

ABSTRACT

AGUDA, REMIL MARTINEZ. Modeling the Solubility of Sclareol in Organic Solvents using Solubility Parameters. (Under the direction of Dr. Peter K. Kilpatrick and Dr. Ruben G. Carbonell.)

This study aimed to obtain the solubility of sclareol, a nutraceutical, in a set of organic solvents and to correlate the experimental data using the Hansen solubility parameter approach. Solubility is an important physical property for designing an extraction process for nutraceuticals, using Generally Recognized as Safe (GRAS) solvents. The solubility of sclareol in a wide variety of organic solvents was measured at 25 ° C by gas chromatography. The extended Hansen solubility parameter equation was used to correlate the experimental data. The model was able to correlate the solubility in the individual solvents at 25 ° C with an average % error up to 110 %, which is comparable with similar systems in the literature.

The temperature dependence of solubility of sclareol in selected GRAS ethyl ester solvents was also measured. However, the model was unable to provide a good fit of the experimental data of sclareol in these solvents over a range of temperatures. The predictive capability of the model decreased as the Hansen solubility parameter of the solvent deviates from the solute.

**MODELING THE SOLUBILITY OF SCLAREOL
IN ORGANIC SOLVENTS USING SOLUBILITY PARAMETERS**

by

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BIOGRAPHY

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1 Introduction

1.1 Motivation of the research

Recently, due to rapidly rising health costs and epidemics in Type II adult onset diabetes and other obesity-related ailments such as cardiovascular disease, the media has strongly promoted healthy lifestyles, such as a balanced diet and exercise, to prevent disease. Part of the preventive medicine health regimen is to take vitamins and other nutraceuticals [1].

Nutraceuticals are bioactive components, found in natural sources and typically in low concentrations, that provide health benefits [2]. Some nutraceuticals are found naturally in plants, which are unfit for human consumption. Some nutraceuticals are also found in food. However, multiple servings of fresh fruits and vegetables are oftentimes needed to meet the recommended amount of a particular nutraceutical and this is often difficult to achieve. That is why nutraceuticals are extracted, concentrated, packaged into capsules, and then labeled as health supplements in the market. The demand for nutraceuticals has grown substantially in recent years [3].

The nutraceuticals market had global sales of 77 billion dollars in 2004 and projected sales reaching 103 billion dollars by 2009 [4;5]. In North Carolina, there are nutraceutical companies, such as Avoca, Inc. (Merry Hills, NC), PhytoMyco Research Corporation (Greenville, NC), Daily Manufacturing Inc. (Rockwell, NC) and Gaia Nutraceuticals (Brevard, NC), which grow herbs, extract nutraceuticals from herbs and sell

these extracts as health supplements. These companies sell the nutraceuticals shown in Table 1.1

Table 1.1 Some nutraceuticals and their potential health benefits

Nutraceutical	Health benefits supported by clinical studies [1]
Coenzyme Q10	boosts immune system lowers blood pressure
Lutein and Zeaxanthin	reduces risk of cataracts and macular degeneration in the eyes
Anthocyanins	antioxidant; prevents and combats cancer
Tocopherol	reduces risk of coronary heart disease promotes healthy gastrointestinal tract
Catechin	anticarcinogenic; anti-atherosclerotic; antimicrobial; antioxidant
Beta carotene	antioxidant; anti-carcinogenic
Resveratrol	protection of cardiovascular system stimulates detoxification chemopreventive agent against melanoma and leukemia cells protection of eyes from macular degeneration

The most commonly used solvent for extraction of nutraceuticals from plants is hexane, despite its inefficiency and toxicity. Various means for enhancing nutraceuticals extraction have been developed, including ultrasound, microwaves, and supercritical fluids. These methods shorten the extraction time, decrease the solvent consumption, increase the extraction yield, and enhance the quality of extracts [6].

Generally recognized as safe (GRAS) solvents are non-toxic and environmentally benign solvents for nutraceutical extraction. In this study, GRAS alkyl alkyl ester solvents

are considered as extraction solvents for nutraceuticals. These are synthesized from carboxylic acids (acetic acid, lactic acid, etc.) and alcohols (ethanol, isopropanol, etc.). The alkyl substituents of these esters can be manipulated to a desired polarity and hydrophobicity to dissolve a targeted nutraceutical.

However, physical properties and solubility data of nutraceuticals in GRAS solvents are needed for designing extraction processes for nutraceuticals.

1.2 Research objectives

To measure the solubility of sclareol in organic solvents

To apply the extended Hansen solubility parameter approach, that has been developed by other studies, on correlating experimental solubility data

To utilize the extended Hansen solubility parameter approach, to correlate the experimental data on the solubility of sclareol, in GRAS ester solvents over a range of temperatures

1.3 Overview of thesis

This study aimed to correlate the solubility of a chosen nutraceutical solid in a set of organic liquids, including GRAS ester solvents. Sclareol was chosen as a model nutraceutical. We chose a thermodynamic model based on the solubility parameter.

The chapter on literature review presented the extraction solvents used for extracting sclareol; and GRAS solvents, which can be used for nutraceutical extraction. The solid-liquid equilibria model was introduced and the solubility parameter approach was selected. The extended Hansen solubility parameter model was chosen for our system because it was implemented in correlating solubility of polar organic pharmaceutical solutes in organic solvents. Hansen solubility parameters can also be extended to conditions above ambient temperature and pressure, which can be applied to solubility modeling.

In the experimental section, the solubility of sclareol in a set of organic liquids with a wide range of polarities was gathered at 25 ° C. The solubility of sclareol in GRAS ethyl ester solvents was determined from 25 to 45 ° C.

In the results and discussion chapter, the physical properties of sclareol were gathered in relation to implementing the extended Hansen solubility parameter model. The Hansen solubility parameters (HSPs) of sclareol were regressed from the solubility data. The regression results are then applied to correlate the solubility of sclareol at 25 ° C and the temperature dependence of sclareol in the selected ester solvents.

Initially, the intention of using this thermodynamic model is to correlate the solubility of sclareol in a GRAS ester solvent, ethyl lactate, and CO₂ at conditions above ambient temperature and pressure. This experimental solubility of sclareol in CO₂-expanded ethyl

lactate was generated by Xenia Tombokan, a NCSU doctoral student. However, using the Hansen solubility parameter approach to model this three-component system was not pursued because (1) the calculated solubility in ethyl lactate over a range of temperatures was unsatisfactory, and (2) as far as literature review has revealed, there has been no study done on the modeling of an organic solute in dense CO₂-organic solvent mixtures using the extended Hansen solubility parameter approach, which could be a basis for comparison.

This thesis presents the equilibrium conditions, which suggests the maximum amount of sclareol that can be dissolved using organic solvents. Overall, the results, gathered from the experiment and the model, would serve as a guide for choosing a GRAS extraction solvent for sclareol.

1.4 References

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2 Literature Review

2.1 Studies on extraction of sclareol

In this study, sclareol was chosen as a model nutraceutical. It has antioxidant [1], antifungal [2], antimicrobial [3] and antidepressant [4,5] properties. It is characterized as a biologically active molecule, due to its cytotoxic and cytostatic effects against human leukemic cell lines [6]. The chemical structure of sclareol is shown in Figure 2.1

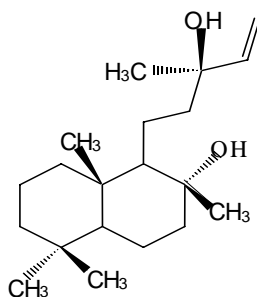


Figure 2.1 Chemical structure of sclareol (from Sigma Aldrich USA website)

Avoca, Inc. (Merry Hills, NC) manufactures sclareol from the Clary sage plant (*Salvia sclarea*) using hexane as the extracting solvent with its patented process [7]. The Clary sage plant is deposited into an extractor using hexane as the solvent. The extract is concentrated by passing it through a falling film evaporator, after which it is filtered to remove any debris and precipitated waxes. This concentrated extract is then subjected to another extractor using a polar solvent (such as a mixture of methanol and water) to selectively remove sclareol from the mixed extract. Sclareol is then further purified by recrystallization from hexane.

Several studies on the extraction of sclareol from Clary sage (*Salvia sclarea*) have been completed using organic solvents and supercritical/liquid CO₂. In studies on the

extraction of sclareol from Clary sage, several organic solvents have been used: petroleum ether [8], ethylene glycol [9], ethanol, methanol, isopropanol, acetone, slaked lime (in ethanol) and diethyl ether [10]. Liu and coworkers [11] investigated the extraction of steam distillation residue of *Salvia sclarea* using supercritical CO₂. These researchers obtained an optimal yield of 40 % at a temperature of 30° C, a pressure of 5000 psia and a flowrate of 5 liters/hour. Ronyai and coworkers [12] produced extracts from *Salvia officinalis* using supercritical fluid extractor (SFE) with 2-stage separators, which produced higher sclareol yields than steam distillation. In these studies, extracts contain sclareol, linalyl acetate and linalool as the principal components. Paraffins and wax esters were also identified as minor components of the extracts.

2.2 GRAS Solvents for nutraceutical extraction

Generally Recognized as Safe (GRAS) solvents, which are safe to use in food, are considered as alternative extraction solvents to hexane and other toxic organic solvents. Some GRAS solvents, which can be used for nutraceuticals extraction, are shown in Table 2.1. This list of solvents is gathered from publications by the Food and Drug Administration (FDA) and the Flavor and Extract Manufacturing Association (FEMA). The FDA lists solvents, which are safe to use as food additives, as established by scientific procedures and the substance's common use in food [13]. FEMA is a panel of industry experts on flavors and extracts which has affirmed 1900 GRAS flavoring substances, published in technical journals [14-16] and food industry handbooks [17].

Since nutraceuticals become ingredients in dietary supplements and nutritional health drinks, GRAS solvents can be used to extract these nutraceuticals with the benefit of being safe as GRAS food additives.

