STATISTICAL OPTIMIZATION OF LACTIC ACID EXTRACTION USING GREEN SOLVENT AND MIXED EXTRACTANTS (TOA AND TOMAC)

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Received: 10 October 2017; received in revised form 14 November 2019

Abstract: Since some previous years, reactive extraction has become more attractive and competitive technique for the separation and purification of lower carboxylic acids from fermentation broth as well as from dilute aqueous streams. This paper shows the results of investigation of reactive extraction of lactic acid (LA) from an aqueous solution using the synergistic mixture of the extractants (TOA (tri-n-octylamine) and TOMAC (Tri-n-octylmethylammonium chloride)) and a non-toxic and biocompatible green solvent (soybean oil). Three-level Box-Behnken design (BBD) under response surface methodology (RSM) was opted for the experimental design and to interpret the mutual effect of seven independent process parameters on the LA distribution coefficient (K_D). The maximum values of LA distribution coefficient (K_D = 2.51) and its extraction efficiency (η=71.5%) were obtained for the optimum values of various process parameters such as 0.02 [M] initial LA concentration (C_1), 0.5 (v/v) extractant ratio (α), 28.66% (v/v) mixed extractants concentration (Ψ), 2 (v/v) phase ratio (Φ), 27 °C temperature (T), 102 rpm stirring speed (ω), and 63 min contact time (τ). This present investigation will provide a noble discussion on LA reactive extraction using green solvent and on various influencing process parameters for gaining the enhanced value of LA distribution coefficient (K_D).

Key Words: *Reactive extraction, Tri-n-octylamine, Soybean oil, Lactic acid, Response surface methodology*

DOI: https://doi.org/10.3329/cerb.v21i1.47369

1. Introduction

Lactic acid (LA) has very wide scope of its application in various industrial sectors such as in polymer sector (for making biodegradable plastic bags and re-absorbable plates), food sector (as an acidulant and buffering agent), chemical sector (as descaling agent, pH regulator, neutralizer, and cleaning agent), and more recently in medical sector (to make screws, plates, and pins) [1, 2]. LA (2-hydroxypropanoic acid) is a lower carboxylic acid with chemical formula $[C_3H_6O_3]$. LA gives the lactate ion $[CH_3CH(OH)COO^-]$ by losing a proton from the acidic group and it is soluble in water and water-miscible organic solvents but not in the organic solvents [1, 3]. LA participates in many industrial chemical reactions such as esterification, condensation, and polymerization [4]. LA is a largely available organic acid in nature [5]. It was discovered by famous Swedish scientist Carl Wilhelm Scheele in 1780. The first large-scale production of LA in the United States of America was the result of the discovery of a French Scientist Fremy who obtained LA by the fermentation process [1, 6].

The conventional method of LA recovery is not so competitive and also environmentally un-friendly since it gives only the salt of LA instead of pure LA because of the addition of alkalis (like NaOH and $Ca(OH)_2$) which are needed for pH adjustment. It increases the production cost of LA up to 50% because of the addition of chemicals as well as some additional separation and purification steps [7]. It mainly depends on the concentration of clarified fermentation liquor and re-acidification with sulphuric acid (H_2SO_4) to give crude LA [8]. Therefore, high cost and environmental pollution posed by the conventional method had led to search other methods for the recovery of LA. The successfully developed methods of LA recovery are as follows solvent extraction, membrane bioreactor, liquid surfactant membrane extraction,

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adsorption and electro dialysis. Each method possesses some own merits and demerits but among all, reactive extraction specifically has added some advantages (such as a clean process due to the complete recovery of the diluents, highly selective, economic, gives high product yield, easy product removal [9], good control on broth pH without use of any base, has high value of both local mass transfer and distribution coefficient, decreasing in equipment size, utility costs, by-product reactions, and better recovery of product with high purity [10]) over the other methods [1, 11]. In the process of reactive extraction, first the reaction takes place between the extractant molecule which presents in the organic phase and the solute (LA) molecule in the aqueous phase and then formed a complex (acid-extractant) which solubilizes in the organic phase [12]. The studies of binary diluents and extractants for the LA extraction from the aqueous solution as well as from the fermentation broth have been become the current topic of research in view of their improved stripping behavior. For example Poposka*et al.*[13] had reported about the reactive extraction of tartaric acid using Hostarex A324 in isodecyl alcohol/kerosene mixtures, Uslu and Inci,[14] have studied the extraction of propionic acid using Aliquat336, five single solvents, and three binary solvents, and Morales*et al.* [15] had examined the effect of various extractants (tri-n-tributyl phosphate (TBP) and tri-n-octylamine (TOA) in 1-decanol/n-dodecane) on the extraction of monocarboxylic acids. Supercritical fluid (CO_2) extraction is another method for the recovery and purification of LA but it involves limitations such as low purity of product due to the co-extraction of fermentation by-products such as acetic and butyric acids and also involvement of huge capital [16].

At present, reactive extraction has become a very promising technique for the recovery of LA from the fermentation broth as well as from the dilute waste streams since this method gives high purity product with the reduced cost and minimum environment pollution but it suffers from the problems of toxicity of the used extractants/carriers and organic diluents [17]. The petroleum-based organic diluents are usually toxic, non-renewable, non-biodegradable, flammable, volatile in nature, and harmful for the aquatic life in case of solvent loss due to entrainment in the aqueous phase. Therefore, there is paramount need to find-out a greener replacement of the petroleum-based organic solvents in order to curb the environment related problems [18]. The problems of toxicity during the reactive extraction of LA can be minimized either by choosing non-toxic diluent i.e. vegetable oils (such as soybean oil, rice bran oil, sunflower oil etc.) for blending with a toxic extractant to yield a biocompatible mixture or a combination of a non-toxic extractant (such as TOA which is non-toxic to anaerobic bacteria [19]) and a diluents [20]. Vegetable oils or green solvents have a great potential as a greener substitute for the organic solvents because of their sound pertinent properties like non-toxicity, non - flammability, non-volatility, renewability, and biodegradability [18]. To the best of our knowledge till date no work has been reported in the literature for the use of soybean oil as a green solvent for LA extraction from the aqueous solution. Soybean oil (kinematic viscosity at 38 ˚C of32.6 mm^2/s, refractive index at 20˚C of 1.47, and density of 0.9138 kg/l [21]) is non-volatile oil extracted from soybean seeds, and contains, mainly linoleic acid (47.2%), oleic acid (25%), saturated acid (19.2%), and linolenic acid (8.46%), in the form of triglyceride [22].

