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Suppression of Coffee-Ring Effect on Nitrocellulose Membrane: Effect of Polyethylene Glycol

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ABSTRACT

In the development of the diagnostic kit, it was favorable to have a low antigen concentration due to the difficulty of antigen preparedness and purification. However, it can cause the coffee-ring effect, producing different pattern formations on the selected membrane. It can lead to a false interpretation of the result. Thus, the immobilization of protein solution (lysozyme) as a model protein for antigen, with the addition of hydrosoluble polymer additive onto a membrane, was evaluated to suppress the coffee-ring effect. This research aims to evaluate the effect of polyethylene glycol on the protein solution for coffee-ring effect suppression and to analyze the image of the coffee-ring effect. From the experimental studies, 5 different concentrations (v/v%) of PEG which are 3.0, 2.0, 1.0, 0.1 and 0.01 v/v% is added at 4.0 mg/mL of lysozyme solution before being spotted onto nitrocellulose membrane. The color intensity of the dried spot, together with the formation of the coffee-ring effect, is analyzed by Image-J software. It is the approach to measure the suppression of the ring effect, in which 0.01 v/v% concentration portrays the most faded ring effect on nitrocellulose membrane. This effect occurs due to a surface tension gradient that causes the solute particles to accumulate at the edge of the droplet. As Marangoni flow has been altered, the coffee-ring effect is successfully suppressed; thus, uniform pattern deposition is achieved.

Keywords: Coffee-ring effect, lysozyme protein, membrane, polyethylene glycol (PEG)

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INTRODUCTION

In the medical field, biosensor technology is developing vastly along with modern discoveries, including protein, fluorescent microarray, and nanomaterials. Although

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it has a wide range of applications, one of the properties that define the performance of the biosensor is the sensitivity, which is the limit of detection (LOD) for the biological analyte. Nevertheless, the traditional method is still applicable, as it is for general mass screening and unrequired specialist & high-end instruments such as the dot-blot technique. However, along with the advantages, this technique also has some drawbacks where the signal produced through colorimetric can be faded or discolored. In addition, less antigen used during the preparation can cause a ring-like structure, leading to misinterpretation of the result (Devineau et al., 2016).

The drawbacks stated before often appear in a ring-like pattern, formally known as the coffee-ring effect (CRE), a ubiquitous event formed during the evaporation of sessile droplets on a flat substrate, thus leading to solute particle distribution. It is initiated through the combination of several natural events, including the formation of capillary flow, followed by the pinning of the contact line of the droplet on the surface (Bansal et al., 2018). Driven by the flow, the particles that cannot evaporate and accumulate at the periphery of the contact line in a ring shape. Much research has been conducted to suppress CRE due to its typical challenges involving reduced accuracy and quality of the product. A common example of this phenomenon is pigment ink and inkjet printing drying, where the end product is low quality.

Some methods to suppress CRE are by introducing Marangoni flow, controlling the temperature of the substrate (Carreón et al., 2021), and generating electric fields in the droplet (Mampallil et al., 2015). However, most of the methods mentioned have a complex requirement where they need thermal conductivity of substrate or require conductive liquid (Eral et al., 2011); thus, the addition of hydrosoluble polymer additive, which is polyethylene glycol (PEG), was chosen as a method to suppress the coffee-ring phenomenon in this study. In general, the previous work has been demonstrated with the PEG incorporated into the liquid to minimize the ring-like effect (Cui et al., 2012; Seo et al., 2017). The experiment was conducted on glass slide surfaces where the ring structure is visible before being characterized using a high-end instrument for better understanding. Here, this research work also utilized such PEG since the PEG molecule in the water solution where the solution becomes a surfactant-like solution (Cao & Kim, 1994) causes a decline in surface tension while increases in solution viscosity (Nowak et al., 2016, Hu et al., 2013). Therefore, adding PEG to protein solution is expected to produce a homogenized protein-surface interaction, resulting in a uniform deposition of a suspended particle on the nitrocellulose membrane.

