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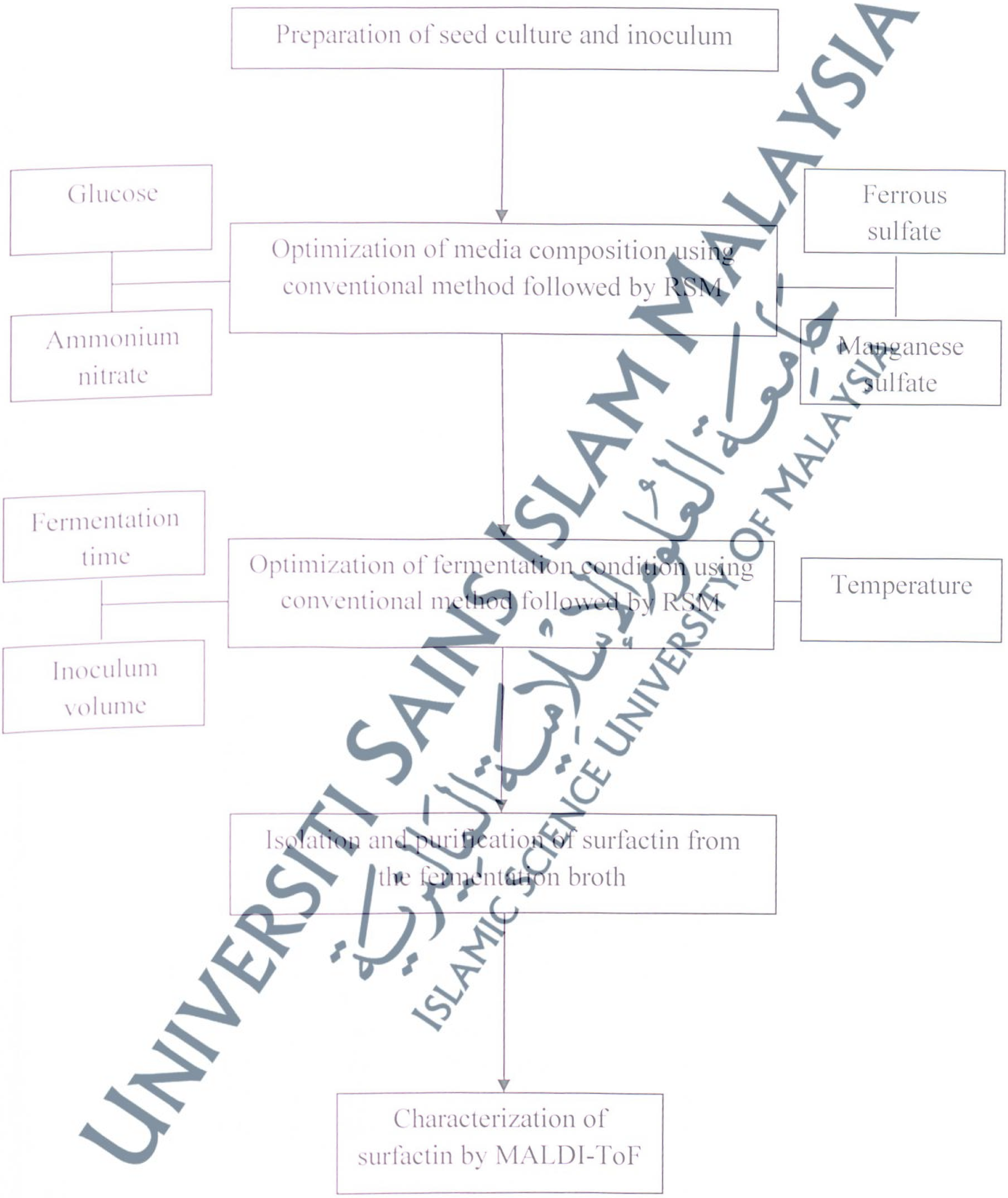
The International Journal of the Japan Society for Analytical Chemistry, *28*(8), 789–93.

