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Optimization of Assay Conditions for Measurement of *t*-RNA: An Experimental Design

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Abstract: A design of an experiment is generally to fictionalize an execution of an experimental process and the term of experimental design strategy usually refers to a two-stage modelling. The first of all named as working strategy is the determination of the experimental execution model and the last one is that a mathematical model for response surface function to define the relationship between the experimental factors. In this work, it was selected optimization of assay conditions for t-RNA using Central Composite Design, the influence of three factors, namely pH, enzyme concentration and amino acid concentration as an experimental design application, and improved an experiment strategy in order to optimize the effective operation factors on this chemical reaction and mathematical solution techniques of the response surface function. As examining the coefficients of the response surface equation and its graphics, we can say that the most effective parameter on the esterification process is enzyme concentration alone and together with pH.

Keywords: Experimental design; statistics; optimization; response surface; t-RNA. © 2022 ACG Publications. All rights reserved.

1. Introduction

When looking at biotechnological processes in general, the first thing that comes to mind is microbial biomass production. Starting from a carbon source (CHO), in the presence of oxygen (O_2) and ammonia (NH_3), biomass (CHNO) is produced together with carbon dioxide (CO_2) and water (H_2O), and some energy (ΔH) is released in the meantime. The content of the biomass formed, Proteins (Enzymes, Nutritional proteins, Functional proteins and CCP-Single cell proteins), Lipids (Glycerides, Fats, Some vitamins and Phospholipids), Nucleic Acids (DNA, RNA and Nucleotides), Minerals (Normal minerals of the cell, Pb , Hg etc. toxic metals and Au, U, Cu accumulation) and Carbohydrates (Saccharides and Emulsifiers). The intermediate or final products of metabolism are called primary metabolites. Whether primary metabolites are intermediates or final products, they have a distinct role in metabolism. In a normally functioning metabolism, especially the accumulation of intermediate products cannot occur. For the production of such metabolites, it is necessary to interfere with the metabolism artificially. The most common primary metabolites are citric acid, fumaric acid, acetic acid, amino acid, vitamin and nucleotide production [1].

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Of these, amino acids are the building blocks of proteins in our body. Amino acids are essential for vital processes such as protein construction, hormone and neurotransmitter synthesis. We normally obtain amino acids from the protein foods we eat. Some amino acids can also be taken in dietary supplement form to naturally support athletic performance or mood. Amino acids are classified as essential, conditionally essential and non-essential amino acids. Our body needs 20 different amino acids. All of these are important for health, but only nine of them are essential and must be taken from outside, so they are not synthesized in the body. Essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Dietary proteins are broken down into amino acids and used in different processes in the body. Such as muscle building, tissue regeneration, neurotransmitter, enzymes, regulation of the immune system. Some non-essential amino acids are classified as conditionally essential. These amino acids are considered essential only in special situations such as illness or stress. For example, even if arginine is not an essential amino acid, the body cannot produce arginine when fighting certain diseases such as cancer. Therefore, in certain situations, arginine is supplemented to meet the body's needs. Amino acids that must be taken from outside under such special conditions are called conditionally essential amino acids. In amino acid production, fermentation proceeds according to the same principle for all amino acids. Here, production technique: deep culture technique, Substrate(C-source): Carbohydrates (10%), Additives: Vitamins and minerals, Fermentation temperature: 35 °C and Nitrogen source: Usually urea. In general, amino acids are used as additives to foods and cosmetics, pharmaceuticals and as a starting material in the synthesis of many compounds. Biologically active L-isomers of amino acids are used to add nutrients and for medical applications [2].

Chemical transformations catalysed by microorganisms or their enzymes are called biotransformation. Enzyme-catalysed reactions have the following important advantages over chemical catalysis [1,2]. (**a**) Specificity: In enzymatic reactions, in principle, by-products do not occur (Effect specificity) and most enzymes catalyse the reaction with a specific substrate (Substrate specificity). In addition, there is regiospecificity and stereospecificity in enzyme-catalysed reactions, (**b**) Moderation of Reaction Conditions: Enzymatic reactions take place in aqueous media around neutral pH and generally at temperatures lower than 40° C, (**c**) Reducing the Activation Energy: As a result of the intermediate catalysis mechanism in enzymatic reactions, the activation energy is very low and the reaction takes place at a high speed.