In the medical sector, the membrane technology has been widely used because it can act as an adsorptive surface for the detection of antigens in the blood sample and as a diagnostic tool to detect protein present in the patient sample due to the simplicity of the design and the speed of the information taken (Ahmad et al., 2016b). A low antigen concentration

was preferable because of the complexity of antigen preparedness and purification, but it can cause CRE to occur, producing a different pattern distribution. In addition, it can lead to false-negative test results. These problems have become an issue since it involves the well-being of a person and the health status that was concerned.

Nitrocellulose, nylon, or polyvinylidene fluoride (PVDF) membranes are commonly used for the detection of protein in the medical industry, but nitrocellulose membrane is preferable to use due to its high protein binding affinity and offers higher efficiency of protein transfer compared to a nylon membrane and PVDF membrane (Kurien & Scofield, 2015). Throughout the discovery of this phenomenon, investigations regarding CRE suppression are widely performed where most are being done on a solid surface such as glass slide and silica. Despite that, the study evaluating CRE suppression on membrane surfaces is still unavailable. Therefore, this experiment was conducted to evaluate the effect of PEG on the protein solution to suppress the ring-like effect along with analyzing the formation of nitrocellulose membrane by Image-J software.

MATEIALS AND METHOD

Material

The membrane used in this experiment is nitrocellulose membrane (Hi-Flow™Plus, Merck) with the pore size of HF135 with 191um thickness. Polyethylene Glycol (PEG) was chosen as a polymer additive with an average molecular weight of 500-600. In contrast, lysozyme from chicken egg white acts as a reference protein. Ponceau S enhances the colorimetric signal since it is compatible with the chosen membrane. All solutions used were supplied from Sigma-Aldrich.

Membrane Characterization

In characterizing the surface morphology and roughness of nitrocellulose membrane, Scanning Electron Microscope, SEM (VP-SEM SU1510, Hitachi, Japan) with accelerating voltage of 5.0kV and Atomic Force Microscopy, AFM (Ntegra NT MDT, Russia), are used respectively. For water contact angle, the images were captured using a live video camera of the Contact Angle Gionometer (VCA-3000S, AST, USA) and the sessile drop method at room temperature with normal humidity. In addition, the contact angles of the membrane were measured using distilled water on the upper side of the membrane, where the live video camera would capture the image immediately.

Protein Immobilization

Immobilization of Protein Solution onto Membrane. The nitrocellulose membrane was cut into several test strips with the dimension of a 1 × 5 cm rectangle where the upper

surface of the membrane was marked. In this experiment, the lysozyme protein is chosen as the reference protein. The lysozyme protein was chosen due to its low price and had more stability (Du et al., 2014). The concentration of reference protein used was constant which was 4.0 mg/mL while different concentration of polyethylene glycol (PEG) concentration (0.01, 0.1, 1.0, 2.0 and 3.0 vol/vol%) were prepared, respectively.

The reference protein is mixed with 0.01vol/vol% of PEG solution in a 1:1 ratio. 1µL of the mixture was then spotted onto the membrane using a micropipette in a triplicate manner. Next, the membrane was let to air-dry in the laboratory with well-circulating air at the normal room temperature between 20–24 °C for about 20 minutes. Once the membrane had completely dried, it was immersed in approximately 10 mL of Ponceau S solution for 5 minutes for signal enhancement. The membrane was then rinsed with distilled water to wash off the undesired membrane background and air-dried at room temperature before proceeding to the analysis. These steps are repeated using different concentrations of PEG.

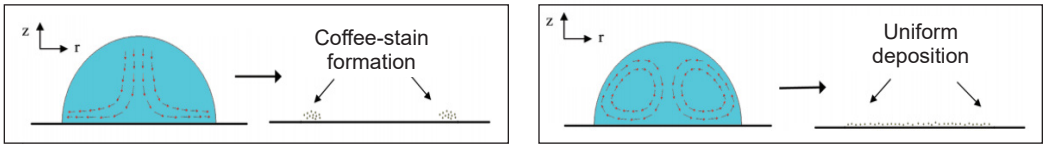
Coffee-Ring Effect (CRE) Analysis

The protein blotting pattern distribution image can be seen using the EPSON Perfection L220 scanner with 16 > 8-bit grayscale and 9600 dpi resolution settings. In addition, the characteristics of the pattern deposition on the surface of the nitrocellulose membrane were analyzed using Image-J software (1.8.0_172, USA) to measure the color intensity of the pattern.

