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Article

Process Yields Improvement of Filter Presses in Rabies Immunoglobulin Production

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Abstract. The objective of this study is to improve the performance of the Equine Rabies Immunoglobulin (ERIG) process by using the caprylic acid as a single precipitated agent and the replacement plan for the filter press machine to support higher demand in ERIG. The experiments based on the face-center central composite design are performed to investigate the relationship of yield recovery with 1. the concentration of caprylic acid used in the purification process, 2. the flow rate, and 3. the pore size of the filter press machine used in the filtration process. The regression analysis shows no relationship on linear, quadratic, or interactions between yield recovery and the three factors. Fortunately, according to the Analysis of Variance (for comparing means), there is a significant effect of interaction between the flow rate and pore size and the main effect of concentration of caprylic acid on the yield recovery at a 90% confidence level. From the interaction plot, at a flow rate of 10.5 ml/s with a filter media pore size of 6-15 micron, the process has the maximum yield recovery of 15.5% that is significantly superior to those of the other two sizes of filter media at the same flow rate. With a flow rate of 16.8 ml/s, the yield recovery is not significantly different for any pore size. At a flow rate of 4.2 ml/s, the yield recovery is higher when using the pore size of 4-9 and 6-15 micron than those from 5-12 micron. Nevertheless, with a 90% confidence interval for average yield recovery by Tukey comparison, the average yield recovery received from 16.8 ml/s at every pore size and 4.2 ml/s at 4-9 micron are not statistically different. Therefore, with the current size, 5-12 micron, of filter media used in the current process, the flow rate is suggested to be 16.8 ml/s whereas, the caprylic acid concentration of 1% is preferred due to the highest yield recovery compared to other concentration levels and the lowest production cost.

Keywords: Equine rabies immunoglobulin, $F(ab')_2$, immunoglobulin, design of experiment, process optimization.

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1. Introduction

Equine Rabies Immunoglobulin (ERIG) is a vital treatment for a risky case of rabies infection. ERIG contains $F(ab')_2$ that provides acquired immunity which neutralized the rabies virus instantly. [1] The traditional biological based ERIG production was developed over a hundred years and improved for higher quality, purity, and yields. Thai Red Cross is the only organization produces ERIG in Thailand by the traditional animal-based method. However, it confronts low yield recovery in a large-scale production. The yield recovery is a process efficiency indicator which calculates from potency and amount of either crude or purified plasma where a potency is a strength unit of rabies medicine in neutralizing rabies virus before it infects the cell. According to in-house study, the potency loss is discovered in the manufacturing process and mostly occurred during the purification process including fractionation by precipitation and filtration process by filter press machine resulting in either yield variation or reduction, which later affects the number of final products from a production line.

Purification process, especially in the fractionation step, has many factors involved such as reagent concentration, plasma pH, and plasma temperature. Many studies were conducted to improve yield recovery in the fractionation process. According to Simsiriwong et al. [2], salt fractionation optimum concentration by conducting factorial experiments report that either 1.5% caprylic acid with 10% ammonium sulfate or only 2% caprylic acid gives the highest result of antibody-titer and specific activity of equine snake antiserum production. Another study is Eursakun et al. [3], reported that 3.5% CA alone is the optimum fractionation condition which give high specific antibody activity, antibody recovery and low turbidity. Caprylic acid was introduced in non-igG precipitation by Santos et al. [4] and Perosa et al. [5]. Using only caprylic acid in precipitation has a benefit over a combination of caprylic acid and ammonium sulfate that may cause a reduction of $F(ab')_2$, yield, and purity. Caprylic acid interacts with large plasma protein by hydrophobic interaction that accumulate the protein to the larger poor soluble particle unlike ammonium sulfate that cause protein salting out from the plasma [6]. Besides, caprylic acid precipitation gives better neutralizing activity, higher yield recovery and less yield loss from aggregation of Immunoglobulin into protein coagulate. The caprylic acid precipitation as one step is also preferred for production optimization [7]. Nudel et al. [8] also studied the effect of the caprylic acid concentration, pH, and temperature on protein precipitation in a small micrometer scale of laboratory filtration by using a full factorial experiment. The regression analysis reported 3% caprylic acid, 37°C, and pH 4.9 as the best combination to produce high protein recovery and content. However, the optimum caprylic acid concentration according to literature reviews varies from one to the other. Consequently, the appropriate amount of caprylic acid is one of the interesting factors of this study.

