The Effect of pH Immobilization of Antibody-B on the GPTMS Functionalized Filter Paper for Detect Antigen B on the Red Blood Cell Surface

Sasinapa Gasipol[1], Chinnawut Pipatpanukul[2,4], Toemsak Srikhirin[3,4] and Nongluck Houngkamhang[1,4*]

[1] *College of Materials Innovation and Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand*

[2] *Office of Education, Faculty of Engineering, Burapha University, Chonburi, 20131, Thailand*

[3] *Materials Science and Engineering Programme, Faculty of Science, Mahidol University, Bangkok, 10400, Thailand*

[4] *Thailand Center of Excellence in Physics, Ministry of Higher Education, Science, Research and Innovation, Bangkok, 10400, Thailand*

Received: 23 May 2021, Revised: 17 June 2021, Accepted:21 June 2021

Abstract

The filter paper-based analytical platform has been interested in recent years due to its availability and inexpensiveness. This work focused on the silane functionalized filter paper which was developed for immobilization of antibody probe, anti-B, for detecting their specific antigen, antigen-B, on red blood cells surface. 3-Glycidoxypropyltrimethoxysilane (GPTMS) was hydrolyzed under a basic solution to conduct anti-B immobilized onto the paper via covalent bonding. The concentration of GPTMS was compared between 0.5% and 5% v/v to obtain the strong signal of red blood cells adhering to the surface. The functionalized surface was characterized with the FTIR technique. Then the pH of the buffer solution was varied to find the suitable pH for linking the antibody onto the silanized filter paper. The results show that using a high concentration of GPTMS can enhance the antibody and antigen interaction. At strong pH (pH 9) solution, the opening epoxide ring can occur, and antibodies were linked onto the surface via this reaction. The interaction of antibody-B and antigen on red blood cells was measured by the mean intensity of the color present on the paper and confirmed red blood cells adhering by scanning electron microscope. The GPTMS functionalized filter paper can enhance the bioactivity of specific interaction of antibody-B and red blood cell-B and the uniformity of the reaction.

Keywords: Antibody-B, Filter paper, Red blood cell, Silane coupling

1. Introduction

Paper-based analytical devices have been interesting in the sensor research field due to the platform is easy-to-use, inexpensive, and can be portable [1]. Determination of blood type is normally based on the principle of haemagglutination between red blood cells and serum antibodies. The currently common techniques used for the identification of blood groups include the slide test, tube test, microplate, and column agglutination [2]. The paper-based technique for ABO blood typing almost combines with wax printing [3] which is needed for the specific chemical and printing machine. 3-Glycidoxypropyltrimethoxysilane (GPTMS) has been used for a wide range of applications by tailor properties as well as increase opportunities for their applications. It is one of the most common precursors for hybrid organic–inorganic materials. Example for optical waveguides [4], coatings for anti-scratch [5], or modification of organic polymer surface [6]. GPTMS can be acted as a grafting agent for the functionalization of silica surfaces. Silane functionalized nanoparticles were used to

Corresponding Author : nongluck.ho@kmitl.ac.th

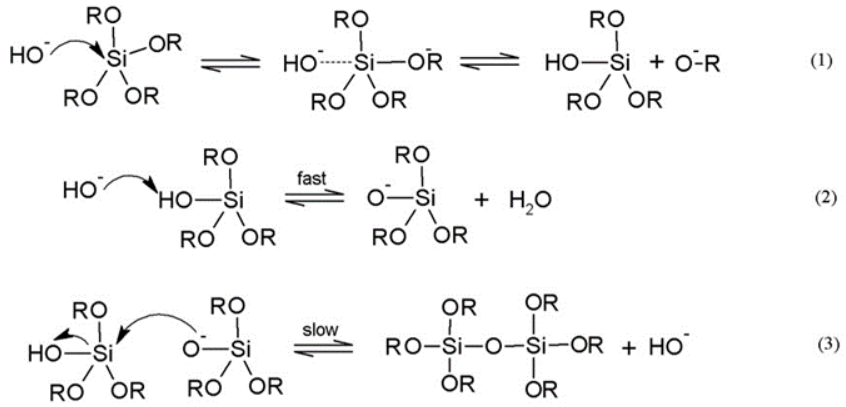

Fig. 1. Hydrolysis reaction (1) and Condensation reaction ((2) and (3)) of alkoxysilane in a basic catalyst

increases detection sensitivity due to the uniformity of the reaction [7]. The alternative way to increase the amount of antibody bound to the paper is using silane coupling onto the paper surface. Alkoxy groups can react with the hydroxyl group on the paper in an aqueous solution via hydrolysis and condensation reactions and then assemble into the silanized papers. The general hydrolysis and condensation mechanisms of an alkoxysilane in a basic catalyst are shown in Fig. 1 [8].

