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Modeling of Gallic Acid Diffusion: Case Study on *Cinnamonum zeylaniccum*

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Abstract

Polyphenols, found in vegetables, fruits and grains, are receiving increasing interest in recent years due to their delaying effects especially on the formation of certain types of cancer. However, in the current literature, there is no information on diffusion kinetics, diffusion coefficients of these materials, and the parameters affect on them. In this study, gallic acid-equivalent polyphenols production from *Cinnamonum zeylaniccum* by classical extraction method in ethanol as solvent was investigated the parameters of extraction temperature (25-55°C) and duration (10-90 min), the stirring speed of the medium (minimum-maximum) and the solid/liquid ratio (0.3-1.5g/40ml). Then, multiple parameter optimization was performed with Design Expert Program. For multiple optimizations, solid/liquid, temperature and time parameters resulted from the single optimization (55°C, 40 minutes, maximum mixing speed and 0.3g *Cinnamonum zeylaniccum*/40mL ethanol) were used in Box-Behnken Design construction. It has been determined by a computer program that the maximum gallic acid diffusion (3.267mg/100g) conditions were at 59°C, 37.6 minutes and 44.4 ml of solvent usage in a quadratic model. The most important single and interactive parameters on the extraction was determined as temperature and solid/liquid ratio, respectively. In the study, in order to define diffusion as a mathematical expression, diffusion kinetics data were obtained by performing experiments at different temperatures, without- or optimum-stirring speed conditions. These data were used in evaluating Peleg, Logarithmic, Page and Mass Transfer models. Molecular, effective diffusion coefficients and activation energy of gallic acid-equivalent of total polyphenols were calculated. It was observed that the increase in temperature and stirring speed increased the diffusion coefficients by decreasing activation energy of diffusion.

Keywords: Gallic acid, Modeling, Polyphenols, Diffusion, *Cinnamonum zeylaniccum*

INTRODUCTION

Today, there are many researches on cancer treatment which is one of the most common disease in the world. Cancer treatment-focused drug delivery systems gain importance in these studies, and the use of plant extracts instead of synthetic chemicals is a major factor in reducing side effects. Cinnamon (Lightning et al., 2016), produced mostly in China, Indonesia and Sri Lanka in the world, is a source of gallic acid (Figure 1), a powerful antioxidant. The ability to bind gallic acid free radicals, a phenolic flavonoid, has attractive properties such as interacting with cancer cells without interfering with healthy cells (Mukarami et al., 2008), (Pavun et al., 2014). Gallic acid found in spices and fruits such as apples, grapes and strawberries has properties such as strong antioxidant, antimutagenic,

anticancer and antiinflammatory (Jeong et al., 2009), (Yena et al., 2011), (Balcerzak et al ., 2008). Gallic acid has been shown to inhibit DNA oxidative reactions of free radicals and chelates formed with heavy ions (Canivenc-Lavier et al., 2009), (Moon et al., 2006), (Ulger, 2016), (Verma et. al., 2013).

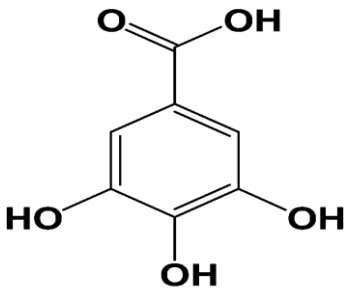

Figure 1. Chemical structure of gallic acid

Since the flavonoids formed by more than four thousand components are obtained by extraction from the plants, the flavonoid content and composition change with the extraction conditions. Both the variability of the extraction method (classical, microwave, ultrasonic, supercritical, etc.) and the solvent diversity cause a world of probability which is considered impossible to realize without optimization methods. For this reason, the response surface method (Turkyılmaz et al., 2014), (Goktas et al., 2015), (Dastianeh et al., 2013), (Levin et al., 2008), (Hesap and Yigitarslan, 2016), (Balci and Yigitarslan, 2017) were developed.

MATERIAL AND METHODS

In the study, the optimization of the extraction process of polyphenols from Cinnamonum zeylaniccum plant in the presence of ethanol as a solvent was carried out in two steps. The plant was supplied from a regional herbalist, and the chemicals such as ethanol, Folin-Ciocalteu and sodium carbonate were bought from Sigma_Alldrich in an analytical purity. In this study, firstly, a single optimization was performed on the parameters and values mentioned in Table 1. Then, in the direction of the results obtained from the single optimization, the Response Surface Method including the three centered-three parameter Box-Benkhen experimental design was used to determine the most effective three parameters optimization has been performed.