These advantages allow many reactions that can be carried out chemically in very complex ways or that cannot be performed at all, with the help of enzymes. These reactions are (**a**) Reacting only one of the similar functional groups, (**b**) Separation of racemic mixtures by selective conversion of one of the enantiomers, (**c**) Inserting a group into the asymmetric centre, (**d**) It is the selective conversion of the inactivated C- atom into a functional group.

The cell is the basic structural, functional, and biological unit of all known living organisms and it is the smallest unit of life. Cells consist of cytoplasm enclosed within a membrane, which contains many biomolecules such as proteins and nucleic acids. Organisms can be classified as unicellular consisting of a single cell; including bacteria, or multicellular including plants and animals. As prokaryotes as single-celled organisms without nucleus include bacteria and archaea Eukaryotes as single- or multi-cellular organisms with nucleus include plants, animals, fungi, protozoa and algae. Organelles are parts of the cell which are adapted and/or specialized for carrying out one or more vital functions, analogous to the organs of the human body. Both eukaryotic and prokaryotic cells have organelles, but prokaryotic organelles are generally simpler and are not membrane-bound. There are several types of organelles in a cell. Some such as the nucleus and golgi apparatus are typically solitary, while others such as mitochondria, chloroplasts peroxisomes and lysosomes can be numerous. The cytosol is the gelatinous fluid that fills the cell and surrounds the organelles. A cell's information centre, the cell nucleus is the most conspicuous organelle found in a eukaryotic cell. It houses the cell's chromosomes, and is the place where almost all DNA replication and RNA synthesis, transcription, occur. The nucleus is spherical and separated from the cytoplasm by a double membrane called the nuclear envelope. The nuclear envelope isolates and protects a cell's DNA from various molecules that could accidentally damage its structure or interfere with its processing [3].

Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes. RNA and DNA are nucleic acids, and, along with lipids,

proteins and carbohydrates, constitute the four major macromolecules essential for all known forms of life. While the specific nucleotide sequence of an mRNA specifies which amino acids are incorporated into the protein product of the gene from which the mRNA is transcribed, the role of *tRNA* is to specify which sequence from the genetic code corresponds to which amino acid. The mRNA encodes a protein as a series of contiguous codons, each of which is recognized by a particular tRNA. One end of the tRNA matches the genetic code in a three-nucleotide sequence called the anticodon. The anticodon forms three complementary base pairs with a codon in mRNA during protein biosynthesis. On the other end of the tRNA is a covalent attachment to the amino acid that corresponds to the anticodon sequence. Each type of tRNA molecule can be attached to only one type of amino acid, so each organism has many types of tRNA. Because the genetic code contains multiple codons that specify the same amino acid, there are several tRNA molecules bearing different anticodons which carry the same amino acid. The covalent attachment to the tRNA is catalysed by enzymes called aminoacyl tRNA synthetases. During protein synthesis, tRNAs with attached amino acids are delivered to the ribosome by proteins called elongation factors, which aid in association of the tRNA with the ribosome, synthesis of the new polypeptide, and translocation (movement) of the ribosome along the mRNA. If the tRNA's anticodon matches the mRNA, another tRNA already bound to the ribosome transfers the growing polypeptide chain from its 3' end to the amino acid attached to the 3' end of the newly delivered tRNA, a reaction catalysed by the ribosome. A large number of the individual nucleotides in a tRNA molecule may be chemically modified, often by methylation or deamidation. These unusual bases sometimes affect the tRNA's interaction with ribosomes and sometimes occur in the anticodon to alter base-pairing properties [4-6].

The process of protein synthesis involves the esterification of the amino acid (eg. arginine) to a specific tRNA (arginyl-tRNA) which transfers the amino acid to the protein assembly site in the cytosol. Arginyl-tRNA synthetase is the enzyme which directs both the activation of arginine and its subsequent covalent linkage to tRNA. Normally, this reaction is highly specific since there is a distinct aminoacyl-tRNA synthetase for linking each amino acid to its correct tRNA and, this reaction could be carried out in vitro conditions in the laboratory. Due to the structural similarity between arginine and canavanine, *both* amino acids are substrates for this enzyme. Arginyl-tRNA synthetase activates and then aminoacylates canavanine to the cognate tRNA for arginine. As a consequence, canavanine rather than arginine is transferred to the protein assembly site and erroneously incorporated into the nascent polypeptide chain. In this way, anomalous, structurally aberrant, canavanyl proteins are synthesized. Several amino acid transfer ribonucleic acid synthetases have been purified from various sources. These enzymes are of obvious interest from many points of view, including their unique role in protein synthesis, in which they are the only enzymes with specificities that are important for the specific incorporation of amino acids into proteins. Since each enzyme of this group is capable of recognizing its own tRNA as well as the correct amino acid, an understanding of protein-nucleic acid interaction requires that the enzymes and the corresponding tRNA species be obtained in pure forms for analyses of their structures and the nature of their combination. During the course of experiments to purify further the prolyl-tRNA synthetase of Escherichia coli, it was found that the arginine-activating enzyme was purified together with the proline enzyme. The two activities were easily separated, however, and we have subsequently continued the purification of the arginyl-tRNA synthetase [6,7].

