Korean J. Chem. Eng., 33(5), 1681-1691 (2016)
DOI: 10.1007/s11814-015-0247-z

pISSN: 0256-1115
eISSN: 1975-7220

Mathematical modeling of supercritical carbon dioxide extraction of methyl eugenol from tuberose flowers

Probir Kumar Ghosh and Paramita Bhattacharjee†

Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata 700 032, India
(*Received 23 July 2015 • accepted 17 November 2015*)

Abstract−Methyl eugenol-rich extracts from dried tuberose flowers (*Polianthes tuberosa* L.) of *Calcutta single* variety were obtained using supercritical carbon dioxide (SC-CO_2) extraction. The optimized conditions for highest yield of methyl eugenol were 50 °C, 300 bar, 135 min with 1 L min^{-1} flow rate of gaseous CO_2. Solubilities of methyl eugenol under different SC-CO_2 extraction conditions were evaluated by Hildebrand solubility parameter and Chrastil equation. The extraction curve of methyl eugenol followed plug flow model. Steady state extraction occurred up to 100 min, followed by unsteady state. Release of methyl eugenol from tuberose flowers followed first-order kinetics (Peppas model) and non-Fickian diffusion. Packed bed characterization was carried out using dimensionless numbers of mass transfer, considering steady and unsteady states of extraction. These findings could be used in the development of the pilot plant and commercial scale extraction of methyl eugenol from floral matrices.

Keywords: Supercritical Carbon Dioxide, Tuberose, Methyl Eugenol, Solubility, Dimensionless Numbers

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) flowers are valued for their sweet fragrance. Besides ornamental value, these flowers have antimicrobial activity against bacteria (Gram positive and Gram negative) and fungi [1]. GC-MS analysis of the supercritical carbon dioxide (SC-CO_2) extracts of these flowers revealed methyl eugenol to be the principal compound [1] that confers the antimicrobial potency [2].

Methyl eugenol (C_{11} H_{14} O_2) is a phenylpropanoid compound, the methyl ether of eugenol. It is also known to be present in floral buds of *Laurus nobilis* [3], flowers of *Ocimum gratissimum* and *Ocimum sanctum* [4] and also in processed products such as rose 'absolute' [5]. It is used in perfumes, toiletries and detergents and as an insect attractant in pesticide formulations [6].

Since methyl eugenol is a phytochemical of prominence, we endeavored to model the SC-CO_2 extraction process to obtain methyl eugenol-rich extracts from tuberose flowers. SC-CO_2 extraction has been employed in this study, since it is a green extraction technique that offers several advantages over conventional extraction techniques of steam distillation and solvent extraction. This technique is environmentally benign; the extracts do not contain solvent residues and there is minimal thermal degradation of phytochemicals during extraction, which makes this process highly amenable to extraction of phytochemicals from botanicals [7].

Mathematical modeling of SC-CO_2 extraction of bioactive compounds has been conducted by several authors for extraction of essential oil from marigold and chamomile flowers [8]; and from seeds of *Echium amoenum* [9] and fennel [10]. We focused on optimization of SC-CO_2 extraction parameters (temperature, pressure and time) for extraction of methyl eugenol from tuberose flowers; determination of solubility of methyl eugenol in different temperature-pressure regimes; analysis of extraction kinetics for mass transfer of methyl eugenol using zero-order and first-order kinetic models and characterization of the hydrodynamic parameters of the packed bed of tuberose flowers by using dimensionless numbers of mass transfer, employing steady state and unsteady state approaches. To the best of our knowledge, this work reports for the first time on modeling of SC-CO_2 extraction of methyl eugenol from tuberose flowers. The data generated from this work can be utilized in scale up of the laboratory SC-CO_2 extraction process to pilot-plant level and for futuristic commercial applications.

MATERIALS AND METHODS

1. Materials

Fresh tuberose flowers (*Polianthes tuberosa* Linn.) of *Calcutta single* variety were procured from cultivators of Barasat, 24 Parganas North, West Bengal (22° 71′ N and 88° 51′ E, at about 13 m elevation above mean sea level, located in the eastern Gangetic plain of India) in March 2015 and were authenticated by West Bengal Food Processing and Horticulture Development Corporation Limited, Kolkata, India. The flowers were cultivated under tropical-temperate climate (24-32 °C, 75-85% RH) in sandy loamy soil of pH 7-8. Specialty chemicals such as methyl eugenol (99% pure), *n*-hexane, petroleum ether (b.p. 60-80 °C), ethanol, toluene and ethyl acetate were purchased from M/s Merck, Germany. All reagents were of AR grade. The procured ethanol was further distilled using $AgNO_3$ before usage. Food grade CO_2 was procured from M/s BOC India Ltd., Kolkata, India. SPE-ED matrix for SFE vessel packing was procured from M/s Applied Separations, USA.

2. Preparation of Tuberose Flowers for Extraction

Fresh tuberose flowers were air dried for 120 h at 23±2 °C in

†To whom correspondence should be addressed.
E-mail: pb@ftbe.jdvu.ac.in

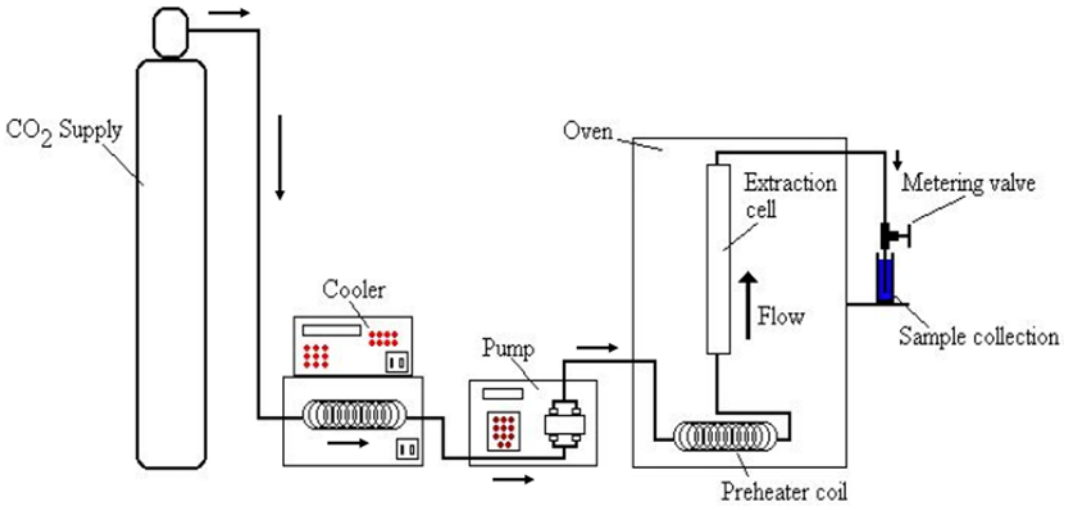

Fig. 1. Flowchart of supercritical carbon dioxide extraction system.

shade to a moisture content of 10% (dry basis), determined by Bidwell-Sterling method (AOAC method 967.19) [11]. This procedure has been reported for air dried, cut rose geranium leaves for SC-CO_2 extraction of its essential oil [12]. The dried flowers were cut using a sharp knife and the dimensions were measured using Vernier Calipers. The cut floret dimensions were determined from the mean of each dimension of ten random cut pieces.