Table 1. The parameters of a single optimization

Temperature (°C)	**Solid/Liquid Ratio (g/mL)**	**Time (min)**	**Mixing rate (rpm)**
25	0.3/40	10	50
30	0.5/40	20	100
35	0.7/40	30	150
40	0.9/40	40	200
45	1.0/40	50	250
50	1.2/40	60	
55	1.5/40	70	
60		80	
		90	

In the single optimization, extraction has been performed for each value of the parameter to be worked on, while keeping the other parameters except the parameter to be worked on in Table 1 as constant value. Then the results of gallic acid analysis on the extracts obtained at these conditions were compared. When the parameter being studied was reached its maximum concentration of GAE, then the other parameter was studied by the same way. Thus optimum values for each parameter for the single optimization, which are valuable if they were taken into consideration alone, were determined.

Multiple optimizations were made using a computer program called Design-Expert. In this section, the parameters specified in Table 2 were encoded as minimum (-1), center (0) and maximum (+1) in the results obtained from the single optimization and they were defined into the program. A second-order polynomial function given in Equation 1 is proposed for expressing the extraction surface. In Equation 1, y represent the predicted response (extraction efficiency), x_i term represents the effect of the corresponding parameter affecting the yield, x_ix_j, x_jx_k, x_ix_k terms express the interactive effects of those parameters, β is the coefficient of the term, and finally ε represents the random error.

$$y = ß_0 + \sum_{j=1}^{k} ß_j X_j + \sum_{j=1}^{k} ß_{jj} X^2{}_j + \sum_{i=1}^{j-1} \sum_{j=2}^{k} ß_{ij} X_i X_j + \varepsilon \qquad \text{Equation 1.}$$

After the desired response the proposed function were entered into the program and, the change interval of the parameters wass defined as given in Equation 2, the Box-Behnken experiment design conditions consisting of 15 sets were obtained. Gallic acid analyzes were performed on the extracts, the yields obtained from experiments realized at these conditions were entered in a program and three-dimensional surface graphs expressing the extraction surface were plotted after carrying out statistical analyses. Equation expressing the surface and the coefficients were determined, and finally optimum values of each parameter was determined with numerical optimization.

$$xi = \frac{xi - xo}{\Delta x} \qquad \text{Equation 2.}$$

Table 2. Box-Benkhen Experimental Design parameters and ranges

Parameters	-1	0	+1
Solid/liquid ratio (g/mL)	0.3/35	0.3/40	0.3/45
Temperature (°C)	50	55	60
Time (min)	35	40	45

For the determination and modeling of gallic acid diffusion coefficients, extractions were carried out for 40 minutes at three different temperatures (35-45-55°C) and two different media (mixed and unmixed) and gallic acid measurements were applied every 5 minutes. Four different models namely Peleg, Mass Transfer, Page and Logarithmic Model have been tested with those results in order to determine the best model that provides the mathematical expression of the extraction.

Peleg's Model: Since the extraction curves (concentration of phenolics vs. time) have similar shape with the sorption curves, all of the extraction processes could be described with a non-exponential equation of Peleg (Peleg, 1988):

$$c_t = c_0 + \frac{1}{K_1 + K_2 t} \qquad \text{Equation 3.}$$

where c_t is the concentration of phenolics at time t (mg GAE/g), c_0 is the initial concentration of phenolics at time t=0 (i.e. c_0=0 in all experiments), t is the extraction time, K_1 is Peleg's rate constant (min.g/mg GAE), and K_2 is Peleg's capacity constant (g/mg GAE). In that equation, K_1 relates to the extraction rate (B_o) at the very beginning of the extraction ($t=t_0$):

$$B_0(mg\ GAE\ g^{-1}) = \frac{1}{K_1}$$ Equation 4.

and K_2 relates equilibrium concentration (c_{eq}) at $t\rightarrow\infty$:

$$c_{eq} = \frac{1}{K_2} \quad (mg\ GAE/mg)$$ Equation 5.

Page's Model: Another model used for the mathematical modeling of the extraction proposed by Page as follows (Jokic *et al.*, 2010):

$$c_t = \exp(-kt^n)$$ Equation 6.

where k and n are the constants of Page's Model, and all the other parameters have the same definitions.