RESULTS AND DISCUSSION

Effect of PEG on Suppression of CRE

The mechanism of droplet deposition induced by specific flow refers to Figure 1, where the schematic diagram shows the droplet deposition induced by CRE and Marangoni flow, respectively. During the evaporation process, the temperature of the surface droplet was reduced in a non-homogenous manner. It is due to the low temperature of liquid-air interaction at the droplet's upper and the high surface tension. Therefore, the flow pattern at the specific area changed into an inward direction near the droplet surface, where the shear stress stabilizes the Marangoni stress (Marin et al., 2016). In the CRE phenomenon, the observed flow was radial outflow that forms due to the absence of the circulating flow.



(a) (b)

Figure 1. Schematic diagram of droplet deposition induced by (a) coffee-ring effect and (b) Marangoni flow (Majumder et al., 2012).

As the particle flow in a droplet has a differential evaporation rate thus, it drives the particle to move to the periphery of the droplet leading to the formation of CRE. As a result, it was observed that strong outflow occurred at the bottom of the surface, as shown in Figure 1(a).

Meanwhile, the suspended particle of PEG solution has a radial inward flow known as Marangoni flow caused by differences in surface tension. Together with the air and liquid surface interfacial, the surface tension will be maximized, which leads to the formation of radial inward flow near the air and liquid surface interfacial. The presence of higher surface tension at the top of the droplet brings the protein particle to the droplet's upper area before it circulates downwards, where the particle either will be absorbed into the substrate or transferred to the edge of the droplet. This produced uniform deposition where the recirculating flow will homogenize the particle concentration (Majumder et al., 2012). As both flows reach equilibrium, a closed-loop flow known as the Marangoni vortex is formed (Seo et al., 2017) as both flows have opposite directional flow characteristics, as shown in Figure 1(b).

Figure 2 shows protein blotting on a nitrocellulose membrane with the constant lysozyme protein solution concentration of 4.0 mg/mL with the addition of manipulated PEG concentration. The blank solution, Figure 2(a), acts as a reference protein to compare with the other PEG-lysozyme solution to measure the level of CRE suppression. By looking at this comparison, adding PEG in the concentrations of 3.0, 2.0, and 1.0 v/v% shows a ring structure similar to the reference protein. These occur due to the uneven evaporation process on the membrane (Zhuang et al., 2019, Nilghaz et al., 2015). As the evaporation process begins at the edge of the droplet, the capillary flow would direct the particle to accumulate from the center to the periphery of the droplet. For the concentration of 0.1 and 0.01 v/v%, the ring structure form is in a lower intensity and almost similar manner. As 0.01 v/v% utilizes a lower concentration of PEG additive, which is cost-effective for a larger-scale implementation, the analysis proceeds with the selected concentration.

Therefore, when the evaporation process is completed, the particles that are now highly concentrated on the droplet's edge create a ring-like shape known as CRE, as in Figure 3(a). In order to suppress the coffee-ring effect, the PEG solution is mixed with the protein liquid to get a uniform pattern deposition, as in Figure 3(b). In general, the Marangoni

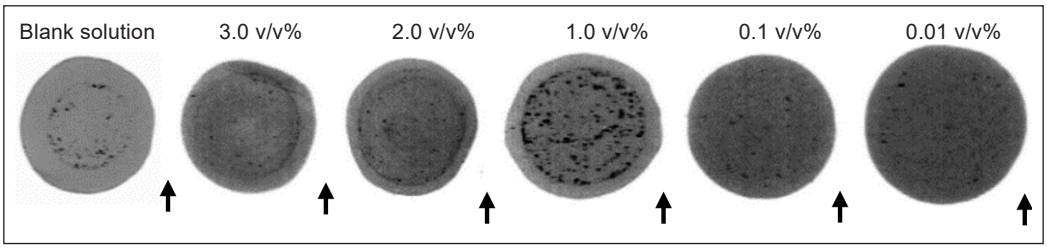
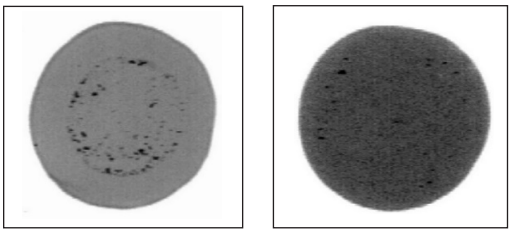