3. Optimization of SC-CO_2 Extraction of Methyl Eugenol from Tuberose Flowers

A laboratory scale 'SCF Green Technology SPE-ED SFE 2' model of M/s Applied Separations, PA, USA was used for SC-CO_2 extractions. A flowchart of the SC-CO_2 extraction process is provided in Fig. 1. The extractions were in a 50 mL cylindrical extraction vessel (SS 316) having length of 304.79 mm and an internal diameter of 13.26 mm. Polypropylene frits were placed in both ends of the sample bed with tight vessel end-fittings.

The optimization of extraction process parameters such as batch size, cut floret dimensions and flow rate of gaseous CO_2 consisted of several preliminary trials. The optimized parameters were found to be 20 g, 5.0 mm×1.0 mm×0.5 mm, and 1 L min^{-1}, respectively. A batch size of 20 g of dried tuberose flowers was required to allow reliable quantification of methyl eugenol content in the extracts. A batch size greater than 20 g restricted loading the filler material (SPE-ED matrix) in the vessel, which is necessary to control moisture content of the packed bed and minimize voidage. It also impeded placement of polypropylene frits and end fittings required for a high pressure vessel. The equivalent channel diameter ($d_{eq.}$) for 20 g of packed bed of tuberose cut flowers was calculated in accordance with the method described by McCabe et al. [13] and from voidage in the packed bed [14]. The $d_{eq.}$ was found to be 0.82 mm.

The flow rate of CO_2 was measured at the collection end of the extraction unit using a bubble flow meter at ambient conditions (1 bar and 23±2 °C). A flow rate greater than 1 L min^{-1} of gaseous CO_2 resulted in loss of extract owing to back-flushing from the solvent trap into the tubings at the extraction vessel end and/or for its carry-over (entrainment) into the outlet tubing. Therefore, the flow rate of CO_2 was maintained constant at 1 L min^{-1} for all experiments.

The yield of methyl eugenol in the extracts was then optimized by using a three-level full factorial design with three parameters of extraction: temperature (40, 50 and 60 °C), pressure (100, 300, 500 bar) and extracting time (static+dynamic) (45, 75 and 135 min). The dynamic time was kept constant at 15 min, since no extract was collected after this time. 20 g batch size, 5.0 mm×1.0 mm×0.5 mm cut floret dimensions and 1 L min^{-1} flow rate of gaseous CO_2 was maintained constant in all extractions. All extracts were collected in 96% ethanol in screw capped glass vials and kept in an ice bath. Post extraction, the ethanol was evaporated using a rotary vacuum evaporator (M/s Eyela Corp., Kyoto, Japan) at 30-40 °C and −45 mbar. The extracts were finally concentrated by purging a gentle stream of nitrogen and weighed gravimetrically. The extracts were subsequently redissolved in 96% ethanol and stored in nitrogen flushed screw capped amber colored glass vials at −18 °C in the dark, until further analyses.

4. Estimation of Methyl Eugenol Content in Tuberose Flowers

The methyl eugenol content in tuberose flowers was estimated by hydrodistillation. However, no essential oil was recovered by this process. Soxhlet extraction using *n*-hexane and petroleum ether (b.p. 60-80 °C) was carried out. All extractions were conducted for 8 h using 20 g tuberose cut flowers ($d_{eq.}$ 0.82 mm).

5. Densitometric Analyses of Methyl Eugenol in Tuberose Flowers Extracts

The solvent and SC-CO_2 extracts were dissolved in ethanol and analyzed for methyl eugenol content by densitometric analysis using high performance thin layer chromatography (HPTLC). The experiments were conducted in accordance with the method reported by Chatterjee and Bhattacharjee [14], with few modifications. Silica gel 60 (F_{254}) coated aluminum plates (M/s Merck, Germany) having dimensions of 200×100 mm were used for densitometric analysis. 30 µl of each extract dissolved in ethanol was spotted on a TLC plate. Toluene : ethyl acetate=4 : 1 was used as the mobile phase at 23±2 °C. The retardation factor (R_f) of methyl eugenol standard in this mobile phase was found to be 0.67±0.02 at its λ_{max} (281 nm).

Methyl eugenol content in the extracts was quantified using standard curve of standard methyl eugenol.

6. Statistical Analysis of Yield of Methyl Eugenol under Different Extraction Conditions

In this study, the yield of methyl eugenol (represented by mean± SD of three independent extractions) refers to methyl eugenol content in the SC-CO_2 extracts determined by densitometric analysis. Statistical analysis of the yields of methyl eugenol was by one-way analysis of variance (ANOVA), response surface methodology and regression modeling. All statistical tests of this work used STATISTICA 8.0 software (Statsoft, OK, USA). A $p \leq 0.05$ was used to verify the significance of the tests.

7. Determination of Solubility of Methyl Eugenol in SC-CO_2

The solubility of methyl eugenol in SC-CO_2 was ascertained by two popular approaches: Hildebrand solubility parameter and the Chrastil equation, in accordance with the reported methods [14-16], with few modifications. 20 g dried and cut tuberose florets ($d_{eq.}$=0.82 mm) were packed into the SC-CO_2 extraction vessel. The extraction temperature was varied at 40 °C and 60 °C and the pressure at 100, 300, 400 and 500 bar. In each run, the extract was collected after 120 min (equilibration/static time) and 15 min collection time (dynamic time) at a flow rate of 1 L min^{-1} of gaseous CO_2.

7-1. Evaluation of Solubility of Methyl Eugenol by Hildebrand Solubility Parameter

The Hildebrand solubility parameter developed by Hildebrand and Scott [17] has been used in this study to evaluate solubilities of methyl eugenol under different temperature-pressure regimes of SC-CO_2. Among the various methods for determination of Hildebrand solubility parameter of a solute, one of the popular methods is the Fedors group contribution method, which is applied when the molecular structure of the solute is known [18,19]. The Hildebrand solubility parameter for a given solute is [20]:

$$\delta\,(\text{cal cm}^{-3})^{1/2}=[\Sigma_i\,(\Delta E_v)_i/\Sigma_i\,(\Delta V_i)]^{1/2} \quad (1)$$

where, $\Sigma_i\,(\Delta E_v)$ is the summation of cohesive energies (cal mol^{-1}) of functional groups in the molecular structure, and $\Sigma_i\,(\Delta V_i)$ is the summation of molar volumes (cm^3 mol^{-1}). The cohesive energy of methyl eugenol was calculated by Fedors group contribution method [21] and molar volumes of SC-CO_2 from Peng-Robinson cubic equation of state [22].

7-2. Evaluation of Solubility of Methyl Eugenol in SC-CO_2 by Chrastil Equation

The Chrastil equation shows a linear relationship between the logarithm of solubility of a solute and the logarithm of SC-CO_2 density [23,24]. It is represented as [25]:

$$\ln S=k \ln \rho+F+G\cdot T^{-1} \quad (2)$$

where, 'S' is the solubility of solute in the gas phase [mg (kg CO_2^{-1})], 'k' is the association constant related to the total number of molecules in the complex [26], ρ is the density of SC-CO_2 (kg m^{-3}), 'F' and 'G' are empirical constants and 'T' is the temperature (K).

8. Study of Kinetics of SC-CO_2 Extraction of Methyl Eugenol from Tuberose Cut Flowers

The kinetics of SC-CO_2 extraction of methyl eugenol was studied to analyze the pattern of release of methyl eugenol from tuberose cut flowers. This study was conducted at the extraction condition at which highest yield of methyl eugenol was obtained.