For the optimization of various process parameters, the previous method of optimization is not useful since this method of single factor optimization by keeping all other parameters at a fixed value does not elucidate the interactive effect of all the involved process parameters [23]. Response surface methodology (RSM) is a very important statistical tool available as software for the optimization of various process variables, and for guessing the best performance conditions with the minimum number of experiments. It is a combination of statistical and mathematical methods which are helpful in the development, improvement, and optimization of the processes [24]. The LA distribution coefficient (K_D) was optimised by using the method of reactive extraction and its experiment run based on the

experimental design received from Box-Behnken design (BBD) with seven variables at 3-levels each using Design Expert 7.16 software (Stat ease Inc., Minneapolis, USA). The main purpose of this present investigation is to study the major process variables (initial LA concentration in aqueous phase (C_1, [M), extractant ratio (α, v/v), mixed extractants concentration (Ψ, % (v/v)), phase ratio (Φ, v/v), temperature (T, ˚C), stirring speed (ω, rpm), and contact time (τ, min)) influencing the LA distribution coefficient (K_D), to optimize the process conditions, and also to find out a suitable and selective combination of non-toxic extractants and green diluent for the LA reactive extraction from the aqueous solution.

2. Materials and Methods

2.1 Reagents

All the reagents were of technical grade and used without any pre-treatment. Tri-n-octylamine (TOA), a tertiary amine, a light colourless liquid with the molar mass 353.66 and density 0.809 $\frac{g}{cm^3}$ was procured from MERCK, Ltd, India. Tri-n-octylmethylammonium chloride (TOMAC), a quaternary amine, with molar mass 404.17 and density 0.888 g/cm^3 was supplied by S.D. Fine-Chem. Ltd, India. Lactic acid (LA) and sodium hydroxide pellets were purchased from S.D. Fine-Chem. Ltd, India. Phenolphthalein solution (pH range, 8.2 to 10) used as an indicator was bought from CDH, Ltd, India. Refined soybean oil as a green solvent was procured from the local market.

2.2 Liquid - Liquid Extraction

The organic phase in this study was composed of extractants/carriers (such as TOA and TOMAC), organic solvent, and green solvent (soybean oil). The aqueous phase with the desired LA concentration was prepared using LA and Milli-Q deionized water (Millipore filtration of MERCK). Both the phases in the desired phase ratio (0.5 - 2, v/v) were mixed into each-other and the resultant solution was shaken for different time intervals in a temperature-controlled incubator (New BrunswickTM Innova® 40) for the rpm in the range of 50 - 200. After mixing of both the phases, these were left for half an hour for exact phase separation. Two phases were formed after settling down of the solution, one phase was settled at the top as an organic phase and other at the bottom as an aqueous phase. Samples were taken from the aqueous layer using a pipette/syringe for titration. The concentration of aqueous phase was determined by using acid-base titration and reconfirmed by the method of colorimetric by using UV/VIS - spectrophotometer (model DR 5000 HACH, USA) and absorbance recorded at 570 nm [25]. The organic phase concentration was determined by the mass balance.

3. Theory of LA Extraction

The overall extraction equilibrium of LA can be described by un-facilitated as well as the facilitated transport as:

3.1 Physical Extraction

The physical extraction of LA has studied for the better understanding of the effect of various extractants (TOMAC and TOA) on the reactive extraction of the LA [26]. In the aqueous phase LA (weak carboxylic acid) molecule exists as a monomer since hydrogen bonding of LA molecule along with the water molecule is much stronger than intermolecular hydrogen bonding between acid. But in the organic solution, maximum part of LA exists as dimers in the low polar or non-polar diluents [27]. LA exists in dissociated form (A^-) in the aqueous phase when the aqueous solution pH is less than its acid dissociation constant ($pK_a = 3.86$). If the pH value of the aqueous solution is less than its dissociation constant then the acid is assumed to be transferred from aqueous phase to organic phase by the three different steps: (i) ionization of acid in the aqueous phase (ii) un-dissociated acid partition in organic phase and, (iii) dimerization of acid in organic phase [28] as discussed below.

Ionization or dissociation of the LA in the aqueous phase:

$$\underbrace{[HA]}_{\text{LA}} \leftrightarrow \underbrace{H^+}_{Hydronium\ \ ion} + \underbrace{A^-}_{Lactate\ \ ion} \quad (1)$$

Partition of the un-dissociated acid molecule:

$$[HA]_{aq.} \leftrightarrow [HA]_{org.} \quad (2)$$

Dimerization of the acid in the organic solvent:

$$[(HA)2]_{org.} \leftrightarrow [HA]^2_{org.} \quad (3)$$

3.2 Lactic Acid Reactive Extraction Stoichiometry

The reactive extraction of LA using a combination of extractants (TOA and TOMAC) and non-toxic diluent (soybean oil) is described by equilibrium reactions which take place between LA molecules and the molecules of extractants at the inter phase of aqueous and organic phase to form LA-extractant complexes. When LA exists as in its un-dissociated form (HA) in the aqueous phase, then the reaction of LA withTOA takes place through hydrogen bond formation and resulting into a complex (1:1) (TOA-LA) at low LA concentration.

$$\underbrace{R_3N}_{TOA} + \underbrace{HA}_{\text{LA}\,(un-dissociated\ \ form)} \leftrightarrow \underbrace{R_3N - HA}_{TOA-LA\ complex} \quad (4)$$

The extracted acid which is extracted by extractants (amines) is not known as an acid for a longer time but as an ammonium salt and the extent of this ion pair association (alkyl ammonium cation and acid radical) quantify the degree of extraction.