For the filtration process, a filter press machine is normally used due to low cost, uncomplicated operation, and easy maintenance. The advantage of using filter press is a large volume batch production that requires a large area of filtration and chamber space to store fractionated protein slurry. The filter press process also involves many parameters such as filter media, pore size, and inlet flow rate [9] that may affect the yield recovery and other process performance.

Due to high ERIG demand, the Thai Red Cross planned to scale up the production by increasing batch production size from 80 kg of starting volume to 120 kg. Therefore, the machine of at least 120 kg production capacity is needed. The current filter press machine reaches its maximum filtration capacity of 80 kg and needs to be replaced for higher filtration capacity which could produce a higher number of finished products in single batch production. With the proposal to buy a new machine along with the objective to improve the yield recovery by using the caprylic acid as a single precipitated agent, the concentration of caprylic acid and the process specification of filter press machine that may affect the yield recovery which include the pore size and flow rate will be considered. Due to the fractionation process and filter press filtration are successive that may have connected effect. Therefore, factors in these processes are included in consideration to inspect their consequences on the yield recovery for ERIG production. The experimental designs as the popular and efficient tools for the process improvement [10] will be conducted for three interested parameters with respect to the highest yield recovery. This paper is organized as follows. The ERIG process operated by the Thai Red Cross are described in next section. The material and method for the experiment are explained in section 3. The experimental results are analyzed and discussed in the section 4. The conclusion and further suggestions are finally presented.

2. ERIG Production Process

As mentioned earlier, the Thai Red Cross is the only organization produces ERIG in Thailand, by the traditional animal-based method as shown in Fig. 1. Traditional production started with plasma pooling and mixing steps. The collected immunized plasma was pooled and mixed to make 80 kg of volume as an active ingredient for single production batch, then a sample was collected to assay strength or potency at starting point of production.

In step 2, called dilution and pepsin digestion, plasma was diluted with water for injection double volume of starting volume. The pH was adjusted, then heated to prepare for pepsin digestion. Pepsin was added to digest the Fc part of Immunoglobulin resulting in $F(ab')_2$, the active neutralizing region. Pepsin digestion has been continuing for an hour before terminated by adjusting pH to neutral. The digested plasma then continued in the purification process. In the next step, thermo-coagulation, plasma was heated to 56°C for an hour to activate the

thermo-coagulation of macro protein such as albumin and globulin, the high molecular weight protein in plasma. Higher size of the protein molecule is occurred (agglomerated) from intermolecular bonding and continued to the next process.

Coagulated proteins were fractionated out of plasma with 14% w/w ammonium sulfate and 1% w/v caprylic acid. Ammonium sulfate was gradually added in the salt-fractionation process while plasma was vigorously stirred. Plasma was continued stirring for an hour for full reaction. Then, caprylic acid was added to plasma and continued stirring for an hour. Ammonium sulfate and caprylic acid react as a coagulating agent to separate fractionated protein and small molecule of protein in plasma.

In the filtration process, fractionated protein was then filtered out from plasma bulk by filter press machine. Filtration media was specified as 5-12 micron in pore size and the flow rate was controlled at 4.2 ml/s. Filtration removes coagulate protein resulting in clear plasma. However, plasma has not been purified yet. Caprylic acid, ammonium sulfate, excess water and fraction of immunoglobulins, remaining in plasma, were able to be removed by diafiltration. In the final step, ultrafiltration can remove impurity by size exclusion resulting in a pure and concentrated bulk of plasma which contains only $F(ab')_2$ as an active pharmaceutical ingredient. The sample was collected for the second potency assay for the %yield of production as shown by Eq. (1). Lastly, concentrated plasma was formulated into ERIG injection solution calculated potency to exact 200 IU/ml and filled in the container as a finished product [11].

$$\%Yield = \frac{Potency\ of\ Conc.\ Bulk \times Conc.\ Bulk\ Weight \times 100}{Potency\ of\ Pooled\ Crude\ Plasma \times Crude\ Plasma\ Weight} \quad (1)$$