The kinetic models such as zero order (cumulative percentage methyl eugenol released vs. time), first order (log cumulative percent methyl eugenol retained vs. time), sub-types of first order such as Higuchi model (cumulative percentage methyl eugenol released vs. √time), Peppas model (log cumulative percent methyl eugenol release vs. log time) and Hixson-Crowell's cube root model [(percentage methyl eugenol retained)$^{1/3}$ vs. time] were used for analysis. The kinetic model that best fitted the extraction data was deduced by comparing the regression coefficient (r) values obtained from these models.

Subsequently, the release exponent (n) was determined from the Peppas model to characterize the different release mechanisms. According to Peppas equation, n=0.45 shows diffusion controlled release, n=0.89 shows swelling controlled release or case II transport and 'n' in the range of 0.45-0.89 depicts superposition of both the above phenomena and is known as non-Fickian or anomalous transport [27].

9. Characterization of Packed Bed during SC-CO_2 Extraction of Methyl Eugenol from Tuberose Flowers during Steady and Unsteady States

SC-CO_2 extraction occurs from a packed bed of sample matrix consisting of cells broken up during communition and a few intact cells [28]. These authors have described two phases of extraction for understanding the extraction process. In the first phase, the solute in the broken cells is extracted, which is easily accessible to SC-CO_2, and the yield increases with extraction time (i.e., during steady state). When the solute in the broken cells is exhausted, the second phase of extraction begins, in which extraction occurs from the walls of the undestroyed cells and controls the remaining part of the extraction. This is the unsteady state. This approach has been adopted for extraction of basil essential oil by liquid CO_2 extraction and SC-CO_2 extraction of essential oil from orange peels [29]. We applied this approach to characterize the SC-CO_2 extraction of methyl eugenol from tuberose cut flowers.

9-1. SC-CO_2 Extraction of Methyl Eugenol from Tuberose Flowers during Steady State

Among the various methods used for modeling of SC-CO_2 extraction, the steady state approach has been extensively studied. This approach has been employed for characterization of SC-CO_2 extraction of essential oil from fennel seeds [10] and for fractionation of edible oils by SC-CO_2 [30].

This study used a two-phase model comprising of the solid flower bed and supercritical fluid (SCF) phase. The basic assumptions of the model were similar to those reported in previous investigations [14,16]. The material flux of methyl eugenol in the extraction conditions, i.e., the net mass out, was fitted to the transport equation at the external surface of the tuberose cut flowers in a packed bed extractor to characterize the mass transfer, in accordance to Chatterjee and Bhattacharjee [14].

The extraction curves were plotted for temperature and pressure conditions of 40 °C and 60 °C and 100, 300 and 500 bar, respectively. Mass transfer ($K_s a$) was determined for each extraction condition from the material flux equation [14,16]. For the mass transfer coefficient K_s, the specific interfacial area (a) of tuberose cut florets was calculated in accordance with Tan et al. [31].

The operating mass transfer coefficients obtained were used to establish the relation between dimensionless numbers such as Sherwood number ($Sh=K_{sl}d_{eq}/D$), Reynolds number ($Re=d_{eq}\rho u/\mu$) and Schmidt number [$Sc=\mu/(\rho D)$] and fitted into a model equation as has been reported for SC-CO_2 extraction of eugenol from clove buds [14].

9-2. SC-CO_2 Extraction of Methyl Eugenol from Tuberose Flowers during Unsteady State

Unsteady state period followed steady state mass transfer during SC-CO_2 extraction of methyl eugenol from tuberose flowers as has been reported for extraction of matricine from chamomile flowers [32]. From our experiments, the maximum yield of methyl eugenol in SC-CO_2 extract was found to be 37% of the total methyl eugenol content (estimated by Soxhlet extraction). This implies that about 63% of methyl eugenol was not amenable for extraction by SC-CO_2 under the experimental conditions employed in this study. Therefore, following each SC-CO_2 extraction, for unsteady state mass transfer calculations, the sample bed in the extraction vessel was subjected to solvent extraction to determine the content of unextracted methyl eugenol. The densitometric quantification of methyl eugenol in these extracts was according to the method described in section 5. The mass transfer in falling rate period phase was analyzed from the following equations [33]:

$$m(\theta)/m_0=\exp(-K\theta) \quad (3)$$

$$F_0=D_s\cdot\theta/d_{eq}^2 \quad (4)$$

where, $K\approx F_0/\theta$,

where $m(\theta)$ is the amount of methyl eugenol [in mg (100 g dry flowers)$^{-1}$] that remains unextracted within the sample bed at time $t=\theta$ (in min), m_0 is the initial amount of methyl eugenol [mg (100 g dry flowers^{-1})] in the sample bed at time t=0, 'F_0' is Fourier number and 'D_s' is the diffusivity in the solid phase (in cm^2 s^{-1}), 'θ' is the time required for the falling phase to start from constant rate period, i.e., in the unsteady state. In this unsteady diffusion-controlled phase of extraction, the mass transfer can be characterized from the plot of ln [$m(\theta)$ m_0^{-1}] versus θ.

The parameter 'D_s' for unsteady mass transfer was determined from experimental data using Eq. (4). For obtaining the dimensionless numbers in unsteady state, 'Sh' and 'Sc' numbers were recalculated using 'D_s' of unsteady state and 'K_{sl}' of steady state.

RESULTS AND DISCUSSION

1. Optimization of SC-CO_2 Extraction of Methyl Eugenol from Tuberose Flowers

The yield of methyl eugenol obtained under different extraction conditions is shown in Table 1. ANOVA study revealed that the yield of methyl eugenol increased significantly with increase of temperature from 40 °C to 50 °C (p=0.0000) and also when temperature increased from 50 °C to 60 °C (p=0.0015). The yield of methyl eugenol changed significantly with change in pressure from 100 to 500 bar (p=0.0038). Increasing extracting time from 45 min to 135 min had a significant effect (p=0.0456) on the yield. The maximum yield of methyl eugenol [4.88±0.08 mg (100 g dry flowers)$^{-1}$] was at 50 °C, 300 bar, 135 min; while its predicted value at this extraction condition was 3.93 mg (100 g dry flowers)$^{-1}$. The increase in yield with temperature at high pressure regimes (>300 bar) is in accordance with the retrograde phenomenon of SCFs, which states that vapor pressure effect is more significant than density effect of SC-CO_2 with increase in temperature, which contributes to higher extract yield. The increase in yield due to retrograde phenomenon also holds good for SC-CO_2 extraction of eugenol from clove buds [14] and squalene from grains of *Amaranthus paniculatus* [16].

Table 1. Densitometric yield of methyl eugenol in SC-CO_2 extracts of tuberose flowers

Temperature (°C)	Pressure (bar)	Time (min)	Methyl eugenol [mg (100 g dry flowers)$^{-1}$][a]
40	100	45	1.24±0.02
40	100	75	1.80±0.03
40	100	135	2.03±0.04
40	300	45	1.26±0.02
40	300	75	1.84±0.03
40	300	135	2.08±0.05
40	500	45	1.28±0.02
40	500	75	1.90±0.04
40	500	135	2.15±0.04
50	100	45	2.20±0.04
50	100	75	2.35±0.05
50	100	135	2.88±0.05
50	300	45	2.32±0.04
50	300	75	3.58±0.06
50	300	135	4.88±0.08
50	500	45	2.24±0.05
50	500	75	2.45±0.05
50	500	135	3.20±0.06
60	100	45	0.56±0.01
60	100	75	0.92±0.01
60	100	135	1.30±0.02
60	300	45	2.56±0.05
60	300	75	3.24±0.06
60	300	135	3.90±0.06
60	500	45	2.88±0.03
60	500	75	3.62±0.04
60	500	135	4.28±0.07

[a]The values are mean±SD of three independent extractions

2. Estimation of Methyl Eugenol content in Tuberose Flowers

The amount of methyl eugenol obtained in Soxhlet extraction was 13.17±0.50 mg (100 g dry flowers)$^{-1}$ using *n*-hexane and 9.50±0.20 mg (100 g dry flowers)$^{-1}$ using petroleum ether (b.p. 60-80 °C). Since a significantly higher amount (p=0.000) of methyl eugenol was obtained with *n*-hexane, this value was considered as the total content of methyl eugenol in the tuberose flowers and the same data was subsequently used for modeling.

