THE ISOLATION OF PROTEIN FROM LATEX *Hevea brasiliensis* AND THE DETERMINATION OF CORROSION INHIBITION ACTIVITY TOWARD CARBON STEEL

UFAIZA ZAIN, SADIJAH ACHMAD, DEANA WAHYUNINGRUM AND BUNBUN BUNDJALI

Chemistry Study Program, Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology

Jl. Ganesha 10, Bandung 40132, Indonesia

Email : ufaizazain@yahoo.com

ABSTRACT

Corrosion is one of serious problems in the petroleum mining. The best method to prevent corrosion that occurred at the inner parts of carbon steel pipelines is the use of organic corrosion inhibitor. Latex, which is an extract isolated from rubber tree *Hevea brasiliensis*, has high economic value. Latex can be obtained by tapping the bark of rubber tree. Latex consisted of rubber particles and non rubber particles such as protein. The corrosion inhibition activity of serum was tested toward carbon steel. Subsequently, the serum was fractionated by the addition of ammonium sulphate solution. The determination corrosion inhibition activities toward carbon steel utilizing Tafel method. The results showed for each fraction, which is the 60%, 80% and 90% fraction, that the efficiency are as much as 66.56%, 44.32% and 33.96%, respectively. The serum showed corrosion inhibition efficiency as much as 87.63%. The corrosion inhibition efficiency of the serum is higher than its fractions because there are synergic effects resulted by all the components of proteins in these fractions. The measurements showed the decrease in corrosion inhibition efficiency of the serum when prolonged for 1, 7 and 21 days, which are 87.63%, 72.27% and 41.25%, respectively.

Keywords: corrosion, corrosion inhibitor, protein, latex, Tafel method.

1. INTRODUCTION

Corrosion is a process of material damage due to a reaction between the materials and its environments (Jones, 1991). Carbon steel pipelines used in gas and petroleum mining

tend to corrode spontaneously. Corrosion might occcur at either inner and outer parts the pipelines. Corrosion that occurred at the inner parts of pipelines can be prevented by adding a corrosion inhibitor along with the flow of other materials injected through the pipelines. Corrosion inhibitor can be defined as a chemical compound when added in small concentration to its enviroment would prevent or slow down corrosion rate (Duda, et.al, 2005). Corrosion inhibitors were made of either inorganic or heteroatom organic compounds. However, recently the heteroatom organic compounds are used more frequent than the inorganic compounds. One of the reasons is the more environmentally friendly properties of heteroatom organic compounds compare to the inorganic compounds when used as corrosion inhibitors (Benadellah et.al, 2007).

One of the organic compounds used as corrosion inhibitor is amino acid. Some research showed that amino acid such as proline, histidine and alanine, gave significant corrosion inhibition activity (Brahma, 2005; Ibrahim, 2007). The research also showed that amino acids which form peptide compounds gave better corrosion inhibition effect than single amino acid (Brahma, 2005; Ibrahim, 2007; Wirman 2007). Protein, which is amino acid polymer, is assumed to have comparable corrosion inhibition effect as much as amino acid and the peptide compounds. Protein used in this research is a protein found in rubber latex of *Hevea brasilliensis*. Some research shows that protein consisted in rubber latex can be allergic to humans, therefore in rubber processing industry the protein must be removed and disposed from latex (Lopez and Romero, 2004; Chen, 1996). This protein waste from latex can be used as a source of corrosion inhibitor, which became the main idea and purpose of this research. Therefore this reseach presents the study of protein isolated from rubber latex of *Hevea brasilliensis* as candidate of potent corrosion inhibitor.

2. MATERIALS AND METHODS

2.1. Materials

All of reagents used in this research are GR grade. All of solvents were distilled prior to use. The material used in this research are: rubber latex of *Hevea brasilliensis*, methanol, aquadest, acetic acid, ammonium sulphate, sodium chloride, acryl amide, *NN-bis*-acrylamide, TEMED and ammonium persulphate, comassie blue, glycerol, *tris* HCl buffer, bromphenol blue and glycine for electrophoresis. Some amino acids were used as standard solution for thin layer chromatography (TLC) analysis: L-Glycine, L-Glutamine, L-Cysteine, L-Alanine, L-Tyrosine, L-Asparagine, L-Valine, L-Phenylalanine, L-

Methionine, L-Proline, L-Threonine, L-Histidine, and L-Tryptophane. The TLC used butanol-acetic acid-water (4:1:1) and methanol-chloroform-water (4:4:1) as developing solvents (eluents).