Figure 2. Protein blotting on nitrocellulose membrane (scanned with the setting of 16 > 8-bit grayscale and resolution of 9600 dpi) at 4.0 mg/mL of protein solution with varying PEG concentration

flow in PEG solution produced a slow-paced inward and outward radial motion, leading to an even distribution of the particle on the membrane surface (Seo et al., 2017).

As PEG solution is highly compatible with nitrocellulose membrane due to existing polar and non-polar function groups, this leads to a high rate of hydrophilic interaction. Using AFM to characterize the membrane surface in Figure 4(b), the rough surface of nitrocellulose aids in a faster evaporation process at the edge of the droplet along with highly porous and homogenous throughout the membrane in Figure 4(a). It is also supported by the low receding angle of the membrane when the water contact angle (WCA) test is performed in Figure 4(c). These factors contribute to the accumulation of lysozyme particles at the contact line of the droplet.

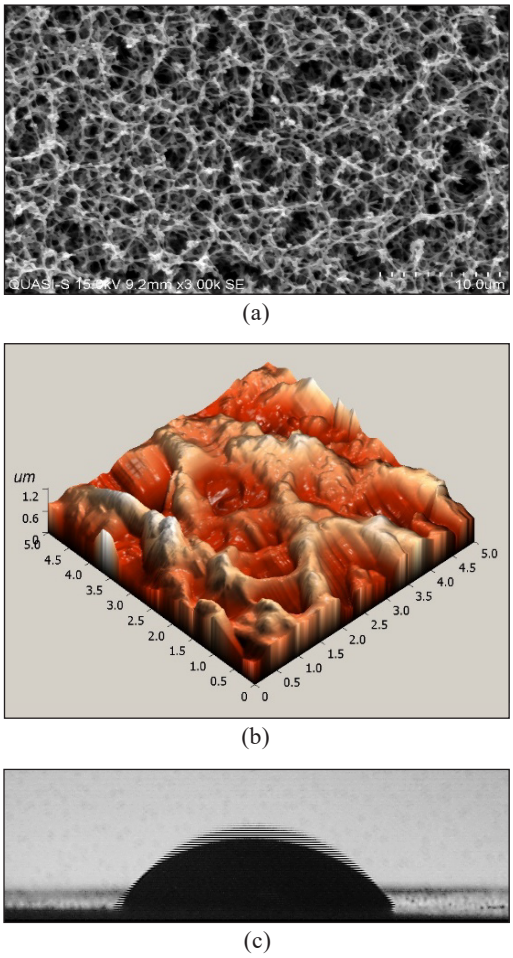


(a) (b)

Figure 3. Image of the deposition pattern of the droplet (a) blank solution (lysozyme solution: 4.0 mg/mL) and (b) PEG solution (0.01 v/v%). The blank solution has the presence of a coffee-ring effect, while the solution with the added PEG does not has a coffee-ring effect.

Image Analysis of CRE Pattern Formation

The spotted solution on the membrane was first scanned before being imported into Image-J software to analyze using the line profile tool. The image analysis was performed to quantitatively measure the intensity of the CRE formed on the nitrocellulose membrane. The graph was plotted with a y-axis denoted as a grey scale value, which stands for the color intensity of the observed spot for the deposition pattern of the particle on the membrane in grey scale value. A higher value of the grey scale represents the low intensity of the spot (Shahruddin et al., 2021). Figure 5 shows the differences in the color intensity profile of



(a)

