Experimental Study and Modeling of Supercritical Extraction of Nicotine from Tabacco Leaves

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ABSTRACT: *In this work, the solubility of nicotine extracted from tobacco leaves, found in the north of Iran, in supercritical carbon dioxide has been measured. Also the effects of pressure, temperature, extraction dynamic time, and the organic co-Solvent on the amount of nicotine extracted from tobacco leaves have been investigated. It should be stressed that in order to reduce significantly the number of experiments, the experiments have been specified based on the Taguchi experimental design. The results obtained from the experiments showed that at the specified pressure, temperature, volume of modifier and dynamic time, the maximum amount of nicotine can be extracted. The experimental data collected in this research has been correlated using the Redlich-Kwong (R.K) equation of state. The results showed that the conventional cubic R.K equation of state can accurately correlate the experimental solubility data with good accuracy.*

KEY WORDS: *Supercritical extraction, Nicotine, Tobacco leaves, Equation of state, RK.*

INTRODUCTION

Nicotine is an alkaloid found in nightshade family of plants (solanaceae), mostly in tobacco, and less in tomato, potato, eggplant and green-pepper. It is also found in coca leaves. Nicotine constitutes 0.3 to 5 % of tobacco plant by dry weight; with biosynthesis taking place in the root and accumulating in the leaves. The molecular structure of nicotine is presented in Fig. 1.

It would noting that the systematic name (IUPAC) of nicotine is(s)-3-(1-Methyle-2-pyrrolidinyl) pyridine. The physical properties of nicotine presented in table 1 [1].

As expounded in Fig. 2, nicotine can exist in a diaprotonated, monoprotonated and in the free base form. The properties of nicotine can be affected by these different types of structure. When it is protonated,

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Table 1: Nicotine's physical properties.

Systematicname(IUPAC):(s)-3-(1-Methyl-2-pyrroli-dinyl)pyridine
Formula:$C_{10}H_{14}N_2$
Molecular mass =162.23 kg/kg mol
Density =1.01 g/cm^3
Melting point =-79$^{\circ C}$(110$^{\circ F}$)
Boiling point =247 $^{\circ C}$
Half life time =2 hr

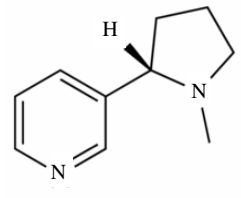

Fig. 1: Nicotine structure.

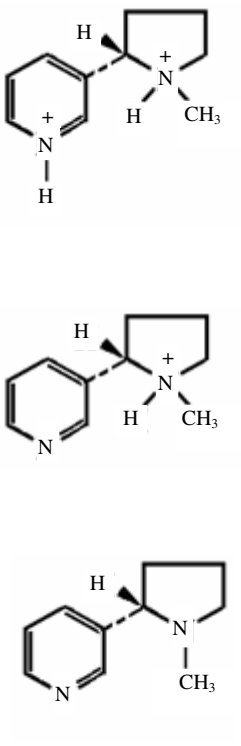

Fig. 2: Different forms of nicotine. a) diporotonated, b) monoporonated, and c) free base.

nitrogen on the pyridine ring is considerably more acidic than that on the pyrrolidine. These different types of structure affect on the nicotine extraction [2]. Nicotine is a kind of oily liquid that miscible with water in its base form. It is very toxic. The LD50 (lethal dose 50 %) is 40-60 mg for human [3]. Nicotine is one of the most addiction substances is known to human. It is metabolized rapidly and extensively to several metabolites in human body. Cotinine (as a major metabolite of nicotine) has a half life of 19.4 hours [4-6]. A large scale application of nicotine is used as an insecticide. As an example, 2800 tons of (s)-nicotine is used as a crop-protectant per year [7-8].