APPENDICES

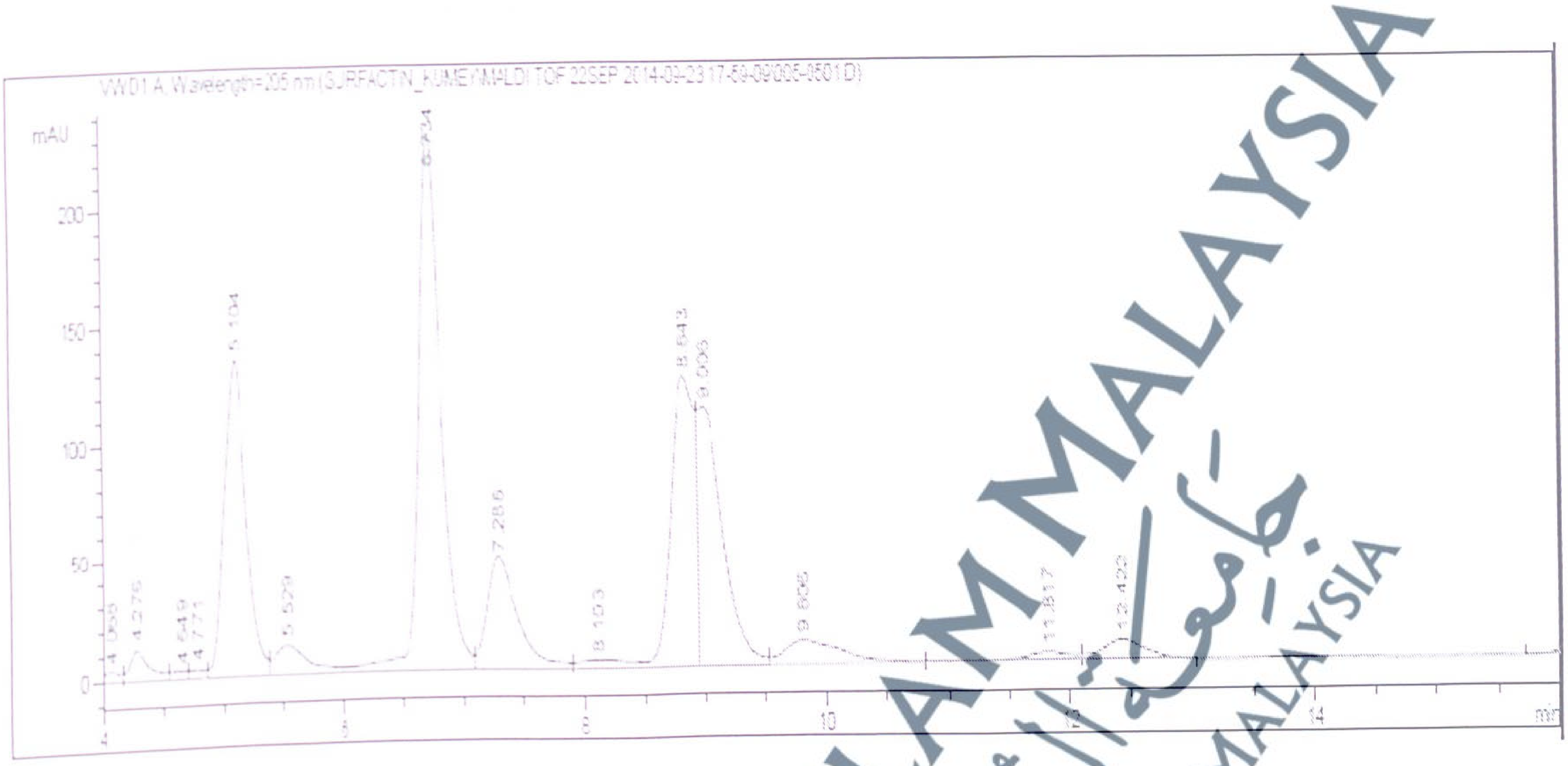
APPENDIX A

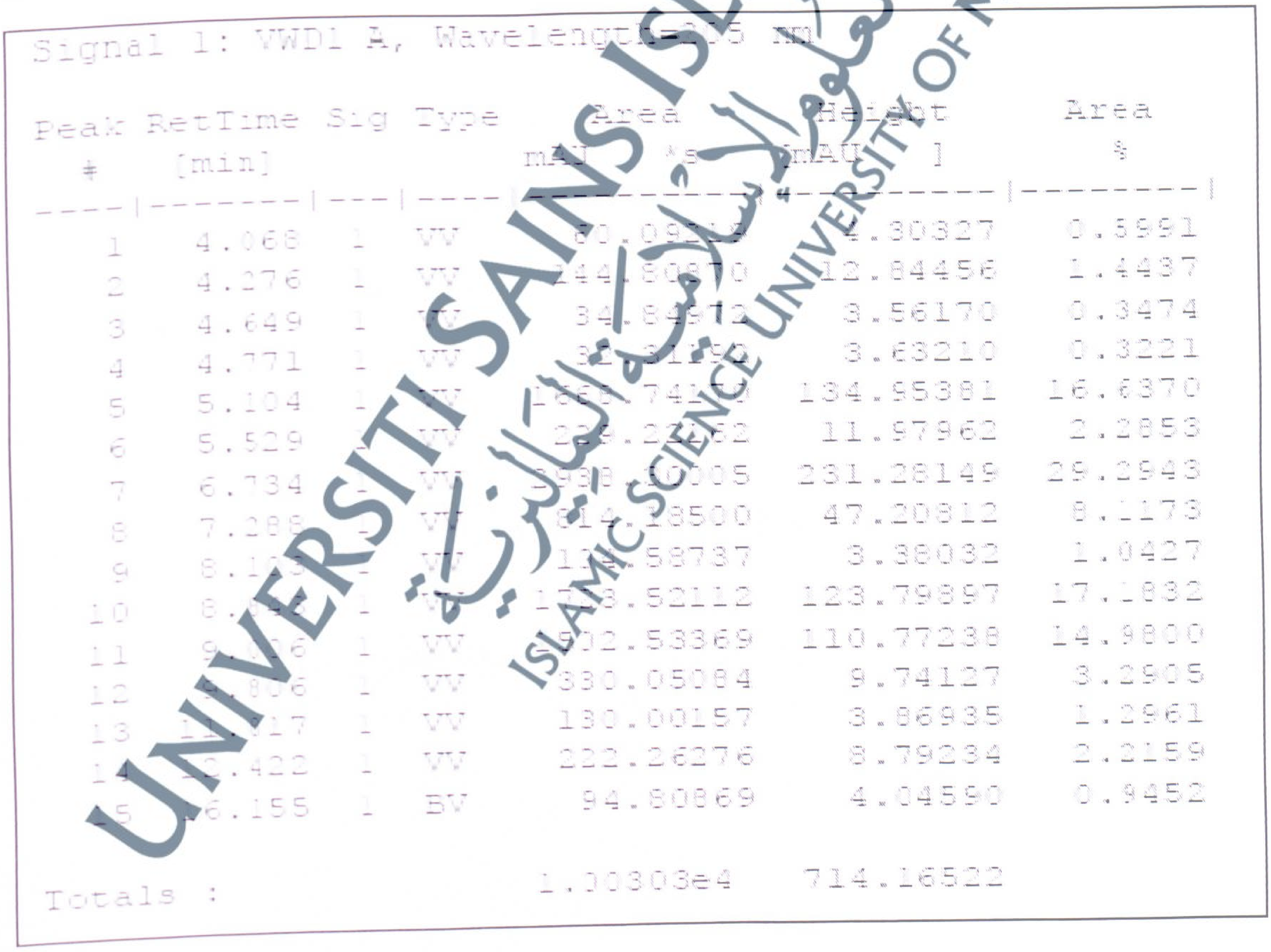
Experimental Design



APPENDIX B

HPLC chromatogram spectrum





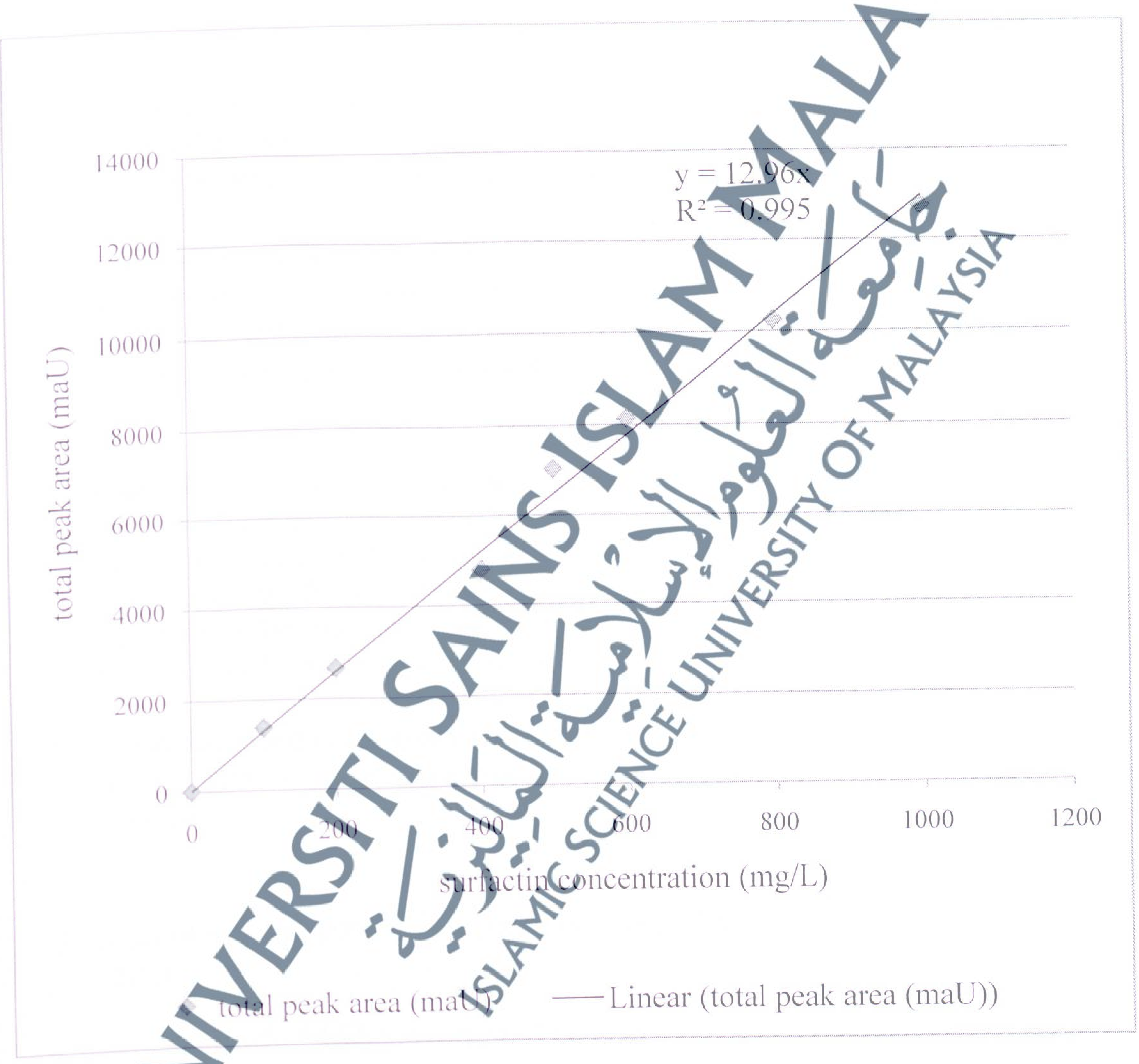
```
Signal 1: VWD1 A, Wavelength=205 nm

Peak RetTime Sig Type    Area       Height      Area
  #   [min]              mAU  *s    [mAU  ]      %
----|-------|---|----|----------|----------|--------|
  1    4.068   1  VV     60.09[illegible]    4.30327   0.5991
  2    4.276   1  VV    144.80870   12.84456   1.4437
  3    4.649   1  VV     34.84912    3.56170   0.3474
  4    4.771   1  VV     32.31[illegible]    3.63210   0.3221
  5    5.104   1  VV   1668.741[illegible]  134.55381  16.6370
  6    5.529   1  VV    229.22[illegible]   11.97962   2.2853
  7    6.734   1  VV   2938.[illegible]0005  231.28149  29.2943
  8    7.288   1  VV    814.18500   47.20812   8.1173
  9    8.193   1  VV    1[illegible].58737    3.38032   1.0427
 10    8.643   1  VV   1723.52112  123.79897  17.1832
 11    9.006   1  VV   1502.53369  110.77238  14.9800
 12    9.806   1  VV    330.05084    9.74127   3.2905
 13   11.817   1  VV    130.00157    3.86935   1.2961
 14   12.422   1  VV    222.26276    8.79234   2.2159
 15   16.155   1  BV     94.80869    4.04590   0.9452

Totals :             1.00303e4  714.16522
```