When LA exists in its dissociated form ($H^+ + A^-$) in the aqueous phase then the complex is formed by ion-pair formation:

$$\underbrace{R_3N}_{TOA} + \underbrace{H^+ + A^-}_{\text{LA}\,(dissociated\ \ form)} \leftrightarrow \underbrace{R_3NH^+A^-}_{TOA-LA\ Complex} \quad (5)$$

Now, LA transportation takes place with the second water-insoluble extractant TOMAC (acts as an ion exchanger), quaternary ammonium salt obtained by methylation of mixed tri octyl/decylamine in which anion of LA is exchanged with an anion of quaternary ammonium salt (or chloride ion) to form oil soluble salts at neutral or slightly alkaline pH, and reaction is given below [29].

$$\underbrace{R_4NH^+X^-}_{TOMAC} + \underbrace{A^-}_{Lactate\ \ ion} \leftrightarrow \underbrace{R_4NH^+A^-}_{TOMAC-LA\ complex} + \underbrace{X^-}_{Chloride\ \ ion} \quad (6)$$

It can be suggested that the synergistic combination of both extractants (TOA + TOMAC) can be led to the formation of various complexes as elucidated below:

$$\underbrace{R_3NH^+A^-}_{TOA-LA\ complex} + \underbrace{R_4NH^+X^-}_{TOMAC} \leftrightarrow \underbrace{R_3NH^+A^-R_4NH^+}_{LA-(TOA+TOMAC)\ complex} + \underbrace{X^-}_{Chloride\ \ ion} \quad (7)$$

or

$$\underbrace{R_4NH^+A^-}_{TOMAC-LA\ complex} + \underbrace{R_3N}_{TOA} \leftrightarrow \underbrace{R_3N - R_4NH^+A^-}_{LA-(TOA+TOMAC)\ complex} \quad (8)$$

The LA distribution coefficient (K_D) is defined as the ratio of the concentration of acid (LA) in its all forms (such as complex, partition, and dimmers) in the organic phase to its concentration in its entirely existing form (such as non-dissociated and dissociated) in the aqueous phase [28].

$$\text{LA distribution coefficient } (K_D) = \frac{[HA]_{org.}}{[HA]_{aq.}} \quad (9)$$

Where $[HA]_{org.}$ and $[HA]_{aq.}$is the concentration of LA in organic and aqueous phaserespectively. Distribution coefficient (K_D) depends on the following factors such as the characteristics of the diluent (act as non-reactive component) used, concentration of the extractant (act as a reactive component in the organic phase), and the number of carbon atoms present in the tertiary amines (usually used as an extractant) which further increases its extractability since the basicity and polarity of the amines increase with an increase in carbon chain length of the amines [1, 30]. The key reaction in the LA reactive extraction is the reaction between solute (LA) and extractants (TOA and TOMAC) which produces a product (complex) with the lowest bond energy (less than 50 kJ/mol) so that solute is easily and economically back extracted for its again use [11]. The diluents are used in reactive extraction to increase the physical properties (surface tension,

viscosity, and density) of these extractants by providing solvation and extraction power through giving specific interaction [31]. The transportation of solute concentration to the organic phase with respect to the sum of the solute concentration in both organic and aqueous phase is known as the degree of extraction (η, %) and is represented in the form of K_D.

$$\text{LA extraction efficiency } (\eta, \%) = \frac{K_D \times 100}{1+K_D} \quad (10)$$

3.3 Experimental Design

LA distribution coefficient (K_D) was calculated by using the method of liquid-liquid extraction and its experimental run based on the experimental design received from Box - Behnken design (BBD) with the seven variables at three levels each using Design Expert 7.16 software (Statease Inc., Minneapolis, USA). The effect of seven independent process parameters (such as LA concentration in aqueous phase (C_1, [M]), extractant ratio (α, v/v), mixed extractants concentration (Ψ, %v/v), phase ratio (Φ, v/v), temperature(T, ˚C), stirring speed (ω, rpm), and contact time (τ, (min)) on the LA distribution coefficient (K_D) has been studied. The form of the original value of each factor (un-coded) and their corresponding levels (coded) has shown in Table 1. This experimental design includes 62 numbers of experiments using various combinations of several process parameters as shown in Table 2.

Table 1: Range of Different Variables for the LA Distribution Coefficient in Coded and un-Coded form

	Factors	Process Parameters	Level			
			Coded variables	-1.00	0.00	1.00
Un-coded variables	X_1	LA concentration	C_1, [M]	0.06	0.02	0.1
	X_2	Extractant ratio	α	0.5	1.25	2
	X_3	Mixed extractants concentration	Ψ (%, v/v)	10	20	30
	X_4	Phase ratio	Φ (v/v)	0.5	1.25	2
	X_5	Temperature	T (°C)	20	35	50
	X_6	Stirring speed	ω (rpm)	50	125	200
	X_7	Contact time	τ (min)	60	90	120

3.4 Statistical Analysis and Optimization

For the different interactions of any given two independent variables while keeping the other variables remain constant at the 0.00 value, response surface and contour figures were created. Such 3-Dsurface elucidates the useful detail information and perfect geometrical representation about the nature of the system inside the experimental design [32]. For gaining the large LA distribution coefficient (K_D), the optimum values of the various variables (such as initial LA concentration in the aqueous solution, extractant ratio, extractant concentration, phase ratio, stirring speed, temperature, and contact time) were determined within the experimental range. The experimental runs are being conducted using the model values of the variables which were generated by the optimization of the predicted values.

4. Results and Discussions

4.1 Regression Model

The following regression equations were generated as a result by using the multiple regressions (along with backward elimination regression with alpha to exist = 0.100) on experimental data. The quadratic model (in the coded forms) equation describes the role of each variable and their quadratic interaction on the LA distribution coefficient (K_D) as follows.

Distribution Coefficient (K_D) $= 1.96 - 0.255\times X_1 - 0.0587\times X_2 + 0.296\times X_3 - 0.457\times X_4 - 0.256\times X_5 - 0.129\times X_6 - 0.06988\times X_7 + 0.548\times X_1\times X_2 - 0.06475\times - 0.12\times X_1\times X_5 - 0.339\times X_2\times X_4 - 0.097\times X_2\times X_6 + 0.132\times X_2\times X_7 + .074\times X_3\times X_4 - 0.198\times X_3\times X_5 - 0.096\times X_3\times X_7 + 0.152\times X_4\times X_7 + 0.193\times X_5\times X_6 - 0.077\times X_1^2 - 0.175\times X_2^2 - 0.421\times X_3^2 - 0.238\times X_4^2 - 0.208\times X_5^2 - 0.448\times X_6^2$ (11)

Where, $X_1, X_2, X_3, X_4, X_5, X_6, and\ X_7$ represent initial LA concentration, extractant ratio, extractant concentration, phase ratio, temperature, stirring speed, and contact time, respectively. The quadric model equation (Eq.11) includes twenty-four terms which comprise seven linear terms, six quadric terms, and eleven two-factorial interactions. Out of these, the insignificant terms are those terms which have probability (p) > Fischer tropsch coefficient (f) value more than 0.05 in Table 3. The values of the probability, p ($p>f$) were used as a tool to decide the importance of each coefficients. Lower the values of p, more the significant was the correlation with the corresponding coefficient. The values of coefficient of determination (R^2) were satisfactory (R^2>0.979) for the response p≤0.05 [33]. These values give a good agreement between the experimental observation and predicted values due to the difference between predicted R^2 (a measure of the value of the reduction in the variability of the response) and adjusted R^2 is very low (0.08). There was no lack of fit in the model equation (p≥ 0.05). This model has good reliability, reproducibility, and accuracy since the coefficient of the variation (CV) has relatively small percentage value (2.51%). The coefficient of the variation elucidates about the range to which data were spread. This model contains adequate precision 105.24 [34]. The three-D plots were generated for the different interaction of any two independent variables on the LA distribution coefficient (K_D), while keeping the values of the other variables constant at zero level.