2.2. Methods

2.2.1. Sampling

The sample used in this research is the latex rubber which is taken from the area of rubber plantations in Purwakarta, West Java, Indonesia.

2.2.2. The isolation of Protein

2.2.2.1. Protein Coagulation

Latex rubber was separated from the serum by coagulation prior to the addition of 500 mL of 1% v/v acetic acid solution into 300 mL of latex rubber, followed by the filtration process.

2.2.2.2. Fractionation of Protein

Fractionation of protein was carried out by adding ammonium sulphate to the protein serum in levels of saturation from 0% to 100%. The deposit was separated using centrifuges at 5300 rpm for 30 minutes.

2.2.3. The Determination of Corrosion Inhibition Activity

Fractions of protein serum were dissolved in 100 mL of 1% w/v NaCl solution at the concentration of 40 ppm whilst the concentration of crude protein serum in 1% NaCl solution is 5% v/v. The 1% (w/v) NaCl solution was also used as blank solution in each measurement. Into 110 mL specialized chamber equipped with magnetic stirrer was introduced 100 mL of blank solution or sample solution. The working electrode (carbon steel), the reference electrode (SCE), and auxiliary electrode (platinum electrode) were immersed into the electrolyte solution. Carbon dioxide gas was introduced into the electrolyte solution until saturation reached, approximately 20 minutes. The carbon steel type used is API 5L X65 with compositions (in percentage, %): Fe (97,9327); C (0,0737); Si (0,2882); S (0,0068); P (0,0153); Mn (1,5353); Ni (0,0129); Cr (0,0224); V (0,0276); Cu (0,0051); W (0,0029); Ti (0,0169); Sn (0,0005); Al (0,0282); Nb (0,0396); Zr (0,0009); Zn (0,0014). The measurement utilizing Potentiostate/Galvanostate PGZ 301 VoltaLab® 30 model and VoltaMaster® software program until the curve of potential measurement towards time was completely formed well. The measurements of each

sample solution should be initiated by the measurement of blank solution. The inhibition activity can be calculated using following equation:

$$\%\ \text{inhibition efficiency} = \frac{\text{Corrotion rate of blank solution (mm/Y)} - \text{corrosion rate of sample (mm/Y)}}{\text{Corrotion rate of blank solution (mm/Y)}} \text{x100\%} \quad (2\text{-}1)$$

or

$$\%\ \text{inhibition efficiency} = \frac{I_{Blank}\ (mA/cm^2) - I_{Sample}\ (mA/cm^2)}{I_{Blank}\ (mA/cm^2)} \text{x100\%} \quad (2\text{-}2)$$

with I_{blank} is corrosion current density of blank solution (uninhibited system), in mA/cm^2, and I_{sample} is corrosion current density of sample solution (inhibited system).

2.2.4. Protein Analysis

Protein that have been shown to have corrosion inhibition efficiency was analyzed by native PAGE electrophoresis using Leamli reagent. The determination of amino acids consisted in each protein fraction utilized the thin layer chromatography (TLC).