APPENDIX C

Surfactin standard calibration curve



APPENDIX D

Formula for Statistical Analysis

(Design Expert Version 7.1.6, *User's Guide*)

1. **Model** represents the terms estimating factor effects.

2. **Residual** are the unexplained variation seen as the difference between the observed response and the value predicted by the model for a particular design point. It is used to estimate experimental error.

3. **Lack of fit** compares the residual error mean square (MS) to the pure error MS and represents the variation of data around the fitted model for a particular design point. It is used to estimate experimental error. If a model has a significant lack of fit it is not good predictor of the response and should not be used.

4. **Pure error** or experimental error is the normal variation in the response which appears when an experiment is repeated. Repeated experiments rarely produce exactly the same results. Pure error is the minimum variation expected in a series of experiments. It can be estimated by replicating points in the design. The more replicated points the better will be the estimate of the pure error. Pure error is used to test the lack of fit terms for possible significance.

5. **Corrected total** is the total sum of squares corrected for the mean. It is the sum of the squared differences between the individual observations and the overall average.

6. **Sum of squares (SS)** is the sum of the squared distances from the mean due to an effect.

    6.1 **Model SS** is the sum of squares for terms in the model.

    6.2 **Residual SS** is the sum of squares for all the terms not included in the model.

Residual SS = Corrected total SS – Model SS

6.3 **Lack of fit SS** is the residual SS after removing the pure error SS.

Lack of fit SS = Corrected total SS – Pure error SS – Model SS

6.4 **Pure error SS** is the pure error SS for replicated points.

6.5 **Corrected total** is the sum of squared deviations of each point from the mean.

7. **Degree of freedom (d.f)** is the number of independent comparisons available to estimate a parameter.

7.1 **Model d.f** is the model which comprises the number of model terms including the intercept minus one.

$$\text{Model d.f} = k + k(k + 1)/2$$

Where, K = Degree of freedom of first order coefficients

7.2 **Residual d.f**is the estimation of variance around the model.

Residual d.f = Corrected total d.f – Model d.f

7.3 **Lack of fit d. f** is the amount of information available from the replicated points.

Lack of fit d.f = Residual d.f – Pure error d. f

7.4 **Pure error d.f**is the amount of information available from replicated points.

$$\text{Pure error} = n_1 - 1$$

Where, n_1 = Total number of center points

7.5 **Corrected total d.f** is the total degrees of freedom for the experiment, minus one for the mean.

$$\text{Corrected total d.f} = n_1 + n_2 - 1$$

Where, n_1 = Total number of center points

n_2 = Total number of axial and fractional points

8. **Mean square (MS)** is the sum of squares divided by the number of degrees of freedom and it is used to estimate the variance.

8.1 **Model MS** is the estimate of model variance.

$$\text{Model MS} = \text{Model SS} / \text{Model d.f}$$

8.2 **Residual MS** is the estimate of process variance.

$$\text{Residual MS} = \text{Residual SS} / \text{Residual d.f}$$

8.3 **Lack of fit MS** is the estimate of lack of fit.

$$\text{Lack of fit MS} = \text{Lack of fit SS} / \text{Lack of fit d.f}$$

8.4 **Pure error MS** is the estimate of pure error variance.

$$\text{Pure error MS} = \text{Pure error SS} / \text{Pure error d.f}$$

9. **F-value** is a probability distribution used to compare variances by examining their ratio. If they are equal then the F value would equal 1. The F value in the ANOVA table is the ratio of model mean square (MS) to the appropriate error mean square. The larger the ratio, the larger the F value and the more likely that the variance contributed by the model is significantly larger than random error.

9.1 **F-value of model** compares model variance with residual variance.

