were tested for their hydrophilicity with uncoated cotton fabric as a comparison. Distilled water (0.05 mL) was dropped on the cotton fabric. Then it was photographed using a camera. The contact angle was measured using a protractor.

3. Results and discussion

3.1. Synthesis of PEDVB and PEDVB-SO$_3$H
As seen in Fig 1, the FTIR of eugenol monomer shows a typical absorption of O-H stretching vibration at the wavenumber 3525.92 cm$^{-1}$, while the C-H sp$^3$ stretching and bending appear at 2976.15 and 1451.34 cm$^{-1}$, respectively. The aromatic C=C bending vibration and the C-O ether stretching are seen with the absorption at wavenumbers 1610.88 and 1267.61 cm$^{-1}$. The C=C stretching of alkenes is shown by the absorption at 1650 cm$^{-1}$.
PEDVB copolymer was fabricated by reacting eugenol with DVB as a crosslinking agent with the ratio of 10:1 in the presence of BF$_3$ as a catalyst. Polymerization takes place through a cationic addition which includes the initiation, propagation, and termination steps. The success of PEDVB synthesis can be confirmed with the disappearance of allyl group absorption (C=C sp$^2$ stretching) at a wavenumber of about 1650 cm$^{-1}$ and a loss of vinyl group absorption DVB (-H$_2$C=CH$_2$ bending) at a wavenumber of about 990 cm$^{-1}$.

The addition of a sulfonate group to the copolymer aromatic ring aims to increase the copolymer molecular weight so that its thermal resistance is also more optimal, and to increase the active -OH group, which is used to form bonds so that the copolymer sticks to the cellulose on the fabric surface. The sulfonated PEDVB copolymer experienced a color change from peach to black with the yield obtained of 88.1%, a molecular weight of 20.8 kDa and a melting point of 158-160 °C.

![Figure 1](image.png)

**Figure 1** FTIR spectra of eugenol (black line), PEDVB (red line), and PEDVB-SO$_3$H (blue line)

| Functional group                      | Wavenumber (cm$^{-1}$) | PEDVB | PEDVB-SO$_3$H |
|---------------------------------------|------------------------|-------|---------------|
| O-H stretching                        | 3526                   | 3434  |               |
| C-H sp$^2$ stretching                 | 2959                   | 2959  |               |
| C=C aromatic bending                  | 1602                   | 1604  |               |
| C-O ether stretching                  | 1218                   | 1221  |               |
| S=O stretching                        | -                      | 1200  |               |
| S-O stretching                        | -                      | 1036  |               |
| C-S stretching                        | -                      | 703   |               |

The success of sulfonation can also be determined by measuring the degree of sulfonation of the copolymer to determine the number of sulfonate groups that have been substituted. The results of determining the degree of sulfonation carried out by the titration method obtained a value of 2.53%. It is also possible that the spectra that appear are similar to the PEDVB copolymer because the substituted sulfonate groups do not reach 100%. The value of the degree of sulfonation obtained is relatively small, indicating that the PEDVB-SO$_3$H copolymer produced is not polar.

3.2. *Antibacterial activity test of PEDVB-SO$_3$H copolymer by disc diffusion method*

The PEDVB-SO$_3$H copolymer antibacterial activity test was carried out to determine the differences in the antibacterial activity of the copolymer by using the disc diffusion method against *E. coli* (gram-
negative) and S. aureus (gram-positive) bacteria. The disc diffusion method is based on the diffusion of antibacterial compounds from disc paper on solid media. The disc diffusion method was chosen as the initial screening to determine the antibacterial activity, and the result was a clear inhibition zone.

The various concentration of the PEDVB-SO$_3$H solutions used to determine MIC, with amoxicillin positive control and DMSO negative control. The two bacteria (E. coli and S. aureus) used for the test must be adjusted to the McFarland standard of 0.5 (equivalent to $1.5 \times 10^8$ CFU/mL) so that the number of bacteria used during the test is the same so the test results can be compared.