3. Generation of Response Surface Curves

The effects of extraction temperature, pressure and time on the yield of methyl eugenol are shown in Fig. 2(a)-(c). The extraction temperature, pressure and time were fixed at their middle values

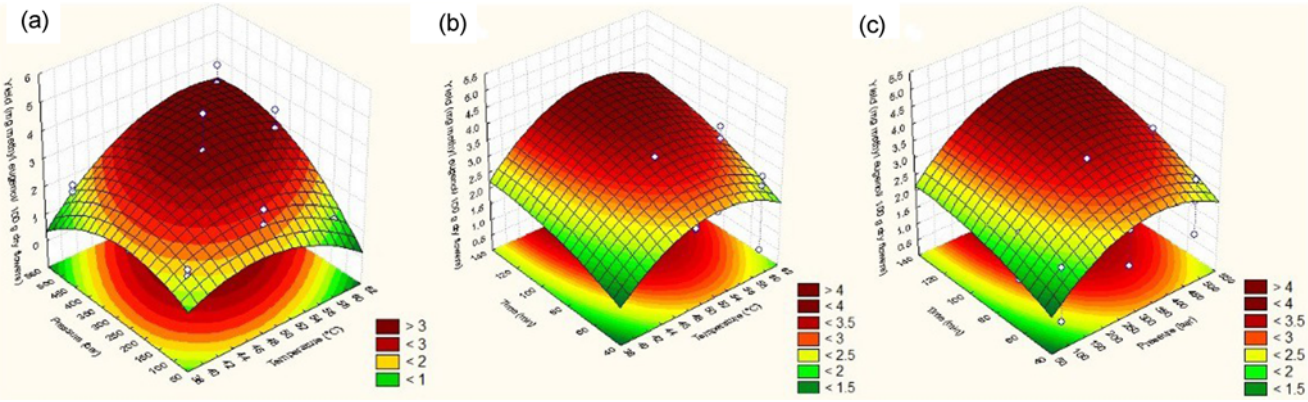

Fig. 2. Plot of response surface indicating methyl eugenol yield (a) as a function of temperature and pressure at 75 min; (b) as a function of temperature and time at 300 bar, and (c) as a function of pressure and time at 50 °C.

of 50 °C, 300 bar and 135 min in Fig. 2(a), (b), (c), respectively. The response surfaces were characterized by regression modeling.

4. Regression Modeling

The second-order polynomial equation that describes the response surface is given below.

$$Y=B_0+\Sigma B_iX_i+\Sigma B_{ii}X_i^2+\Sigma B_{ij}X_iX_j \quad (5)$$

where, Y represents the experimental response (yield of methyl eugenol), B_0, B_i, B_{ii} and B_{ij} are constants and regression coefficients of the model, and X_i and X_j are independent variables in coded forms. The expanded model includes linear, quadratic and cross-product terms as shown below (with intercept):

$$Y=2.4666+0.4384\,X_1+0.4916\,X_2+0.5666\,X_3+0.3722\,X_1^2 +0.3355\,X_2^2+0.1000\,X_3^2+0.6483\,X_1X_2+0.0860\,X_1X_3+0.0850\,X_2X_3 \quad (6)$$

where, X_1, X_2 and X_3 are the extraction temperature, pressure and time, respectively. This equation explains the effects of all three variables on the response Y. All the second-order and first-order terms showed significant effects for temperature and pressure; while only first-order terms showed significant effect for extraction time. The linear dependence of yield on extraction time in our studies indicates the influence of static time on extract yield, since dynamic time was kept constant.

The two-level interactions showed that interdependence of pressure and temperature (i.e., the cross product term X_1X_2) had more significant effect (p=0.0003) than any other combination, with X_1X_3 (p=0.5491) and X_2X_3 (p=0.5540) having insignificant effects on yield of methyl eugenol. ANOVA analysis of the model revealed high F values (Fisher's variance ratio) in the range of 10-23, indicating significant interactions among the variables (Table 2).

To establish the adequacy of the above regression model and violations of the basic assumptions of the same, a residual analysis was performed with the experimental data in accordance with Chatterjee and Bhattacharjee [14]. Analysis of the residuals showed the residuals to be 'structure less', having no obvious pattern, which proved the adequacy of the model [34]. A plot of the predicted vs. the observed values of responses Y [mg methyl eugenol (100 g dry flowers)$^{-1}$] showed a close fit and good correlation (r=0.92) (Fig. 3). Thus, a statistically significant multiple regression relationship between the independent variables and the responding variables was obtained. This model showed that the yield of methyl eugenol from tuberose flowers could be increased by variations in temperature

Table 2. ANOVA study of the response surface methodology

Effect	Degree of freedom	Yield of methyl eugenol [mg (100 g dry flowers)$^{-1}$] SS	Yield of methyl eugenol [mg (100 g dry flowers)$^{-1}$] MS	Yield of methyl eugenol [mg (100 g dry flowers)$^{-1}$] F	Yield of methyl eugenol [mg (100 g dry flowers)$^{-1}$] P
X_1	1	3.32	3.39	13.77	0.00
X_1^2	1	3.39	3.32	13.47	0.00
X_2	1	4.27	4.27	13.32	0.00
X_2^2	1	2.70	2.70	10.95	0.00
X_3	1	5.78	5.78	23.42	0.00
X_3^2	1	0.23	0.23	0.93	0.34
X_1X_2	1	5.04	5.04	20.43	0.00
X_1X_3	1	0.09	0.09	0.37	0.54
X_2X_3	1	0.08	0.08	0.36	0.55
Error	17	4.19	0.24		
Total	26	28.72			

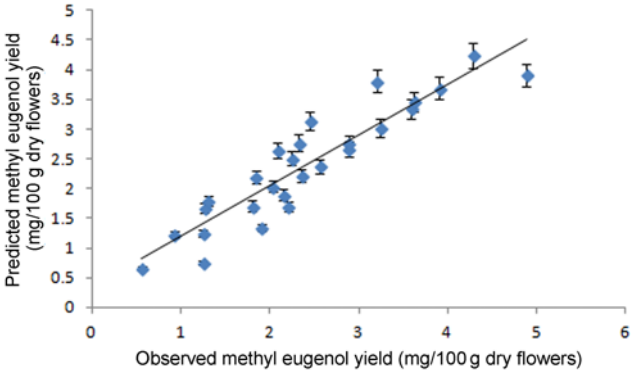

Fig. 3. Plot of predicted vs. observed values of methyl eugenol yield mg (100 g dry flowers)$^{-1}$.