Nicotine enhances concentration, learning and memory due to increase of acetylcholine. Nicotine and its metabolites are considered for treatment of a number of disorders, such as, Alzheimer's disease (AD), Parkinson's disease (PD), and Schizophrenia. It is estimated that 4.5 million American people have AD and by 2050 its range will be increased from 11.3 to 16 million. PD affects over 1 million people only in the United States [8]. The extraction of a compound from a solid matrix is a two step process. While the first step is the desorption of the analyte from the surface which is dependent on the extraction temperature, pressure, the size of particles and analyte-matrix-solvent interactions ,the second step is the salvation of the analyte by the solvent molecules [9-12]. In tobacco, nicotine partly is in the free state and partly is combined with the tobacco constituents. The large amount of nicotine is in the tobacco leaf and partly combined with the tobacco constituents. The latter type of nicotine must be liberated if extraction is to be completed [13]. A treatment that helps in liberation of combined nicotine is to be moistened with water [14]. In comparison with other extraction methods, such as maceration [15], liquid-liquid extraction [16-18], steam distillation, soxhlet, and ultra sonic extraction [9,19], supercritical extraction could have a higher efficiency because of the plausible diffusivity, lower density, viscosity, and surface tension. On the other hand the properties of supercritical fluids can be varied over a wide range by changing the operational conditions.

Carbon dioxide (CO_2) with P_c =7.28 MPa and T_c=304.1 K, is the most frequently used solvent for supercritical fluid extraction [20], because of its practical advantages including its nontoxic and nonflammable character, environmental safety, huge availability, low

cost at high purity, and natural compounds with low volatility and polarity. When the extracts are recovered in the separators, CO_2 is easily separated because of its high volatility.

Supercritical Fluid Extraction (SFE) allows the extraction of active ingredients from herbs and plants with a better reproduction of flavor or fragrance than conventional operations. Thermal degradation and decomposition of labile compounds are avoided, due to the operation at reduced temperature, whereas the absence of concentrated oxygen prevents oxidation reaction [20]. A number of workers have studied on the extraction of nicotine from tobacco [21, 22], tobacco waste [13], cigarette tobacco [9], and snuff [23]. All report that CO_2 is a non-polar solvent for the extraction of nicotine; for increasing the extraction performance a polar co solvent should be added.

Equations of state are applied widely in many industries including separation, oil and gas industry and supercritical extraction. Van der waals [24] developed the first scubic equation of state that is a two-parameter equation. In this equation of state the effect of intermolecular forces and size of molecules are considered. *Redlich* and *Kwong* [25] modified the attractive term of van der waals equation of state. *Soave* [26] and *Peng-Robinson* [27] proposed an equation of state that the parameters were defined as functions of reduced temperature and acentric factor.

Cubic equations of state are commonly used for calculating phase behavior of pure and multi component fluids. These equations have two terms, a repulsive term and an attractive term. According to the statistical Thermodynamics, the compressibility factor, Z, is presented as follows [28] :

$$Z = Z^{hs} + Z^{pert} \tag{1}$$

Where the first term is the repulsive forces and the second term is the attractive forces. In general, cubic equations of state have the following form:

$$P = \frac{RT}{v-b} - \frac{aRT}{v^2 - uv - w^2} \tag{2}$$

In the above equation, parameters *a* and *b* are function of critical properties. In Eq. (2), the first term indicates the repulsive term and the second term indicates the attractive term. Choosing u=b and w=0, the Redlich-Kwong equation of state is obtained. By imposing critical point constrains, parameters a and b at the critical point can be found as a function of T_c and P_c:

$$a_c = 0.47448\frac{R^2 T_c^2}{P_c^2} \tag{3}$$

$$b = 0.08663\frac{RT_c}{P_c} \tag{4}$$

In general, parameter a is considered as a function of reduced temperature and parameter b is considered constant. For R.K EOS the temperature dependency of parameter a is defined as follows:

$$a = a_c\, T_r^{-1/2} \tag{5}$$

For mixtures the van der waals mixing rules can be used.