Inhibition zone measurements were carried out after 12 hours and 24 hours to determine the effectiveness of copolymers in inhibiting bacterial growth. The diameter of the inhibition zone decreases with time, indicating that the bacteria are experiencing growth. Following are the observations of the inhibition zone diameter of the PEDVB-SO$_3$H copolymer against E. coli and S. aureus in Table 2.

| Sample          | Inhibition zone (mm) |          |          |          |          |          |          |          |          |
|-----------------|----------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                 |                      | E. coli (G-) | S. aureus (G+) |          |          |          |          |          |          |
|                 |                      | 12 hours  | 24 hours | 12 hours | 24 hours | 12 hours | 24 hours | 12 hours | 24 hours |
| Amoxicillin 30 ppm | 19.0  | 13.0  | 11.0  | 6.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| DMSO            | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| PEDVB-SO$_3$H 1 ppm | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| PEDVB-SO$_3$H 2 ppm | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| PEDVB-SO$_3$H 3 ppm | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| PEDVB-SO$_3$H 4 ppm | 6.0  | 5.2  | 6.5  | 5.3  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| PEDVB-SO$_3$H 5 ppm | 6.0  | 5.5  | 6.5  | 5.5  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| PEDVB-SO$_3$H 10 ppm | 6.5  | 6.0  | 7.0  | 6.5  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| PEDVB-SO$_3$H 20 ppm | 7.0  | 6.3  | 7.5  | 7.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| PEDVB-SO$_3$H 30 ppm | 7.5  | 7.0  | 8.0  | 7.5  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |

As seen on Table 2, DMSO as a negative control does not show any inhibition zone in the disc diffusion method. The PEDVB-SO$_3$H copolymer with various concentrations showed different antibacterial activity. The higher the copolymer concentration, the greater the antibacterial activity. Therefore, for the next stage in copolymer coating on cotton fabric, it is necessary to determine the minimum inhibitory concentration (MIC), which is the smallest concentration capable of inhibiting bacterial growth. According to the data obtained, the MIC of the PEDVB-SO$_3$H copolymer produced was 4 ppm against E. coli and S. aureus bacteria. The diameter of the inhibition zone in E. coli is smaller than the inhibition zone of S. aureus. This is influenced by differences in the structure of the bacterial cell walls. Gram-positive bacteria (S. aureus) have a cell wall that has lots of peptidoglycans and polysaccharides (teichoic acid), as well as fewer lipids compared to gram-negative bacteria (E. coli), which have a membrane, so that copolymer molecules do not easily diffuse through the cell walls. Polysaccharides in the cell walls of gram-positive bacteria (S. aureus) are polar polymers, so that the cell walls are also relatively polar. The cell wall structure of gram-positive bacteria (S. aureus) is simpler, single-layered with a low lipid content (1-4%) so bioactive easily enter the cell. Meanwhile, the cell wall structure of gram-negative bacteria (E. coli) is more complex, layered 3 consisting of an outer layer (lipoprotein), a middle layer (lipopolysaccharide) as a barrier to the entry of antibacterial bioactive materials, and an inner layer (peptidoglycan) with a high enough lipid content (11-12%) [18]. This also confirms that the presence of C benzene bonds with S in the sulfonate group (-SO$_3$H) and the hydroxyl group (-OH) on eugenol is able to bind hydrogen better to the cell walls of gram-positive bacteria (S. aureus).
3.3. Synthesis of AgNPs

Synthesis of AgNPs in this study using a solution of AgNO₃ and sodium citrate (Na₃C₆H₅O₇) as a reducing agent as well as a stabilizer. Sodium citrate is a strong reducing agent. Stabilizers is employed to prevent aggregation, which causes large chemical bonds between particles, then forms a strong electric dipole so it can agglomerate. The following is the chemical reduction reaction for the formation of AgNPs [19]:

$$4\text{Ag}^+ + \text{Na}_3\text{C}_6\text{H}_5\text{O}_7 + 2\text{H}_2\text{O} \rightarrow 4\text{Ag}^0 + \text{H}_3\text{C}_6\text{H}_5\text{O}_7 + 3\text{Na}^+ + \text{H}^+ + \text{O}_2$$

The reaction of Ag⁺ ion with citrate (C₆H₅O₇⁻) can form [Ag⁺......citrate⁻] complex ([Ag₃(C₆H₅O₇)ₙ⁻]ₙ⁻) which has a more dominant role in reducing Ag⁺ ions to Ag⁰ slowly so that the reaction can occur. One indicator of the formation of AgNPs is marked by turning the solution to yellowish. The formation of AgNPs can also be seen through the maximum absorption on the UV-Vis spectrophotometer. The results of the measurement of synthesized AgNPs were carried out on day four and had a maximum absorption at a wavelength of 437 nm with an absorbance of 0.462. According to [20], the absorbance peak of AgNPs at a wavelength of 438 nm has a range of particle sizes of 60-80 nm.