	Process Steps	Material Flows	Process Details	Tests
	1. Plasma Pooling and Mixing	Crude Plasma	Pool and Mix the Plasma	
		Pooled Crude Plasma		Potency Assay
	2. Dilution		Add Water for Injection	
Purification Processes		Diluted Crude Plasma		
	3. Pepsin Digestion		Add Pepsin	
		Digested Plasma	Adjust pH	
	4. Thermo-coagulation		Add Ammonium Sulfate	
	and Salt fractionation	Salt-fractionated Plasma		
	5. Precipitation		Add Caprylic Acid	
		Precipitated Plasma		
	6. Filtration	Filtered Plasma (Filtrate)	Filter with Filter Press and Discard Precipitate	
	7. Diafiltration	Concentrated Bulk		Potency Assay & Turbidity
	8. Formulation and Sterile Filtration		Formulate the Injectable ERIG	
		Final Bulk		
	9. Filling and Capping		Fills in Vials.	
		Finished Products		

Fig. 1. Process chart of ERIG production by Thai red cross [11].

3. Materials and Methods

3.1. Design of Experiment

Design of experiment (DOE) [12] was used in this study to plan the experiment in order to collect and analyze data using appropriate statistical methods. In addition, the appropriate design was chosen to save resources by using a reasonable number of the experiment and keeping the superior result implementation. In this study, plasma was sampled from the production batch. Potency assay as the primary response is sophisticated using high price equipment and time-consuming. Therefore, the experiment should be carefully designed with respect to the number of experiments. A central composite design (CCD) is the most popular class of designs used for fitting a first and second-order model.

Generally, a CCD consists of a 2^k factorial runs used for fitting the main effect. 2k axial runs (with the distance α of the axial from the design center) to allow for the quadratic terms to be incorporated into the model and 3 to 5 replications of center runs. Note that k is the number of factors involved in the study. Box and Hunter [13, 14] suggested that a second-order response surface design should be rotatable. The rotatability of the prediction model indicates the consistent and stable variance of the predicted response at points of interest. The CCD is made rotatable by choice of α of the axial run. The CCD has benefits over other designs due to a high range of rotatability performance [12]. Extending the range of factors to the axial point of CCD is appropriate for the continuous factor only. For this study to consider the pore size of filter press as the categorical factor, the face-center central composite design (CCF), as the alternative of CCD in which α=1, consisting of 2^3 factorial runs plus 2×3 axial runs and 3 replications of center points with 17 total numbers of runs represented by points shown in Fig. 2 is preferred. CCF uses a full range of factor levels that give higher space of experiment while maintaining the rotatability benefits. The benefit of the CCF is that the design has repeated center point at least 3 runs to make result confirmation which normally uses recent process setting [15].

In this study, the CCF design was selected to find the optimum condition of ERIG production with respect to high potency. The effect of caprylic acid (CA) concentration used in precipitation protein, filter pore size (PS) and filtration flow rate (FL) in the filtration process by filter press on the percentage of yield recovery is investigated. The turbidity was experimented to make sure that the purity of the product is satisfied. The CA concentration levels used in the experiment are set at 1, 3, and 5% according to the studies of Simsiriwong et al. [2]. Filtration pore size was selected based on previous screening experiment for filter selection which higher pore size than 20 μm was unable to retain precipitated protein particle. Therefore, pore size of 4-9, 5-12, and 6-15 micron were included in the experiment. Current filtration flow rate (4.2 ml/s) was set at the first level of factor. The fourth times of the current flow rate which roughly the maximum of machine handle ability was set at the third level, then the middle was selected by the average of both level and set as the second level of the flow rate factor.

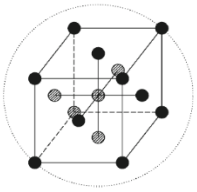

Fig. 2. Face-center central composite design (CCF).

3.2. Immunized Plasma

Immunized horse plasma was received from the Queen Saovabha Memorial Institute (QSMI). The plasma was measured the potency as the potency of pooled crude plasma, the starting point, which then used in yield calculation.

3.3. Pepsin Digestion and Thermocoagulation

Plasma was diluted with water for injection (WFI) two times of starting volume then heated to 30 ◦C and kept constant during digestion. Plasma's pH was adjusted to 3-4 with 1N HCl then Pepsin enzyme was added and stirred for an hour. Digestion reaction of the enzyme was ended by readjusting pH to 5 with 2N NaOH. Plasma was heat to 56 ◦C for an hour to coagulate non-immunoglobulin protein. Finally, the pH was adjusted to 7 to completely stop the enzyme reaction.