In this work, the GPTMS functionalized filter paper in basic condition was successfully demonstrated. The antibodies can be covalently linked onto the paper surface via an opening epoxide ring for detection of their specific antigen. The antibody-B was used as a protein immobilization probe which will have specific interaction with antigen-B on the red blood cells surface. The low and high concentration of GPTMS was treated on the paper to compare the results. The pH of the buffer was varied to find the most optimum pH for antibody immobilization onto the silanized surface. The color intensity of antibody-antigen interaction was compared between silanized paper and without silanization.

2. Experimental details

2.1 Materials

The 3-Glycidoxypropyltrimethoxysilane (GPTMS) was purchased from Dow corning (Thailand) LTD. Commercial filter paper (industrial grade) with a pore size of 10 µm was cut at 1 cm^2 sized. Antibody-B and red blood cells (RBC-A, -B, and -O) were purchased from The National Blood Centre Thai Red Cross Society, Thailand. The chemical used for preparing phosphate buffer saline pH 7.4 from Sigma-Aldrich. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) was used to adjust the pH buffer obtained from Sigma-Aldrich and Carlo Erba, respectively. The chemical structure of GPTMS showed in Fig. 2.

2.2 Functionalization of filter paper

The 0.5% and 5% of GPTMS were prepared in deionized (DI) water for hydrolysis for 30 minutes. The pH was adjusted in basic condition (pH13) by NaOH. The filter paper was immersed in the solution for 3 hours then dried at room temperature. The filter paper was then baked at 70 ºC for 6 hours to condense the silane on the paper surface. The functionalized filter paper was characterized by the FITR technique compared with the non-functionalized.

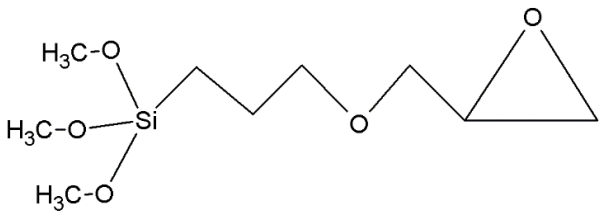

Fig. 2. Chemical structure of GPTMS

2.3 Antibody immobilization and red blood cell detection

The functionalized filter paper was rinsed with DI water before used. The PBS buffer at various pH (<4, 5, 6, 7, 8, 9, and >9) was dropped onto each piece of paper followed with 10 µL of antibody-B and waited for 20 minutes. A red blood cell sample at 5 µL was dropped on the antibody area and wait for the reaction for around 2 minutes. The paper was rinsed with PBS buffer to wash unbound red blood cells from the paper surface and observed the red color on the paper that determined the reaction of antibody-B and antigen on the red blood cells surface. The results were scanned by EPSON L210 scanner for recording the picture. The spot intensity was analyzed by the Image J program. The specific interaction of RBC-B adhered on the immobilized antibody-B surface was measured by a scanning electron microscope.

3. Results and discussion

3.1 GPTMS functionalized filter paper

In this study, *3*-glycidoxypropyltrimethoxysilane (GPTMS)organosiloxane can be used as a coupling agent to immobilize biomolecule (antibody) onto the paper surface to increase the detection signal of antigen on red blood cells. GPTMS can couple organic molecules through the reactive epoxide functionality and coat the paper surface through the hydrolyzed Si-OH groups. FTIR spectra of non-functionalized (non-FN) paper and functionalized (FN) paper were compared in Fig. 3. As expected, the bands of interest are weak and they could be overlapped by characteristics of cellulose when functionalized GPTMS on filter paper. The peak at 3,335 cm^{-1} is characteristic for O-H stretching and 2,897 cm^{-1} for C-H stretching of polysaccharides or cellulose fiber. The typical bands represented to cellulose also were shown in the range of 1,630-900 cm^{-1} that belong to stretching and bending vibrations of -CH_2 and -CH, -OH, and C-O bonds [9], [10]. In the spectra of GPTMS functionalized paper, the Si-O-Si bands were noticed at 852 cm^{-1} and 790 cm^{-1} (inset graph) for bending and stretching, respectively [11]. The peak of epoxide ring [12] (C-H of the three-membered ring) at 3,045 cm^{-1} was not observed which concordant to the previous work [11] may be due to the overlapping of the O-H board band of the cellulose paper. By the way, the strong specific band of Si-O-Si around 1,150 cm^{-1} may be overlapped with the same region of an intense C-O-C vibration band of cellulose. Hence, these signals are not easily measured by the FTIR technique.