![Figure 2. UV Visible spectra AgNPs](image)

3.4. Effect of AgNPs on PEDVB-SO₃H

AgNPs were contacted into the 4 ppm PEDVB-SO₃H polymer matrix obtained from MIC measurements from the antibacterial test results with the disc diffusion method by stirring. AgNPs possibly interact with the polymer matrix through electrostatic interactions of Ag-S via sulfate functional groups and Ag-O via hydroxyl groups. The porosity of the sulfonated polymer allows AgNPs to diffuse throughout the polymer matrix allowing electrostatic interactions with sulfonate functional groups. The proposed interaction of AgNPs with PEDVB-SO₃H is shown in figure 3.
Figure 3. Proposed interaction of AgNPs and PEDVB-SO$_3$H

Table 3. Bacteria inhibition zone of polymer/AgNP

| Sample                     | Inhibition zone (mm) |  |
|----------------------------|----------------------|--|
|                            | $E.\ coli$ (G-)      | $S.\ aureus$ (G+) |
|                            | 12 hours 24 hours    | 12 hours 24 hours |
| Amoxicillin 30 ppm         | 17.0 10.0            | 13.0 6.0          |
| DMSO                       | 0.0 0.0              | 0.0 0.0           |
| PEDVB-SO$_3$H 4 ppm        | 6.0 5.2              | 6.5 5.3           |
| PEDVB-SO$_3$H 4 ppm/AgNP   | 6.5 5.5              | 7.0 6.5           |
| PEDVB-SO$_3$H 30 ppm       | 7.5 7.0              | 8.0 7.5           |
| PEDVB-SO$_3$H 30 ppm/AgNP  | 7.5 7.5              | 8.5 8.5           |

The results of the antibacterial activity test are shown in Table 3. The inhibition zone diameter of PEDVB-SO$_3$H/AgNPs is higher than that of PEDVB-SO$_3$H alone for both $E.\ coli$ and $S.\ aureus$. This indicates that the presence of AgNPs distributed into the polymer matrix plays a role in inhibiting bacterial growth. Visual observations and manual measurements of the inhibition zone diameter of the PEDVB-SO$_3$H 30 ppm and PEDVB-SO$_3$H 30 ppm/AgNPs were insignificant or even similar. This can occur because there is a possibility that the surface of PEDVB-SO$_3$H polymer matrix will have the potential to attack bacteria first, while the distributed and stabilized AgNPs in the polymer matrix will contribute to a longer inhibition time. The resulting inhibition zone diameter is in the range of 6-10 mm so it can be stated that both copolymers and composites have moderate inhibitory activity.
3.5. The coating on Cotton Fabric
Copolymer solutions of PEDVB-SO$_3$H and composite PEDVB-SO$_3$H/AgNPs as antibacterial materials are sprayed on the cotton fabric using an airbrush to produce smaller droplets of the compound. The advantages of using an airbrush is that it is faster, does not require a large amount of copolymer solution, and the attachment of copolymer to the fabric is stronger because it uses pressure compared to the dyeing method. The size of the droplets can range from hundreds of micrometers to several tens of nanometers. The droplet size distribution is also almost monodisperse. Cured on the coated fabric is also carried out so the active copolymer compound is more bonded in the fabric fibers.

3.5.1. Characterization using SEM-EDX. The cotton fabric coated with the PEDVB-SO$_3$H/AgNPs composite was analyzed using SEM-EDX to determine the morphology and the composition of the fabric surface. Based on the results of SEM characterization, the uncoated fabric (Figure 4a) has a clean structure and no thickening on the surface. Meanwhile, the cotton coated with PEDVB-SO$_3$H 30 ppm/AgNPs (Figure 4b) shows thicker fiber properties, and a layer appears to form on the surface of the fabric. This proves that the composite has been coated on the fiber. The EDX analysis results on the PEDVB-SO$_3$H 30 ppm/AgNPs coated cotton (Figure 5b) showed the presence of Ag peak with the composition contained was 0.15% by mass. This shows that the composite has been successfully coated on the fabric.

![SEM characterization results: a) uncoated fabrics, b) coated fabrics with PEDVB-SO$_3$H 30 ppm/AgNPs](image-url)
3.5.2. Hydrophilicity test of coated cotton fabric. Cellulose as raw material for cotton fabric is hydrophilic and naturally able to absorb water. Apart from its hydrophilicity properties, cellulose has advantages as a hydrophobic material substrate due to its abundance, biodegradability, and unique physical, chemical and mechanical properties [21]. The wettability of the fabric was evaluated by measuring the contact angle (θ) to determine the success of coating and sticking polymer and composite material on the fabric. The contact angle is obtained using the sessile drop technique. The contours of the droplets is seen from the front look symmetrical. Figure 6 shows the contact angle between the water drops and the substrate surface, while the data for the contact angle values of the coated cotton fabric are shown in table 4.