4.2 Distribution Coefficient (K_D)

The predicted model briefly elucidates the effect of the seven independent parameters on the LA distribution coefficient (K_D). The 3-D graphical representation of the response surfaces as illustrated in the figures (1-8) needs to describe the interactive effect of the independent variables on the LA distribution coefficient (K_D). Each response curve signifies the variation in the level of two factors with the other five factors kept at zero level. The significant interactions between the independent variables have been observed as X_1&X_4, X_1&X_5, X_2&X_4, X_2&X_6, X_3&X_4, X_3&X_5, X_3&X_7, and X_5&X_6.

The effect of initial LA concentration (C_1, [M]) and phase ratio (Φ, v/v) on LA distribution coefficient (K_D) was shown in (Fig.1). Fig.1 has predicted that with the increase in the phase ratio and initial LA concentration, LA distribution coefficient decreases continuously. LA distribution coefficient (K_D) has been found to be decreased with the increase in the initial LA concentration irrespective of phase ratio because of attainment of saturation of the organic phase with the increase in the value of LA in the aqueous phase [35]. It may be due to the less availability of the extractants molecules in the organic phase at the high concentration (C_1= 0.1 [M]) of LA in the aqueous phase which further decreases the number of complexes formed between acid-extractant [27]. The LA distribution coefficient (K_D) decreases with an increase in phase ratio irrespective of LA concentration since the inadequate mixing (due to the improper ratio of the phases) of both phases (organic and aqueous phases) reduces the tendency of the complex formation which further decreases the interfacial rate of mass transfer [36]. The maximum value of LA distribution coefficient (K_D) was obtained for the low value of the phase ratio (Φ = 0.5 v/v) as well as LA concentration (C_1= 0.02 [M]).

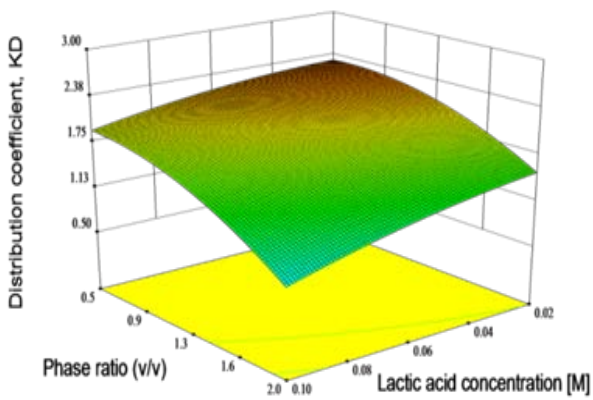

Fig.1 Effect of the phase ratio and LA concentration on the LA distribution coefficient (K_D)

Table 2: Experimental Design of the Coded Process Parameters for the LA Distribution Coefficient by Using Soybean Oil

Coded Process Variables for Experiments							
LA concentration	Extractant ratio	Mixed extractants concentration	Phase Ratio	Temperature	Stirring speed	Contact time	Distribution coefficient
(X_1)	*(X_2)*	*(X_3)*	*(X_4)*	*(X_5)*	*(X_6)*	*(X_7)*	K_D
0	-1	-1	0	0	-1	0	0.72
0	0	1	1	0	0	-1	1.22
-1	-1	0	-1	0	0	0	2.42
0	0	0	0	0	0	0	1.94
-1	1	0	1	0	0	0	0.5
1	1	0	-1	0	0	0	2.58
0	1	0	0	-1	0	-1	1.69
0	0	1	1	0	0	1	1.19
0	1	0	0	-1	0	1	1.83
0	-1	1	0	0	1	0	1.25
0	-1	-1	0	0	1	0	0.67
0	0	1	-1	0	0	1	1.69
0	0	0	-1	-1	1	0	1.47
0	0	0	0	0	0	0	1.99
-1	0	-1	0	-1	0	0	1.15
0	0	0	0	0	0	0	1.97
0	0	-1	1	0	0	1	0.63
-1	0	0	0	0	1	1	1.48
1	0	0	0	0	-1	-1	1.4
0	0	0	-1	-1	-1	0	2.13
0	1	1	0	0	-1	0	1.37
0	1	-1	0	0	-1	0	0.79
0	0	-1	-1	0	0	1	1.43
-1	-1	0	1	0	0	0	2.27
0	0	0	1	-1	1	0	0.53
0	0	-1	-1	0	0	-1	1.68
0	0	0	0	0	0	0	1.95
0	0	0	0	0	0	0	1.98
1	-1	0	-1	0	0	0	0.8
1	0	-1	0	-1	0	0	0.9
1	0	1	0	-1	0	0	1.89
0	0	0	-1	1	-1	0	1.23
1	-1	0	1	0	0	0	0.58
0	-1	0	0	-1	0	-1	2.1
-1	0	0	0	0	1	-1	1.64
1	0	1	0	1	0	0	0.74
0	0	-1	1	0	0	-1	0.28
0	0	0	1	-1	-1	0	1.18
-1	1	0	-1	0	0	0	1.82
0	-1	1	0	0	-1	0	1.29
-1	0	0	0	0	-1	-1	1.9
0	1	-1	0	0	1	0	0.35
0	0	0	1	1	1	0	0.43
-1	0	0	0	0	-1	1	1.72
0	-1	0	0	1	0	1	1.21
-1	0	1	0	-1	0	0	2.14
0	1	1	0	0	1	0	0.94
0	-1	0	0	1	0	-1	1.59
1	0	0	0	0	-1	1	1.27
0	1	0	0	1	0	-1	1.19
0	-1	0	0	-1	0	1	1.71
0	0	0	-1	1	1	0	1.31
1	0	-1	0	1	0	0	0.54
1	0	0	0	0	1	1	0.98
1	0	0	0	0	1	-1	1.12
0	1	0	0	1	0	1	1.33
-1	0	1	0	1	0	0	1.46
0	0	1	-1	0	0	-1	2.33
1	1	0	1	0	0	0	0.82
0	0	0	1	1	-1	0	0.27
-1	0	-1	0	1	0	0	1.27
0	0	0	0	0	0	0	1.98