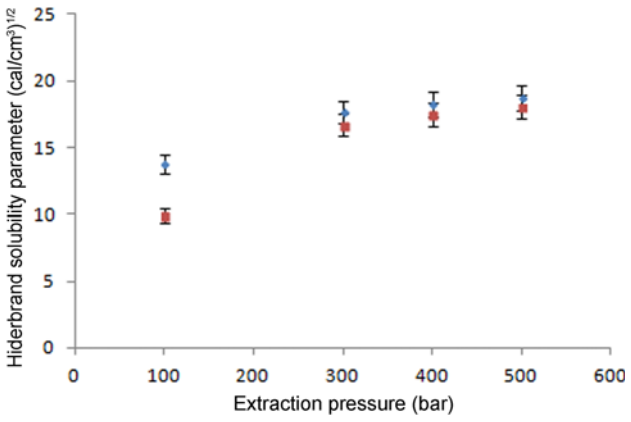

Fig. 4. Dependence of Hildebrand solubility parameter of methyl eugenol in SC-CO_2 on extraction pressure. Blue diamond, at 40 °C; red square, at 60 °C.

and pressure (linear and quadratic, respectively) and time (linearly).

5. Analysis of Response Surfaces

The response surfaces are shown in the stereoscopic figures (Fig. 2(a), (b), (c)). From the test statistics for the regression models (F-test and one way-ANOVA) as discussed above, the combined effects of extraction temperature and pressure had the most significant effect on the yield of methyl eugenol than any other combination of extraction parameters.

6. Optimum Extraction Conditions of Methyl Eugenol from Tuberose Flowers using SC-CO_2

To determine the optimal processing conditions of extraction of methyl eugenol from tuberose cut flowers, i.e., to determine the optimal values of X_1, X_2 and X_3, the first partial derivative of the regression equation was conducted with respect to X_1, X_2 and X_3 and set to zero. This was done by putting the second-order regression equation in matrix form as described by Montgomery [35] and Ge et al. [36]. The point thus obtained is known as the stationary point, X_{1S}, X_{2S}, X_{3S} (X_{1S}=57.43 °C, X_{2S}=464.7 bar and X_{3S}= 160.06 min). The amount of methyl eugenol extracted in this stationary point (X_s) was found to be 4.37 mg (100 g dry flowers)$^{-1}$.

7. Characterization of Response Surface

The response surfaces were characterized by analyzing whether the stationary point obtained was a point of maximum response, minimum response or a saddle point. The regression equation was transformed to the canonical form and the eigenvalues were determined in accordance with the method described by Montgomery [35]. The eigenvalues obtained were –0.000013, –0.000110, –0.007449. Since the eigenvalues obtained were all negative, X_S was a point of maximum response.

8. Hildebrand Solubility Parameter (δ) of Methyl Eugenol in SC-CO_2

The values of cohesive energies of various functional groups in methyl eugenol and molar volumes of SC-CO_2 used for evaluation of Hildebrand solubility parameter are shown in Table 3. The graphical representation for solubility (δ) of methyl eugenol as a function of extraction temperatures and pressures is shown in Fig. 4. We observed that δ decreased significantly from 40 to 60 °C (p= 0.0200). However, δ increased insignificantly with pressure (at constant temperature) (p=0.0700). Highest δ [(18.67±0.05 cal cm^{-3})$^{1/2}$] for methyl eugenol was obtained at 40 °C, 500 bar.

This observation was in agreement with the retrograde phenomenon (decreased solubility effect) for SCFs. Since the δ values

Table 3. Cohesive energy values of functional groups of methyl eugenol by Fedors group contribution method and molar volume of SC-CO_2 by peng robinson cubic equation of state

Functional group	Number of units in structure	ΔE_i unit^{-1a}	Energy (cal mole^{-1})	Temperature (°C)	Pressure (bar)	Molar volume of SC-CO_2 (cm^3 mole^{-1})^b
=CH_2-	1	1030	1030	40	100	77.69
=CH-	4	1030	4120	40	300	47.37
-CH_2	1	1180	1180	40	400	44.32
C=	3	1030	3090	40	500	42.28
-O-	2	800	1600	60	100	150.01
-CH_3	2	1125	2250	60	300	52.87
Conjugated double bond	3	410	1230	60	400	48.14
Six member ring	1	250	250	60	500	45.25
		Total	14750			

[a]Energy values of functional groups obtained from Fedors group contribution method
[b]Calculated from Peng Robinson cubic equation of state

Table 4. Density of SC-CO_2 and experimental solubility of methyl eugenol determined under different extraction conditions

Temperature (°C)	Pressure (bar)	Density of CO_2 (kg m^{-3})[a]	Solubility of methyl eugenol [mg (kg CO_2)$^{-1}$][b]
40	100	566.35	0.028±0.002[b]
40	300	928.85	0.029±0.002
40	400	992.77	0.029±0.003
40	500	1040.68	0.027±0.002
60	100	293.31	0.059±0.006
60	300	832.23	0.062±0.008
60	400	914.00	0.061±0.008
60	500	972.37	0.063±0.006

[a]Calculated from Peng Robisnon Cubic Equation of state (PR-CEOS)
[b]The solubility of methyl eugenol is mean±SD of three independent extractions

did not follow the SCF phase equilibrium principle at higher pressure regimes (30-50 MPa), we did not consider these values for further analyses. A similar trend on solubility of SC-CO_2 extracted eugenol from clove buds has been reported by Chatterjee and Bhattacharjee [14].

9. Determination of Solubility of Methyl Eugenol in SC-CO_2 by Chrastil Equation

The solubility of methyl eugenol in SC-CO_2 varied significantly with conditions of extraction (Table 4). The two most important factors that affect the solubility of a solute in supercritical state are solvent density and temperature [14], and our data agreed with this phenomenon. We observed that the solubility of methyl eugenol in SC-CO_2 increased significantly (p=0.0000) with increase in temperature from 40 °C to 60 °C. The maximum solubility of methyl eugenol was at 60 °C, 500 bar [0.063 mg (kg CO_2)$^{-1}$]. From the values of solubility of methyl eugenol, a linear regression equation was developed according to the Chrastil equation as follows:

$$\ln S = 8.88 + 0.03 \ln \rho - 3948.41/T \quad (7)$$

where, S is the solubility of methyl eugenol in SC-CO_2 [mg (kg CO_2)$^{-1}$]. The regression coefficient 'r' obtained using this equation is 0.99, and the standard error of the equation is 0.03. The validity

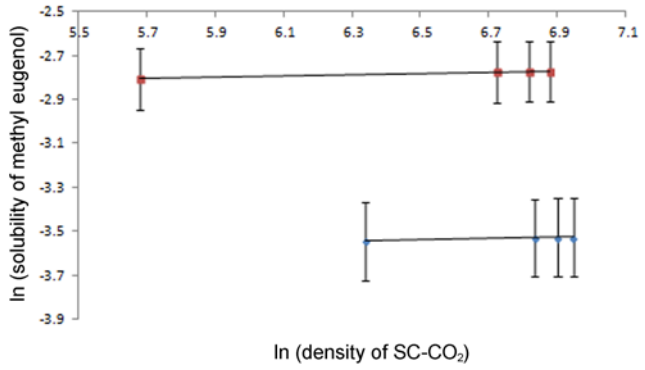

Fig. 5. Solubility of methyl eugenol in SC-CO_2 as a function of solvent density. Blue diamond, at 40 °C; red square, at 60 °C.