A contact angle value <90° indicates that the hydrophilicity of the material is high or hydrophobicity is low. The reported water contact angle of the fine cellulose film varied between 27° and 47°. Based on table 4, the water contact angle measured on the cotton without coating is 41.5° which indicates a hydrophilic character. PEDVB-SO$_3$H and PEDVB-SO$_3$H/AgNPs coated fabrics have a contact angle value >90° which indicates hydrophobic character. The greater the concentration of the PEDVB-SO$_3$H copolymer coated on the fabric, the higher the hydrophobicity as the contact angle value increases. Increased contact angle implies reduced adhesion. The presence of AgNPs on the PEDVB-SO$_3$H copolymer decreased the contact angle value due to reduced hydrophobic character and increased adhesion.
Figure 6. The angle of contact between the water drops and the substrate: a) cotton fabric only, b) cotton fabric coated with AgNPs, c) cotton fabric coated with PEDVB-SO$_3$H 4 ppm, d) cotton fabric coated with PEDVB-SO$_3$H 4 ppm/AgNPs, e) cotton fabric coated with PEDVB-SO$_3$H 30 ppm, d) cotton fabric coated with PEDVB-SO$_3$H 30 ppm/AgNPs

### Table 4. Contact angle value of the fabric

| Sample                                | Contact angle |
|---------------------------------------|---------------|
| Cotton fabric                         | 41.5          |
| Cotton fabric + AgNPs                 | 56.0          |
| Cotton fabric + PEDVB-SO$_3$H 4 ppm   | 117.0         |
| Cotton fabric + PEDVB-SO$_3$H 4 ppm/AgNPs | 115.5       |
| Cotton fabric + PEDVB-SO$_3$H 30 ppm  | 121.0         |
| Cotton fabric + PEDVB-SO$_3$H 30 ppm/AgNPs | 118.0       |

3.5.3. **Antibacterial activity test using turbidimetric method.** The test for the antibacterial activity of copolymer coated cotton fabric was carried out using the turbidimetric method. The principle of the turbidimetric method is based on the turbidity of the liquid media (reference), which is measured the absorbance value using a UV-Vis spectrophotometer at a wavelength of 600 nm. The lower the absorbance value, the lower the turbidity, indicating that the higher the antibacterial activity in inhibiting bacterial growth. The antibacterial activity test for cotton fabric is shown in Table 5.

Uncoated fabrics do not exhibit antibacterial properties, whereas coated fabrics exhibit varying percent inhibition. Cotton fabrics coated only with AgNPs have stronger antibacterial activity than PEDVB-SO$_3$H polymer coated fabrics. Antibacterial activity is increased in the coated fabrics with PEDVB-SO$_3$H 30 ppm/AgNPs compared to coated fabrics with AgNPs only, PEDVB-SO$_3$H only, and PEDVB-SO$_3$H 4 ppm/AgNPs. This indicates that AgNPs are stabilized in the polymer matrix with a high enough concentration so a greater percentage of inhibition is obtained. This is possibly due to the surface of the PEDVB-SO$_3$H polymer matrix, which still has the potential to attack bacteria first, and the AgNPs that are distributed and stabilized in the polymer matrix will participate in the inhibition of bacterial growth and contribute to a longer inhibition time.
Table 5. Data on percent inhibition of coated cotton fabrics

| Sample                                | Inhibition (%) |             |             |
|---------------------------------------|----------------|------------|------------|
|                                       |                | E. coli (G-) | S. aureus (G+) |
| Cotton fabric                         | 0              | 0          |            |
| Cotton fabric + AgNPs                 | 50.8           | 77.1       |            |
| Cotton fabric + PEDVB-SO₃H 4 ppm      | 10.4           | 30.0       |            |
| Cotton fabric + PEDVB-SO₃H 4 ppm /AgNPs | 31.5           | 43.5       |            |
| Cotton fabric + PEDVB-SO₃H 30 ppm     | 35.1           | 53.1       |            |
| Cotton fabric + PEDVB-SO₃H 30 ppm /AgNPs | 51.5           | 91.6       |            |

4. Conclusion
This study shows that the sulfonated poly-(eugenol divinylbenzene) nanosilver composite was successfully synthesized. The resulting inhibition zone diameter of copolymers and composites shows moderate inhibitory activity. The data shown by SEM-EDX reveals that the spray coating method is successful in making the composites coated well on cotton fabrics. Coated cotton fabric has hydrophobic properties and exhibits good antibacterial activity against E. coli and S. aureus bacteria.

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