$$a = \sum_i^n \sum_j^n x_i x_j a_{ij} \tag{6}$$

$$b = \sum_i^n x_i b_i \tag{7}$$

$$a_{ij} = (a_{ii} a_{jj})^{1/2} \tag{8}$$

The fugacity coefficient of component *i* in a mixture can be obtained using the R.K equation of state as below:

$$\ln \hat{\varphi}_i^v = \frac{b_i}{b}(z-1) - z\times\ln\left(1-\frac{b}{v}\right) + \frac{a}{bRT^{3/2}}\left[\frac{b_i}{b} - \frac{2(aa_i)^{1/2}}{a}\right]\times\ln(1+b/v) \tag{9}$$

The fugacity of component i in a mixture can be calculated as follows;

$$f_i = P y_i\, \varphi_i \tag{10}$$

The aims of the present work are investigating on the effects of different parameters; such as pressure, temperature, modifier volume, and dynamic extraction time; on the solubility of nicotine in supercritical carbon dioxide, optimizing the operating conditions of the supercritical carbon dioxide extraction, finding the crossover point of supercritical extraction, obtaining the interaction operating parameters, and correlating the experimental data using the RK equation of state.

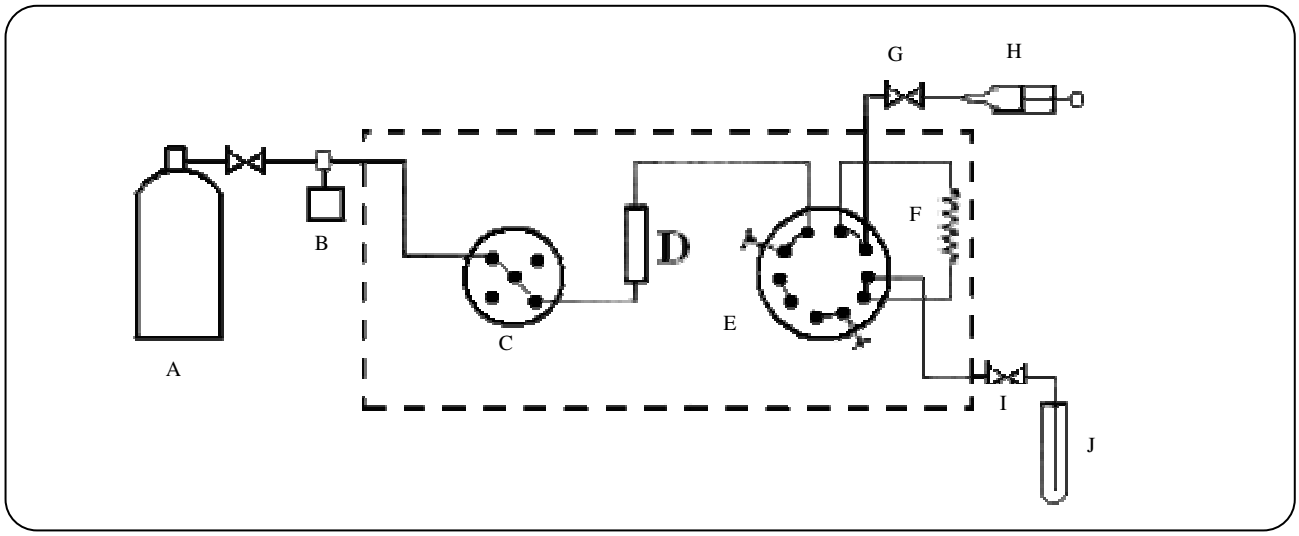

Fig. 3: Schematic diagram of experimental apparatus for SFE: (A) CO_2 gas tank ; (B) Supercritical fluid pump; (C) 5-port, 4-position valve; (D) 10 mL extraction cell; (E) 10-port,2-position valve; (F) injection loop; (G) on/off valve; (H) syringe; (I) micro adjust valve; (J) collection vial.

The tobacco leaves of Mazandaran province of IRAN, cutter grade 3 of Burley (C3B) type, were used for nicotine extraction purpose.

EXPERIMENTAL

The purpose of the experiments was to measure the solubility of nicotine extracted from the tobacco leaves in supercritical carbon dioxide.