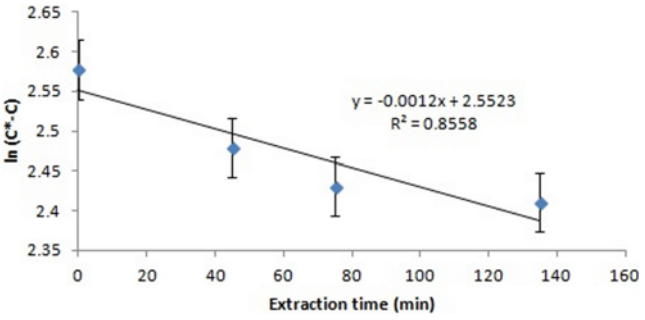

Fig. 6. Yield of methyl eugenol from tuberose cut flowers by SC-CO_2 at 50 °C, 300 bar as a function of extraction time.

of the fitted model was indicated by the insignificant lack of fit (p=0.4500) for this equation. The plots of ln S vs. ln ρ at 40 °C and 60 °C showed the isotherms to be linear and parallel, which confirmed the suitability of the Chrastil equation in our work (Fig. 5). Thus, this correlated Chrastil equation can be used to predict the solubility of methyl eugenol from tuberose flowers in SC-CO_2 at different conditions of extraction.

10. Study of Kinetics of SC-CO_2 Extraction of Methyl Eugenol from Tuberose Flowers

The mass transfer phenomenon that explains the SC-CO_2 extraction of methyl eugenol from tuberose flowers has been explained by plotting the yields of methyl eugenol at 50 °C, 300 bar (where highest yield was obtained) with extraction time. From the extraction curve, we observed that the first part of the curve followed first-order kinetics, where the yield increased almost linearly with time; while the second part was an asymptote (Fig. 6). The extraction curve indicated 'plug flow' behavior which has been commonly reported for SC-CO_2 extraction of essential oils from secretory ducts of flowers [8,37].

Our experiments showed an increase in yield of methyl eugenol with time of extraction (Table 1). The first phase of extraction showed increase in yield with time. The yield plateaued after 100 min. This meant that in the first 100 min of extraction, methyl eugenol from cut ends of the florets were extracted by SC-CO_2 and subsequently, the extraction occurred from the lateral surfaces of the florets during the unsteady state. In the unsteady state (i.e., post 100 min of extraction), owing to comparatively lesser availability of methyl eugenol in the tuberose cut florets, the yield curve plateaued.

Since dynamic time was kept constant in our experiments, increased yield was owing to higher equilibration time (i.e., the time for equilibration of concentration of methyl eugenol inside the flowers to that in the bulk SC-CO_2). Essential oils are located in specialized secretory glands called osmophores [38]. These glands swell and rupture under SC-CO_2 conditions, releasing methyl eugenol, which diffuses on to the surface of the cut flowers and forms a film. The film eventually solubilizes into the bulk SC-CO_2 phase. This phenomenon has also been reported for SC-CO_2 extraction of essential oil from-glands of chamomile and marigold flowers [9], glands of *Thymus serpullum* leaves, and from secretory ducts of celery fruit and valerian root [39].

The kinetics of SC-CO_2 extraction of methyl eugenol from tuberose flowers was studied using zero-order and first-order kinetic

models. We found that the SC-CO_2 extraction of methyl eugenol from the packed bed of tuberose cut flowers was best described with highest correlation (r=0.99) by the first-order kinetics model, known as the Peppas model. The mechanism of release of methyl eugenol from tuberose florets during extraction can be explained by the Korsmeyer and Peppas equation [40]:

$$M_t/M_a = k \times t^n \quad (8)$$

where, M_t/M_a is the fractional release of methyl eugenol in time 't', 'n' is the diffusion release exponent, indicative of mechanism of release and 'k' is the release rate constant. Since in this study the value of 'n' was determined to be 0.74, the mechanism of release was by 'anomalous transport', which is the case when 'n' lies between 0.45 and 0.89, establishing that the release mechanism was a superposition of swelling and diffusion controlled phenomena [40]. This finding was in agreement with the literature reports [38,40]. The value of 'k' for extraction of methyl eugenol sample was found to be 1.01 (Table 5).

11. Packed Bed Characterization of SC-CO_2 Extraction of Methyl Eugenol from Tuberose Flowers

11-1. SC-CO_2 Extraction during Steady State

In this study, the order of magnitude of viscosity of SC-CO_2 was 10^{-4} P, in agreement with that reported for SCFs [14] (Table 6). The values of diffusivities of methyl eugenol estimated by the Wilke-Chang equation were in the range of 6.82×10^{-5} to 1.12×10^{-4} $cm^{-2}s^{-1}$ (Table 6), in agreement with Kotnik et al. [32], who reported diffusivities of essential oil of chamomile flower heads in SC-CO_2 to be in the range of 2.66 to 10.90×10^{-5} $cm^{-2}s^{-1}$ during steady state. The Schmidt numbers (Sc) were found to be in the range of 6-10, in agreement with Tan et al. [31] and Paulitis et al. [41], who reported 'Sc' of around 10 for SCFs.

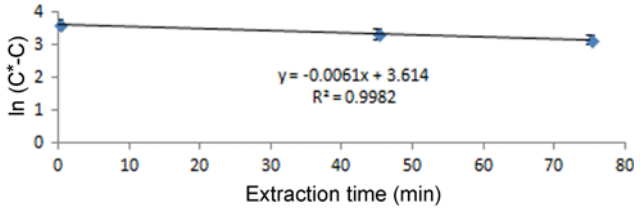

Fig. 7. Plot of logarithm of concentration gradient of methyl eugenol in packed bed of tuberose cut florets with time during SC-CO_2 extraction at 40 °C and 100 bar.

The mass transfer coefficient of methyl eugenol from tuberose cut flower packed bed was evaluated by plotting graphs of ln (C*-C) vs. time for each extraction condition (e.g., in Fig. 7, for 40 °C, 100 bar). The values of mass transfer coefficient (K_d) were on the order of 10^{-4} cm s^{-1}, in agreement with reports on solid-fluid systems (10^{-4} to 10^{-3} cm s^{-1}) [31] and that of eugenol from clove buds (10^{-4} cms^{-1}, Chatterjee and Bhattacharjee [14]). In the pressure-temperature regimes, where most SC-CO_2 extractions are conducted, there are generally no accepted correlations for mass transfer coefficients. The Reynolds number (Re) values in our study were in the range of 1066-1222, which is the zone where turbulent forced convection prevails in most SC-CO_2 extractions (i.e., at Re>150) [42].

The general correlation for relating the dimensionless numbers Sh, Sc and Re is [43]:

$$Sh = A\,(Re)^a\,(Sc)^{1/3} \quad (9)$$

Table 5. Kinetics of release of methyl eugenol from tuberose cut flowers by SC-CO_2

Correlation coefficient (r)					Diffusion release exponent (n)	Release constant (k)	Type of transport
Zero order	First order						
		Higuchi	Peppas	Hixson Crowell			
0.95	0.53	0.97	0.99	0.51	0.74	1.01	Anomalous

Table 6. Physical properties of SC-CO_2, diffusivity of methyl eugenol in SC-CO_2 and dimensionless numbers in steady and unsteady states

Temperature (°C)	Pressure (bar)	Viscosity of SC-CO_2 (10^{-4} P)[a]	Diffusivity of methyl eugenol in SC-CO_2 in steady state (cm^2s^{-1})[b]	Mass transfer coefficient (10^{-4} cms^{-1})	Reynolds number (Re)	Schmidt number (Sc) (steady state)	Sherwood number (Sh) (steady state)	Diffusivity of methyl eugenol in SC-CO_2 in unsteady state (10^{-4} cm^2s^{-1})[c]	Schmidt number (Sc) (unsteady state)	Sherwood number (Sh) (unsteady state)
40	100	4.38	1.12×10^{-4}	3.24	1206.72	6.89	0.23	1.11	6.92	0.23
40	300	7.36	6.70×10^{-5}	3.21	1179.70	11.82	0.39	1.11	7.07	0.23
40	500	9.12	5.40×10^{-5}	3.20	1065.85	16.23	0.48	1.10	7.83	0.24
60	100	2.03	2.64×10^{-4}	3.23	1345.70	2.63	0.10	1.12	6.20	0.23
60	300	6.36	8.45×10^{-5}	3.22	1221.97	9.04	0.31	1.11	6.83	0.24
60	500	7.89	6.82×10^{-5}	3.21	1151.89	11.89	0.38	1.11	7.25	0.23