Table 2.1 List of some GRAS solvents that can be used for nutraceutical extraction

Solvent	Common Uses in Food [17]
Acetic acid	curing and pickling agent, flavor enhancer, flavoring agent
Anisole	flavoring agent
Butyl butyrate	flavoring agent
1,3-butylene glycol	extraction of natural and synthetic flavoring
Ethanol	beverage
Ethyl acetate	decaffeination of coffee and tea
Ethyl benzoate	flavoring agent
Ethyl butyrate	flavoring agent
Ethyl decanoate	flavoring agent
Ethyl formate	flavoring agent
Ethyl hexanoate	flavoring agent
Ethyl lactate	flavoring agent
Ethylene dichloride	extraction of oleoresins from spice
Glycerin	solvent for flavoring agents, emulsifier
Glyceryl monooleate	flavoring agent, additive in non-alcoholic beverages
Glyceryl palmitostearate	formulations use in tablets
Isoamyl acetate	flavoring agent
Isobutyl acetate	flavoring agent
Isopropyl acetate	flavoring agent
Isopropyl alcohol	extraction of hops and spice
Isopropyl citrate	solvent for extraction, flavor enhancer, acidity regulator
Lactic acid	solvent, flavor enhancer, anti-microbial agent, acidity regulator
Linoleic acid	flavoring agent, dietary supplement for heart health
Methyl acetate	flavoring agent
Octanoic Acid	flavoring agent
Propionic acid	flavoring agent, anti-microbial agent, preservative
Propyl acetate	flavoring agent
Stearic acid	naturally found in cooking oil
Water	beverage
Ethyl vanillin	flavoring agent
Limonene	flavoring agent

In this study, GRAS ester solvents are considered as extraction solvents for nutraceuticals. These are synthesized from carboxylic acids and alcohols. The parent alcohols and carboxylic acid of these esters can be manipulated to a desired polarity and hydrophobicity to dissolve a targeted nutraceutical, such as sclareol.

Dense CO₂ is also considered as a GRAS extracting solvent. CO₂ at high pressure has been used for extraction of a wide variety of natural products from plants [18]. CO₂ pressure and/or temperature can be manipulated to achieve a density close to those of an organic extraction liquid to solubilize a solid. Studies on bench-scale nutraceutical separations using supercritical CO₂ have been completed [18-20]. The critical temperature of CO₂ (31 °C) makes it a moderate condition for the extraction of thermally labile compounds, which is characteristic of most nutraceutical compounds.

2.3 Solid-liquid equilibria models using solubility parameters

Solubility is the basic information needed for an extraction process. A thermodynamic model can be used in fitting the experimental solubility data, checking the consistency of the data and predicting trends outside the range of the data.

The following equation relates solubility of a solid in a liquid [21].

$$\ln \gamma_2 x_2 = \frac{\Delta H_{fusion}}{R} \left(\frac{1}{T_{melting}} - \frac{1}{T} \right) \quad (1)$$

where

γ_2 is the activity coefficient of the solute

x_2 is the mole fraction of the solute

ΔH_{fusion} is the heat of fusion of the solute, J/mol

T_{melting} is the melting temperature, K

T is the temperature of the solution, K

R is the ideal gas constant = 8.314 J/mol-K

In this equation, the difference of the heat capacities of the solute and solvent are negligible.

When the solute and solvent are of the same polarity, the solution is ideal and activity coefficient is equal to unity, the solid-liquid solubility equation results in the ideal solubility

$$\ln x_2^{ideal} = \frac{\Delta H_{fusion}}{R} \left(\frac{1}{T_{melting}} - \frac{1}{T} \right) \quad (2)$$

The ideal solubility depends on the properties of the solute and is independent of the solvent's properties. The solute's properties are the heat of fusion, which is independent of temperature, ΔH_{fusion} , and melting temperature, $T_{melting}$. In the ideal solution, the difference in the heat capacities and molar volumes of the solute and solvent are negligible.

The activity coefficient accounts for the departure of the solution from ideality. This accounts for the difference in the chemical properties of the solute and solvent.

The activity coefficient can be determined using the solubility parameter.

The Hildebrand solubility parameter, δ , is a property of a pure substance evaluated from the energy of vaporization, E, and the molar volume, V :

$$\delta = \left(\frac{E}{V} \right)^{1/2} \quad (3)$$

E is evaluated from heat of vaporization, $\Delta H_{\text{vaporization}}$, for liquids: $E = \Delta H_{\text{vaporization}} - RT$ or for solids, by group contribution method (based on the chemical structure of the compound)

[22]. The heat of vaporization is assumed to be constant from 25 degrees Celsius to the boiling point of the substance. V is pressure and temperature dependent. δ is expressed in $\text{MPa}^{1/2}$ or $(\text{Cal}/\text{cm}^3)^{1/2}$ where $1 \text{ MPa}^{1/2} = 2.0455 (\text{Cal}/\text{cm}^3)^{1/2}$.

This relationship of the Hildebrand solubility parameter, δ and molar volume, V , was applied to the Regular Solution Theory (RST) in modeling experimental solubility data. In the RST, the general equation is

$$\ln x_2 = \frac{\Delta H_{\text{fusion}}}{R} \left(\frac{1}{T_{\text{melting}}} - \frac{1}{T} \right) - \frac{V_2 \phi_1^2 (\delta_1 - \delta_2)^2}{RT} \quad (4)$$

where

subscript 2 is for the solute and subscript 1 is for the solvent ,

V_2 = molar volume of solute as a subcooled liquid

ϕ_1 = volume fraction of the solvent, calculated as

$$\phi_1 = \frac{x_1 V_1}{x_2 V_2 + x_1 V_1} \quad (5)$$

In a regular solution, there is no entropy change when a small amount of its components is transferred to it from an ideal solution of the same composition and the total volume remains unchanged upon mixing.

The use of the Hildebrand solubility parameter in calculating for the solubility gives good predictions for non-polar solutes in non-polar solvents. According to a review of solubility studies for organic compounds by Grant and Higuchi [23], the RST cannot satisfactorily fit and predict solubility of polar solutes in polar solvents, which is the case for sclareol dissolved in esters and other polar organic solvents. The RST uses solubility

parameters, which captures the non-polar (London dispersion) interactions, not the polar and hydrogen bonding interactions of the molecules in the mixture. The Hansen solubility parameters account for these interactions.

Hansen [24] partitioned the Hildebrand solubility of a pure substance into contributions representing three types of forces: dispersion forces, δ_d , polarity, δ_p and hydrogen bonding, δ_h , with the following relationship:

$$\delta_t^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \quad (6)$$

where δ_t is the overall solubility parameter.

Hansen defines each solubility parameter as follows:

The dispersion solubility parameter, δ_d , describes the non-polar interactions between molecules. This type of interaction is common to all molecules and is caused by the temporarily induced dipoles in molecules. All molecules contain this type of interaction.

The polar solubility parameter, δ_p , expresses the interactions of the dipole-dipole and dipole-induced dipole in the interacting molecules. These are present in polar molecules.

The hydrogen bonding solubility parameter, δ_h , is generally called the electron exchange parameter. Hydrogen bonding is an attraction among molecules because of hydrogen bonds and resembles polar interactions. This also captures Lewis acid-base interactions, which are the electron-donor and electron-acceptor interactions between the solute and solvent. However, this is not only the hydrogen bonding interaction that occurs between molecules with a hydrogen atom bonded to an electronegative atom such as nitrogen, oxygen and fluorine, but also to other atoms.

The Hansen solubility parameters (HSPs) are gathered from experimental solubility data or correlations based on the chemical structure of the compound [22, 24] .

The extended Hansen solubility parameter equation for modeling of solubility of solid solutes in liquid solvents was proposed by Martin and coworkers [25-29] to correlate experimental solubility in a series of solvents and to predict the solubility in untested solvents.

The general form of the equation is

$$\left(\frac{RT}{V_2 \phi_1^2} \right) \left\{ \ln \frac{x_2^{ideal}}{x_2} \right\} = C_1(\delta_{d1} - \delta_{d2})^2 + C_2(\delta_{p1} - \delta_{p2})^2 + C_3(\delta_{h1} - \delta_{h2})^2 + C_0 \quad (7)$$

where
subscripts

1 – solvent

2 – solute

d - dispersion

p - polar

h – hydrogen bonding

R = ideal gas constant = 8.314 MPa cm³ /mol K

T = absolute temperature, K

V₂ = molar volume of solute as a subcooled liquid, cm³/mol

x₂ = experimental solubility of solute (mole fraction)

x₂^{ideal} = ideal solubility of solute (mole fraction), calculated from the heat of fusion, ΔH_{fusion} , and melting temperature, T_m, of the solute

$$\ln x_2^{ideal} = \frac{\Delta H_{fusion}}{R} \left(\frac{1}{T_{melting}} - \frac{1}{T} \right)$$

φ₁ = volume fraction of the solvent

$$\phi_1 = \frac{(1-x_2)V_1}{x_2V_2 + (1-x_2)V_1}$$

- δ_{d1} = solvent dispersion solubility parameter term, MPa^{1/2}
 δ_{p1} = solvent polar solubility parameter term, MPa^{1/2}
 δ_{h1} = solvent hydrogen bonding solubility parameter term, MPa^{1/2}
 δ_{d2} = solute dispersion solubility parameter term, MPa^{1/2}
 δ_{p2} = solute polar solubility parameter term, MPa^{1/2}
 δ_{h2} = solute hydrogen bonding solubility parameter term, MPa^{1/2}

$C_1, C_2, C_3, A, B, C, D$ = regression coefficients obtained from multiple linear regression
 C_o = arbitrary constant obtained by completing the square in equation 7

In the standard form in the above equation, the quadratic terms of the right hand side of the equation are expanded to give the multiple linear regression formula for the response in the left hand side:

$$\left(\frac{RT}{V_2\phi_1^2} \right) \left\{ \ln \frac{x_2^{ideal}}{x_2} \right\} = C_1\delta_{d1}^2 + C_2\delta_{p1}^2 + C_3\delta_{h1}^2 + A\delta_{d1} + B\delta_{p1} + C\delta_{h1} + D \quad (8)$$

where the matching terms show that, for example,

$$\begin{aligned}
 A &= -2C_1\delta_{d2} \\
 B &= -2C_2\delta_{p2} \\
 C &= -2C_3\delta_{h2}
 \end{aligned} \quad (9)$$

It then follows that the solubility parameters of the solute, δ_{d2} , δ_{p2} and δ_{h2} are ratios of the regression coefficients:

$$\begin{aligned}
\delta_{d2} &= \frac{-A}{2C_1} \\
\delta_{p2} &= \frac{-B}{2C_2} \\
\delta_{h2} &= \frac{-C}{2C_3}
\end{aligned}
\tag{10}$$

In order to estimate the Hansen solubility parameters of a solute, the multiple regression (by method of least squares) in equation 8 is first fitted to experimental data of a set of solubility data points.