Materials

Nicotine (MERK NO.820877) was purchased from Merck Company with the purity of more than 98 %. The molecular weight and density of nicotine are respectively, 162.24 g mole and 1.01 Kg/L.

HPLC grade methanol and Dichloromethane were provided from Aldrich Company.

CO_2 was purchased from Sabalan Company (Iran) with purity of 99.99 %.

Samples of tobacco leaves were brought from the farms in the north of IRAN. All the chemicals were used as received without further purification.

The supercritical extraction experiments were done in the Chemistry Department of Tarbiat modarress University.

Equipment

A Suprex MPS (Pittsburgh, PA) in the SFE mode was used for all experiments. A schematic diagram of the equipment used to conduct the experiments was given in figure 3.0. The extraction vessel was a 10 mL stainless steel vessel. Sintered stainless steel filters (5 μm) were used to prevent any carryover of the solutes. A Dura flow manual variable restrictor (Suprex) was used in the SFE system. In order to prevent sample plugging, the restrict point was warmed electrically.

In order to measure the amount of nicotine extracted from the tobacco leaves, the injection of extracted samples was carried out using a 25 μL Hamilton Syringe model 702 NR. Chromatographic analyses were carried out on a HP SERIES 1100(U.S.A) HPLC system. The chromatographic system consisted of injector equipment with a 20 μL sample loop, a HP SERIES 1100 pump and a HP SERIES 1100 UV-Vis spectrophotometer detector. A column (250 × 4.6 mm ID) from water (CA.U.S.A) packed with C18 (μ Bond Pak ^{TM}) was used to perform the sample analyses.

Procedure

At the outset 3 grams of powdered tobacco was thoroughly mixed with 5 grams of glass beads, and then packed into the extraction cell. The beads prevent channeling in the cell and increase the contact surface between the sample and the SCF and consequently reduce the phase equilibria time. The size of particle is less than 180 μm, for better mass transfer accomplishment. Extraction takes place at pressure, temperature, modifier

Table 2: Three levels Taguchi experimental design.

Con. / Run	P(atm)	T(ºC)	t-dyn(min)	V_{mod} (µL)
1	100	35	5	0
2	100	50	10	100
3	100	65	25	250
4	250	35	10	250
5	250	50	25	0
6	250	65	5	100
7	350	35	25	100
8	350	50	5	250
9	350	65	10	0
Total	18	18	18	18

volume and dynamic time ranges of; 100-350 atm, 35 - 65 °C, 0-250 µL, and 5-25 min, respectively. The solvent passed through the 5 ports, 4 position valve and entered in the cell. Then the feed and the solvent were pressurized in the cell. When the extraction cell reached to the desired pressure and temperature, extraction was performed within a 20 min static time followed by the dynamics extraction time. When the dynamic extraction time was finished, the loop was depressurized into the collection vial. The extraction experiments were performed under Taghuchi method. Table 2 shows the operating conditions of each run.

The extracted fluid was collected in a 5 mL volumetric flask. The final volume of the extracted fluid was adjusted to 5.0 mL with dichloromethane at the end of the extraction. In order to improve the collection efficiency, the 5.0 mL volumetric flask was placed in an ice bath during the dynamic extraction stage. In order to study the effect of using the modifier on the extraction, methanol was spiked into the extraction vessel with charged sample prior to the extraction. Analysis was performed with HPLC system as described before. Data were collected and processed by Chem. Station (Agillent Technologies, USA) data analysis software. A flow rate of 1.0 mL/min was applied in laboratory temperature of 20(±2) °C. The mobile phase was methanol phosphate buffer (60:40; v/v; pH=6) and the detection wavelength was 240 µm.