[a]Calculated from Richenberg equation

[b]Calculated from Wilkie-Chang equation

[c]Caluclated by Hong et al. (1990) method

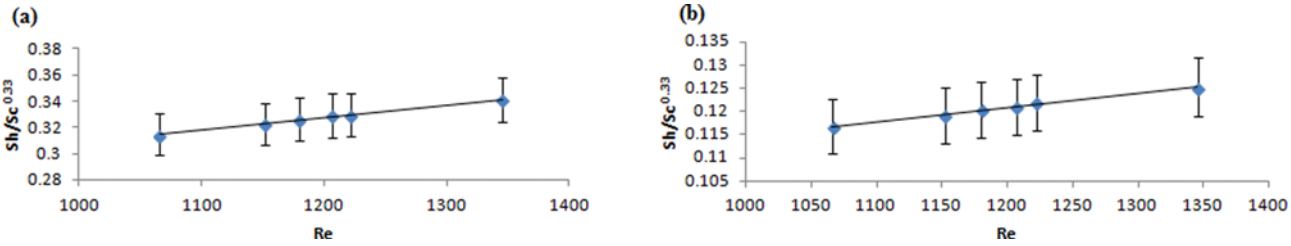

Fig. 8. Dependence of Sherwood number and Schmidt number on Reynolds Number in (a) steady state and (b) unsteady state.

Upon substitution of the calculated Sh, Sc and Re numbers in Eq. (9) and application of regression analysis we have:

$$\ln (Sh\ Sc^{-1/3}) = -3.5789 + 0.3474 \ln Re \quad (10)$$

The correlation coefficient (r) of this equation was 0.99 and standard error was 0.03.

Hence, the mass transfer equation correlating the dimensionless numbers was found to be:

$$Sh = 2.7\times10^{-2}\ Sc^{0.33}\ Re^{0.35} \quad (11)$$

The plot of $Sh\ Sc^{-1/3}$ vs. Re showed a very good fit (r=0.99) and a linear relationship among the dimensionless numbers [Fig. 8(a)]. Therefore, we can conclude that the mass transfer of methyl eugenol in packed bed conditions in SC-CO_2 is explained by this equation.

11-2. SC-CO_2 Extraction during Unsteady State

In the unsteady state, we observed higher values of diffusivity for methyl eugenol in unsteady state (1.10 to 1.12×10^{-4} $cm^2\ s^{-1}$, Table 6), compared to SC-CO_2 extractions of matricine from chamomile flower heads (2.49 to 4.89×10^{-8} $cm^2\ s^{-1}$) reported by Kotnik et al. [32]. This difference could possibly be attributed to differences in temperature-pressure regimes of extractions conducted by different authors. The increase in values of diffusivities of methyl eugenol was in agreement with those reported for eugenol in SC-CO_2 during extraction from clove buds [14].

The modified diffusivity values were used to obtain 'Sh' and 'Sc' numbers in the unsteady state (Table 6). Upon substitution of the calculated 'Sh' and 'Sc' numbers in Eq. (9) along with the unchanged 'Re' and application of regression analysis we obtained:

$$\ln Sh = -4.5923 + 0.3543 \ln Re \quad (12)$$

The correlation coefficient (r) for this equation was 0.98 and standard error was 0.03. Therefore, the mass transfer equation correlating the dimensionless numbers in unsteady state was found to be

$$Sh = 1.0\times10^{-2}\ Sc^{0.33}\ Re^{0.35} \quad (13)$$

The plot of $Sh\ Sc^{-1/3}$ vs. Re showed a very good linear fit (r=0.98) and a linear relationship among the dimensionless numbers [Fig. 8(b)]. We observed that steady and unsteady state approaches could successfully characterize the extraction of methyl eugenol from tuberose flowers.

CONCLUSION

Methyl eugenol was extracted from tuberose cut flowers using SC-CO_2 extraction. Highest yield of methyl eugenol, 4.88±0.08 mg methyl eugenol (100 g dry flowers)$^{-1}$, was obtained with 20 g dry tuberose flowers ($d_{eq.}$=0.82 mm) at the optimized condition of 50 °C, 300 bar and 135 min at a constant flow rate of CO_2 at 1 L min^{-1}. Statistical analyses revealed that temperature and pressure (in quadratic and linear form) and time (in linear form) showed significant effects on yield of methyl eugenol.

The solubility of methyl eugenol in SC-CO_2 was estimated by using the Chrastil equation. The yield of methyl eugenol increased with time of extraction (in steady state) in the first 100 min, and thereafter the yield plateaued (in unsteady state). The extraction curve of methyl eugenol followed plug flow model. The release of methyl eugenol was by first-order kinetics (Peppas model) and the mechanism of release was by non-Fickian diffusion. The hydrodynamic parameters of the packed bed of tuberose flowers were characterized by using an empirical correlation developed from dimensionless Reynolds, Schmidt and Sherwood numbers. High correlations (r=0.99 in steady state and r=0.98 in unsteady state) of these empirical equations suggest that the extraction process was satisfactorily modeled. These modeling aspects would find use in pilot plant and commercial scale extractions of methyl eugenol from floral matrices.

This study is expected to benefit the bioprocess industries at large who may find an alternative use of tuberose flowers as a source of methyl eugenol. SC-CO_2 extraction of both fresh flowers and which have lost their ornamental appeal (owing to drying) may be conducted to obtain methyl eugenol. This would enable complete commercial utilization of tuberose flowers.

ACKNOWLEDGEMENT

The first author acknowledges DST-INSPIRE (IF: 131031, JRF-P), Government of India, for providing financial support for the study.

NOMENCLATURE

a : specific interfacial area of tuberose cut florets for mass transfer (cm^2cm^{-3} of particle) (SI unit: 1 m^2m^{-3}=0.01 cm^2cm^{-3})
C : concentration of methyl eugenol in supercritical fluid phase at time t (expressed as % of total methyl eugenol)
C* : saturated or equilibrium concentration of methyl eugenol (expressed as % of total methyl eugenol)
D : molecular diffusivity of methyl eugenol in SC-CO_2 (cm^2s^{-1}) (SI unit: 1 m^2s^{-1}=10,000 cm^2s^{-1})

$d_{eq.}$: equivalent channel diameter [mm]
ΔE_v : cohesive energies (cal mol^{-1}) (SI unit: 1 J mol^{-1}=0.2390 cal/mol)
F : empirical constant in density correlation
F_0 : Fourier's number
G : empirical constant in density correlation
K_{sl} : operating run particle to fluid phase mass transfer coefficient (cms^{-1}) (SI unit: 1 ms^{-1}=100 cms^{-1})
k : association constant related to the total number of molecules in the complex
$m(\theta)$: amount of methyl eugenol remaining in the flowers (for unsteady state) (mg) (SI unit: 1 g=1,000 mg)
m_0 : total methyl eugenol in the flowers [mg]
r : correlation coefficient
Re : Reynolds number [$d_{eq}\rho u/\mu$]
R_f : retardation factor
S : solubility of solute in the gas phase [g kg^{-1}]
Sc : Schmidt number [$\mu/\rho D$]
Sh : Sherwood number [$K_{sl}d_{eq.}/D$]
T : temperature [^oC]
t : time [min]
V : volume (cm^3) (SI unit: 1 m^3=1,000,000 cm^3)
$\Sigma_i(\Delta V_i)$: summation of molar volumes of the contributing groups (cm^3/mol) (SI unit: m^3 mol^{-1} 1,000,000 cm^3 mol^{-1})
Y : yield of methyl eugenol [mg (100 g dry tuberose flowers)$^{-1}$]