3.4. Caprylic Acid Precipitation

The variables of interest were CA concentration (%), filter media pore size (micron), and filtration flow rate (ml/s). After cooling plasma down to 30 ◦C, plasma was divided into 3 samples according to CA% (1, 3 and 5%). Caprylic acid was slowly added to the desired concentration under vigorously and continuously stirring for an hour. Precipitated plasma was left to settle overnight as shown in Fig. 3.

Fig. 3. Precipitated plasma with 1, 3 and 5% of CA.

3.5. Depth Filtration

Before filtration, plasma was reconstituted by a stirrer to homogenous suspension. Filtration was set up by order of the experiments as shown in Table 1, filter media and feed flow rate were set by the assigned level of each experiment. Selected filter media was placed in the filter holder (shown in Fig. 4) and wet thoroughly before the holder was securely close by the top part. Flow rate calculation [18] were done and converted to peristaltic pump working unit as round per minute (rpm).

The calibration of peristaltic pump used rpm in the conversion of a milliliter per second (ml/s) which was done by the separate experiment. A feeding tube was filled with homogeneously mixed plasma before connected to the filter holder. The amount of plasma fed in the filter system was restricted to 20 ml per experiment. Therefore, feeding time was calculated and set according to the flow rate which was set to the designed level. Throughput was measured by weighing filtered plasma output. The sample was taken for potency assay as potency after filtration. The equipment for the filtration process is set as shown in Fig. 4.

Fig. 4. Equipment for the filtration process.

3.6. Turbidity Measurement

Glassware and measurement cuvette were rinsed with WFI and dried with particle free compressed air. Plasma was poured into quart cuvette, then was measured for 5 times in a unit of NTU (Nephelometric turbidity unit). The average value of turbidity was recorded.

3.7. Potency Assay

The potency of the sample after filtration was assayed by Rapid Fluorescence Focus Inhibition Test (RFFIT) conducing by QSMI (Bangkok) under WHO guidelines. [1, 19] RFFIT experiment is based on viral neutralization of test samples in the cell-based model. The potency is calculated by probit program which uses the number counted of survived in the calculation.

4. Results and Discussion

The measurable responses of the study are %yield and turbidity. Yield recovery in percentage was calculated following Eq. (1). Table 1 shows the %yield and turbidity of 17 experiments. Turbidity, as mentioned before, is another concern that has been investigated. Since the turbidity is the reflex residue of protein fraction left in plasma after filtration. The lower value of turbidity is preferred to get a high purity. The results of all turbidity

test for each of the experiments shown in Table 1 are less than 10 NTU. It can be concluded that all plasma after filtration were under production specification. Therefore, we assured that depth filtration could produce a clear ERIG filtrate which passed specification each time of production.

Table 1. Yield recovery and turbidity from 17 experiments.

Run order	Caprylic acid (%)	Flow Rate (ml/s)	Pore Size (micron)	Yield Recovery (%)	Turbidity (NTU)
1	1	16.8	A (4-9)	13.063	2.074
2	3	10.5	C (6-15)	15.505	0.921
3	1	4.2	A	13.164	1.457
4	5	4.2	A	12.439	0.877
5	5	16.8	C	11.917	0.956
6	3	4.2	B (5-12)	10.376	1.280
7	3	10.5	B	12.387	2.081
8	1	16.8	C	14.554	0.971
9	3	16.8	B	13.442	1.860
10	5	16.8	A	13.692	1.245
11	3	10.5	A	10.591	1.761
12	3	10.5	B	10.733	1.127
13	1	4.2	C	12.818	1.220
14	1	10.5	B	13.949	1.149
15	5	10.5	B	12.772	1.457
16	3	10.5	B	11.501	1.555
17	5	4.2	C	12.194	1.511

Note: Pore size A denotes for pore size 4-9 micron.
Pore size B denotes for pore size 5-12 micron.
Pore size C denotes for pore size 6-15 micron.