In this work it was examined optimisation of assay conditions for tRNAs in vitro using Central Composite Design by DesignExpert 700 software [8,9].

2. Materials and Methods

A design of an experiment is generally to fictionalize an execution of an experimental process *and* the term of experimental design strategy usually refers to a two-stage modelling. The *first of all* named as working strategy is the determination of the experimental execution model *and the last one* is *that* a linear or a non-linear mathematical model for response surface function to define the relationship between the experimental factors. From the literature studies it is understood that there would be some design options such as full factorial design, fractional factorial design and central composite design used for experimental design strategies. The simplest one of the scan designs is full factorial design and this

method has two levels for the factors and it is used to determine which factors are affected on the result of the experiment. For example, if a chemical reaction is affected by pH and temperature a full factorial design for the reaction could be done for two-factors in two-levels. Experiment number is calculated by formula 2^k. Here k is the number of factors and the number of levels is 2 coded as -1 and +1. The number of experiments is 4 for 2 factors. [10, 11, 12].

In the evaluation step of the experimental design is to model a response surface function to define the relationship between the factors and the experimental result. For the response surface function as the yield of a reaction depending on the factors of pH and catalyst concentration it should be firstly to propose a mathematical model of this surface and a simulation graphic of the model would be plotted. For the response surface function can be offered some linear or non-linear models. Here a linear model not consisting of the parabolic effects of the factors was proposed only as an example.

$$y = b_0 x_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 \quad (1)$$

Calculating of the coefficients in the mathematical model based on an optimization process covers minimization of the sum of the distances between factors calculated from model and experimental values and this optimization are named as least square method. Written optimization function for the response surface is as follows. Analysis of this optimization function could be carried out by some numerical techniques [8].

$$J = \sqrt{\sum_{i=1}^{n} d_i^{\,2}} = \sqrt{\sum_{i=1}^{n} (y_{i-\exp erimental} - y_{i-calculated})2} \rightarrow Minimum \quad (2)$$

In our design for optimisation of assay conditions for tRNAs using Central Composite Design, the influence of three factors, namely (1) pH, (2) enzyme concentration and (3) amino acid concentration, on the esterification of tRNA arginyl-tRNA synthetase is to be studied by counting the radioactivity of the final product, using ^{14}C-labelled arginine. The higher is the count, the better are the conditions. A central composite design is set up to perform the experiments. True values of the factors and the results of the experiments are given in Table 1 and Table 2 [12].

Here, the total number of experiments, *N* (=20), equals the sum of

• 2*k* (=8) factorial points, often represented as the corners of the cube,

• 2*k* + 1 (=7) star points, often represented as axial points on (or above) the faces ofthe cube plus one in the center,

• and *R* (=5) replicate points, in the centre or in equation form

$$y = b_0 x_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{11} x_1^{\,2} + b_{22} x_2^{\,2} + b_{33} x_3^{\,2} + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 \quad (3)$$

Optimization of assay conditions for tRNAs by central composite design was conducted on following steps by DesignExpert 700 software [9].

3. Results and Discussion

For statistically evaluation procedure of selected experimental design model, Central Composit Design menu under Responce Surface menu is set by using Design Expert Run Menus. (Figure 1.a):

1. Selection of response surface model (Figure 1.b).
2. Three factors and response value for each run were introduced to the program in real and actual coded. (Table 2).
3. Getting of statistical evaluation by ANOVA results (Table 3, 4 and 5).