Greek Letters

δ : solubility parameter (cal $cm^{-3})^{1/2}$ [SI unit: $MPa^{1/2}$=2.0455 (cal $cm^{-3})^{1/2}$]
ε : void fraction of the extractor
μ : viscosity of SC-CO_2 (Poise=g$cm^{-1}s^{-1}$) (SI unit: 1 Pa·s=10 Poise)
ρ : density [kg m^{-3}]
θ : extraction time (min) in unsteady state

Subscripts

eq. : equivalent
f : factor

SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

REFERENCES

1. P. K. Ghosh, P. Bhattacharjee and S. Das, *Int. J. Pharm. Sci. Res.*, **5**, 1279 (2014).
2. A. K. Anand, M. Mohan, S. Z. Haider and A. Sharma, *Int. J. Pharm. Sci.*, **3**, 223 (2011).
3. B. Nabiha, E. Abdelfateh, K. Faten, W. J. Paul, M. Michel and C. M. Moncef, *J. Essent. Oil Bear. Pl.*, **12**, 694 (2009).
4. R. K. Joshi, *Indian J. Pharm. Sci.*, **75**, 457 (2013).
5. F. Ayci, M. Aydinli, Ö. A. Bozdemir and M. Tutaş, *Flavour Frag. J.*, **20**, 481 (2005).
6. N. T. P. *Reports on Carcinogens*, **11**, 153, PMID: 15326674 (2002).
7. M. Mukhopadhyay, *Fundamentals of supercritical fluids and phase equilibria*, Natural Extracts using Supercritical Carbon dioxide, CRC Press, Florida, USA (2000).
8. I. Žižvoić, M. Staminić, A. Orlović and D. Skala, *J. Supercrit. Fluids*, **39**, 338 (2007).
9. S. M. Ghoresihi and E. Bataghva, *Korean J. Chem. Eng.*, **31**, 1632 (2014).
10. E. Reverchon, J. Daghero, C. Marrone, M. Mattea and M. Poletto, *Ind. Eng. Chem. Res.*, **38**, 3069 (1999).
11. A. O. A. C.: AOAC method 967.19, in: Helrich, K. Ed., Official Methods of Analysis of AOAC International, 15th Ed., AOAC International, Arlington, VA, USA (1990).
12. P. B. Gomes, V. G. Mata and A. E. Rodrigues, *J. Supercrit. Fluids*, **41**, 50 (2007).
13. W. L. McCabe, J. C. Smith and P. Harriot, *Flow past immersed objects,* Unit Operations of Chemical Engineering, 7th Ed., McGraw Hill, Ohio, USA (2005).
14. D. Chatterjee and P. Bhattacharjee, *Food Bioprocess Technol.*, **6**, 2587 (2013).
15. G. F. Silva, F. M. C. Gamarra, A. L. Oliveira and F. A. Cabral, *Braz. J. Chem. Eng.*, **25**, 419 (2008).
16. P. Bhattacharjee, D. Chatterjee and R. S. Singhal, *Food Bioprocess Technol.*, **5**, 2506 (2012).
17. J. H. Hildebrand and R. L. Scott, The Solubility of Non electrolytes, 3rd Ed., Dover, New York, USA (1950).
18. R. F. Fedors, *Polymer Eng. Sci.*, **14**, 147 (1974).
19. M. G. Sajilata, M. V. Bule, P. Chavan, R. S. Singhal and M. Y. Kamat, *Sep. Purif. Technol.*, **71**, 173 (2010).
20. J. W. King, *LWT-Food Science Technol.*, **28**, 190 (1995).
21. R. J. Reid, J. M. Prausnitz and B. E. Poling, *Pure component constants*, The Properties of Gases and Liquids, 4th Ed., Mc-Graw-Hill, New York, USA (1987).
22. D. Y. Peng and D. B. Robinson, *Ind. Eng. Chem. Fund.*, **15**, 59 (1976).
23. S. Jafari Nejad, H. Abolghasemi, M. A. Moosavian and M. G. Maragheh, *Chem. Eng. Res. Des.*, **88**, 893 (2010).
24. S. Ismadji and S. K. Bhatia, *J. Supercrit. Fluids*, **27**, 1 (2003).
25. O. J. Catchpole and J. C. Von Kamp, *Ind. Eng. Chem. Res.*, **36**, 3762 (1997).
26. D. Westerman, R. C. D. Santos, J. A. Bosley, J. S. Rogers and B. Al-Duri, *J. Supercrit. Fluids*, **37**, 38 (2006).
27. S. Song, Z. Wang, Y. Qian, L. Zhang and E. Luo, *J. Agric. Food Chem.*, **60**, 4388 (2012).
28. H. Sovová, R. Komers, J. Kucera and J. Jez, *Chem. Eng. Sci.*, **49**, 2499 (1994).
29. E. Reverchon, *J. Supercrit. Fluids*, **10**, 1 (1997).
30. A. Martin and M. J. Cocero, *J. Supercrit. Fluids*, **39**, 304 (2007).
31. C. S. Tan, S. K. Liang and D. C. Liou, *Chem. Eng. J.*, **38**, 17 (1988).
32. P. Kotnik, M. Skerjet and Z. Knez, *J. Supercrit. Fluids*, **43**, 192 (2007).
33. I. K. Hong, S. W. Rho, K. S. Lee and K. P. Yoo, *Korean J. Chem. Eng.*, **7**, 40 (1990).
34. D. C. Montgomery, *Experiments with a single factor: the analysis of variance*, Design and Analysis of Experiments, John Wiley and Sons, New York, USA (2001).
35. D. C. Montgomery, *Response surface methods and other approaches to process optimization*, Design and Analysis of Experiments, John

Wiley and Sons New York, USA (2001).
36. Y. Ge, Y. Ni, H. Yan, Y. Chen and T. Cai, *J. Food Sci.*, **67**, 239 (2002).
37. H. Sovová, *J. Supercrit. Fluids*, **66**, 73 (2012).
38. L. J. Cseke and P. B. Kaufman, *How and why these compounds are synthesized by plants*, Natural Products from Plants. In: L. J. Cseke, C. R. Lu, A. Kornfield, P. B. Kaufman and Kirakosyan, A. Eds., CRC Press, Florida, USA (2006).
39. M. Stamenić, I. Zizović, A. Orlović and D. Skala, *J. Supercrit. Fluids*, **46**, 285 (2008).
40. R. W. Korsmeyer, R. Gurny, E. Doelker, P. Buri and N. A. Peppas, *Int. J. Pharm.*, **15**, 25 (1983).
41. M. E. Paulaitis, V. J. Krukonis, Y. Kurnik and R. C. Reid, *Rev. Chem. Eng.*, **1**, 179 (1983).
42. F. Stuber, A. Ma, V. Ma, A. Larrayoz and F. Recasens, *Ind. Eng. Chem. Res.*, **35**, 3618 (1996).
43. I. Norhuda and A. K. Mohd Omar, *Int. J. Chem. Bio. Eng.*, **2**, 10 (2009).