The regression analysis and the response surface method were widely used in various experimental studies to model the relationship of the dependent variable to the independent variable in a statistical way [20-22]. The regression analysis was performed to identify the relationship of %yield as the response of the study with three factors which are CA, PS, and FL where PS_B =1 for pore size B (5-12 micron), and PS_C = 1 for pore size C (6-15 micron). Equations (2)-(4) present the regression equation based on the linear, quadratic and two-level interaction terms of all factors for the pore size of A (4-9 micron), B (5-12 micron) and C (6-15 micron) consequently. P-values of all terms as shown in Table 2 are not less than 0.10 illustrating that each term is not statistically significant in predicting %yield in the presence of the others. Stepwise approach [16] was then employed to determine which terms are useful. Unfortunately, there is no linear, quadratic and interaction relationship between %yield and each factor at a 90% confidence level.

$$\text{Yield} = 12.26 - 1.34\,CA + 0.341\,FL + 0.233\,CA^2 - 0.0131\,FL^2 - 0.0065\,CA\times FL \quad (2)$$

$$\text{Yield} = 10.82 - 1.62\,CA + 0.539\,FL + 0.233\,CA^2 - 0.0131\,FL^2 - 0.0065\,CA\times FL \quad (3)$$

$$\text{Yield} = 14.12 - 1.73\,CA + 0.353\,FL + 0.233\,CA^2 - 0.0131\,FL^2 - 0.0065\,CA\times FL \quad (4)$$

The EGIG is a biological product that is less predicted than other manufacturing products. Therefore, it is difficult to determine the mathematical prediction model. As shown in the previous studies of ERIG [2-3], instead of performing the regression analysis, the analysis of variance (ANOVA) [17] was applied to study the effect of the studying factors on %yield. The ANOVA and DOE are useful tools for experiment's optimize result inspection [23, 24]. In this study, they were used to investigate the effect of various factor-level combinations of three factors on %yield. The objective of ANOVA is to see whether %yields from different levels of the factors have a common means or not rather than to determine the relationship equation between %yield and factors.

Table 3 presents the ANOVA table of three main effects and one interaction effect analyzed from 17 experimental results. Note that with 17 experimental runs, only one interaction effect can be studied at a time due to an insufficient degree of freedom. With a 10% significance level, among the three interaction effects of CA×FL, CA×PS and FL×PS, the interaction effect between FL×PS is the only one that is statistically significant on %yield as shown by its p-value of 0.036. Meanwhile, the p-value 0.067 of CA illustrates that there is the effect of CA on %yield at the same significance level. Therefore, at a 10% significant level, it can be concluded that there is the interaction effect of FL×PS and the main effect of CA on %yield.

Table 2. Response surface regression: %yield versus CA, Fl, PS.

Term	Coef	SE Coef	T-Value	P-Value
Constant	12.26	3.36	3.65	0.015
CA	-1.34	1.73	-0.78	0.473
FL	0.341	0.596	0.57	0.591
PS_B	-1.44	3.57	-0.40	0.703
PS_C	1.87	2.96	0.63	0.556
CA^2	0.233	0.265	0.88	0.420
FL^2	-0.0131	0.0267	-0.49	0.643
CA×FL	-0.0065	0.0487	-0.13	0.899
$CA\times PS_B$	-0.282	0.751	-0.38	0.723
$CA\times PS_C$	-0.396	0.613	-0.64	0.547
$FL\times PS_B$	0.198	0.238	0.83	0.445
$FL\times PS_C$	0.012	0.195	0.06	0.953
Model Summary				
S	R-sq	R-sq(adj)	R-sq(pred)	
1.73487	51.33%	0.00%	0.00%	

Table 3. Analysis of variance: %yield versus CA, FL, PS.

Source	Df	SS	MS	F	P-value
CA	2	6.033	3.0165	4.37	0.067
FL	2	6.957	3.4783	5.04	0.052
PS	2	5.009	2.5045	3.63	0.093
FL*PS	4	14.601	3.6503	5.29	0.036
Error	6	4.139	0.6898		
Lack of Fit	4	2.768	0.6920	1.01	0.553
Pure Error	2	1.371	0.6854		
Total	16	30.923			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
0.830537	86.62%	64.31%	*		

