

Kinetic study of superheated water extraction of berberine from *Berberis vulgaris* root

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The kinetics of superheated water extraction of berberine from *Berberis vulgaris* root was investigated. Response surface methodology was employed to reduce the experimental efforts of the kinetic study. A response surface model was successfully developed for berberine extracted concentration on the basis of the experimental data that gathered according to a central composite design and it was employed to generate several simulated data. The simulated response surface data was then employed to develop a kinetic model for berberine extraction. It was found that a second order kinetic model is able to fit the experimental/simulated data. The activation energy for the superheated water extraction kinetics of berberine with sample/solvent ratio of 0.03 and 0.02 was found to be $E_a = 61.4 \text{ kJmol}^{-1}$ and 37.93 , respectively.

Key Words: Kinetic modeling, superheated water Extraction, Berberine, Design of Experiments

INTRODUCTION

Extraction of bioactive materials from herbal plants may be interesting due to the growing trend of using herbal remedies and food additives instead of chemical ones. For the chemical standardization of botanicals and herbal preparations, the extraction of bioactive components is an essential step. Extraction is the process of separating active portions of plant (or animal) tissues using selective solvents through standard procedures [1, 2]. The products obtained from plants are relatively complex mixtures of metabolites, either in liquid or semi liquid or dry powder [2, 3 and 4]. The seedless barberry plant (*Berberis vulgaris* L. family *Berberidaceae*), is one of the commercial shrub trees. The fruit and various extracts obtained from the plants of this family are widely used in commercial scale and as food additive in folk food systems and medicines. All species of *Berberidaceae* produce alkaloids. The main component of *Berberis vulgaris* (barberry) stem and root extract is berberine which is a quaternary ammonium salt derived from the protoberberine

group of isoquinoline alkaloids. Berberine (BBR) is the major compound identified in the root, rhizome and stem bark of many plants. Berberine has been used for over 2000 years in traditional Eastern medicine and is also effective in the prevention and treatment of some illnesses [5-9]. These properties were summarized in recent reviews [5, 6, 8]. The conventional extraction techniques include maceration, infusion, percolation, digestion, decoction, Soxhlet, counter-current extraction, aqueous- alcoholic extraction. For a general extraction such as solvent extraction, there are few adjustable parameters to control the selectivity of extraction processes. Consequently, the application of alternative extraction techniques with higher selectivity and efficiency is desirable [10, 12]. Pressurized solvent extraction (PES) is a solid-liquid extraction (SLE) technique which has been developed as an alternative to current extraction methods. When water is used as the extraction solvent, the technique is named as Pressurized Hot Water Extraction (PHWE), Sub-critical Water Extraction or super heated water extraction [4, 10-12]. SCW extraction, using water under external pressurization above its boiling point offers an efficient, non-toxic, and environmental-friendly

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alternative to extract polar or slightly polar compounds. The polarity of subcritical water is much less than that of water at ambient condition ($\epsilon = 79$ at 298 K). The dielectric constant of SCW is in the range of 20-40 depending on temperature and pressure. It is very similar to the dielectric constant of methanol ($\epsilon = 33$ at 298 K) or ethanol ($\epsilon = 25$ at 298 K). In addition, the densities of subcritical water are high (0.82- 0.98g/cm³ at 4.0MPa). Thus, desired polar or slightly polar components, that are not soluble well in water at ambient conditions, can be much more soluble in subcritical water [10-13]. The effect of general operating parameters such as the extraction solvent, solvent to feed ratio, sample particle size, time and temperature extraction are mentioned as important factors for both conventional and non conventional techniques. The non-conventional extraction techniques are presumed to replace conventional solvent techniques, which make studying their kinetics mechanisms and modeling essential. Such studies enable prediction of the extraction behavior which is considered to be useful for scaling up of the process [14-16].