Table 3: ANOVA for Response Surface Reduced Quadratic Model Analysis of Variance

Source	Sum of Squares	Degree of freedom (DOF)	Mean Square	*f*- value	p-value Prob. > F	
Model	20.5	24	0.856	736.19	< 0.0001	Significant
LA						
Concentration (X_1)	1.56	1	1.566	1346.7	< 0.0001	
Extractant ratio (X_2)	0.08	1	0.0826	71.082	< 0.0001	
Mixed extractants						
Concentration (X_3)	2.10	1	2.105	1810.21	< 0.0001	
Phase ratio (X_4)	5.02	1	5.02	4319.75	< 0.0001	
Temperature (X_5)	1.58	1	1.581	1359.11	< 0.0001	
Stirring speed (X_6)	0.39	1	0.399	343.37	< 0.0001	
Contact time (X_7)	0.11	1	0.112	100.730	< 0.0001	
(X_1). (X_2)	2.40	1	2.409	2070.83	< 0.0001	
(X_1). (X_4)	0.03	1	0.033	28.832	< 0.0001	
(X_1). (X_5)	0.12	1	0.114	98.204	< 0.0001	
(X_2). (X_4)	0.92	1	0.918	789.14	< 0.0001	
(X_2). (X_6)	0.07	1	0.075	65.248	< 0.0001	
(X_2). (X_7)	0.13	1	0.138	119.37	< 0.0001	
(X_3). (X_4)	0.04	1	0.043	37.658	< 0.0001	
(X_3). (X_5)	0.31	1	0.315	270.96	< 0.0001	
(X_3). (X_7)	0.07	1	0.073	63.048	< 0.0001	
(X_4). (X_7)	0.18	1	0.184	158.88	< 0.0001	
(X_5). (X_6)	0.30	1	0.300	258.15	< 0.0001	
$(X_1)^2$	0.08	1	0.0830	71.378	< 0.0001	
$(X_2)^2$	0.42	1	0.421	71.37	< 0.0001	
$(X_3)^2$	2.45	1	2.452	362.51	< 0.0001	
$(X_4)^2$	0.78	1	0.784	2108.42	< 0.0001	
$(X_5)^2$	0.60	1	0.6009	674.22	< 0.0001	
$(X_6)^2$	2.78	1	2.785	516.65	< 0.0001	
Residual	0.04	37	0.0011			
Lack of Fit	0.04	32	0.00129	4.437	0.0512[a]	Non-significant
Pure Error	0.00	5	0.00029		< 0.0001	
Cor. Total	20.5	61	0.856		< 0.0001	

Standard deviation=0.034	R^2=0.9979
Mean=1.35	Adjusted R^2=0.979
Coefficient of variation=2.511	Predicted R^2=0.968
Press=0.242	Adequate precision= 105.24

The effect of initial LA concentration (C_1, [M]) and temperature (T, ˚C) on the LA distribution coefficient (K_D) has shown in Fig.2. It has been elucidated in Fig.2 that the LA distribution coefficient (K_D) decreases with an increase in both the temperature and initial LA concentration in the aqueous phase, respectively. With the rise in temperature, the LA distribution coefficient (K_D) decreases at fast rate for the high value of the LA concentration (C_1= 0.1[M]), since with rise in the temperature the increment in the thermal energy of the solution (i.e. increasing randomness between the molecules) which is responsible for the very less interactions between extractants (such as TOA and TOMAC) and solute (LA) molecules. During the formation of complex, system should have very less entropy or it should be more and more ordered but, in this case, it is disturbed due to the increased system entropy with the continuous increment in extraction system temperature which further disturbed the formation of the complexes [37]. At high temperature (T = 50˚C), the decrement in LA distribution coefficient (K_D) is more with respect to low temperature (T = 20˚C) with the increase in the initial lactic LA concentration. The LA distribution coefficient (K_D) decreases with the rise in initial LA concentration due to the presence of saturation of organic phase with LA molecules at a high value of concentration [38].

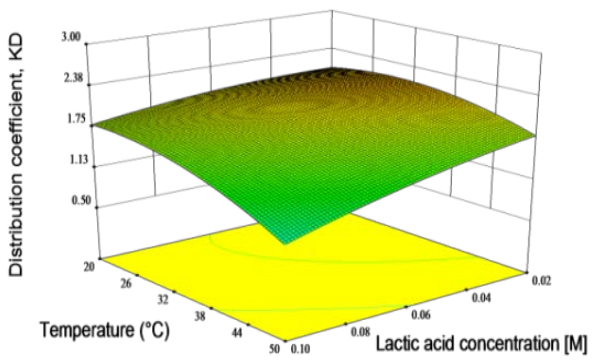

Fig.2 Effect of the temperature and LA concentration on the LA distribution coefficient (K_D)

The interaction between phase ratio (Φ, v/v) and extractant ratio (α, v/v) has shown in Fig.3. It has been explained in Fig. 3 that at the low value of phase ratio (Φ = 0.5 v/v), the LA distribution coefficient (K_D) increase with the rise in the extractant ratio (α, v/v) since as the value of extractant ratio increases, the amount of TOMAC (quaternary amine) decreases with respect to TOA (tertiary amine), which is very viscous (1500 mpa.s at a temperature of 30C) in nature and creates a hindrance for the interfacial mass transfer between the phases. TOMAC having an organic cation along with the chloride ion which makes it possible to work as an anion exchanger reagent [37].