Table 1. The factor levels of the reaction

Factors	Levels	−1.7	−1	0	1	1.7
Factor 1:	**enzyme (µg protein)**	3.2	6.0	10.0	14.0	16.8
Factor 2:	**arginine (µmol)**	860	1000	1200	1400	1540
Factor 3:	**pH**	6.6	7.0	7.5	8.0	8.4

Table 2. The results of the experiments

Factor 1	Factor 2	Factor 3	Counts
−1	−1	−1	4422
1	−1	−1	4983
−1	1	−1	4599
1	1	−1	4810
−1	−1	1	4573
1	−1	1	5128
−1	1	1	4599
1	1	1	4930
−1.7	0	0	4704
1.7	0	0	4891
0	−1.7	0	4695
0	1.7	0	4566
0	0	−1.7	4773
0	0	1.7	4872
0	0	0	5063
0	0	0	4968
0	0	0	5035
0	0	0	5122
0	0	0	4970
0	0	0	4925

4. Plotting of response surface graphics related with process factors (Figure 2, 3 and 4).
5. Plotting normal plot of residuals (Figure 5).

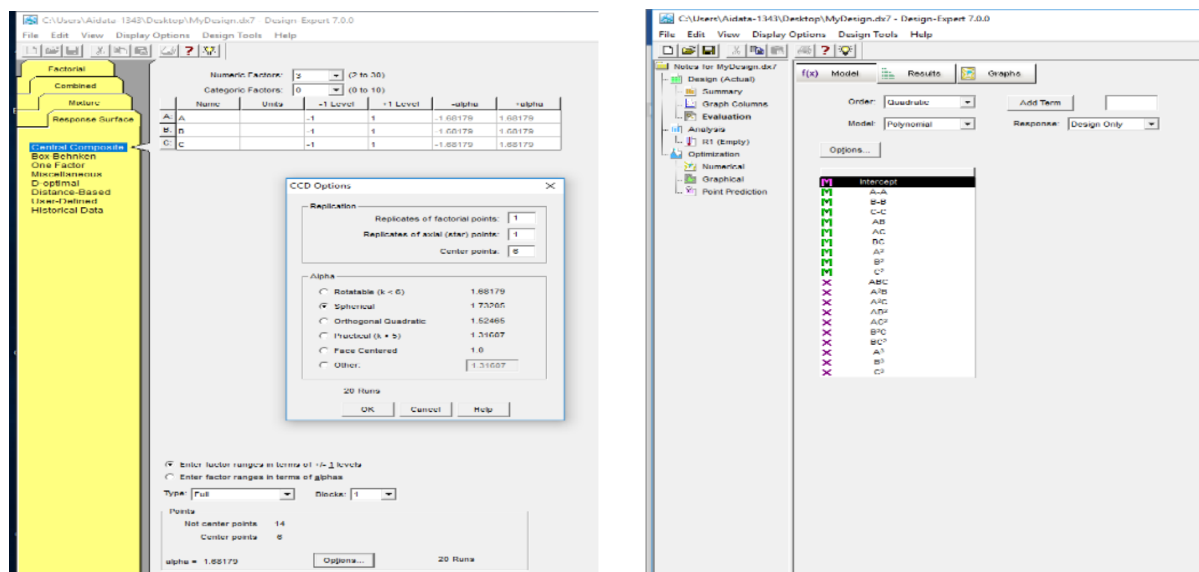

Figure 1.a. Design Expert Run Menus and **b.** Selection of response surface model

Table 3. Getting of statistical evaluation by ANOVA results

Response	**1**		**Counts**			**ANOVA**
			ANOVA for Response Surface Quadratic Model			
			Analysis of variance table [Partial sum of squares - Type III]			
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	***p*-value Prob > F**	
Model	6,95E+08	9	77197.01	43715	0.0026	Signific.
A-enzyme	2,85E+08	1	2,85E+08	26.18	0.0005	
B-arginine	10850.78	1	10850.78	1.00	0.3415	
C-pH	24844.91	1	24844.91	46784	0.1617	
AB	41184.50	1	41184.50	28550	0.0803	
AC	1624.50	1	1624.50	0.15	0.7073	
BC	3872.00	1	3872.00	0.36	0.5641	
A2	74676.57	1	74676.57	31564	0.0256	
B2	2,47E+08	1	2,47E+08	22.74	0.0008	
C2	57463.75	1	57463.75	46874	0.0444	
Residual	1,09E+08	10	10881.37			not
Lack of Fit	82334.85	5	16466.97	43772	0.1193	signif.
Pure Error	26478.83	5	5295.77			
Cor Total	8,04E+08	19				

Table 4. Getting of model evaluation by ANOVA results

The Model F-value of 7.09 implies the model is significant. There is only a 0.26% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant.
In this case A, A2, B2, C2 are significant model terms.
Values greater than 0.1000 indicate the model terms are not significant.