Many studies have been carried out on the kinetic modeling of continuous solvent extraction such as supercritical fluid extraction. Following the experimental determination of the extraction yield, using non-conventional extraction techniques modeling is the next important step, not only for predicting the extraction yield at unmeasured operational conditions but also for optimizing the process and determining the best process parameters [15]. Nowadays, there is a wide spectrum of mathematical models that a number of studies have applied in order to describe the process of extraction from plant materials. Based on the last approach, these models can be classified into three groups. The first is empirical models which are used when there is no information about the mass transfer mechanisms for extraction process. In the second group models, second Fick's law of diffusion is combined with the heat-mass transfer in order to obtain analytical solutions of the model equations. The third groups of models consist of models based on setting and solving the mass balance equations [16]. So far, berberine has been extracted from various plant sources using conventional and non-conventional methods. Considering the accessible literature data, there is no reported kinetic study of berberine extraction. This study was aimed to develop a kinetic model for berberine extraction by sub critical water.

MATERIAL AND METHODS

Preparation of plant material

The barberry root was collected from Barberry gardens around Birjand (South Khorasan Province, Iran) at the end of the harvest season, subsequently dried in Vacuum oven at 50°C for 72hrs and ground into fine powder. In order to avoid any sort of confusion between samples, the powder was classified in 3 groups with three kinds of sieves (mesh No. 24, mesh No. 35, mesh No. 45 sieves)

SCW Extraction Instrumentation

Subcritical water extraction processes were performed using a reactor that was designed and manufactured in the novel technologies laboratory of Research Institute of Food Science and Technology, Mashhad, Iran. This reactor was made from stainless steel 316. The temperature values were measured by a digital thermometer. Pressure was controlled by pressure gauge manometer (with an accuracy of 2 bars). Thermal supply was an electrical element fed with 220V alternative current (AC). The glycerol was an intermediate to transfer stable heat.

Design of Experiments

In this study, a three factors central composite design (CCD) was employed for SCW modeling. The factors and their levels are as follows:

Temperature: 110-170 °C, time: 30-70 mins, Sample/Solvent Ratio: 0.01-0.03. Totally 20 points were designed and extraction was performed by subcritical water according to each experiment condition. Design of experiments as well as statistical analysis was performed by MINITAB 16.

HPLC Conditions

Preparation of reference standards. Some standard berberine solutions (10, 50, 100, 250 and 500 µg/ml) were prepared in methanol. Calibration curve was prepared by plotting respective peak areas of berberine against concentration. Then Quantization was done using standard berberine calibration curve.

Measurement of berberine concentration. The crude extracts were passed through Whatman No. 1 filter and were analyzed by Knauer HPLC including HPLC pump (k-1001), C₁₈ Column, UV-detector (k-2600) measuring wavelengths used in 330 nm. Injection volume was 20 micro liters. The conditions of solvent mixtures (methanol + water) were supposed to run as follows.

The 20% methanol /80% water solution becomes a 100% methanol solution in 10 mins and

remains for 5 mins in this mode. It gets back to its earlier mode in 5 mins and stays the same for another 5 mins. It took 25 minutes altogether. The extracts obtained from each batch of subcritical water extractions were quantitatively analyzed by HPLC and berberine concentration $\mu\text{gr/ml}$ (extract) was calculated. As indicated in the chromatogram (Fig.1) the berberine peak is visible in the chromatogram after approximately 9.4 minutes.

Response surface models

In this study, berberine concentration was analyzed by multiple regressions through the least squares method to fit the following equation:

$$Y = B_0 + \sum B_i x_i + \sum B_{ii} x_i^2 + \sum B_{ij} x_i x_j, (1)$$

Where, Y is the predicted response variable; B_0 , B_i , B_{ii} , and B_{ij} are constant regression coefficients of the model, and x_i , x_j ($i = 1,2,3$; $j = 1,2,3$; $i \neq j$) represent the independent variables in the form of coded values according to the following transformation equation:

$$x_i = \frac{X_i - X_0}{\Delta X}, (2)$$

Where x is the dimensionless coded value of the variable X_i , X_0 is the value of X_i at the center point, and ΔX is the step change [17-19].

The second-order polynomial coefficients were calculated using the software MINITAB 16 to estimate the responses of the dependent variable.

Kinetic modeling of berberine extraction

The aim of kinetic study is to develop a kinetic model for berberine extraction by superheated water. The kinetic data were employed to fit several kinetic rate equations and it was found the following kinetic rate equation is able to fit the data:

$$C = \frac{t}{A + Bt}, (3)$$

C : Concentration of Berberine in the solution at any time.