To determine the suggested levels of each factor, the interaction and main effect plot were further constructed as presented in Fig. 5 and 6 respectively. The interaction plot shows the cross of each other flow rate line in every

pore size, confirming that the pore size and flow rate significantly interact with each other. From the interaction plot, the maximum point of the average %yield of 9 treatments (all combination of FL and PS) is at the upper-right corner when the flow rate is set at 10.5 ml/s and pore size C (6-15 micron) is used in the filtration process. Additionally, the plot shows an increasing trend of %yields when the flow rate is set at 10.5 ml/s and the pore size is getting larger (A to B to C). At the smallest flow rate of 4.2 ml/s, %yield is relatively low comparing to other flow rates disregarding to the pore size of filtration media as seen in the main effect plot of Fig. 6. Since depth filtration needs a certain minimum amount of pressure to push filtrate through filter media, the lower pressure could not give the good filtration output. As well as the protein accumulated on the filter surface could rise the resistance on the filtrate to pass through. By these reasons, could consequence in the low yield recovery. At the flow rate of 4.2 ml/s, there is no significant difference in %yield between pore size A and C while B gives lower yield recovery. As seen from the interaction plot, the increasing flow rate is able to increase the %yield recovery for only B type of filtration media. Otherwise, the increasing flow rate is not improving yield for A and C that could be limited by other factors of filtration such as filtration area and characteristic of precipitated protein that could block the filtration channel in filter media such as compressibility of precipitated protein [25].

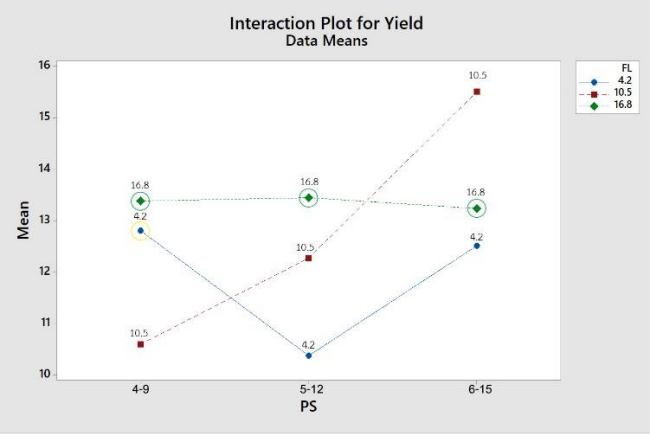

Fig. 5. Interaction plot for yields of F(ab')$_2$.

The flow rate of 16.8 ml/s has pretty much the same average %yield recovery for any pore size. It means that the variation from different pore sizes is very small when the flow rate is set at 16.8 ml/s. Filter media blockage could happen in the unsuitable filtration situation with e.g. a high pressure apply could put large particles into filtration media, high elasticity of particle could penetrate through pore or form pore-less cake which retains the filtrate in the filter holder chamber. In this situation, high pressure applied and high elasticity precipitated protein were able to block the filter which reduce yield of filtration. Therefore, from the interaction plot, the suggested filtration condition is 10.5 ml/s of flow rate and pore size of 6-15 micron. However, the Tukey pairwise comparison [17] presented in Fig. 7 for a different treatment means of the suggested condition (FL of 10.5 ml/s - pore size C) with other treatments according to the interaction between FL and PS shows that the average %yield from four following treatments/conditions are not statistically different from the suggested condition (since the confidence intervals contain the value of 0) at 90% confidence level.

- 4.2 ml/s of flow rate and pore size A (4-9 micron)
- 16.8 ml/s of flow rate and pore size of A (4-9 micron)/ B (5-12 micron)/ C (6-15 micron)

With a low flow rate of 4.2 ml/s, the smallest pore size (4-9 micron in this case) is preferred whereas with the moderate flow rate of 10.5 ml/s, the largest pore size of 6-15 micron is suggested. With the high flow rate of 16.8 ml/s, the pore size of filtration does not matter. Even though with the moderate flow rate of 10.5 ml/s, the largest pore size of 6-15 micron is suggested. However, with a pore size of 5-12 micron currently used in the filtration process, the flow rate is suggested to set at 16.8 ml/s.

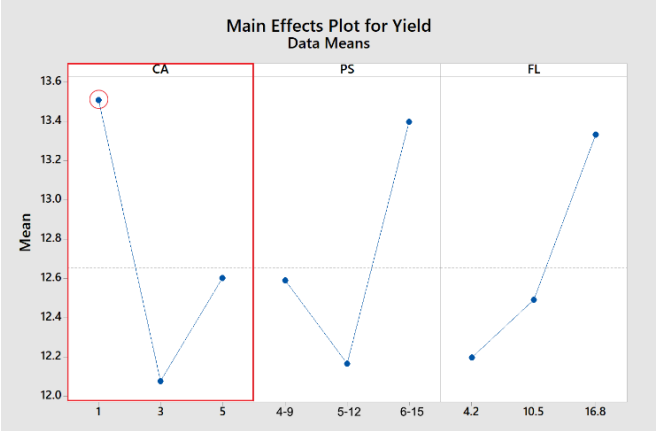

Fig. 6. Main effect plot for yield of F(ab')$_2$.