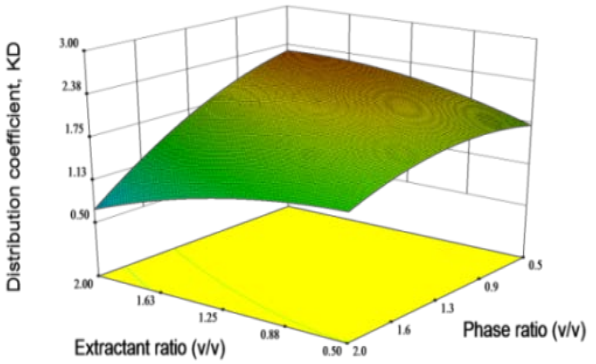

Fig.3 Effect of the phase ratio and extractant ratio on the LA distribution coefficient (K_D)

Alone TOMAC does not give high LA distribution coefficient (K_D) since it is very viscous in nature which further limits rate of mass transfer but due to the synergetic effect of the extractants (TOMAC+TOA) and the presence of natural solvent (also has good solvation for the complex), it gives high value of LA distribution coefficient (K_D) [17]. At high value of phase ratio (Φ = 2 v/v), the value of LA distribution coefficient (K_D) decreases with an increase in the extractant ratio since here phase ratio is dominating not the extractant ratio. These extractants (TOA and TOMAC) are toxic in nature to microbes so they should be mixed with the natural solvent (soybean oil) which helps in both reducing the toxicity as well as increase the solvation for the complex (acid-extractant) and also in making the system eco-friendly [39]. The maximum value of LA distribution coefficient (K_D) has been gained for low value of phase ratio (Φ = 0.5 v/v) and extractant ratio value (for α = 1.6 (approximate) v/v).

Fig.4 shows the interaction between extractant ratio (Φ, v/v) and stirring speed (ω, rpm). It is explained from Fig.4 that at high extractant ratio (Φ = 2 v/v), the LA distribution coefficient (K_D) increases with an increase in the stirring speed up-to-the optimum value (ω = 105 (approximate) rpm), then decreases since with an increase in the stirring speed, because of the disturbance in the interfacial layer at the interface increases more with the stirring speed which further reduces the thickness of the layer and increases the rate of transfer of LA molecules from aqueous phase to the organic phase [28].

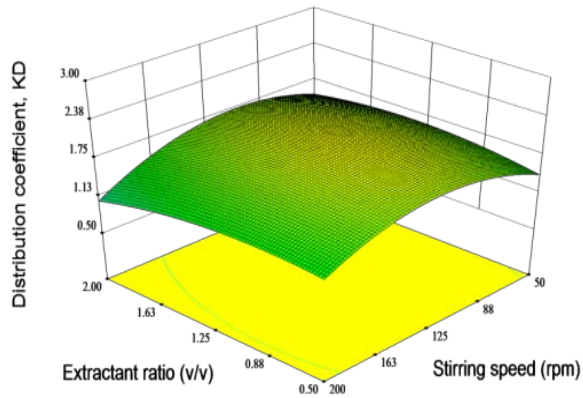

Fig.4 Effect of the stirring speed and extractant ratio on the LA distribution coefficient (K_D)

Beyond the optimum stirring speed (ω = 105 (approximate) rpm), the LA distribution coefficient (K_D) decreases with increase in stirring

speed because of the large displacement of extractant molecules from the interface due to a high level of turbulence hence very fewer molecules of extractants are present at the interface to form various complexes (acid-extractant) [40]. At high stirring speed (ω = 200 rpm), the value of LA distribution coefficient (K_D) changes very less and levelled-off for further increase in the extractant ratio but at low stirring speed (ω = 50 rpm), the value of LA distribution coefficient (K_D) first increases up to extractant ratio value (for α = 1.3 (approximate) v/v) then reduces with further increase in the value of extractant ratio since with the rise in extractant ratio the loading rate of the extractants reduces [41]. The maximum value of LA distribution coefficient (K_D) has been obtained at stirring speed of 105 (approximate) rpm and extractant ratio value (for α = 1.3 (approximate) v/v).

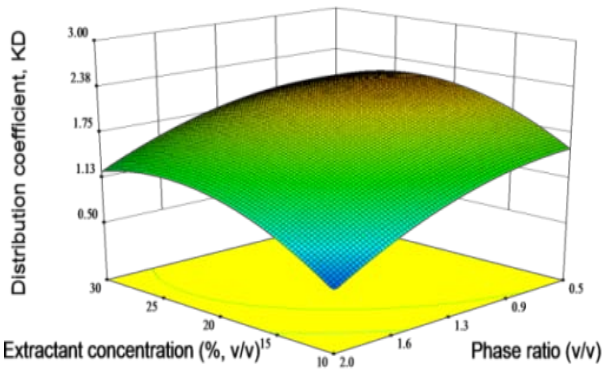

Fig.5 Effect of the phase ratio and mixed extractants concentration on the LA distribution coefficient (K_D)

Fig.5 shows the interaction between the phase ratio (Φ, v/v) and mixed extractants concentration (Ψ, % v/v). Fig.5 shows that at high values of phase ratio (Φ = 2 v/v), with the increase in the mixed extractant concentration, the value of LA distribution coefficient (K_D) increases but levelled off at high mixed extractants concentration because the concentration for the formation of complex (acid-extractant) also increases with the increase in the mixed extractants concentration [12]. At low phase ratio (Φ = 0.5 v/v), the value of LA distribution coefficient (K_D) first increases up to the maximum value with an increase in mixed extractants concentration up to (Ψ = 25% v/v (approximate)) then decreases since with increasing in extractant concentration, the extractability of the extractant increases which favors the rate of formation of complexes [35]. The LA distribution coefficient (K_D) reduces for high extractant concentration, may be due to less availability of the solute molecules at a low value of phase ratio. The other plausible reason may be due to the high viscosity of the organic phase increases the interfacial resistance between the phases which further reduces the rate of mass transfer of LA molecules from the aqueous phase to the organic phase [36]. The maximum value of LA distribution coefficient (K_D) has been obtained at low value of phase ratio (Φ = 0.5 v/v) and mixed extractants concentration (Ψ = 25% v/v (approximate)). The effect of mixed extractants concentration (Ψ, v/v) and temperature (T, ˚C) on LA distribution coefficient (K_D) was shown in Fig.6.