The above kinetic rate has been derived according to the assumption of a second order rate law for berberine extraction:

$$\frac{dC}{dt} = k(C - C_s), (4)$$

k is the second-order extraction rate constant, $\text{mL} \cdot \mu\text{g}^{-1} \cdot \text{min}^{-1}$.

C_s is the extraction capacity (concentration of berberine at saturation in g L^{-1})

By considering the boundary condition $t = 0-t$ and $C_t = 0-C_t$, the integrated rate law for a second-order extraction was obtained:

$$C = \frac{C_s^2 kt}{1 + C_s kt}, (5)$$

or

$$C = \frac{t}{\frac{1}{C_s^2 k} + \frac{t}{C_s}}, (6)$$

$$A = C_s^2 k, (7)$$

$$B = \frac{1}{C_s} (8)$$

For a second order system, the rate constants increase with temperature and may be described by the Arrhenius law:

$$k = k_0 e^{-E/RT}, (9)$$

Where: k is the extraction rate constant ($\text{L mg}^{-1} \text{min}^{-1}$), k_0 is the temperature independent (pre-exponential) factor ($\text{L mg}^{-1} \text{min}^{-1}$), E is the activation energy (J mol^{-1}), R is the gas constant ($8.314 \text{ J mol}^{-1} \text{K}^{-1}$), T is the absolute suspension temperature (K).

RESULTS AND DISCUSSION

Development of the response surface model

Table 1 shows the experimental data of SCW berberine extraction in different process conditions. The estimated regression coefficients of response surface model were presented in Table 2. The estimated regression coefficients of polynomials in coded unit, the standard errors of coefficients and their t-statistic and P-values are shown in Table 2. The correlation coefficient and adjusted correlation coefficient of the fitted model are also presented in the Table.

The principal model analysis is based on analysis of variance (Table 2), which provides numerical information for the F -value and the P -value. A summary of the analysis of variances (ANOVA) is given in Table 2. The ANOVA of regression model reveals that the model is highly significant as evident from Fisher's F-test value being 68.84 with a low probability value ($P_{\text{model}} < 0.0001$). The goodness of the fit of the model was checked by determination coefficients, $R^2 = 98.41\%$ and $R^2_{\text{adj}} = 96.98\%$ that revealed that there is a good agreement between experimental and predicted value of concentration. The RSM predicted data are presented in Table 1.

Table 1. Design of Experiments (CCD) for barberry root and prediction of berberine concentration by RSM.

Points	Temperature	Time (min)	Ratio	Concentration Experimental Data (µg/ml)	Concentration Predicted Data (µg/ml)
1	170	70	0.03	281.128	256.385
2	140	50	0.02	77.683	66.748
3	170	30	0.01	90.739	76.129
4	170	50	0.02	141.572	144.120
5	140	50	0.03	110.906	119.066
6	110	30	0.01	20.739	28.600
7	170	30	0.03	226.072	215.056
8	140	50	0.02	80.239	66.748
9	110	70	0.01	31.183	25.822
10	110	50	0.02	31.906	18.175
11	140	50	0.02	75.294	66.748
12	140	50	0.01	54.906	36.256
13	140	70	0.02	76.128	77.820
14	110	70	0.03	54.461	52.604
15	140	50	0.02	75.80	66.748
16	140	50	0.02	78.80	66.748
17	170	70	0.01	84.572	77.233
18	140	30	0.02	70.406	58.299
19	110	30	0.03	23.628	14.576
20	140	50	0.02	66.683	66.748

Table 2. Estimated regression coefficients of response surface model, the standard errors of estimated coefficient, the statistics and ANOVA results

Term	Coef	SE Coef	T	P
Constant	74.102	3.840	19.299	0.000
Temperature	66.2167	3.532	18.748	0.000
Time	9.5889	3.532	2.715	0.022
Ratio	41.4056	3.532	11.723	0.000
Temperature* Temperature	14.7468	6.735	2.190	0.053
Time* Time	1.2745	6.735	0.189	0.854
Ratio *Ratio	10.9134	6.735	1.620	0.136
Temperature* Time	0.9514	3.949	0.241	0.814
Temperature* Ratio	38.2153	3.949	9.678	0.000
Time* Ratio	10.2014	3.949	2.583	0.027