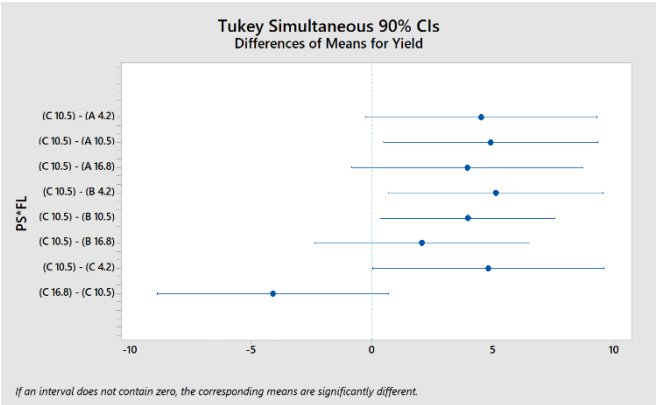

Fig. 7. Tukey pairwise comparison for yields of F(ab')$_2$ by interaction of pore size and flow rate.

Based on the main effect plot of CA on the response, %yield, the maximum point is on the upper-left corner when the concentration of caprylic acid used in the purification process is 1%. The main effects plot of CA shows 1% of CA giving the highest %yield by an average of 13.5% whereas 5% and 3% of CA produce the average yield approximately 12.6% and 10.6%, consecutively. In Fig. 8, with Tukey comparison between 1% and 3% of CA

shows that the average %yield for 3% of CA is significantly lower than those of 1% of CA presented by the negative value of the confidence interval for the difference between the population mean of yield produced form 3% and 1% of CA. While the confidence interval for the difference between the population mean of %yield produced from 1% and 5% of CA contains the value of 0. Therefore, it is possible that there is no difference in average %yield for 1% and 5% of CA. However, the 1% CA concentration used in the purification process is preferred due to the minimum amount used resulting in the lowest cost.

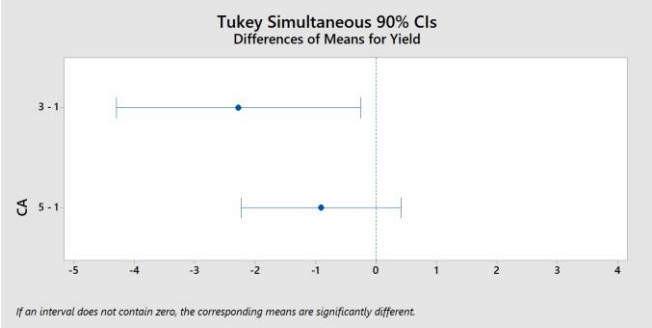

Fig. 8. Tukey pairwise comparison for yields of F(ab')$_2$ by % caprylic concentration.