For immobilization of antibody-B onto filter paper, 0.5% and 5% (v/v) GPTMS in water which represented the low and high content of linker molecule were compared for the covalent link of antibody onto the surface. The hydrolysis and condensation reaction of GPTMS were done in basic catalysis for the formation of silica. An increase of GPTMS content can increase antigen binding to the antibody surface which was seen by the more intense red color of the paper as shown in Fig. 4. Specific reaction of antibody-B and red blood cells-B (RBC-B) was observed compared with unspecific binding with RBC-A and

RBC-O. The unspecific signal was around 2.7±0.7% while the specific interaction showed the signal more than 2.7% on both functionalized GPTMS (0.5% and 5%). The intensity of 8.0±0.6% to 11.8±1.6% was obtained on the specific interaction of RBC-B and antibody-B on the functionalized surface. Although the non-functionalized surface showed a specific signal of 6.4±1.2% which was lower than the functionalized surface. Functionalized paper with 5% GPTMS can increase the signal for 45.7% and 32.2% when compared with non-functionalized paper and 0.5% GPTMS functionalized paper, respectively. From this result can be noted that a modified surface with a suitable amount of GPTMS can enhance the bioreactivity and uniformity of the reaction.

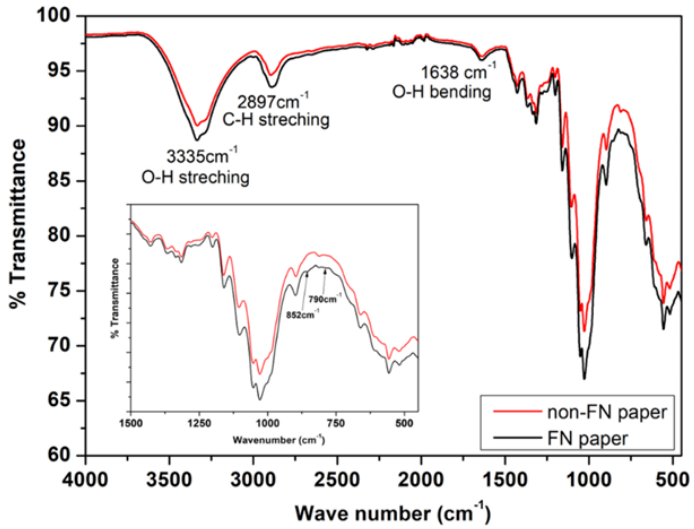

Fig. 3. FTIR spectra of non-functionalized paper and functionalized paper with GPTMS

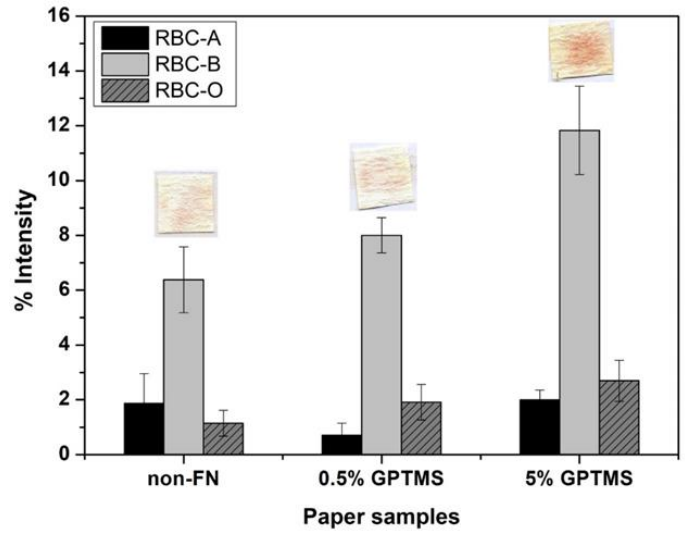

Fig. 4. The adhering of red blood cell-B on immobilized antibody-B on the non-functionalized (non-FN) paper and functionalized paper with 0.5% and 5% GPTMS

3.2 pH effect on the antibody immobilization

Filter paper functionalized with 5% GPTMS was used to immobilized antibody-B by varying the pH of a solution in the range of 4 to 9. To find the optimum immobilizing pH, red blood cell samples were dropped onto the surface and the red color was measured after loosely bound RBCs were washed off. The intensity of the unspecific signal of antibody-B and RBC-O was lower than the specific signal on both non-functionalized and functionalized surfaces as shown in Fig. 5. The specific signal of antibody-B and RBC-B on non-functionalized paper still exhibit too low at pH range 4 to 7 but it showed a little bit increase when pH more than 8. The higher signals were clearly seen in the case of antibody-B and RBC-B interaction on functionalized paper at all immobilizing pH.