F. Regression= 68.84; R-Sq= 98.41%; P_value = 0.000; R-Sq(adj) =96.98%

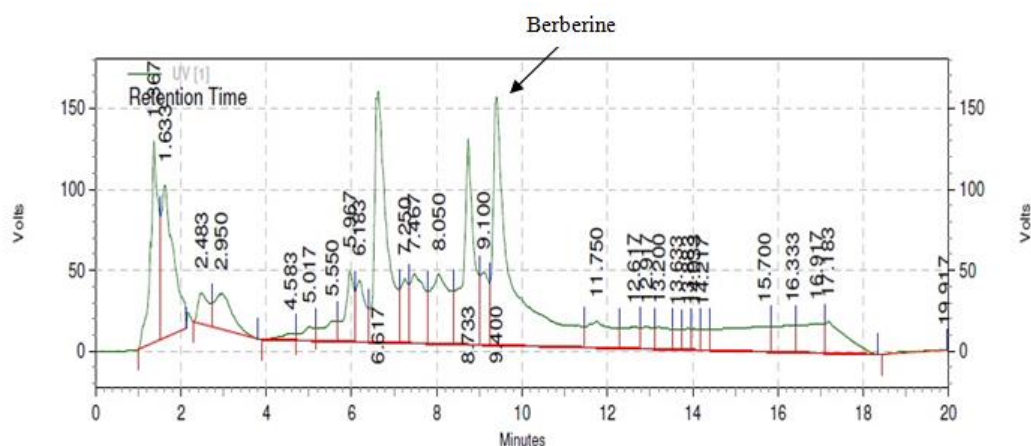


Fig.1. Chromatogram obtained for berberine in barberry root by SCW extraction.

All three factors (time, temperature and sample/liquid ratio) have statistically significant effect on the berberine concentration according to the t-student test and corresponding P-value of each coefficient. However, the higher value of estimated regression coefficient for temperature indicates that temperature has more effect.

Kinetic study

According to the second order nature of the kinetic model, temperature should have an increasing effect on the berberine concentration. However, this behavior is not observed in low temperatures in the range of 110-120°C. To reduce the errors of parameter estimation, the kinetic study was limited to temperature range of 120-170°C. In addition, the dependency of berberine concentration on time is significantly affected by the ratio according to the response surface results. So, the ratio has the significant effect of the kinetics of extraction and the kinetic study should be conducted in fixed ratios. If we develop a kinetic model separately in each ratio, the number of experimental points that are necessary for an efficient parameter estimation of the kinetic model significantly decreases. For example, the number of experimental points in ratio of 0.03 is 5 according to the Table 1. So, to increase the points we employed response surface model to generate several simulated data and conduct the kinetic study with simulated data. So for the purpose of the kinetic study, the response surface model was first employed to generate several simulated data, sample/water ratio of 0.03 at temperatures 120, 130, 140, 150, 160 and 170°C, time 20-70 mins (stepwise 1 min). A total of 246 points were produced for berberine concentration (41 points in each temperature). This procedure was repeated for ratio of 0.02 and 246 points were produced from a similar manner. Each data set was employed to fit the second order kinetic model (Eq. 6). The estimated coefficients of the kinetic model with their associated 95% confidence intervals as well as the statistical criteria are presented in the Table 3. The high R^2 and R^2_{adj} values of above 0.99 reveals that the regressed rate model fits the data well in agreement with the low values of RMSD and Variances as presented in Table 3 in different temperatures.

Calculation of activation energy

The estimated k values in the six temperatures are $2.69E-05$ (120°C), $1.05E-04$ (130°C), $1.71E-04$ (140 °C), $2.19E-04$ (150°C), $2.54E-04$

(160°C) and $2.79E-04$ (170°C) in the case of sample/water = 0.03.

Linearization of Eq. 9 as shown below will give the value of the activation energy (E) and the temperature independent factor (k_0) from $\ln(k)$ against $1/T$ plot:

$$\ln k = \ln k_0 - \left(\frac{E}{RT} \right), \quad (11)$$

Fig. 2 presents the plot of $\ln k$ against $1/T$. The activation energy for berberine extraction is calculated as 61.4 kJ mol^{-1} from the slop of the fitted line (see Fig. 2).