Then the corresponding ratios of the fitted coefficients are calculated.

The last term of the right-hand side of equation 8 appears to be a free constant and is fitted as such to the experimental data.

$$C_0 = D - C_1\delta_{d2}^2 - C_2\delta_{p2}^2 - C_3\delta_{h2}^2 \tag{11}$$

From these constants, C_1 , C_2 , C_3 and C_0 , the solubility is backcalculated by iteration.

2.4 Studies on solubility modeling using solubility parameters

Several studies have used this model for studying solubility behavior of organic solutes, as shown in the Table 2.2. According to Martin and coworkers [26], the following conditions must apply for a regression procedure to be successful: (a) constant $C_0 < 1.0$ or 2.0, (b) regression equation must successfully predict solubilities of the solute in the solvent systems employed, and (c) regression must be obtained by a sufficient number of solvents (20 is good, 40 is much better) with the solubility parameters both below and above the

solubility parameters of the solute. The number of solvents suggests that more solubility data give a better chance of obtaining reasonably comparable solute solubility parameters with the known solubility parameters of other organic molecules, as the solubility data is regressed over a variety of solubility parameters of the solvents. The R^2 value account for the % variability of solubility that can be explained by the set of Hansen solubility parameters.

Table 2.2 Studies on extended Hansen solubility parameters for solubility of organic compounds

Compound	Source	Number of solvents	R ²	Regression Coefficients		Hansen Solubility Parameters, MPa ^{1/2}	
naphthalene	J. Pharm. Sci, Vol 70, No 11, 1981 page 1260-1264	24	0.9765	C ₁	1.05	dispersion	19.2
				C ₂	-0.31	polar	2.0
				C ₃	0.23	hydrogen bonding	3.9
				C ₀	0.05	overall	19.7
	J. Pharm. Sci Vol 71, No 11 , 1982 page 1285-1287	26	0.9860	C ₁	0.67	dispersion	20.6
				C ₂	-0.14	polar	4.0
				C ₃	0.13	hydrogen bonding	1.9
				C ₀	0.37	overall	21.1
		26	0.9800	C ₁ (fixed)	1.00	dispersion	19.2
				C ₂	-0.15	polar	4.2
				C ₃	0.13	hydrogen bonding	1.6
				C ₀	0.86	overall	19.7
benzoic acid	J. Pharm. Sci. Vol. 73, No 2, 1984 page 179-188	40	0.7100	C ₁	5.49	dispersion	17.3
				C ₂	0.47	polar	16.1
				C ₃	0.16	hydrogen bonding	4.2
				C ₀	-18.27	overall	24.0
sulfamethoxypyridazine	J.Pharm. Sci. Vol 78, No 7, 1989 page 567 - 573	30	0.8350	C ₁	-4.89	dispersion	15.6
				C ₂	0.29	polar	23.0
				C ₃	0.11	hydrogen bonding	7.3
				C ₀	-12.83	overall	28.7
temazepam	Int. J. Pharm. Vol 78, 1992, page 189-198	29	0.8990	C ₁	0.32	dispersion	35.1
				C ₂	0.17	polar	14.1
				C ₃	0.09	hydrogen bonding	7.2
				C ₀	-24.55	overall	38.5

The regression coefficients, C_1 , C_2 and C_3 can either be curve fitting parameters or descriptive constants for the molecular interaction. If these are considered as curve fitting parameters, then the model can only be applicable over the range of solvents in which the solute is tested. Hence, the coefficients do not give any physical meaning to the system under study.

On the other hand, the regression coefficients, C_1 , C_2 and C_3 are physically interpreted as approximately the fractions of the nearest neighbor solvent molecules where the solute molecule has the dispersion, polar and hydrogen bonding interactions. Then their values should be less than 1 and always positive. However, as shown in Table 2.2, not all the C_1 , C_2 , C_3 and C_0 values are positive and less than 1, which is a result of the multiple linear regression (by the method of least squares) but does not necessarily relate the physical interactions of the solute molecule with the neighboring solvent molecules.

But the C_1 value can be set to 1, which means that all the parts of the molecule experience London dispersion (non-polar) forces. In the case of naphthalene, when C_1 was fixed to 1, then from multiple linear regression, other coefficients changed yet C_2 became negative. For naphthalene, the extended Hansen method gave less than 100 % difference between the experimental and calculated solubility in individual solvents. Investigators [30], who have applied this model for pharmaceutical solutes in organic solvents, thought that naphthalene is a poor model for a drug because it lacks polar side chains and functional groups. Polar solutes would be a better solute model for pharmaceutical solutes and, likewise, for nutraceutical solutes.

The C_0 values are far from zero for the polar solutes such as benzoic acid, sulfamethoxypyridazine and temazepam. Martin and coworkers [28] thought that a high C_0 value would suggest that Hansen solubility parameters are not sufficient to capture and explain the interactions between solute and solvent. C_0 would also account for non-ideal entropy of mixing, which is due to the volume and size differences between the solute and solvent.

Modifications in the model has improved the correlation coefficient and lowered the % error between the experimental and calculated values. These were: (1) inclusion of the Flory-Huggins size correction term for non-ideal entropy of mixing, which accounts for the difference between the size of the solute and solvent molecules [31; 32]; (2) using a four solubility parameter approach ($\delta_d, \delta_p, \delta_a, \delta_b$) for solute molecules which are acids and bases. This approach involves the proton-donor parameter, δ_a and the proton-acceptor parameter, δ_b , instead of using the hydrogen bonding solubility parameter, δ_h [28].

In summary, the extended Hansen solubility approach is used to analyze the solubility data and to obtain the Hansen solubility parameters of the solute. Different approaches can be used in fitting the experimental solubility data to obtain regression equations, which aim to provide a reasonable prediction of solubility of the solute in untested solvents.

2.5 Studies on Hansen solubility parameter above ambient temperature and pressure

In extending the HSPs to higher temperatures and pressures, a thermodynamic framework on determining Hansen solubility parameters at high pressure and temperature for pure fluids was proposed by Williams and coworkers [33].

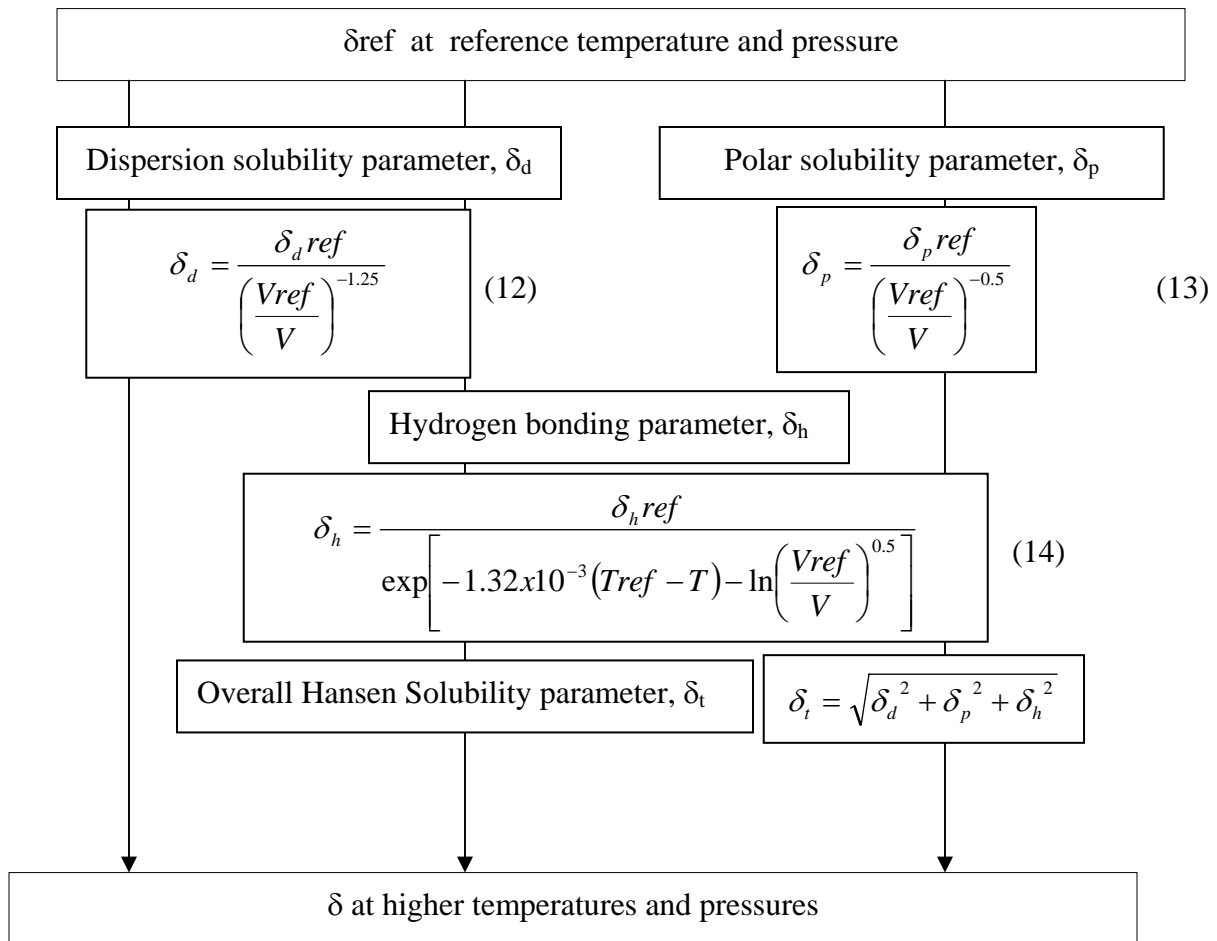


Figure 2.2 Extending Hansen solubility parameters above ambient temperature and pressure.