We noticed that the signal was higher in low and high pH it means that the reaction occurs in strongly acidic and basic conditions which can open the epoxide ring and link the antibody onto the surface via the amine group. The opening of epoxides ring under acidic conditions occurs in 2 steps, first, the epoxide is protonated by H^+, and second, the nucleophile (NH_2 from antibody) attacks at the carbon-oxygen atom (C-O) in the epoxide ring. The ring-opening reaction occurs to form an antibody linkage to the surface. In case of strong basic condition, ring-opening of epoxides proceed by a nucleophile (NH_2 from antibody) attacks the epoxide at the carbon of the C-O atom and result in a transfer of proton to the alkoxide (RO^-) providing neutral alcohol with having antibody link at the end chain. The epoxide ring-opening reaction in basic and acid conditions can be seen in Fig. 6. From this context, the highest signal of antibody-B and RBC-B showed at pH 9 of antibody immobilization then the immobilizing solution was chosen at pH 9 which was an optimum immobilizing pH in this work.

3.3 SEM characterization of the interaction of antibody and red blood cell

SEM image can confirm the interaction of immobilized antibody-B and RBC-B on GPTMS functionalized paper compared with non-functionalized paper as shown in Fig. 7. The results show that less amount of red blood cells adhered on the non-functionalized surface while the functionalized surface showed a higher amount of red blood cells adhering. Because GPTMS acts as the grafting agent to anchor the functionality which linking the antibody onto the surface. This can enhance the bioactivity between immobilized antibodies and antigens on the red blood cell surface. For a non-functionalized surface, the antibody could be physically adsorbed onto the filter paper then loosely bound molecules may be washed off easily leading to the low signal of red blood cells adhered on the surface.

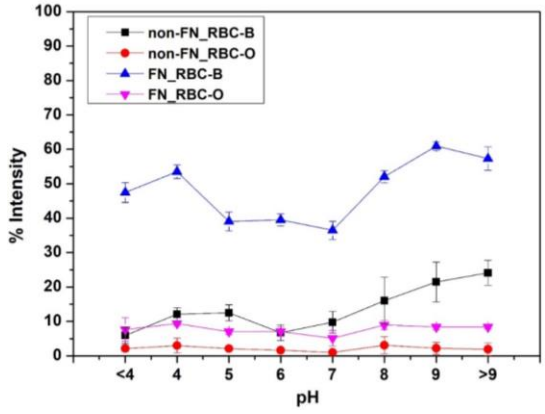

Fig. 5. Measurement signal of antibody-B with RBC-B and RBC-O interaction by immobilizing antibody with different pH in the range of 4 to 9

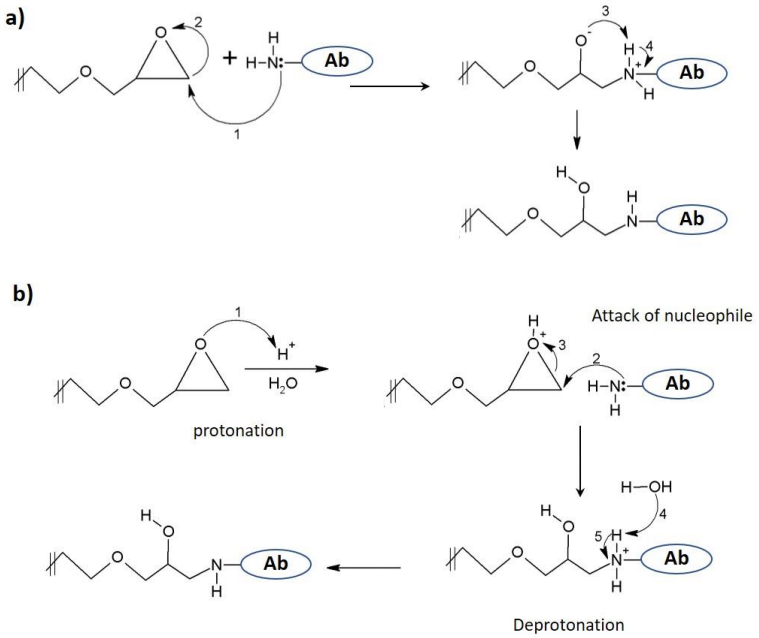

Fig. 6. Immobilization of antibody via epoxide ring opening of GPTMS under a) basic and b) acidic conditions