The pre-exponential factor of this extraction (k_0) is evaluated as 6734.5 from interception of fitted line (see Fig. 2)

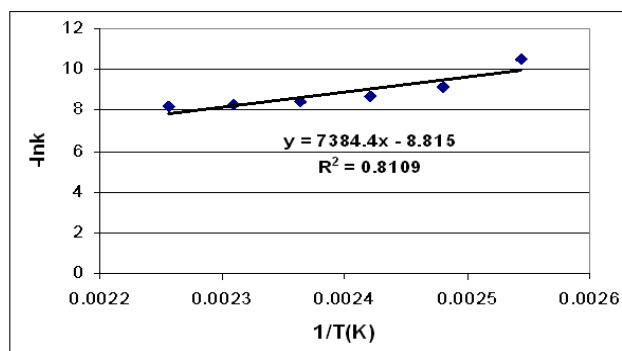


Fig. 2. Linear relationship between second order extraction constant, $\ln(k)$ and temperature for superheated water extraction of berberine (sample/ratio=0.03).

The estimated k values of ratio=0.02 are $1.50E-04$ (120 °C), $3.19E-04$ (130 °C), $4.43E-04$ (140 °C), $5.26E-04$ (150 °C) , $5.81E-04$ (160 °C) and $6.15E-04$ (170 °C) as presented in Table 3.

The plot of $\ln k$ against $1/T$ is presented in Fig. 3. The slop of the fitted plot presents the E/R value and the activation energy is 37.93 kJ/mol . It is clear that the activation energy significantly decreases (about 24 kJ/mol) when sample/water ratio decreases from 0.03 to 0.02. So the activation energy depends to the ratio. Further kinetic study (not presented here) showed that activation energy approaches to zero in lower ratios (0.02 to 0.015). This means that temperature dependency of berberine extraction decreases significantly.

When sample/liquid ratio was low, the concentration gradient was high and, thus, the transfer rate increased. Goula [20], Qu et al. [21], Rakotondramasy-Rabesiaka et al. [22] , Zhang et al. [23] and Li et al. [24] reported the same effect of sample/liquid ratio on concentration of pomegranate seed oil, antioxidant from pomegranate, protopine, oil from flaxseed and chlorogenic acid from *Ecommia ulmodies*.

Table 3. Parameter estimates with associated confidence intervals for Equation 3.1 regressed from the simulated/ experimental data set.

	T	A	B	Cs	k	R ²	R ² _{adj}	RMSD	Variance
Ratio=0.03	120	0.5785±0.01867	0.0039±34E-05	253.6	2.69E-05	0.995	0.995	0.156	1.074
	130	0.2630±0.0128	0.0053±25E-05	190.1	1.05E-04	0.992	0.992	0.249	2.747
	140	0.1423±0.0082	0.0049±16E-05	202.8	1.71E-04	0.992	0.992	0.304	4.09
	150	0.0858±0.0054	0.0043±11E-05	230.7	2.19E-04	0.993	0.993	0.341	5.14
	160	0.0557±0.0037	0.004±7.6E-05	266.0	2.54E-04	0.995	0.995	0.368	5.98
	170	0.0382±0.0026	0.0033±5.5E-05	306.4	2.79E-04	0.996	0.996	0.389	6.69
Estimated Activation Energy, E(kJ/mol):61.4					Pre-exponential Factor, k ₀ :6734.5				
Ratio=0.02	120	0.7908±0.0448	0.0109±0.00085	91.9	1.50E-04	0.988	0.987	0.132	0.7659
	130	0.3686±0.0251	0.0108±0.00049	92.1	3.19E-04	0.988	0.988	0.168	1.2397
	140	0.1962±0.0146	0.0093±0.00029	107.2	4.43E-04	0.990	0.990	0.192	1.6192
	150	0.1148±0.0090	0.0078±0.00019	128.6	5.26E-04	0.993	0.993	0.2094	1.9330
	160	0.0721±0.0058	0.0065±0.00012	154.5	5.81E-04	0.995	0.995	0.2236	2.2039
	170	0.0478±0.0039	0.0054±8.27E-05	184.4	6.15E-04	0.996	0.996	0.2355	2.4473
Estimated Activation Energy, E(kJ/mol): 37.93					Pre-exponential Factor, k ₀ : 22.18				