Logarithmic Model: In mathematical modeling of extraction processes, Logarithmic model can also be used as follows:

$$c_t = a\ Logt + b$$ Equation 7.

where a and b are the logarithmic model constants.

Mass Transfer Model: Extraction occurs through two steps; Firstly, the solvent penetrates into the solid to dissolve the extractable material, and then the extractable material diffuses from inside the solid to the bulk liquid. The rate determining step of the overall process is the diffusion (Cheung *et al.*, 2012). The rate of this step under unsteady-state conditions is defined by Fick's second law as:

$$\frac{\partial c}{\partial t} = D\ \frac{\partial^2 c}{\partial x^2}$$ Equation 8.

where, c is the concentration of the solute (mg/g), t is time (min), D is the diffusion coefficient (m^2/min), and x is the distance of diffusion. This equation is valid when very dilute solution is used in the extraction and the diffusivity is assumed to be constant (Cacae and Mazza, 2003). If the shapes of the solid particles are assumed to as perfect spheres having the same properties and also if the perfect mixing of the solid-liquid medium occurs, the time of mass transfer at infinity, the general solution of this equation becomes:

$$Ln\left(\frac{c_\infty}{c_\infty - c}\right) = 0.498 + \frac{9.87Dt}{R^2}$$ Equation 9.

where, c is the concentration of the extracted material in the solution at time t (mg/g), c_∞ is the concentration of the extracted material at time t=∞, and R is the characteristic distance (m); i.e. for spheres it is equal to the radius. This equation can be rewritten as:

$$Ln\left(\frac{c_{eq}}{c_{eq} - c}\right) = a + K_{obs}t$$ Equation 10.

Since c_∞ is considered as equilibrium concentration, a is a constant (0.498), and

$$K_{obs} = \frac{9.87D}{R^2}$$ Equation 11.

In this research, Equation 10 was used to fit the experimental data and to obtain a, K_{obs} and diffusion coefficient values.

The model constants were calculated by applying these models to the experimental data and then the estimated gallic acid amount was calculated by using the model constants with these model constants. Furthermore, for each model, the correlation coefficient values were calculated using Equation 12 and these values were compared.

$$r^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - y_{model})^2}{\sum_{i=1}^{n}(y_i - y_{mean})^2}$$ Equation 12.

In the study, Arrhenius law was used to determine energy barriers that must be overcome (activation energy) for the gallic acid extraction from this plant (Equation 13):

$$k = k_{obs}\exp(\frac{-E_a}{RT})$$ Equation 13.

where, k is the extraction rate constant (L/g.min), k_0 is the temperature-independent factor (L/g.min), E_a is the activation energy of the extraction (j/mol), R is the universal gas constant (8.314 j/mol.K) and T is the absolute temperature of the extraction medium (K). Thus, after linearization the plot of ln k versus 1/T, activation energy and k_0 can be determined from the equation:

$$Ln\ k = Ln\ k_0 + (\frac{-E_a}{RT})$$ Equation 14.

At the end of each extraction, the samples were filtered using 110 mm Whatman filter paper. In order to determine the amount of gallic acid in the extract, 50 µL of the extract was mixed with 0.5 mL of Folin-Ciocalteu, 450 µL of purified water, 1.5 mL of Na_2CO_3 (20% by weight), respectively, and again 5 mL of purified water was added and incubated in an incubator for 2 hours at 25°C. Once the color formation was complete, the mixture was analyzed at 765 nm wavelength on the UV_VIS spectrophotometer (Carry 60). These absorbance values were inserted into the calibration curve (Absorbance = 0.001532 * Concentration; R^2 = 0.9174) and the concentrations of gallic acid in the extracts were calculated.

RESULTS AND DISCUSSION

In this study, the most suitable model for the extraction of gallic acid at all temperatures studied was found as the mass transfer model (Figures 2-7). As shown in Figures, the mass transfer coefficient increased as the temperature increased, resulting in a more yield obtained in the mixed condition compared to the unmixed medium. In the mass transfer model, the optimum conditions for this study were found as extractions at 55°C in a mixed environment. Also, it was observed that, the experimental and calculated concentration values were getting closer as the temperature increased. Thus, it was concluded that mass

transfer model can be used effectively at high temperatures. The other models applied did not fit to the experimental data as much as mass transfer model, and thus they were not given.