CORRELATION THE EXPERIMENTAL DATA

The obtained experimental data was correlated using the Redlich-Kwong (R.K) equation of state. Considering the equality of fugacities of nicotine in solid and extracted phases by applying equations (2) to (10), the critical properties of nicotine were considered as adjusting parameters and they were obtained by fitting the solubility experimental data for nicotine in supercritical CO_2 data. In order to measure the accuracy of the RK equation of state in correlating the experimental solubility data, the average absolute relative deviation (AARD) between the experimental and the calculated solubility of nicotine in CO_2 at different operating conditions, was defined as below.

$$AARD = (1/N)\sum \left| (y_n^{cal} - y_n^{exp})/y_n^{exp} \right| \quad (11)$$

Where, N is the number of experimental data, y_n^{cal} and y_n^{exp} are calculated and measured solubility of nicotine in the supercritical extracted respectively.

RESULTS AND DISCUSSION

Several parameters have to be optimized in order to extract the analytes of interest quantitatively, in a short period of time. Among them the pressure, the temperature, the volume of the modifier and the dynamic time are generally considered as the most important factors. The experimental design was carried out by using three levels Taguchi method (Roy, 1990). The results are analyzed by statistical Qualitek-4 software. The influences of each of the studied parameters on the extraction of nicotine from tobacco leaves using supercritical carbon dioxide are shown in Figs. 4 to 7.

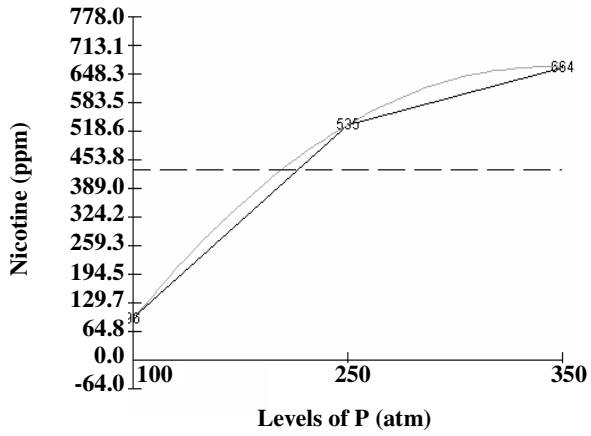

Fig. 4: The functionality of nicotine extraction with pressure at 3 level.

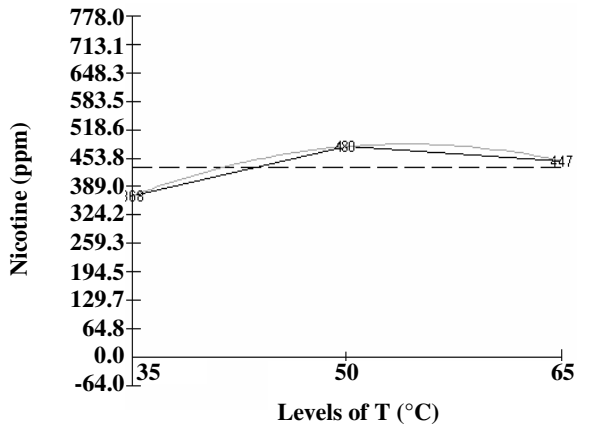

Fig. 5: The functionality of nicotine extraction with temperature at 3 level.

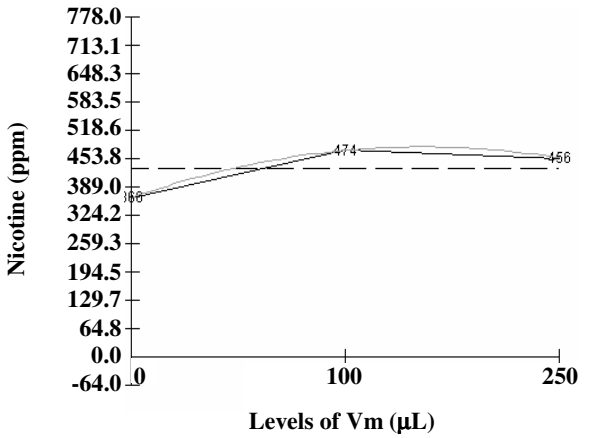

Fig. 6: The functionality of nicotine extraction with modifier volume at 3 level.