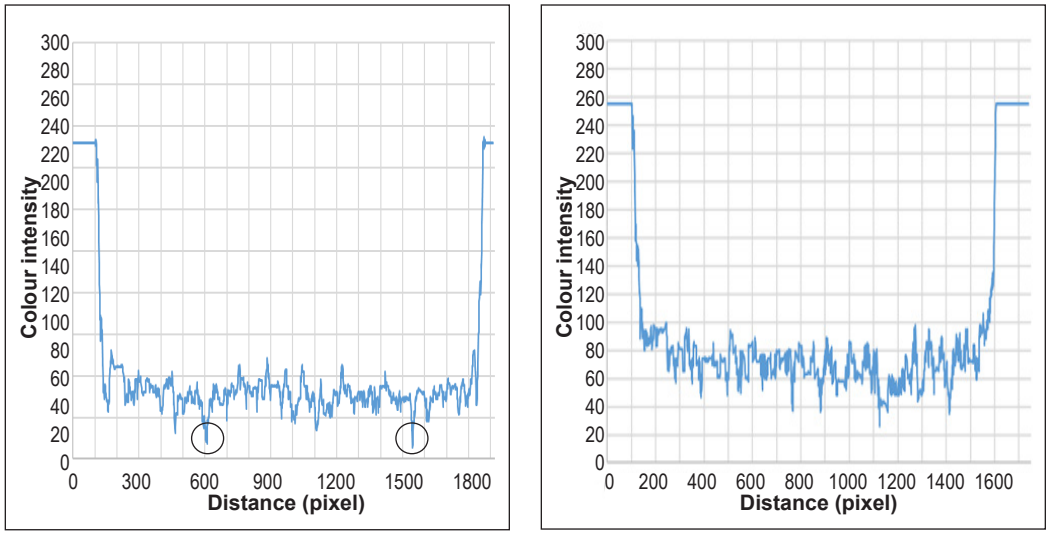
(b)

(c)

Figure 4. Nitrocellulose membrane images under (a) SEM, (b) AFM, and (c) water-contact angle test

the references protein solution and the addition of 0.01 v/v% PEG, respectively. For the reference protein, the formation of two valley-like shapes is denoted with a circle in Figure 5(a), indicating CRE's presence. The low grey scale value with a sharp peak shows the presence of CRE, while the grey scale value with a smooth line profile and the absence of apparent valley formation represents no CRE formation on the nitrocellulose membrane. This formation of CRE is due to the suspension of a particle at the edge of the droplet that leads to the formation of CRE at a given time. As for the concentration of 0.01 v/v% of PEG produces a high grey scale value without the obvious valley-like formation. It indicates no deposition of lysozyme protein particles at the edge of the dried spot.

In Figure 5(a), the reading of the grey scale value that portrays the formation of the valley structure in the graph was recorded at 107.574 and 105.694. Besides that, in Figure 5(b), the lowest and highest value of the grey scale value were 27 and 96, respectively. Comparing these readings, the low value obtained from the graph for a solution with a PEG profile supports the image of dried the spots where the CRE is formed in a reference protein. The deposition of protein particles in Figure 5(a) is mostly concentrated at the edge of the spot, whereas in (b), the evaporation is evenly throughout the spot, resulting in a more uniform and dark appearance. It also shows that the protein particles do not accumulate as much at the edge of the spot as at the earlier spot (Ahmad et al., 2016a). However, the CRE produced was not significant enough in Figure 5(b), even though naked eye can be observed the ring-like effect. In addition, the membrane surface roughness can also affect the deposition pattern of the particle. Therefore, it was preferable to use a smooth surface area to increase the even distribution of particles.



(a) (b)

Figure 5. Side profile of greyscale intensity of dried protein spot for (a) blank solution and (b) addition of 0.01 v/v% of PEG solution

CONCLUSION

In conclusion, the uniform pattern deposition can be achieved by adding hydrosoluble polymer additive such as PEG into the lysozyme protein solution before spotting it on the nitrocellulose membrane. The varying concentration of PEG will induce the main contributor to the formation of CRE, which is the Marangoni flow; thus, the CRE can be suppressed. The low concentration of PEG, which was 0.01 v/v%, was favorable to be used in diagnostic testing as it proves to aid in forming a more uniform protein spot with even evaporation. Moreover, the low concentration of PEG may increase the number of diagnostic tools employed in resource-limited countries to include the number of transmittable disease cases, thus improving global healthcare. This study serves as the pipeline for other researchers to include additional high-end equipment for a more detailed study of the interaction of polymeric membrane and polymer additives. Both show excellent results in suppressing this phenomenon.

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