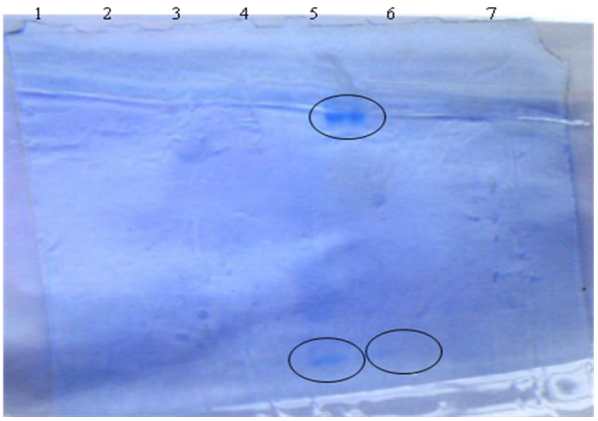
3. RESULTS AND DISCUSSION

The coagulation of rubber latex in 1% v/v acetic acid solution resulted 540 mL of protein serum. The determination of protein serum by means of biuret reagent which gave purple solution that indicated the presence of protein. The fractionation of protein used ammonium sulphate gave 3 fractions of protein which are 60 %, 80 % and 90 %. The increase in the concentration of ammonium sulphate solution caused the precipitation of protein through the dehydration process of protein molecules. If protein was present in solution, then water molecules will form a layer around the protein. The formations of this layer tend to maximize the hydrogen bond, which is less preferred condition because it requires energy. A high concentration of salt in water causes the breaking off the hydrogen bond and water molecules prefer to interact with the ion salt than with the protein molecules. The absence of water molecules around the protein molecule made the protein molecules tend to increase the interaction between themselves that caused the precipitation. In this process, the protein molecules that have greater molecular weight will be the first one to be precipitated.

The analysis of protein using native PAGE electrophoresis showed that the protein fraction of 60% gave two bands with Retention mobility (Rm) of 0.86 and the protein fraction of 80% gave two bands with Rm 0.86 and 0.25.

Figure 1 The protein serum of rubber latex

The results indicated that the protein molecules of fraction of 60% are more compact and larger. On the other hand, the fraction of 80% has two types of protein. One of proteins is the same as the protein found in fraction of 60% and the other one has larger molecular structure. In the fraction of 80% the protein type has stronger intensity than the more compact structure protein that found in the fraction of 60%.



1 = The solution of BSA 50 µg/mL
2 = Serum after prolonged for 3 days
3 = Fresh serum
4 = The protein fraction of 60 %
5 = The protein fraction of 80%
6 = The protein fraction of 90%
7 = The solution of BSA (bovine serum albumin) 50 µg/mL

Figure 2 The electrophoregram of proteins using Native PAGE analysis

The results of the TLC analysis showed that both of the fractions of 60% and 80% have similiar amino acid composition, whilst the amino acid composition of the fraction of 90% is slightly different. In addition, it was found the presence of amino acid that has different Rf (Retention factor) value from the thirtienth amino acids used as standards in TLC, which gave orange spot on the TLC of the fraction of 60% and 80%. This amino acid was suspected to be the main component of the fraction of 60% and 80% since the intensity of orange colored spot on the TLC of the fraction of 60% and 80% is more dominant than other spots. This amino acid should be a polar compound because it showed a high Rf value on polar solvents (butanol-acetic acid-water (4:1:1)) as eluents but a low Rf when more non polar solvents used as developing solvents (methanol-chloroform-water (4:4:1)). Based on these results, it can be concluded that the protein fraction of 60% and 80% contain more polar amino acids than the fraction of 90%.

The results of the determination of corrosion inhibition activity of crude protein serum and the isolated proteins toward carbon steel in 1% w/v NaCl solution are presented on Table 1. The corrosion inhibition activity of protein fraction of 60 % is the largest, followed by fraction of 80% and 90%. This result showed that corrosion inhibition efficiency is influenced by molecular size and molecular weight of proteins found in each fraction. The corrosion inhibition efficiency is also influenced by the molecular structure of the protein. The electrophoresis analysis showed that the fraction of 60%, which has the largest corrosion inhibition efficiency, consisted of a more compact protein molecules than the other fractions. It can be seen from the Rm value of protein found in fraction of 60% is 0.86%. In the native PAGE analysis of protein showed that the more compact of protein molecule will have a higher mobility compared to the larger but less compact protein. The data also showed that the fresh protein serum has greater corrosion inhibition efficiency (in %) towards carbon steel than the isolated proteins. It can be assumed that the corrosion inhibition activity of protein serum was caused by the presence of other components consisted in crude protein serum that also provides corrosion inhibitor activity. The presence of other components gave synergistic effect with the corrosion inhibition activity of isolated proteins. However, most of the components in protein serum are relatively unstable. It can be seen from the results of the determination of corrosion inhibition activity of protein serum which is decreased along with the prolonged storage time (Table 2 and Figure 3).