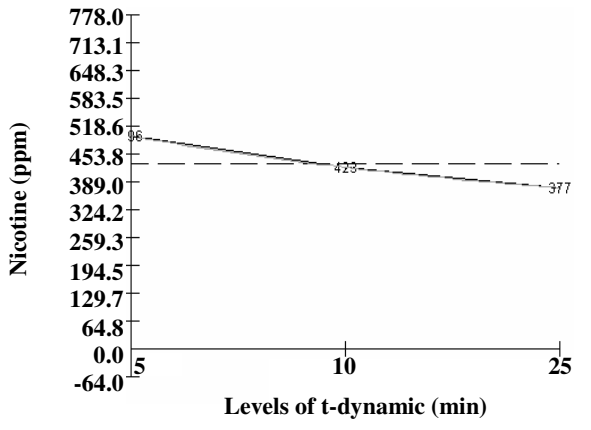

Fig. 7: The functionality of nicotine extraction with dynamic time at 3 level.

Fig. 4 shows the variation of the amount of nicotine extracted from the samples of tobacco leaves with the system pressure. As seen from Fig. 4 the amount of extracted nicotine increases as the pressure of system increases. As a matter of fact, by increasing the pressure in the extraction cell, the fugacity coefficient of CO_2 decreases appreciably and in turn the solubility of nicotine in the supercritical CO_2 increases. Fig. 5 shows the variation of nicotine extracted from tobacco leaves as a function of the temperature. As this figure shows, the temperature has a cross over point. This means that the temperature has two opposite effects on extraction. The cross over point is in the range of 60 to 65 °C. Before the cross over point by increasing the temperature the amount of nicotine extracted increases and after that with increasing the temperature the amount of nicotine extracted decreases. Fig. 6 shows the variation of the amount of nicotine extracted from tobacco leaves as a function of the modifier (co-solvent) volume. As seen from this figure, after adding 100 µL of modifier (level 2), the addition of modifier hasn't considerable effect on the extraction. Fig. 7 shows the effect of dynamic times on the nicotine extraction. As seen in this figure, the amount of extracted nicotine increasingly decreases with increase in the dynamic time. When the exit line becomes open, the pressure decreases and by decreasing the pressure the amount of extracted nicotine decreases. This result shows that the nicotine extraction decreases by increasing the dynamic time.

Fig. 8 shows the effective influence of the studied parameters in the extraction. The pressure has the maximum influence, 89.638 %, the temperature, the dynamic time, and the modifier volume has approximately 3.4 % influence.

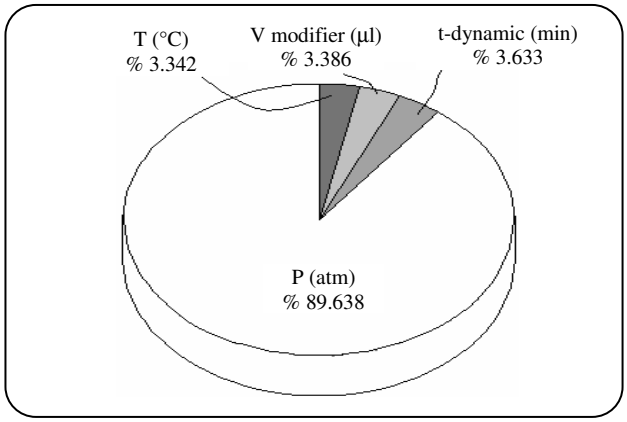

Fig. 8: The effective percents of the parameters in the extraction.