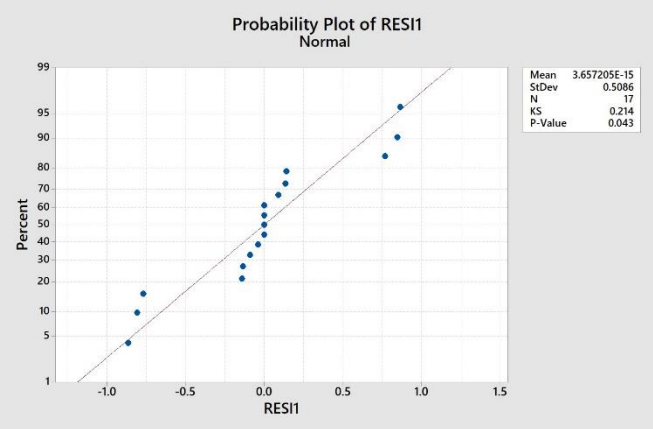

Fig. 9. Normality plots of the residuals

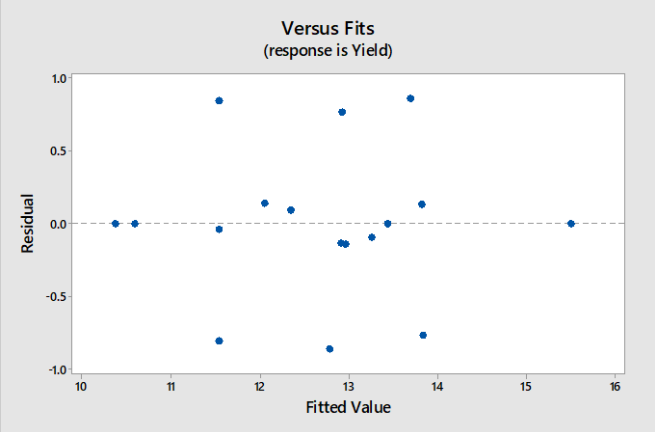

Fig. 10. Plot of residual versus fitted value

The normality plot of residuals for yield presented by Fig. 9 shows that the residuals are random distributed around the straight line. Nevertheless, with a small p-value of 0.043, the residual can be assumed to follow a normal distribution at a 99% confidence level and valid the statistical meaning and stand for a mean of the population. The plot of residuals versus fitted values illustrated in Fig. 10 shows that the symmetric distribution of data points around a zero line with the same variation. Therefore, the assumptions required by the analysis of variance are satisfied.

5. Conclusion

Equine Rabies Immunoglobulin contains F(ab')$_2$ which neutralized the rabies virus instantly for a risk case of infection. Thai Red Cross, the only organization produces ERIG in Thailand using an animal-based method, confronts a low yield recovery and a higher product demand. From the in-house study, potency loss in manufacturing mostly occurred during the purification process including fractionation and filtration. Therefore, the objective of this study is to improve the yield recovery by using the caprylic acid as a single precipitated agent in the fractionation process along with the replacement plan for the larger filter press machine to support higher demand in ERIG. The design of the experiment was applied to determine the optimum concentration of caprylic acid and filtration parameter, the filter media pore size and the flow rate. In addition, the highest yield recovery is targeted in the optimization. A face-central composite design was chosen for a pore size of a filter press machine as a categorical factor and a small number of experiments. A total number of 17 experiments with the parameter of a caprylic acid concentration (1, 3 and 5%), a flow rate (4.2, 10.5 and 16.5 ml/s) and a pore size (4-9, 5-12, 6-15 micron) are implemented with the yield recovery, calculated based on the potency of filtrated plasma, as a response of the experiment. From the regression analysis, there is no linear, quadratic and interaction relationship between the yield recovery and each factor at a 90% confidence level. This non-significance relationship can occur because the range of the experiment may not be wide enough to demonstrate the relationship between the yield recovery and the factors. Since the experiments have been performed in the laboratory and controlled manually, therefore, the imprecision and the learning curve to the experiment procedure caused by the experimenter might affect the response value. However, these uncontrollable factors were minimized by randomized experiments. Besides, the response might be affected by other factors such as temperature and pH that were not included in this study.

Fortunately, with the analysis of variance, there is the main effect of caprylic acid concentration and an interaction effect between the flow rate and the pore size of filter press machine on the yield recovery at a 90% confidence level. From the interaction and main effect plot, the highest average yield recovery occurs if the flow rate is set at 10.5 ml/s with the pore size 6-15 micron and 1% caprylic acid is used. However, as discussed in Section 4, four combinations of the setting can be used as an

alternative. Therefore, the current pore size of 5-12 micron, the flow rate of 16.5 ml/s is suggested, while 1% of caprylic acid is preferred due to the lowest cost.

This experimental design was planned for a small number of experiments at the beginning due to the high cost of experiments, therefore, the results were statistically analyzed with a limited degree of freedom. Hence, more numbers of experiments are suggested to investigate all factor combinations together. More number of factors are also suggested to determine the relationship model to find the optimal value of all settings. For the pharmaco-biological experiment, to handle the variation from the organic cell base test, the test should be done by a masterful experimenter to assure robust results. This study is conducted in a laboratory-scale mentioned before that may have some difference from the production scale. Therefore, the future study needs to scale up to a higher scale level. The number of experiments and designs of the experiment should be carefully considered and selected whether it is suitable for an experiment.

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