Equations 12, 13 and 14 calculate for the Hansen δ from the molar volume, V , as a function of temperature and pressure. Reference values for the solubility parameter, δ_{ref} , molar volume, V_{ref} , and temperature, T_{ref} , are applied to this model. These equations have been applied to CO_2 by Williams and coworkers [33] up to the supercritical region. They determined HSPs for CO_2 to see what liquid solvent HSPs are comparable with liquid/supercritical CO_2 for extraction purposes. Similar HSPs for CO_2 and liquid solvent means miscibility at a particular temperature and pressure (based on the principle of 'like dissolves like'). Over a range of temperature and pressure, similarity of the HSPs of CO_2 , with an organic solvent would indicate if an organic solvent can act as a cosolvent in an extraction process. A cosolvent enhances the solubility of a desired nutraceutical in dense CO_2 .

As far as the literature search has revealed, there has been no study on the use of the calculated HSPs using this approach at conditions above ambient temperature and pressure in mixtures of organic liquid + CO_2 or organic solute + organic solvent + CO_2 systems because equations 12, 13 and 14 in Figure 2.2, have been recently proposed.

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3 Experimental Section

3.1 Materials

Sclareol (> 95 % purity) from Sigma-Aldrich (St. Louis, MO), n-hexane, isohexane, ethanol, 1-propanol (99.9 % purity, HPLC Grade) from Sigma-Aldrich, toluene, acetonitrile, tetrahydrofuran, cyclohexane, methanol (99.9 % purity, HPLC Grade) from Fisher Scientific (Atlanta,GA), heptadecane, heptadecanol (99 % purity) from Fisher Scientific, ethyl-s-lactate, ethyl acetate, ethyl hexanoate (98 % purity) from Sigma-Aldrich, acetone, triethanolamine, formamide, decahydronaphthalene, N, N- dimethylformamide, dimethyl sulfoxide (99% purity) from Sigma-Aldrich, butyl butyrate (99.9 % purity) from Eastman and carbon dioxide (>99.99 % purity) from National Welders, were used without further purification. Decahydronaphthalene (DHN) is a mixture of 76 % trans-DHN and 24% cis-DHN. A Teflon membrane (with pore size of 0.45 μm , contained in a Whatman syringeless autovial) from Fisher Scientific was used for filtering the samples.

3.2 Gas chromatography analysis of sclareol

Gas chromatography (GC) has been widely used in the quantitative analysis of sclareol and natural product extracts containing sclareol [1-5]. GC analysis is the method of choice in identifying and quantifying sclareol in mixtures. Pure sclareol (95 % by GC) standard was obtained from Sigma Aldrich.

An Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a 20 meter DB-5HT column (0.32 mm diameter, 0.25 micron thickness, J & W Scientific) was used for analysis. 0.5 microliter of a solution containing the sample was analyzed using splitless injection and an injector temperature of 275°C. Flame ionization

detection was utilized with a detector temperature of 310°C. The column flow of helium carrier gas was set to a linear gas velocity of ~ 35 cm/sec. The initial oven temperature was 160°C, followed by a 4 °C/min temperature increase to 310°C as the final temperature. The oven temperature increase began upon injection and the final temperature was maintained for 30 minutes. Data were collected using the Perkin-Elmer Total Chrom 6.2 chromatographic analysis system. Quantitation of sclareol was done using the external standard method. Heptadecane and heptadecanol in toluene (52 mg/L and 56 mg/L, respectively) were used as internal standards to check reproducibility of the injection of the sample. This protocol is similar to the gas chromatographic method for the analysis of allelopathic natural products in rye (*Secale cereale L.*), developed by Danehower and Finney [6]. A sample chromatogram is shown in Figure 3.1 with a summary of the operating conditions.

In preparing the sclareol solution prior to GC analysis, 0.200 mL of the sclareol solution was combined with 1.0 mL of the internal standard, vortexed for about 10 seconds and transferred into 2 mL autosampler vials containing 250- μ L sample vial inserts. In the analysis of pure sclareol samples, no derivatization of sclareol solution was done. A calibration curve for each solvent was prepared.

The calibration curve is the basis for determining the concentration (in g/L) of sclareol in an unknown sample (refer to Figure 3.2). The peak area of the sample calculated based upon the formula of the standard curve. The R^2 values typically are in the range of 0.90 to 0.99. A blank run was included in the analysis of each batch of samples. The blank consisted of the pure solvent and the internal standard.

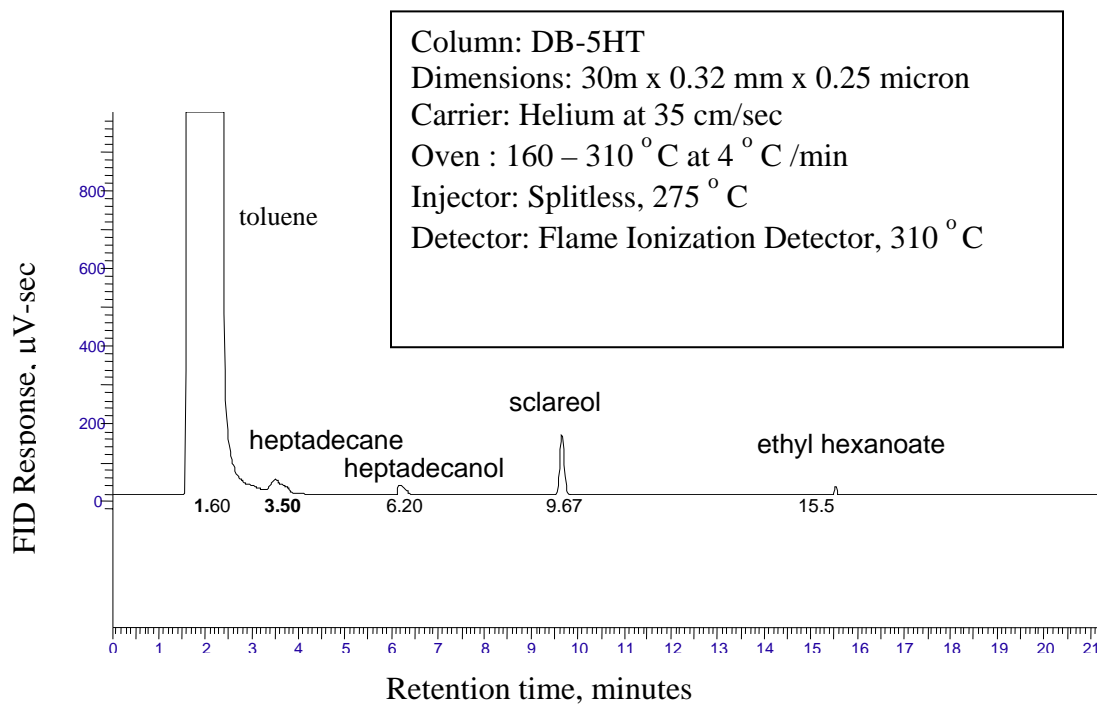


Figure 3.1 Sample chromatogram of sclareol in ethyl hexanoate with internal standards, heptadecanol and heptadecane. The gas chromatography operating conditions are shown.

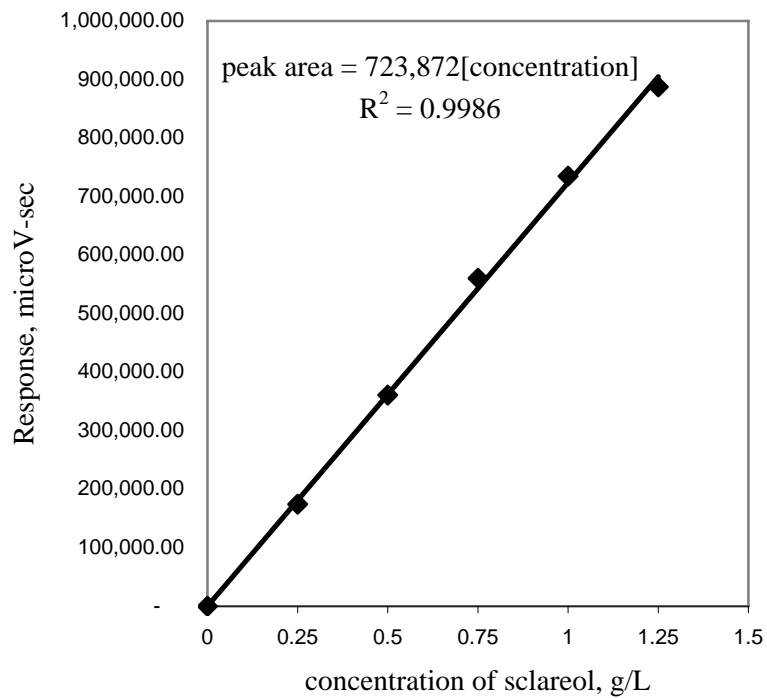


Figure 3.2 Sample calibration curve of sclareol in ethyl lactate. The concentration of an unknown is interpolated on the graph from its flame ionization (FID) response, also known as peak area.

3.3 Solubility of sclareol in organic solvents at 25 ° C

A slurry of the organic solvent and sclareol is stirred for a day at room temperature (25 ± 2 ° C), filtered, diluted and analyzed by gas chromatography (GC).

3.4 Temperature dependence of solubility of sclareol in selected ester solvents

To determine the solubility of sclareol in a variety of ethyl ester solvents as a function of temperature, ethyl lactate, ethyl acetate and ethyl hexanoate were chosen. These ethyl esters are GRAS. These solvents show separate peaks from sclareol in the gas chromatograms, which is necessary for quantifying the solution composition. Ethyl lactate and ethyl acetate have been used with dense carbon dioxide for nutraceutical extraction [7, 8]. Ethyl hexanoate was chosen to see the effect of increasing the hydrocarbon chain length of the ethyl ester solvent on the solubility of sclareol.

Mixtures of sclareol in the ethyl ester were prepared at different initial concentrations. The mixtures were stirred for 4 hours in an oven maintained at the desired temperature (accurate up to ± 2 ° C), filtered, diluted and analyzed by gas chromatography (GC). From the plot of the concentration of filtered mixture of sclareol against the initial concentration of the sclareol-ester mixture (with undissolved solids), the concentration of the filtered mixture reached a constant value, which is assumed to be the saturation concentration.