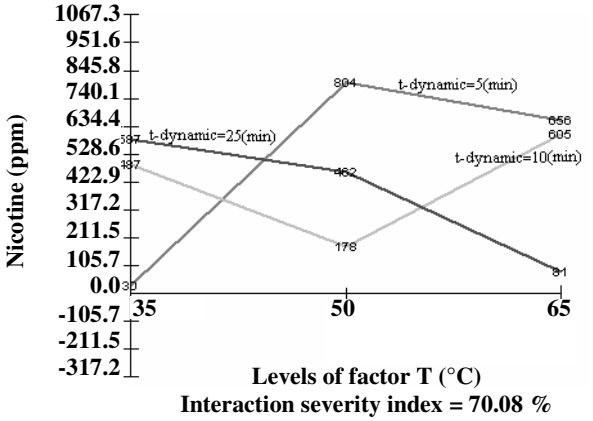

Fig. 9: The effect of interaction between the temperature and the dynamic time on the extraction.

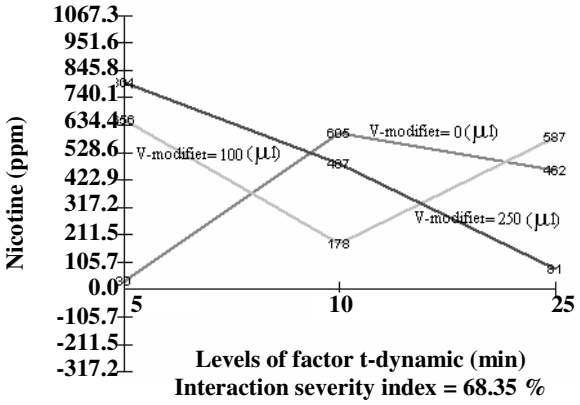

Fig. 10: The effect of interaction between the dynamic time and the modifier volume time on the extraction.

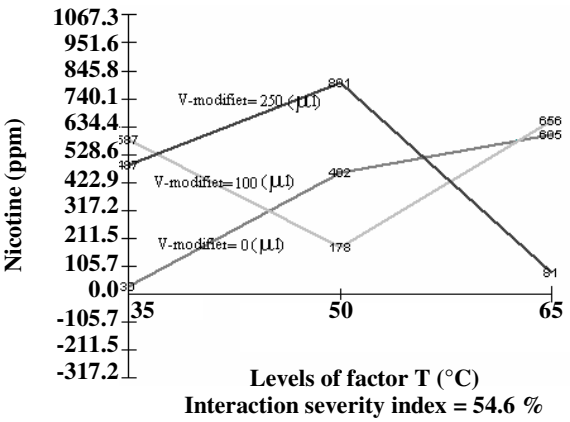

Fig. 11: The effect of interaction between the temperature and the modifier volume on the extraction.

Fig. 9 shows the effect of interaction between the temperature and the dynamic time. This interaction has the most interaction severity index, expressed in percent and corresponds to angle between the lines between 0 and 90 degrees, its value is nearly 70 %. As seen, in the first level of dynamic time, first the nicotine extraction increases as temperature increases then the nicotine extraction decreases. In the second level of dynamic time, the nicotine extraction decreases with increasing in temperature and then the nicotine extraction increases. In the third level of dynamic time, the amount of nicotine extracted falls with increasing the temperature. Fig. 10 shows the effect of interaction between dynamic time and modifier volume on the nicotine extraction with approximately 68 % interaction severity index. As seen in Figs. 9 and 10, the same trend was observed for the interaction between dynamic time and modifier volume on the nicotine extraction as it was observed for the interaction between the temperature and the dynamic time. Fig. 11 shows the interaction between the temperature and modifier volume. This interaction has nearly 55 % interaction severity index. In the first level of modifier volume, by increasing the temperature, the nicotine extraction increases. In the second level of modifier volume, increasing in temperature causes first increase and then decrease in the nicotine extraction. The third level effect is inversed of the second level effect.

Table 3 shows the analysis of variance (ANOVA) for the studied parameters. As shown in Table 3, the variation of pressure has the most influence in the nicotine extraction. Table 4 represents the optimum conditions for the studied parameters in extracting nicotine from tobacco leaves with supercritical carbon dioxide.

The RK equation of state was used to correlate the

Table 3: ANOVA Parameter.