If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 3.11 implies the Lack of Fit is not significant relative to the pure error. There is a 11.93% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Std. Dev.	104.31	R-Squared	0.8646
Mean	4831.40	Adj R-Squared	0.7427
C.V. %	Şub.16	Pred R-Squared	0.1704
PRESS	6,67E+08	Adeq Precision	7.615

The "Pred R-Squared" of 0.1704 is not as close to the "Adj R-Squared" of 0.7427 as one might normally expect. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response tranformation, outliers, etc.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 7.615 indicates an adequate signal. This model can be used to navigate the design space.

Table 5. Getting of response surface evaluation by ANOVA results

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Final Equation in Terms of Actual Factors:						
Counts =	501314470					
	14.443.262	* enzyme				
	-2.818.740	* arginine				
	4.265.239	* pH				
	-7.175.000	* enzyme * arginine				
	1.425.000	* enzyme * pH				
	-2.200.000	* arginine * pH				
	-7.198.479	* enzyme 2				
	-13.102.821	* arginine 2				
	-6.314.595	* pH2				

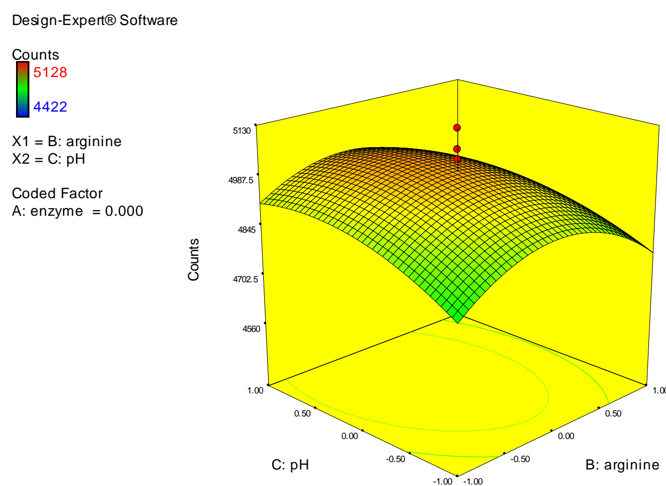

Figure 2. Plotting of response surface graphics related with process factors (Factor B and A)

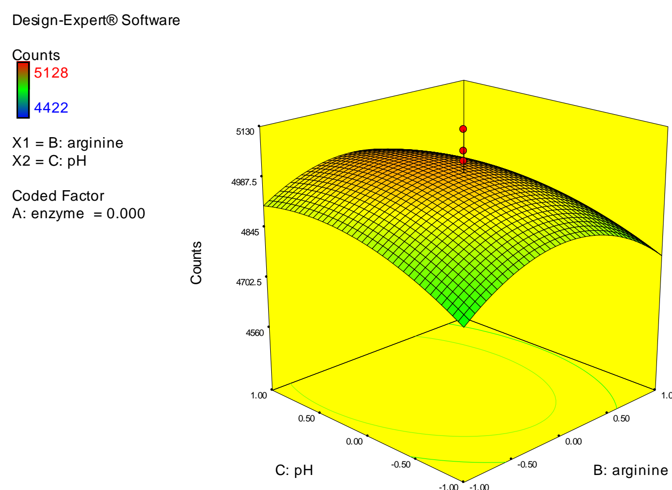

Figure 3. Plotting of response surface graphics related with process factors (Factor C and B)

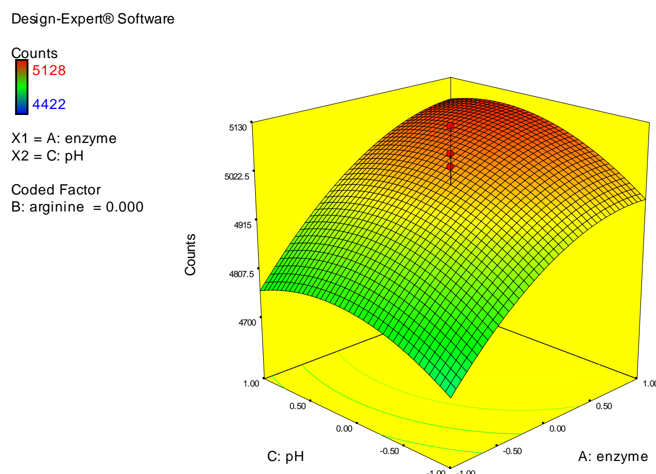

Figure 4. Plotting of response surface graphics related with process factors (Factor A and C)