3.5 References

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4 Results and Discussion

4.1 Physical properties of sclareol

For implementing the thermodynamic model for solubility, the physical properties of sclareol were needed. Table 4.1 presents the physical properties of sclareol. The heat of fusion and melting temperature of sclareol was measured using differential scanning calorimetry (Model Q100, TA Instruments, New Castle, DE). The density of sclareol was determined experimentally using a helium-displacement pycnometer (Micrometrics AccuPyc 1330, Micrometrics, Norcross, GA).

Table 4.1 Physical properties of sclareol

Physical property		Source
Melting temperature, T_{melting}	103.3 °C	Differential scanning calorimetry
Heat of fusion, ΔH_{fusion}	28.7 kJ/mol	Differential scanning calorimetry
Density at room temperature, T	1.058 g/cm ³	Gas pycnometry
Molar volume at room temperature, V_S	291.6 cm ³ /mol	Gas pycnometry
Molar volume as a subcooled liquid, V_L	337.8 cm ³ /mol	Goodman and Rackett equation
Critical temperature, T_C	845.5 K	Group contribution method
Critical pressure, P_C	1.58 MPa	Group contribution method
Rackett parameter, Z_{RA}	0.806	Rackett equation

The critical pressure and temperature of sclareol were estimated using group contribution method (Joback method). The Rackett parameter was backcalculated from the saturated liquid density and critical properties. The density of the sclareol as a subcooled liquid was calculated from the density at the melting temperature. The Goodman equation and the Rackett equation calculate the molar volume of the solute as a subcooled liquid.

The Goodman equation [1] relates the density of an organic solid with the density of the liquid at the triple point

$$\rho_s(T) = \left(1.28 - 0.16 \frac{T}{T_t} \right) \rho_L(T_t) \quad (15)$$

where ρ_s is the solid density at temperature, T and the ρ_L is the liquid density at the triple point, T_t . In most cases, the triple point is very close to the normal melting temperature. Thus, substitution of the melting temperature in place of the triple point temperature is an assumption in the calculation.

Then the saturated liquid density at 25 °C is calculated using Rackett equation [2]

$$\log \rho_L = \log \left(\frac{P_c}{RT_c} \right) - \left[1 + (1 - T_r)^{2/7} \right] \log Z_{RA} \quad (16)$$

where the ρ_L is the saturated liquid density, P_c is the critical pressure, T_c is the critical temperature, $T_r = T/T_c$, which is the reduced temperature and Z_{RA} is the Rackett parameter, a correlating parameter unique to each compound. Thus, the molar volume of sclareol as a subcooled liquid at 25 °C is 337.8 cm³/mol.

4.2 Solubility of sclareol in organic solvents

The solubility of sclareol in various organic solvents were determined at 25 °C as shown in Figure 4.1. Chemical structures of the tested solvents are in Figure 4.2. Solubility values have less than 10 % relative standard deviation. The solubility in ethanol was confirmed by repeating the solubility measurement – the old data point was 0.0218 while the new data point was 0.0222. The highest solubility of sclareol is in butyl butyrate and the lowest solubility is in formamide. Of these 19 solvents tested, the GRAS solvents are ethanol, ethyl acetate, ethyl lactate, and ethyl hexanoate and butyl butyrate.

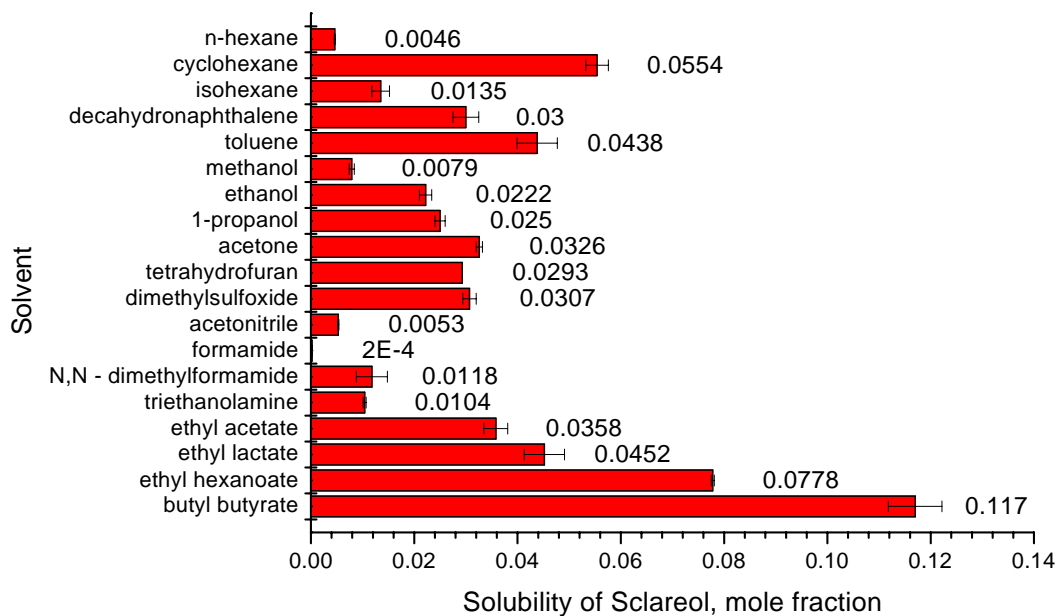
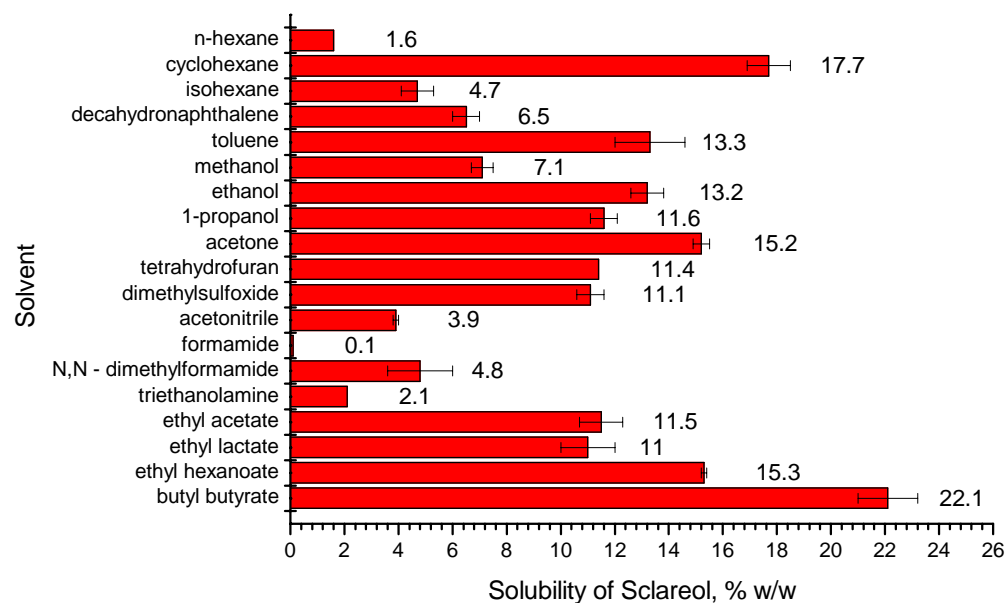


Figure 4.1 Solubility of sclareol in organic solvents at 25 °C

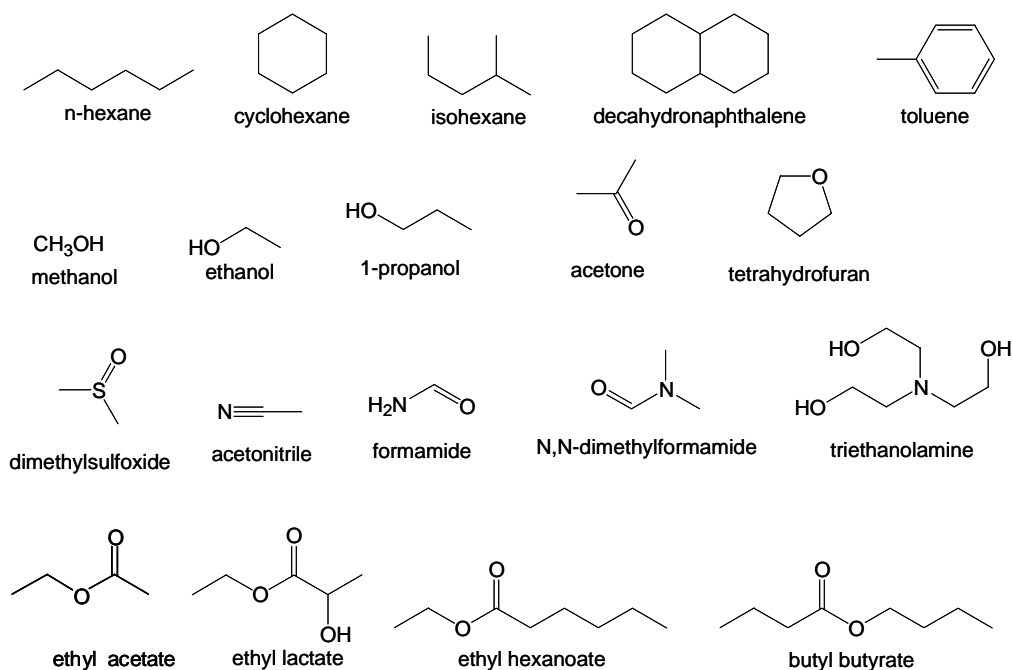


Figure 4.2 Chemical structures of solvents

Table 4.2 shows the solvents' Hansen solubility parameters (HSPs), from a handbook of solubility parameters by Charles Hansen [3]. The HSPs of isohexane and ethyl hexanoate are not available in the literature. The solvents were chosen because of the wide range of HSPs. Hydrocarbons (solvent number 1-5) only have dispersion parameters (δ_d). The alcohols (solvent number 6 – 8, 15) have the highest hydrogen bonding parameter. In this set, triethanolamine, a hydroxyl group containing amine, has the highest hydrogen bonding solubility parameter. The nitrogen-containing and sulfur-containing compounds have the

highest polar solubility parameters (solvent number 11-15). Formamide has the highest polar parameter among all the solvents. Other solvents such as acetone (a ketone, solvent number 9) and tetrahydrofuran (an ether, solvent number 10) were also considered. Alkyl esters (solvent number 16-19) were also tested. The Hansen solubility parameters (HSPs) for decahydronaphthalene (DHN) were calculated from the mole fraction weighted values of the mixture of its isomers (76 % trans-DHN and 24 % cis-DHN). The dispersion solubility parameters of trans-DHN and cis-DHN are 18.0 and 18.8 MPa^{1/2}, respectively. The polar and hydrogen bonding parameters of trans-DHN and cis-DHN are both 0.0 MPa^{1/2}.