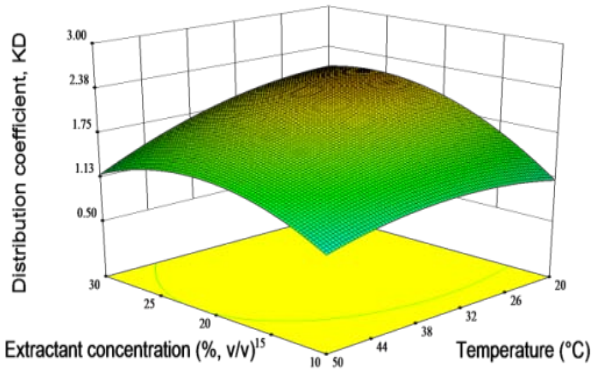

Fig.6 Effect of the temperature and mixed extractants concentration on the LA distribution coefficient (K_D)

Fig.6 elucidates that with an increase in extraction the temperature, the value of LA distribution coefficient (K_D) decreases irrespective of the mixed extractants concentration but this decrease is sharp at high mixed extractants concentration (Ψ = 30 % v/v) and smooth at low mixed extractants concentration (Ψ = 10 % v/v) due to the high viscosity of the organic phase at high mixed extractants concentration [26]. At high temperature (T = 50˚C), the LA distribution coefficient (K_D) increases with an increase in the mixed extractants concentration but less than at low temperature (T = 20˚C) due to the dominance of the temperature factor is more than the mixed extractants concentration. At low temperature (T = 20˚C), with

the increase in the mixed extractants concentration, the value of LA distribution coefficient (K_D) increases up to a maximum value then leveled-off for high values of mixed extractants concentration. It may be due to the non-polar nature of the TOA and itself a poor solvating media for the polar complex [38]. The maximum value of the LA distribution coefficient (K_D) was obtained for the mixed extractants concentration (Ψ = 26 % v/v (approximate)) and extraction temperature (T = 26˚C (approximate)). The effect of mixed extractants concentration (Ψ, v/v) and contact time (τ, min) on LA distribution coefficient (K_D) has shown in Fig.7.

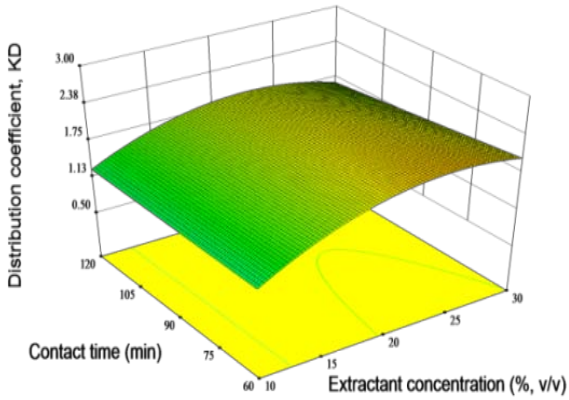

Fig.7 Effect of the contact time and mixed extractants concentration on the LA distribution coefficient (K_D)

Fig.7 illustrates that for high values of mixed extractants concentration (Ψ = 30% v/v), the value of LA distribution coefficient (K_D) decreases with an increase in the contact time. It may be due to the low contact time (60 min (approximate)) is adequate for the efficient and effective mixing of both the phases which further leading to the high rate of interfacial mass transfer [36]. With the increase in contact time, the value LA distribution coefficient (K_D) decreases which may be due to the beginning of the back extraction for higher values of contact time. The synergistic effect of the mixed extractants (TOMAC+TOA) gives higher values of the LA distribution coefficient (K_D) than the individual ones which make extraction process more and more effective and efficient [17, 37]. At the low values of mixed extractants concentration (Ψ = 10 % v/v), the LA distribution coefficient (K_D) does not change so much or remains constant with the contact time because the dominating factor is mixed extractant concentration, not the contact time and therefore further increment in contact time does not have any influence on the LA distribution coefficient. The maximum value of LA distribution coefficient (K_D) was obtained at low contact time (τ = 60 min) and high mixed extractants concentration (Ψ = 27% v/v (approximate)). The effect of extraction temperature (T, 26˚C) and stirring speed (ω, rpm) on the LA distribution coefficient (K_D) has elucidated in Fig.8.

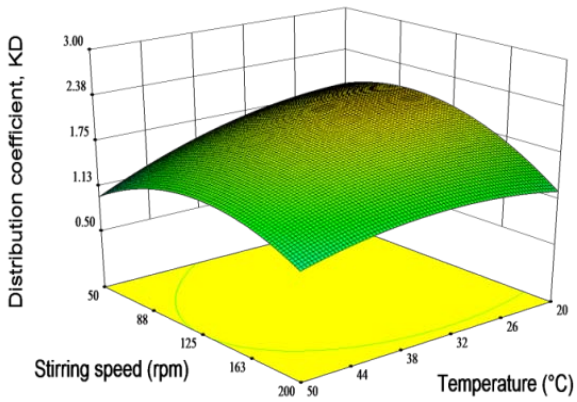

Fig.8 Effect of the stirring speed and temperature on the LA distribution coefficient (K_D)

Fig.8 has been explained that LA distribution coefficient (K_D) increases with the increase in the stirring speed up-to an optimum value then reduces because the high stirring speed responsible for the poor interaction between the extractant molecules and solute molecules which further resulting into the formation of a weak or unstable (means dissociated at very fast rate) acid-extractant complex and hence, responsible for the poor values of LA distributions [42]. With the decrease in the temperature, the value of the LA distribution coefficient (K_D) increases since the molecules of the solution becomes more and more ordered (means decreasing in entropy of the solution) with reducing the temperature which will further helps for strong interaction between acid-extractant [29]. The maximum value of LA distribution coefficient was achieved for the optimum values of extraction temperature (T = 26˚C (approximate)) and stirring speed (ω = 105 rpm (approximate)).

The optimized values of various process parameters are as follows: 0.02 [M] initial LA concentration (C_1), 0.5 (v/v) extractant ratio (α), 28.66% (v/v) mixed extractants concentration (Ψ), 2 (v/v) phase ratio (Φ), 27 ˚C temperature (T), 102 rpm stirring speed (ω), and 63 min contact time (τ). After the process parameter optimization using response surface methodology, the optimized value of LA distribution coefficient (K_D) have been achieved 2.51. The final LA extraction efficiency under the obtained optimized process conditions was found to be near 71.5%. The synergism of these extractants (TOMAC+TOA) has been provided the higher LA extraction efficiency than their individual applications during the extraction process [17, 37]. This extraction efficiency has been found better than the individual extractants [43, 44].