F-value of model = Model MS / Residual MS

9.2 **F-value of lack of fit** compares lack of fit variance with pure error variance.

F-value of lack of fit = Lack of fit MS/ Pure error MS

10. **Prob>F** is the probability value that is associated with the F Value for this term. It is the probability of getting an F Value of this size if the term did not have an effect on the response. In general, a term that has a probability value less than 0.05 would be considered a significant effect. A probability value greater than 0.10 is generally regarded as not significant.

11. **Coefficient of determination (R^2)** is an estimate of the overall variation in the data around the mean accounted for by the model. A value of 1.00 represents the ideal case at which 100 % of the variation in the observation value can be explained by the chosen model.

$$R^2 = 1- [\text{Residual SS} / (\text{Model SS} + \text{Residual SS})]$$

APPENDIX E

Structural data for the isolated surfactin isoforms and their mono- and dimethyl esters (Kowall et al., 1998)

Fraction number[a]	Surfactin species	Molecular mass in daltons	Fatty acid chain length[b]	Chain type[c]
	Surfactins (Series 1)			
1	[Leu7]—	1008	C_{13}	anteiso*
2	[Val7]—	994	C_{13}	anteiso
3	[Ile2,Val7]—	994	C_{13}	n.d.
4	[Leu7]—	1022	C_{14}	iso*
	[Ile7]—	1008	C_{13}	
5	[Val7]—	1008	C_{14}	iso/n
6	[Ile2,Val7]—	1008	C_{14}	iso*
7	[Leu7]—	1036	C_{15}	anteiso
8	[Ile7]—	1022	C_{14}	iso*
9	[Val7]—	1022	C_{15}	anteiso*
10	[Ile2,Val7]	1022	C_{15}	anteiso
11	[Ile7]—	1036	C_{15}	anteiso*
12	[Ile2,Ile7]—	1036	C_{15}	n.d.
	Surfactin, Monomethyl Ester (Series 2)			
14	[Val7]—	1008	C_{13}	anteiso*
15	[Leu7]—	1022	C_{13}	anteiso*
	[Val7]—	1008	C_{13}	
16	[Leu7]—	1022	C_{13}	anteiso*
	[Ile2,Val7]—	1008	C_{13}	
18	[Ile7]—	1022	C_{13}	anteiso*
	[Val2,Val7]—	1008	C_{14}	
19	[Ile7]—	1022	C_{13}	anteiso*
20	[Val7]—	1022	C_{14}	iso
21	[Leu7]—	1036	C_{14}	iso
22	[Val7]—	1022	C_{14}	iso/n
	[Ile2,Val7]—	1022	C_{14}	
	Surfactin, Monomethyl Ester (Series 2)			
23	[Leu7]—	1036	C_{14}	iso/n
24	[Ile7]—	1036	C_{14}	iso
25	[Val7]—	1036	C_{15}	anteiso*
	[Ile7]—	1036	C_{14}	
25	[Leu7]—	1050	C_{15}	anteiso*
	[Val7]—	1036	C_{15}	
27	[Leu7]—	1050	C_{15}	anteiso*
	[Ile2,Val7]—	1036	C_{15}	
28	[Ile7]—	1050	C_{15}	anteiso*
29	[Ile7]—	1050	C_{15}	anteiso*
30	[Ile2,Ile7]	1050	C_{15}	n.d.
	Surfactin, Dimethyl Ester (Series 3)			
34	[Val7]	1022	C_{13}	anteiso*
35	[Leu7]—	1036	C_{13}	anteiso*
35	[Ile7]—	1036	C_{13}	anteiso*
37	[Val2,Val7]—	1022	C_{14}	iso
38	[Val7]—	1036	C_{14}	iso/n
39	[Leu7]—	1050	C_{14}	iso
40	[Ile7]—	1050	C_{14}	iso/n
	[Ile2,Val7]—	1036	C_{14}	
41	[Val7]—	1050	C_{15}	anteiso*
42	[Leu7]—	1064	C_{15}	anteiso
43	[Ile7]—	1064	C_{15}	anteiso
	[Ile2,Val7]—	1050	C_{15}	
44	[Ile2,Ile7]—	1064	C_{15}	n.d.