Table 4.2 Hansen solubility parameters of solvents tested for sclareol

Solvent number	Solvent	Hansen solubility parameters				
		V ₂ molar volume cm ³ /mol	δ _d dispersion MPa ^{1/2}	δ _p polar MPa ^{1/2}	δ _h hydrogen bonding MPa ^{1/2}	δ _t overall MPa ^{1/2}
1	n-hexane	131.6	14.9	0.0	0.0	14.9
2	cyclohexane	108.7	16.8	0.0	0.2	16.8
3	isohexane	131.9	NA	NA	NA	NA
4	decahydronaphthalene	156.9	18.2	0.0	0.0	18.2
5	toluene	106.8	18.0	1.4	2.0	18.2
6	methanol	40.7	15.1	12.3	22.3	29.6
7	ethanol	58.5	15.8	8.8	19.4	26.5
8	1-propanol	75.2	16.0	6.8	17.4	24.6
9	acetone	74.0	15.5	10.4	7.0	19.9
10	tetrahydrofuran	81.7	16.8	5.7	8.0	19.5
11	dimethylsulfoxide	71.3	18.4	16.4	10.2	26.7
12	acetonitrile	52.6	15.3	18.0	6.1	24.4
13	formamide	39.8	17.2	26.2	19.0	36.7
14	N, N - dimethylformamide	77.0	17.4	13.7	11.3	24.9
15	triethanolamine	133.2	17.3	22.4	23.3	36.7
16	ethyl acetate	98.5	15.8	5.3	7.2	18.2
18	ethyl hexanoate	166.5	NA	NA	NA	NA
19	butyl butyrate	166.7	15.6	2.9	5.6	16.8

* NA : not available

In the regression, solvent pairs can segregate the effect of one type of solubility parameter for obtaining the HSPs of sclareol.

Table 4.3 Solvent pairs which have similar Hansen solubility parameters

	Solvent	Hansen solubility parameters			Solubility of sclareol mole fraction
		δ_d dispersion MPa ^{1/2}	δ_p polar MPa ^{1/2}	δ_h hydrogen bonding MPa ^{1/2}	
similar δ_p and δ_h	decahydronaphthalene	18.2	0.0	0.0	0.0300
different δ_d	n-hexane	14.9	0.0	0.0	0.0046
	triethanolamine	17.3	22.4	23.3	0.0104
similar δ_d and δ_h	methanol	15.1	12.3	22.3	0.0079
different δ_p	formamide	17.2	26.2	19.0	0.0002
	1-propanol	16.0	6.8	17.4	0.0250
	acetonitrile	15.3	18.0	6.1	0.0053
	butyl butyrate	15.6	2.9	5.6	0.1170
similar δ_d and δ_p	acetone	15.5	10.4	7.0	0.0326
different δ_h	ethanol	15.8	8.8	19.4	0.0222
	ethanol	15.8	8.8	19.4	0.0222
	ethyl lactate	16.0	7.6	12.5	0.0452
	ethyl lactate	16.0	7.6	12.5	0.0452
	1-propanol	16.0	6.8	17.4	0.0250

The hydrocarbons, the n-hexane and decahydronaphthalene only have δ_d , which isolates the effect of the dispersion parameter on the regression. The dispersion parameter and hydrogen bonding parameter of the solvent pairs: formamide and 1-propanol; and acetonitrile and butyl butyrate are close to each other. These pairs have different polar parameters, which isolated the effect of the polar parameters. These solvent pairs have closely similar δ_d and δ_p but different δ_h values: acetone and ethanol, ethanol and ethyl lactate, ethyl lactate and 1-propanol. These solvent pairs have different hydrogen bonding parameters, which isolated the effect of the hydrogen bonding parameter.

The solubility values between these solvent pairs show that similarity between any of the two Hansen solubility parameters does not mean close values in the experimental solubility. The difference in one solubility parameter would account for the difference between experimental solubility.

The results from multiple linear regression, using equation 8, are shown in Table 4.4.

$$\left(\frac{RT}{V_2 \phi_1^2} \right) \left\{ \ln \frac{x_2^{ideal}}{x_2} \right\} = C_1 \delta_{d1}^2 + C_2 \delta_{p1}^2 + C_3 \delta_{h1}^2 + A \delta_{d1} + B \delta_{p1} + C \delta_{h1} + D \quad (8)$$

There were two approaches for the regression: unconstrained and constrained. In the unconstrained regression, all coefficients can take any value. In the constrained regression, using equation 17, $C_1 = 1$, based on the assumption that all molecules in the mixture experience London dispersion interactions in all directions [8].

$$\left(\frac{RT}{V_2 \phi_1^2} \right) \left[\ln \frac{x_2^{ideal}}{x_2} \right] - \delta_{d1}^2 = C_2 \delta_{p1}^2 + C_3 \delta_{h1}^2 + A \delta_{d1} + B \delta_{p1} + C \delta_{h1} + D \quad (17)$$

The regression coefficients for the constrained and unconstrained coefficients are given in Table 4.4. The coefficient for the dispersion term, C_1 , is higher than the other terms in the equation. HSPs of sclareol from the unconstrained and constrained regression are close to each other. The HSPs of sclareol are within the ranges of the HSPs of the solvents tested.

Table 4.4 Results from multiple linear regression and the Hansen solubility parameters of sclareol from 17 solvents

		unconstrained	constrained
regression	C_1	2.23	1.00 (fixed)
coefficients	C_2	0.11	0.09
	C_3	-0.03	-0.01
	A	-77.01	-35.90
	B	-1.71	-1.32
	C	0.99	0.46
	D	669.25	329.09
	C_0	6.51	6.13
	R^2	0.85	0.99
Hansen solubility parameters, $\text{MPa}^{1/2}$	δ_d	17.2	17.9
	δ_p	8.1	7.1
	δ_h	16.1	16.9
sum of squared residuals		0.0115	0.0121
average % error		61	110

The R^2 accounts for the % variability of solubility that can be explained by the set of Hansen solubility parameter values. For the unconstrained coefficients, 85 % of the variability of $x_2^{\text{experimental}}$ can be explained by δ_d , δ_p and δ_h . For the constrained minimization, 99 % of variability of $x_2^{\text{experimental}}$ can be explained by δ_p and δ_h . A larger R^2 value means that the variation in the solubility values can be explained by δ_p and δ_h . The δ_p range of values is 0.0 to 26.2 while the δ_h range of values is 0.0 to 23.3. The δ_d range of values is 14.9 to 18.4 , which suggests that the variation in the non-polar properties of the solvents is relatively small. Thus, fixing $C_1 = 1$ lets the $x_2^{\text{experimental}}$ depend on δ_p and δ_h in the correlation.

The regression coefficient and HSPs of sclareol show that the dominant interaction for sclareol with the solvents is the London dispersion forces, followed by hydrogen bonding

and polar interaction ($\delta_d > \delta_h > \delta_p$). The HSPs of sclareol are similar to the alcohols, 1-propanol and ethanol, attributed to the presence of hydroxyl groups in their chemical structures. The low solubility in highly polar solvents such as formamide and acetonitrile suggest that polar interactions are less significant in sclareol solubility. The dispersion interactions are more important. This is manifested in increasing the hydrocarbon chain length in the alcohol and esters, which results in increasing the δ_d and a corresponding increase in the experimental solubility of sclareol.

Comparing the HSPs of sclareol with other drug molecules, the sclareol HSPs (from the unconstrained fit) fall within the range of the HSPs of the drugs, as shown in Table 4.5. In the studies on the HSPs of drugs [4-7], the differences in solubility are largely a result of differences in δ_p and δ_h values of the solute. Figure 4.3 shows the chemical structures of these drug molecules as compared to sclareol.

Table 4.5 Hansen solubility parameters of some drug molecules

Compound	Solubility parameters		
	δ_d dispersion MPa ^{1/2}	δ_p polar MPa ^{1/2}	δ_h hydrogen bonding MPa ^{1/2}
piroxicam	16.8	21.4	6.6
paracetamol	16.6	14.3	18.4
naproxen	17.4	12.1	9.9
ibuprofen	16.4	6.4	8.9

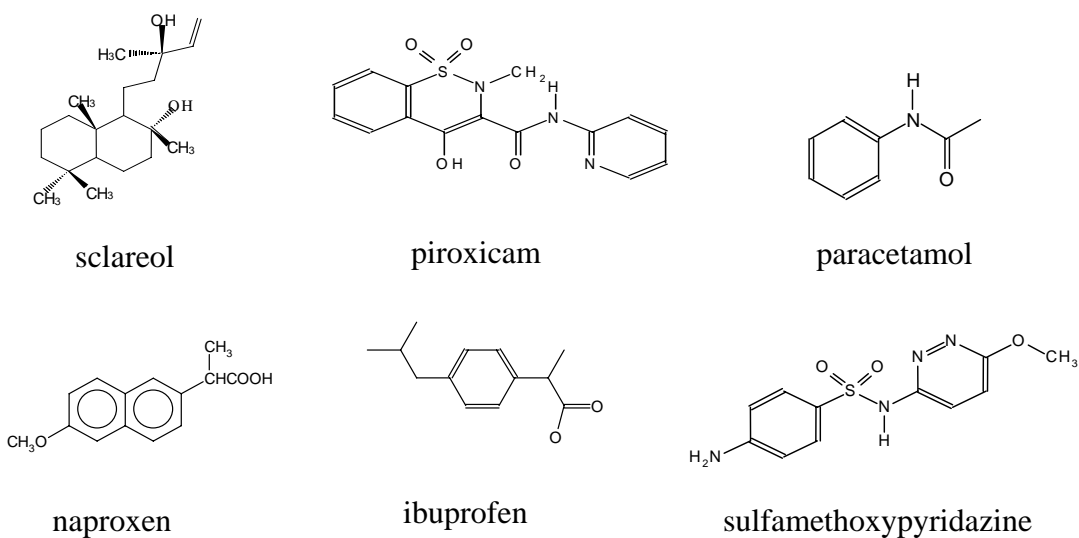


Figure 4.3. Chemical structures of the drug molecules compared with sclareol

The backcalculated solubility values from the HSP model (equation 7) using the constrained and unconstrained coefficients are shown in Table 4.6. The HSP model gives an error greater than 100%. Generally, a criterion set by previous studies [5, 6] for the HSP model to provide a good fit for the data is an error less than 30%. For the solubility data, 7 out of the 17 data points are less than 30 % error for the unconstrained fit while 5 out of 17 data points are less than 30 % error for the constrained fit.