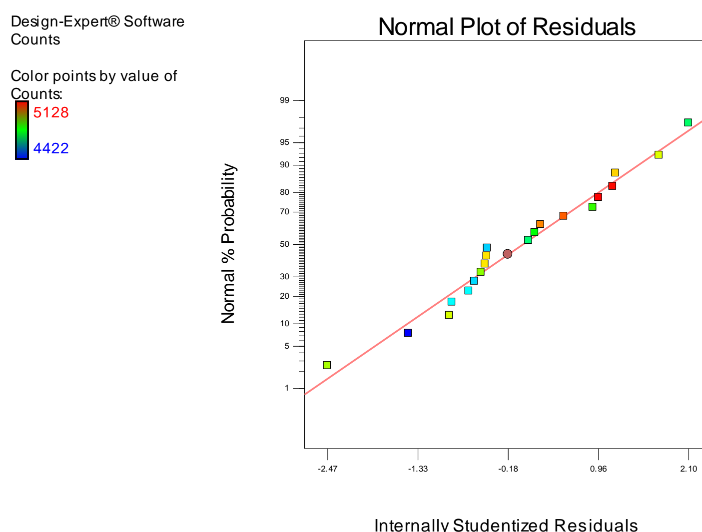

Figure 5. Normal plotting of residuals

Many designs for use in chemistry for modelling are based on the central composite design, sometimes called a response surface design. In our design for optimisation of assay conditions for tRNAs using Central Composite Design, the influence of three factors, namely (1) pH, (2) enzyme concentration and (3) amino acid concentration, which is to be optimised, on the esterification of tRNA. arginyl-tRNA synthetase is to be studied by counting the radioactivity of the final product, using ^{14}C-labelled arginine. A central composite design is set up to perform the experiments. Here, the total number of experiments, *N* (=20), equals the sum of 2*k* (=8) factorial points, often represented as the corners of the cube, 2*k* + 1 (=7) star points, often represented as axial points on (or above) the faces of the cube plus one in the centre, and *R* (=5) replicate points in the centre, degrees of freedom for the lack-of-fit, or in equation form;

$$y = b_0 x_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3$$

Following introduction, the percentage recoveries as response of the extraction to the program it was selected the response surface for e model statistical evaluation. As examining of the coefficients of the response surface equation and its graphics, we can say that the most effective parameter on the esterification process is enzyme concentration alone and together with pH.

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References

[1] J.E. Bailey and D.F. Ollis (1996). Biochemical engineering fundamentals, McGraw Hill International Editition, Newyork, U.S.A.
[2] A. Telefoncu (1995). Biyoteknoloji, Ege Üniversitesi, Fen Fakültesi Yayınları, 152İzmir, Türkiye.
[3] N.A. Campbell, B. Williamson and R.J. Heyden, (2006). Biology: Exploring Life, Pearson Prentice Hall. Boston, Massachusetts, United States of America.
[4] L.H. Dolph, J.L. Byeong and M.P.Robert, (2017). Transfer RNA in Protein Synthesis, 1st Edition, CRC Press and Taylor & Francis Group, London, England
[5] S. Cheriyedath. (2019). https://www.news-medical.net/life-sciences/-Types-of-RNA-mRNA-rRNAand-tRNA.aspx, 21 June.
[6] Index of Garose. (1997). http://www.uky.edu/~garose/Link101.htm, Arginly-tRNA Synthetase, 31 July.
[7] S.K. Mitra and A.H. Mehler (1967). The arginyl transfer ribonucleic acid synthetase of *Escherichia coli*, *J. Biol. Chem.* **242**, 5490-5494.
[8] O. Akpolat (2016). Experimental design methods with practical applications, *J. Ongoing Chem. Res.* **2**, 64-80.
[9] D.C. Montgomery, (2005). Design and Analysis of Experiments, Student Solutions Manual, Wiley, EB-Books, Santa Ana, CA, U.S.A.
[10] G. Özevci, N. İpteş and O. Akpolat, (2013). Statistical design of experiments, *I. Central Anatolia Region Agriculture, Food, Environment and Biology Congress*, TARGID 2013, 2-4 Oct., Niğde, Türkiye
[11] D. Özdemir (2012) Kemometri temel eğitimi ders notları, *Kimya Akademisi Press*, Antalya, Turkiye
[12] R.G. Brereton (2003). Chemometrics: Data analysis for the laboratory and chemical plant, John Wiley & Sons Ltd., Provide the city and Country, Chichester West Sussex, United Kingdom.

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