The diffusion coefficients for different temperatures and different media were calculated by mass transfer model were summarized in Table 3. According to results, the diffusion coefficient increased with the increase in temperature, and, es expected, the effective diffusion coefficients were higher than that of molecular ones, since the agitation produced an extra velocity thus the molecules could move faster, thus increased mass transfer was observed.

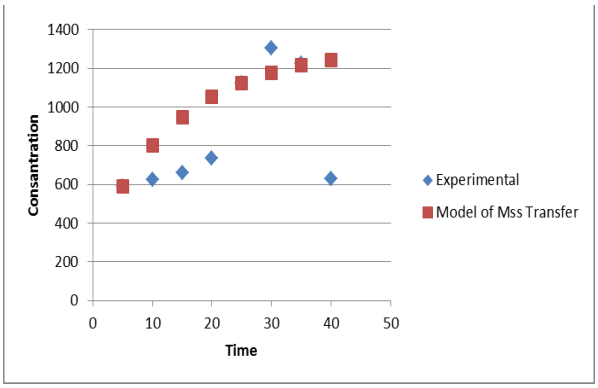

Figure 2. Moleculer Diffusion at 35°C

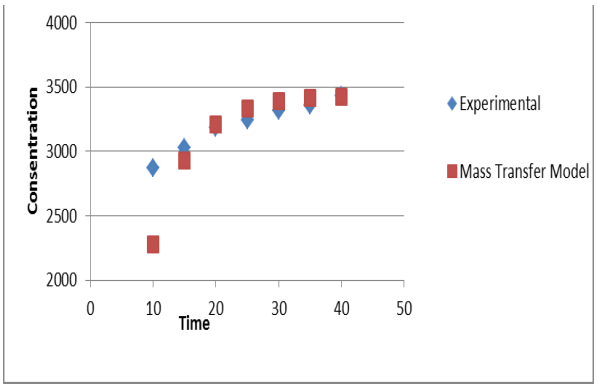

Figure 3. Convective Diffusion at 35°C

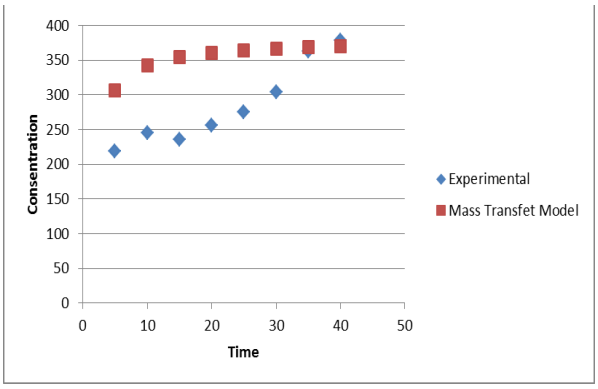

Figure 4. Moleculer Diffusion at 45°C

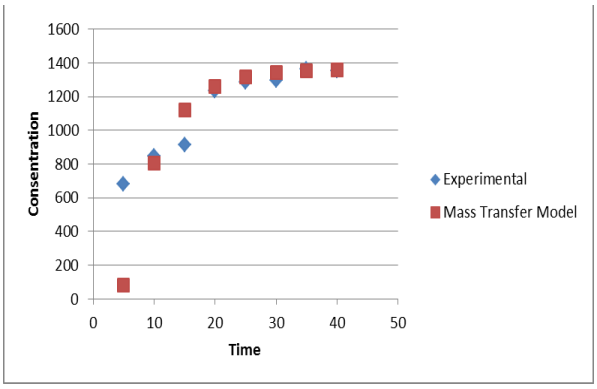

Figure 5. Convective Diffusion at 45°C

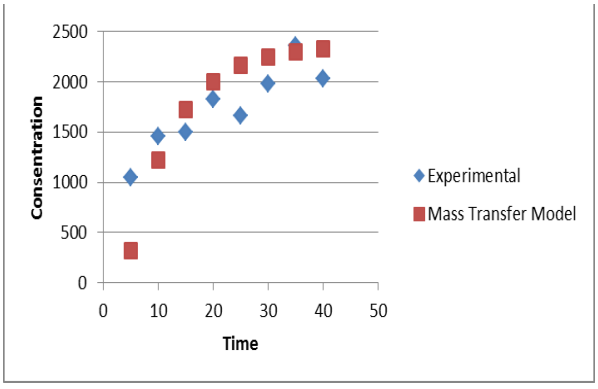

Figure 6. Moleculer Diffusion at 55 °C

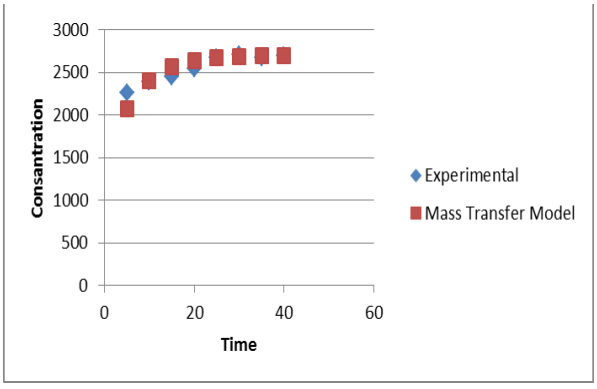

Figure 7. Convective Diffusion at 55°C

Table 3. Diffusion Coefficients

T (°C)	$D_{moleculer}$ (m^2/s)	$D_{convective}$ (m^2/s)	$D_{effective}$ (m^2/s)
35	0.1118	0.1563	0.2681
45	0.1644	0.1053	0.2698
55	0.1861	0.1037	0.2898

In this study, the data given in Table 4 were applied to the proposed design in the Design-Expert program and then statistical tests were applied to each of the proposed functions. The model with the highest regression coefficient and the lowest incompatibility was chosen as the best predicted function for the response surface. For the proposed second-order model, these values were 0.9174 and 0.7686, respectively. The predicted R^2 value was found to be acceptable, since the actual and the calculated data were in agreement confirming this. As a result, according to the statistical analysis, the most suitable model was selected as the quadratic model.

The variance analysis (Table 5) for the quadratic model was applied by ANOVA table of the Design - Expert program; where A was the volume of solvent, and B was the temperature, and C was the extraction time. Taking into consideration of the reality that as the magnitude of F value increases and the p-value decreases the affect of that parameter increases, the most effective single parameter was determined as temperature (as approved also with mass transfer model) and the most effective binary parameters were determined as time and temperature.