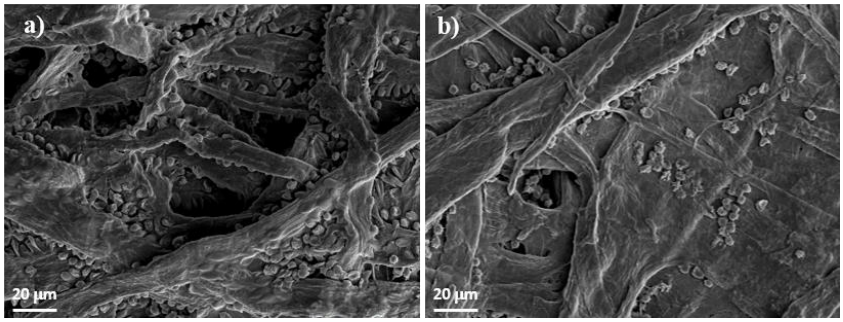

Fig. 7. SEM image of specific interaction of immobilized antibody-B and RBC-B on a) GPTMS functionalized filter paper and b) non-functionalized filter paper

4. Conclusion

In this study, surface functionalization of filter paper is accomplished to modify property as well as increase the opportunity for antibody immobilization. By using 3-Glycidoxypropyltrimethoxysilane (GPTMS) in an aqueous solution through the hydrolysis and condensation reaction to form silica in high pH conditions. The efficiency of GPTMS grafting showed in the high signal intensity of red blood cells adhered onto the surface more than that of non-functionalize. Moreover, the functionalized surface provided uniformity of the reaction and signal enhancement. GPTMS functionalized filter paper may offer the application in biochemistry by probing different types of protein reactions.

Acknowledgment

The authors would like to thank Thailand Center of Excellence in Physics for financial support.

References

[1] Chaiyo S, Siangproh W, Apilux A, Chailapakul O. Highly selective and sensitive paper-based colorimetric sensor using thiosulfate catalytic etching of silver nanoplates for trace determination of copper ions. Anal. Chim. Acta 2015;866:75-83.

[2] Estridge BH, Reynolds AP, Walters NJ. Basic medical laboratory techniques. Boston: Cengage Learning; 2000.

[3] Noiphung J, Talalak K, Hongwarittorrn I, Pupinyo N, Thirabowonkitphithan P, Laiwattanapaisal W. A novel paper-based assay for the simultaneous determination of Rh typing and forward and reverse ABO blood groups. Biosens. Bioelectron. 2015;67:485-9.

[4] Innocenzi P, Martucci A, Guglielmi M, Armelao L, Pelli S, Righini GC, Battaglin GC. Optical and surface properties of inorganic and hybrid organic–inorganic silica–titania sol–gel planar waveguides. J. Non-Cryst. Solids 1999;259(1-3):182-90.

[5] Philipp G, Schmidt H. New materials for contact lenses prepared from Si-and Ti-alkoxides by the sol-gel process. J. Non-Cryst. Solids 1984;63(1-2):283-92.

[6] Watling JD, Lewis DA. Condensation of glycidoxypropyltrimethoxysilane to afford siloxane pre-polymers useful in the preparation of hardcoating resins. J. Sol-Gel Sci. Technol. 2003;28(2):167-74.

[7] Evans E, Gabriel EF, Benavidez TE, Coltro WK, Garcia CD. Modification of microfluidic paper-based devices with silica nanoparticles. Analyst. 2014;139(21): 5560-7.

[8] Brinker CJ, Scherer GW. Sol-gel science: the physics and chemistry of sol-gel processing. Academic press; 2013.

[9] Rosa MF, Medeiros ES, Malmonge JA, Gregorski KS, Wood DF, Mattoso LH, Glenn G, Orts WJ, Imam SH. Cellulose nanowhiskers from coconut husk fibers: Effect of preparation conditions on their thermal and morphological behavior. Carbohydr. Polym. 2010;81(1):83-92.

[10] Poletto M, Pistor V, Zeni M, Zattera AJ. Crystalline properties and decomposition kinetics of cellulose fibers in wood pulp obtained by two pulping processes. Polym. Degrad. Stab. 2011;96(4):679-85.

[11] Guido E, Colleoni C, De Clerck K, Plutino MR, Rosace G. Influence of catalyst in the synthesis of a cellulose-based sensor: Kinetic study of 3-glycidoxypropyltrimethoxysilane epoxy ring opening by Lewis acid. Sens. Actuators B Chem. 2014 ;203:213-22.

[12] Launer PJ. Infrared analysis of organosilicon compounds: spectra-structure correlations. Silicone compounds register and review. NY:Burnt Hills;1987. p.100-3.