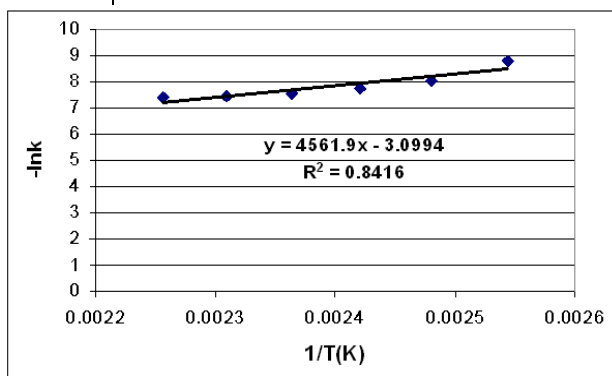


Fig. 3. Linear relationship between second order extraction constant, $\ln(k)$ and temperature for superheated water extraction of berberine (sample ratio = 0.02)

Fig. 4 and Fig. 5 present the predicted kinetic model in different temperatures in sample/water ratio 0.03 and 0.02 respectively. The experimental points and some RSM simulated data are also presented. The graphs clearly show the capability of the kinetic model for demonstration of experimental and simulated data. It is important to note that the model is applicable in sample/water ratio of 0.02-0.03 and the temperature range of 120°C -170°C.

Generally, the solid-liquid extraction process can be considered as the reverse of an adsorption process. Due to this, the kinetic equations of

adsorption process can be applied to solid-liquid extraction. So, the second-order law gives the best fits for the extraction rate. Whereas, mechanistic models provide more from a basic understanding of a given system, a greater basis for extrapolation and a representation of a response function that is more precise than one attained empirically. [14, 15]

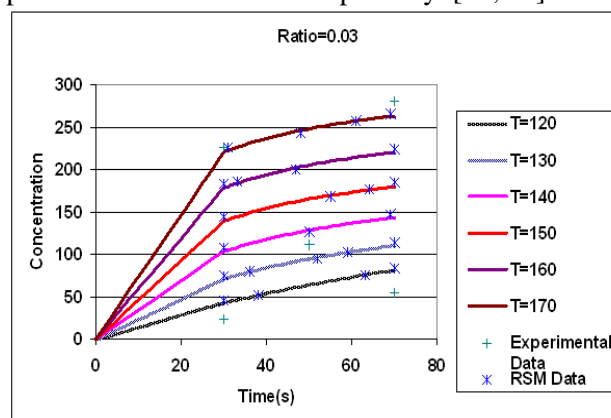


Fig. 4. Extraction of berberine using superheated water at different temperatures and sample/ratio of 0.03: comparison between experimental data, response surface simulated data and second order kinetic models.

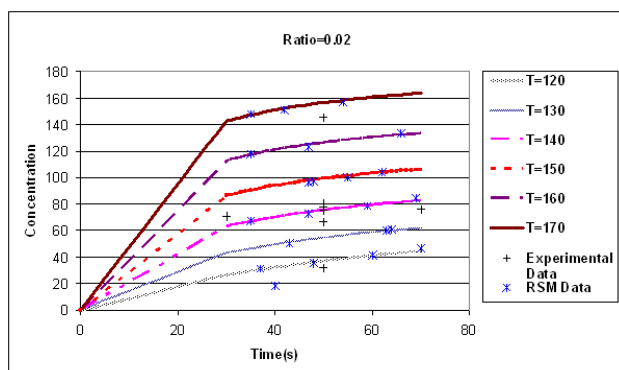


Fig. 5. Extraction of berberine using superheated water at different temperatures and sample/ratio of 0.02: comparison between experimental data, response surface simulated data and second order kinetic model

CONCLUSION

Combined RSM/kinetic modelling was employed to study the rate of berberine extraction from *Berberis vulgaris* root using superheated water at various temperatures, times and sample/solvent ratios. It was found that a second order kinetic model can describe the berberine extraction process. The experimental trials showed that k has high values at high temperatures. The activation energy depends on sample/solvent ratio, as increase of sample/solvent ratio increases the activation energy of extraction. Superheated water extraction technique is a notably faster extraction method than conventional extraction techniques, as well as being clean, cheap, and environment-friendly.

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