Table 4. Design Expert Data

		Factor 1	**Factor 2**	**Factor 3**	**Response 1**
Std	**Run**	**A:Volume of solvent**	**B:Temperature**	**C:Time**	**R1 (Yield)**
		mL	C	dk	mg/100g
15	1	0	0	0	2038.64
8	2	1	0	1	2789.85
14	3	0	0	0	2063.71
1	4	-1	-1	0	1369.04
5	5	-1	0	-1	1455.96
13	6	0	0	0	2067.19
3	7	-1	1	0	2784.32
10	8	0	1	-1	3267.19
4	9	1	1	0	2994.56
12	10	0	1	1	3205.57
7	11	-1	0	1	2569.97
6	12	1	0	-1	3171.80
2	13	1	-1	0	1271.28
9	14	0	-1	-1	1590.95
11	15	0	-1	1	1069.45

Table 5. ANOVA Table

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	7,647E+006	9	8,497E+005	6,17	0,0296	significant
A-Hızc Hacmi	5,244E+005	1	5,244E+005	3,81	0,1086	
B-sicaklik	6,039E+006	1	6,039E+006	43,83	0,0012	
C-Sre	2773,02	1	2773,02	0,020	0,8927	
AB	23716,10	1	23716,10	0,17	0,6954	
AC	5,595E+005	1	5,595E+005	4,06	0,1000	
BC	52871,63	1	52871,63	0,38	0,5628	
A^2	63311,17	1	63311,17	0,46	0,5280	
B^2	25227,82	1	25227,82	0,18	0,6865	
C^2	3,535E+005	1	3,535E+005	2,57	0,1701	
Residual	6,890E+005	5	1,378E+005			
Lack of Fit	6,885E+005	3	2,295E+005	946,14	0,0011	Not significant
Pure Error	485,12	2	242,56			
Core Total	8,336E+006	14				

3D Surface graphs were plotted to better analyze the interaction between the parameters. It was seen in Figure 8 that the amount of concentration increased when the temperature and time were increased simultaneously. In the case of decreased tempareures and extraction times, the yield was minimum. The yield would not be affected by increasing the time course of extraction when the temperature was kept at the minimum level, while in the reverse case the yield was increased. If the temperature and the time were at maximum values, the extraction yield reached its maximum. In Figure 9, binary effect of solvent volume and time was investigated on 3D surface. When the graphic is interpreted, it had been observed that, the concentration reached its maximum value at the maximum values of the time and solvent volume.