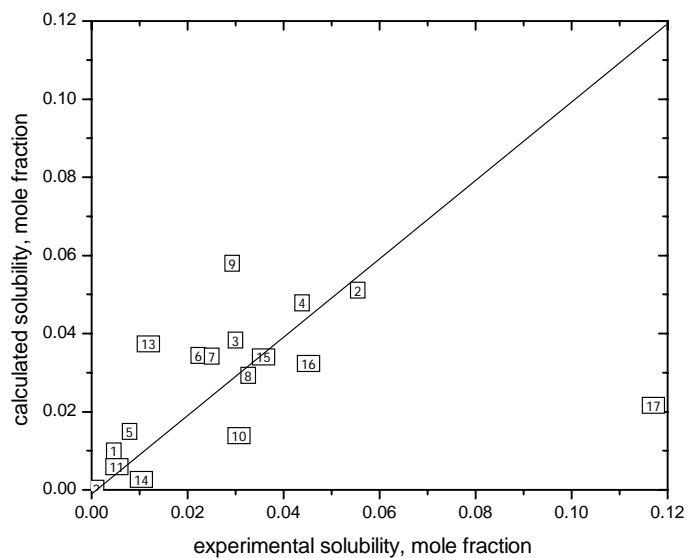
In solubility studies on naphthalene [8], the extended HSP model provided % error less than 30%, although naphthalene is a poor model for drug molecules because of its lack of functional groups and side chains. For the model's application to polar drug solutes, such as benzoic acid [9] and sulfur-containing compounds, such as sulfonamides [10] and sulfamethoxypyridazine [16], the % error in several solvents also exceeded 100 %. The

average % error for benzoic acid in 40 solvents is 59 % [9] while for sulfamethoxypyridazine in 30 solvents is 62 %. Thus, for sclareol, it is not expected to have % error less than 100% for all the solvents.

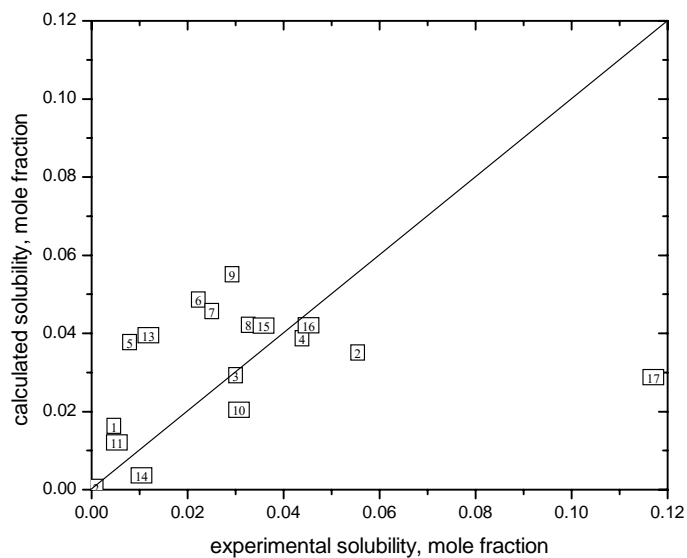
Table 4.6 Results of backcalculation from the HSP model compared to the experimental data in 17 solvents

Solvent number	Solvent	Solubility of sclareol, mole fraction			% error	
		experiment	HSP model		unconstrained	constrained
1	n-hexane	0.0046	0.0099	0.0163	119	258
2	cyclohexane	0.0554	0.0511	0.0351	8	37
3	decahydronaphthalene	0.0300	0.0383	0.0293	28	2
4	toluene	0.0438	0.0479	0.0387	9	12
5	methanol	0.0079	0.0149	0.0377	88	376
6	ethanol	0.0222	0.0344	0.0486	55	119
7	1-propanol	0.0250	0.0342	0.0457	37	83
8	acetone	0.0326	0.0293	0.0422	10	29
9	tetrahydrofuran	0.0293	0.0580	0.0551	98	88
10	dimethylsulfoxide	0.0307	0.0139	0.0204	55	34
11	acetonitrile	0.0053	0.0059	0.0121	11	128
12	formamide	0.0002	0.0004	0.0007	118	308
13	N, N - dimethylformamide	0.0118	0.0373	0.0395	215	234
14	triethanolamine	0.0104	0.0027	0.0036	74	65
15	ethyl acetate	0.0358	0.0340	0.0420	5	17
16	ethyl lactate	0.0452	0.0323	0.0421	28	7
17	butyl butyrate	0.1170	0.0216	0.0288	82	75
				average	61	110

$$\% \text{ error} = \frac{|\text{experimental value} - \text{HSP model value}|}{\text{experimental value}} \times 100$$



(a) unconstrained regression

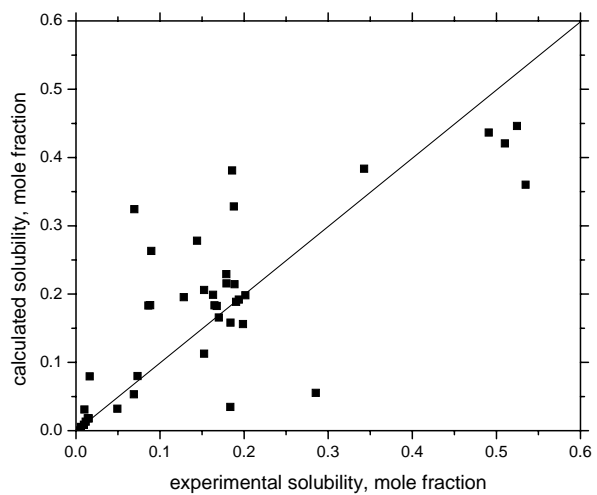


(b) constrained regression

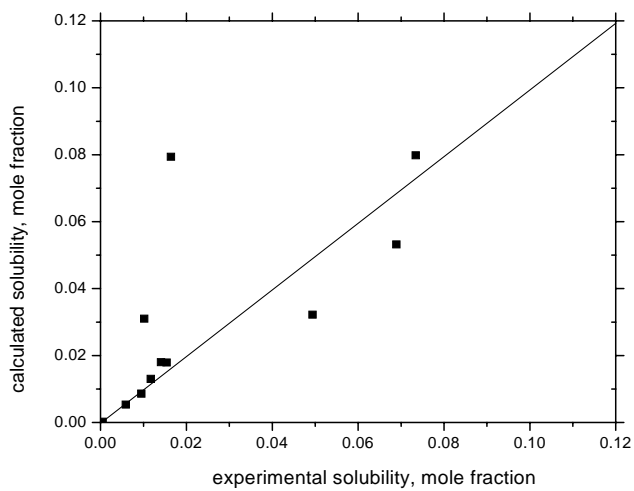
Figure 4.4 Results of the extended HSP model in (a) unconstrained and (b) constrained regression for solubility of sclareol at 25 °C in 17 solvents. The corresponding solvent numbers are in Table 4.6.

The graph of the calculated versus the experimental solubility for sclareol (in Figure 4.4) show the points as distributed along the 45-degree line, which is a measure of how the calculated values agree with the experimental solubility. The data point for butyl butyrate (solvent number 17) is an obvious outlier. In regression, the method of least squares is used. The goal of the method of least squares is to minimize the sum of the squared residuals. This approach is sensitive to outliers because an outlier can result in large residuals, which can pull the 'fitted' model away from the most of the experimental data. This is a limitation of the regression procedure.

The plot for sclareol can be compared to the scatter plot for benzoic acid [9], a polar solute. A portion of the data is gathered and plotted on the same range for comparison. Some data points are also far from the 45-degree line.



(a)



(b)

Figure 4.5 Results from the HSP model using benzoic acid (from literature): (a) in 40 solvents [9]; (b) a portion of the data is taken for comparison with the solubility of sclareol in the same range of values

Initially, the multiple linear regression was done on 12 solvents. This set did not include the following 5 solvents: decahydronaphthalene, methanol, formamide, N, N-dimethylformamide and triethanolamine. The regression results are shown in Table 4.7.