Table 1 The Determination of corrosion inhibition activity of protein serum and isolated proteins

No.	**Sample**	**Concentration**	**I_{corr} (µA/cm²)**	**% Inhibiton Efficiency**
1.	Blank solution (NaCl 1% w/v)	-	80,1027	
2.	Protein serum	5 % (v/v)	9,9113	87,63
3.	Fraction of 60%	40 ppm	26,7853	66,56
4.	Fraction of 80%	40 ppm	44,5995	44,32
5.	Fraction of 90%	40 ppm	52,9002	33,96

Table 2 The Determination of corrosion inhbition activity of protein serum based on its storage time

No.	Sample	concentration	Storage Time (days)	I_{corr} (µA/cm²)	% Inhibiton Efficiency
1.	Blank solution (NaCl 1%)	-		80,1027	
2.	Serum 1	5% (v/v)	1	9,9113	87,63
3.	Serum 2	5% (v/v)	7	22,2111	72,27
4.	Serum 3	5% (v/v)	21	47,0574	41,25

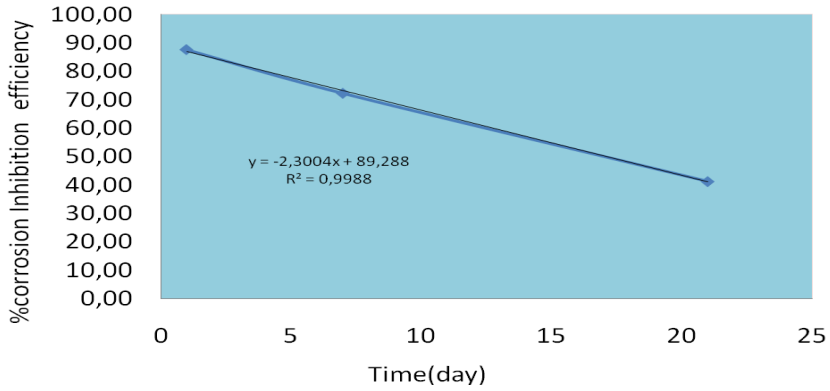


Figure 3 The plot between the corrosion inhibition efficiency and the prolonged storage time of crude protein serum solution

Based on all of the data obtained, it can be concluded that molecular structure and molecular weight of protein as well as amino acid composition of proteins play an important role in the corrosion inhibition process of carbon steel in 1% NaCl solution. In addition, the stability of molecular structure of protein also determines the corrosion inhibition efficiency of protein serum and the isolated proteins. Therefore this study showed the potent of protein isolated from the waste of rubber latex processing as corrosion inhibitor towards carbon steel in a mild electrolyte condition.

4. ACKNOWLEDGEMENTS

1. The Ministry of National Education of Republik Indonesia for the scholarship to the first author

2. The Head of NADFC for permitting the first author to study in Chemistry Study Program, Faculty of Mathematics and Natural Sciences, ITB
3. The Ministry of Research and Technology of Republik Indonesia for partially granted the research
4. To all parties associated to this research that can not be mentioned one by one

REFERENCES

Benabdellah, M., Dafali, A., Hammouti, B., Aounti, A., Rhomari, M., Senhaji, O., and Robin, J.J. 2007. *Chem. Eng. Comm., 194, 1328 – 1341.*

Brahma, R. 2005 *Sintesis Ester Histidin dan Amida Histidin sebagai Senyawa Inhibitor Korosi pada Baja Karbon,* Tesis Program Magister, Institut Teknologi Bandung.

Chen, Z., Posch, A., Lohaus, C., Heimsoth, M.R., Meyer, H.E. and Bauer, X.1996. *J. Allergy Clin. Immunol., 99 (3), 402 -408.*

Duda, Y., Rueda, G.R., Galicia, M., Beltran, H.I., and Rivera, L.S.Z. 2005. *J. Phys. Chem. B, 109, 22674 – 22684*

Ibrahim, P.A. 2007 *Sintesis Senyawa Dipeptida Ester Metil Histidil Prolin sebagai Inhibitor Korosi pada Baja Karbon dalam NaCl 1 %, ,* Tesis Program Magister, Institut Teknologi Bandung.

Jones, D.A., 1991. *Principles and Prevention of Corrosion,* Maxwell Maxmillan International Publishing Group, New York, 1 – 7, 475 – 508.

Lopez, C.A.S.R. and Romero A.R. 2002. *Alegia, Asma e Inmunologia Pediatricas, 11 (3), 92 – 100.*

Wirman, A.P., 2007 : *Sintesis Hsitidinal sebagai Inhibitor Korosi pada Baja Karbon dalam Larutan NaCl 1%,* Tesis Program Magister, Institut Teknologi Bandung.