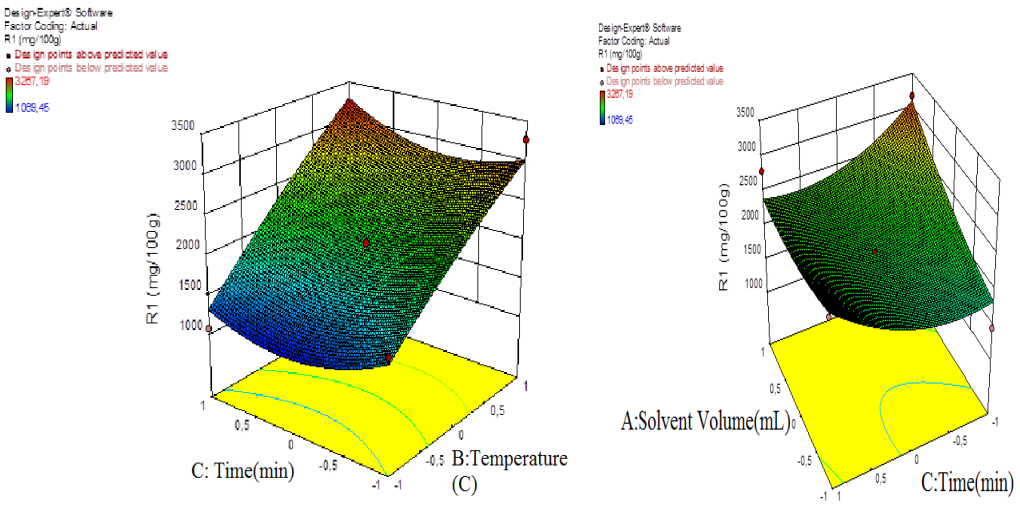

Figure 8. Binary effects of time-temperature (a) and solvent volume-time (b) parameters on the yield of extraction

In Figure 8, the binary effects of solvent volume and temperature parameters were investigated. In that, the yield of extraction reached the maximum in the case of the temperature and solvent volume were at maximum.

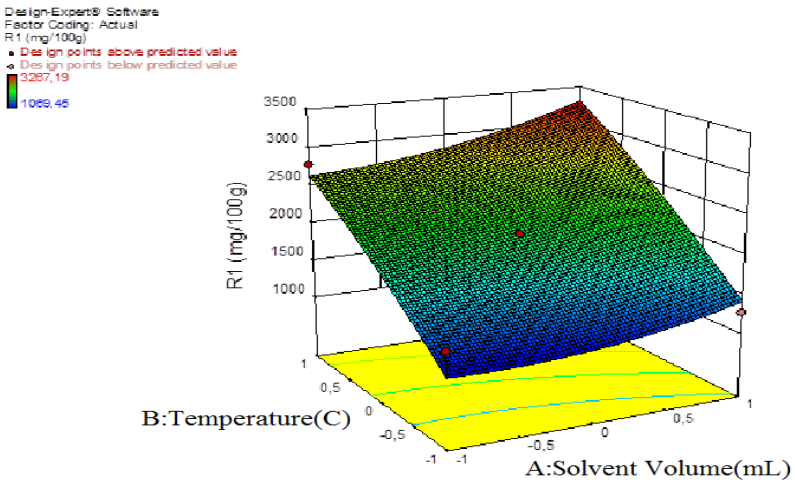

Figure 9. Binary effects of temperature–solvent volume parameters on the yield of extraction

CONCLUSIONS

As a result of the study, the response surface method model equation applicable for the industrial productions was found as in Equation 15:

$$GallicAcid=2056.51+256.02(A)+868.86(B)+18.62(C)+77(A)(B)-373.99(A)(C)+114.97(B)(C)+130.95(A^2)-82.66(B^2)+309.44(C^2)$$

Equation 15.

100 different numerical solutions of the mathematical model were proposed by the Design-Expert program. The highest amount of gallic acid (3219.67 mg/100g) production conditions were determined as 45mL of ethanol used extraction at maximum temperature (60°C) and at maximum mixing speed during 45 minutes. This result was also confirmed with the experimental run realized at those conditions.

Additionally, Mass transfer model was found as the best model representing the experimental data at all conditions. The diffusion coefficients were in the range of 0.1-0.3, and the activation energies of extraction were calculated as 292,776 J/mole İn molecular extractions, 81,9760 J/mole in convective transport.

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