Table 4: Thermodynamic Parameters of Lactic Acid Reactive Extraction

Extractants	T (K)	1000/T (K^{-1})	D	log K_D	ΔH (kJ mol^{-1})	ΔG(kJ mol^{-1})	ΔS(J mol^{-1})
TOA	293.15	3.41	1.515	0.180	-6.045	-1.095	-16.885
	298.15	3.35	1.526	0.183			
	303.15	3.29	1.492	0.173			
	308.15	3.24	1.410	0.149			
	313.15	3.19	1.283	0.108			
TOMAC	293.15	3.41	0.252	-0.598	-30.945	2.965	-115.68
	298.15	3.35	0.263	-0.58			
	303.15	3.29	0.228	-0.642			
	308.15	3.24	0.147	-0.832			
	313.15	3.19	0.119	-925			
TOA +TOMAC	293.15	3.41	2.548	0.406	-3.567	-2.329	-4.223
	298.15	3.35	2.560	0.408			
	303.15	3.29	2.525	0.402			
	308.15	3.24	2.443	0.387			
	313.15	3.19	2.316	0.364			

4.3 Effect of the Temperature on the LA Reactive Extraction

Temperature is considered as one of the most important process parameter which has a direct influence on the equilibrium of liquid-liquid extraction process. By keeping all other process parameters constant, the effect of temperature on the LA extraction using the synergistic effect of the mixed extractants (TOA and TOMAC) has been studied from 293.15 to 313.15 K in a temperature-controlled shaker. The following thermodynamic properties (change in enthalpy (ΔH), entropy (ΔS), and Gibbs free energy (ΔG)) have been calculated by using the following equations [45, 46].

$$\frac{\Delta log\, K_D}{\Delta \frac{1}{T}} = \frac{-\Delta H}{2.303 R} \quad (12)$$

$$\Delta G = \Delta H - T\Delta S \quad (13)$$

Where R the universal gas constant (8.314 Jmol^{-1}K^{-1}) and K_D is the LA distribution coefficient. The graph between log K_D vs 1000/T is

linear (as shown in Fig.9). The value of the change in enthalpy (ΔH) is found to be negative (as shown in Table 4) for LA extraction with TOA, TOMAC, and (TAO+TOMAC) which elucidates that the LA reactive extraction process is exothermic in nature. The determined values of ΔH, ΔS and ΔG for the LA reactive extraction have elucidated in Table 4. The value of Gibbs free energy for the mixed extractants system (TOA+TOMAC) is negative which indicates the feasibility of the extraction process and also tells about the spontaneity of the extraction reaction. The negative value of entropy confirms that the nature/state of the molecules within the system is more and more ordered during the extraction process [45, 46].

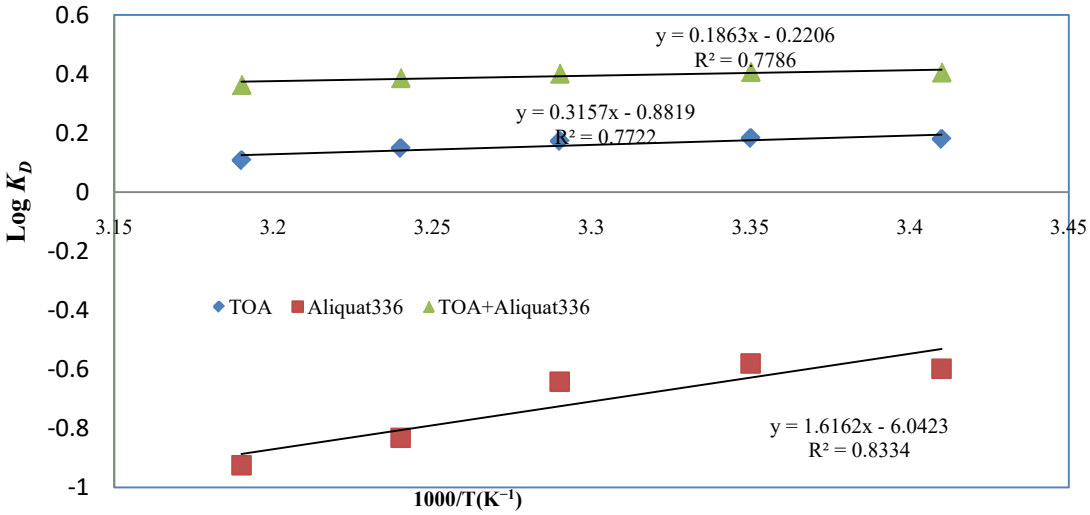

Fig.9 Effect of temperature on reactive extraction of LA with the mixed extractants (TOA+ TOMAC) [LA concentration (C_1) = 0.02 [M], extractant ratio (α) = 0.5 (v/v), extraction concentration (Ψ) = 28.66% (v/v), phase ratio (Φ) = 2 (v/v), stirring speed (ω)= 102 rpm, and agitation time (τ) = 63 min]

5. Conclusions

The numerical optimization technique has been used to optimize the quadratic model equation (Eq.11) for gaining the optimum value of the process parameters which further used to obtain the maximum value of LA distribution coefficient (K_D). The maximum value of LA distribution coefficient (K_D) was found 2.51 for the optimum values of various process parameters such as 0.02 [M] initial lactic acid concentration (C_1), 0.5 (v/v) extractant ratio (α), 28.66% (v/v) mixed extractants concentration (Ψ), 2 (v/v) phase ratio (Φ), 27˚C temperature (T), 102 rpm stirring speed (ω), and 63 min contact time (τ). The maximum LA extraction efficiency has been obtained 71.5% under these optimized conditions. The synergistic effect of the mixed extractants (TOMAC+TOA) has shown the higher value of LA distribution coefficient than the individual extractants. The problem of toxicity in the LA reactive extraction can be reduced either by the use of triglycerides of fatty acids (natural diluents) such as soybean oil along with the extractants or a combination of non-toxic extractant-diluent. The results obtained from this current investigation will be very useful for the design of reactive extraction unit for the separation of lower carboxylic acids from the real fermentation broth as well as from the dilute waste streams of various fermented based industries. Further, research will be need for the better insights of the synergistic extraction mechanisms during the separation of lower carboxylic acids.

ACKNOWLEDGMENT

Authors are thankful to Sant Longowal Institute of Engineering and Technology, Longowal India for providing the lab facilities and all necessary help to perform this research work.

Symbols

DOF	Degree of freedom
CV	Coefficient of variation
Std. Dev.	Standard deviation
PRESS	Predicted residual error of sum of squares
[M]	Molarity
ANOVA	Analysis of variance

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