Col # Factor	DOF (f)	Sum of Sqrs. (S)	Variance (V)	Pure Sum (S')	Percent P (%)
1- P(atm)	2	531840.5	265920.3	531840.5	89.6
2- T(°C)	2	19829.2	9914.6	19829.2	3.3
3- t-dynamic (min)	2	21557.1	10778.5	21557.1	3.6
4-V-modifier (μ *l*)	2	20090.5	10045.2	20090.5	3.4

Table 4: Optimum conditions for extraction of nicotine.

Column # Factor	Level Description	Level	Contribution
1- P(atm)	350	3	232.44
2- T(°C)	50	2	48.226
3- t-dynamic(min)	5	1	63.736
4-V-modifier(μ *l*)	100	2	41.786

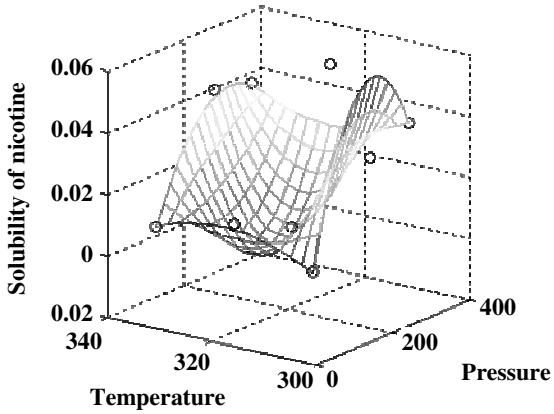

Fig. 12: Solubility of nicotine in super critical CO_2 , ○ experimental data.

measured solubility of nicotine from tobacco leaves in supercritical carbon dioxide. The average absolute relative deviation (AARD) between the experimental and the calculated solubility of nicotine in CO_2 at different operating conditions has an acceptable value of 0.205.

While exact values of critical properties of nicotine are not available, the critical temperature and pressure were assumed as adjustable parameters in correlating the solubility of nicotine in supercritical carbon dioxide with RK equation of state. The adjusted critical values for nicotine were obtained as: T_c=750 (K) and P_c=27.5 (atm).

Fig. 12 compares the experimental values of solubility of nicotine in terms of temperature and pressure with those calculated with the RK equation of state. As seen in Fig. 12, the RK EOS correlated with good accuracy the experimental solubility of nicotine in supercritical carbon dioxide.

CONCLUSIONS

The supercritical extraction of nicotine from tobacco leaves, prepared in the north of IRAN, by carbon dioxide has been studied experimentally. Also the effects of pressure, temperature, extraction dynamic time and organic co-solvent on the amount of nicotine extracted from tobacco leaves and the interactions between them have been investigated. It should be stressed that the number of experiments has been specified based on the Taguchi design. The results showed that the pressure has the maximum effect (86.9 %) on the nicotine extraction. Raising the pressure increases the nicotine extraction. Optimum condition for supercritical extraction of nicotine by carbon dioxide was obtained at pressure of 350 atm, temperature of 55 °C, dynamic time of 5 min, and modifier volume of 100 μL.

The experimental solubility data of nicotine in supercritical carbon dioxide was correlated using the R.K equation of state. While exact values of critical properties of nicotine are not available, the critical temperature and critical pressure of nicotine was considered as adjustable parameters. The results show that the RK equation of state correlate with accepted accuracy the experimental solubility of nicotine in supercritical carbon dioxide.

Nomenclatures

AARD	Average absolute relative deviation
a	Energy parameter in cubic equations of state
b	Size parameter in cubic equations of state
f	Fugacity
N	Number of experimental data
P	Absolute pressure
P_c	Critical pressure
R	Universal gas constant

T	Absolute temperature
T_c	Critical temperature
u	Constant in cubic equations of state
v	Molar volume
w	Constant in cubic equations of state
x_i	Mole fraction of component *i* in condense phase
y_i	Mole fraction (solubility) of nicotine in supercritical phase
Z	Compressibility factor
φ	Fugacity coefficient

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