Table 4.7 Results from multiple linear regression (least squares method) from solubility of sclareol in 12 solvents

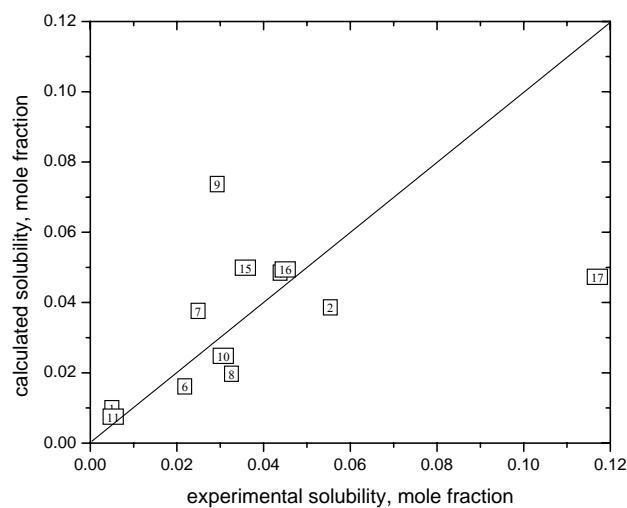
		unconstrained	constrained
regression	C ₁	1.5607	1.00 (fixed)
coefficients	C ₂	-0.0376	-0.0325
	C ₃	0.1160	0.1189
	A	-54.0378	-35.3359
	B	1.6577	1.6194
	C	-2.6589	-2.7453
	D	476.0261	321.0401
	C ₀	11.3084	13.2096
	R ²	0.6077	0.9873
Hansen solubility parameters, MPa ^{1/2}	δ _d	17.3	17.7
	δ _p	22.1	24.9
	δ _h	11.5	11.5
sum of squared residuals		0.0078	0.0076
average % error		48	47

Table 4.8 Results from backcalculation from the HSP model compared to the experimental data in 12 solvents

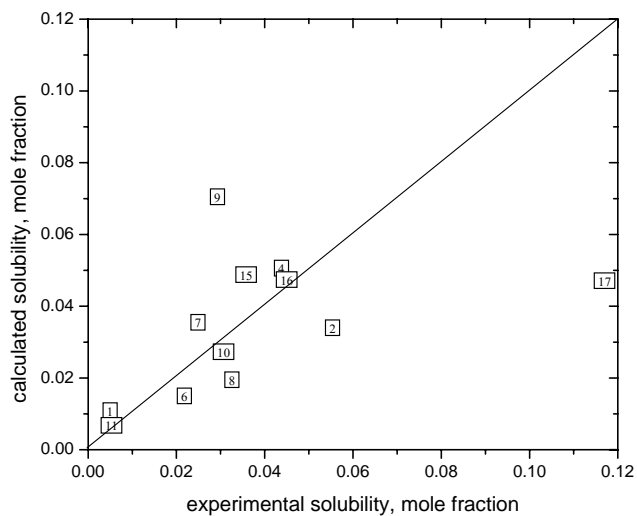
Solvent number	Solvent	Solubility of sclareol, mole fraction			% error	
		experiment	HSP model		unconstrained	constrained
			unconstrained	constrained		
1	n-hexane	0.0050	0.0099	0.0109	98	117
2	cyclohexane	0.0554	0.0386	0.0340	30	39
4	toluene	0.0438	0.0485	0.0506	11	15
6	ethanol	0.0218	0.0161	0.0150	26	31
7	1-propanol	0.0249	0.0376	0.0355	51	42
8	acetone	0.0326	0.0197	0.0195	40	40
9	tetrahydrofuran	0.0293	0.0737	0.0705	152	141
10	dimethylsulfoxide	0.0307	0.0248	0.0273	19	11
11	acetonitrile	0.0053	0.0075	0.0068	42	29
15	ethyl acetate	0.0358	0.0499	0.0488	39	36
16	ethyl lactate	0.0450	0.0494	0.0474	10	5
17	butyl butyrate	0.1170	0.0473	0.0471	60	60
				average	48	47

In this set of solvents, the δ_p of sclareol is very high (22.1 and 24.9 MPa^{1/2}) and out of the range of the tested solvents. These are comparable with the δ_p of nitrogen-containing compounds such as triethanolamine (22.4 MPa^{1/2}) and formamide (26.2 MPa^{1/2}). These compounds are not similar to the chemical structure of sclareol. There is a dependence of the solute solubility parameters on solvent polarity, as studied by Martin and coworkers [11]. In this study, it was observed that the solubility parameters of certain non-ionic solutes in individual solvents tend to vary with the polar and hydrogen bonding character of the solvents.

The % error is lower in the set of 12 solvents as shown in Table 4.8. The solvents with the highest % error in this set are n-hexane, tetrahydrofuran and butyl butyrate. In the set of 17 solvents, the solvents with the highest % error are also in these solvents in addition to formamide, N, N – dimethylformamide and triethanolamine. In the set of 17 solvents, which included the solubility data for the nitrogen-containing solvents, there is a wider range of δ_h and δ_p values. This suggests that the added solvents increase the % error because of a wider range of δ_d , δ_p and δ_h .



(a) unconstrained regression



(b) constrained regression

Figure 4.6 Results of the extended HSP model in (a) unconstrained and (b) constrained regression for solubility of sclareol at 25° C in 12 solvents. The corresponding solvent numbers are in Table 4.8.

Table 4.9 Calculation of the HSPs of sclareol using the group contribution method of van Krevelen and Hoftyzer [17]

Group	Number of groups	F_d $J^{1/2} cm^{3/2} mol^{-1}$	F_p^2 $J cm^3 mol^{-2}$	U_h $J mol^{-1}$	ΣF_d $J^{1/2} cm^{3/2} mol^{-2}$	ΣF_p $J cm^3 mol^{-3}$	ΣU_h J/mol
- CH ₃	5	420	-	-	2100	0	0
- CH ₂	7	270	-	-	1890	0	0
- CH	2	80	-	-	160	0	0
>C<	4	-70	-	-	-280	0	0
= CH ₂	1	400	-	-	400	0	0
= CH	1	200	-	-	200	0	0
- OH	2	210	250,000	20,000	420	500,000	40,000
				sum	4890	500,000	40,000

Molar volume of sclareol at 25 °C, $V = 291.6 cm^3/mol$

$$\delta_d = \frac{(\sum F_d)}{V} = \frac{4890}{291.6} = 16.8 MPa^{1/2}$$

$$\delta_p = \frac{(\sum F_p)^{1/2}}{V} = \frac{(500,000)^{1/2}}{291.6} = 2.4 MPa^{1/2}$$

$$\delta_h = \left(\frac{\sum U_h}{V} \right)^{1/2} = \left(\frac{40,000}{291.6} \right)^{1/2} = 11.7 MPa^{1/2}$$

Compared with the HSPs obtained from experiment, shown in Table 4.4 and 4.7, the HSPs from group contribution show that the dispersion parameter does not vary, the polar parameter is uncertain, and the hydrogen bonding parameter is similar to the HSPs regressed from the set of 12 solvents. As shown in previous studies [16, 4, 6], the HSPs from experiment and from group contribution method are not similar.

Table 4.10 HSPs of drug molecules from group contribution method and experimental solubility data

drug molecule	Hansen solubility parameter, MPa ^{1/2}						
	using van Krevelen group contribution method			using regression from solubility data			
	δ_d dispersion	δ_p polar	δ_h hydrogen bonding	δ_d dispersion	δ_p polar	δ_h hydrogen bonding	
sulfamethoxypyridazine	18.1	12.2	12.2	15.6	23.0	7.3	
piroxicam	19.7	6.2	8.7	16.8	21.4	6.6	
naproxen	19.1	3.3	8.5	17.4	12.1	9.9	

From the chemical structure of sclareol, it is expected that the polar parameter is not high because of the absence of nitrogen and sulfur containing groups in the chemical structure. The solids, with high δ_p , are the nitrogen and sulfur containing compounds, such as piroxicam and sulfamethoxypyridazine, as shown in Figure 4.3.

The difference in solubility parameters, whether obtained experimentally from solubility data or by group contribution method, may be attributed to the following: (a) possible inaccuracy of the group contribution increments, (b) experimental errors in the determination of the mole fraction solubilities of the solute, and (c) the number and nature of solvents can also influence the experimental solubility parameters, as a result of the inter- and intramolecular interactions of the solute in certain solvents.

The % error between the calculated and experimental solubility suggest that the HSP model is not sufficient to explain all the solute-solvent interactions in the formation of the solution. The HSP model does not account for the arrangement and geometry of interaction of the side chains or functional groups in the sclareol molecule with the solvent molecule.

4.3 Temperature dependence of solubility of sclareol in selected ester solvents

The solubility of sclareol from 25 to 45 °C in three GRAS ethyl esters are shown below. Ethyl hexanoate gave the highest solubility.

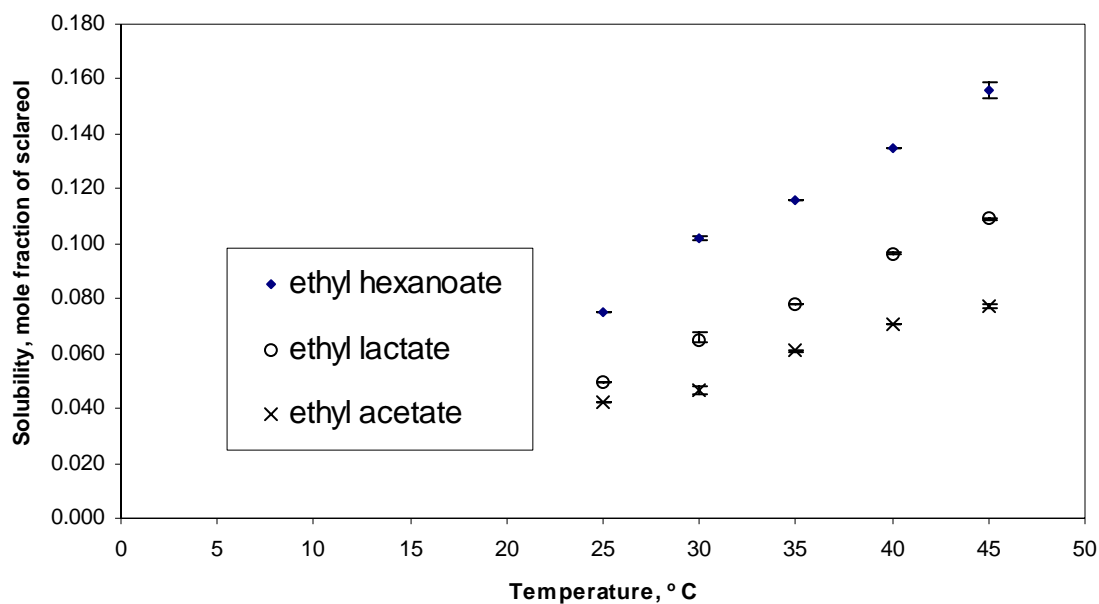


Figure 4.7 Solubility (in mole fraction) of sclareol in ethyl ester solvents from 25 to 45 °C.

A plot of the experimental solubility, $\ln x$ vs. $(1/T)$, known as the van't Hoff plot, shows the linearity of the solubility data as a function of temperature.

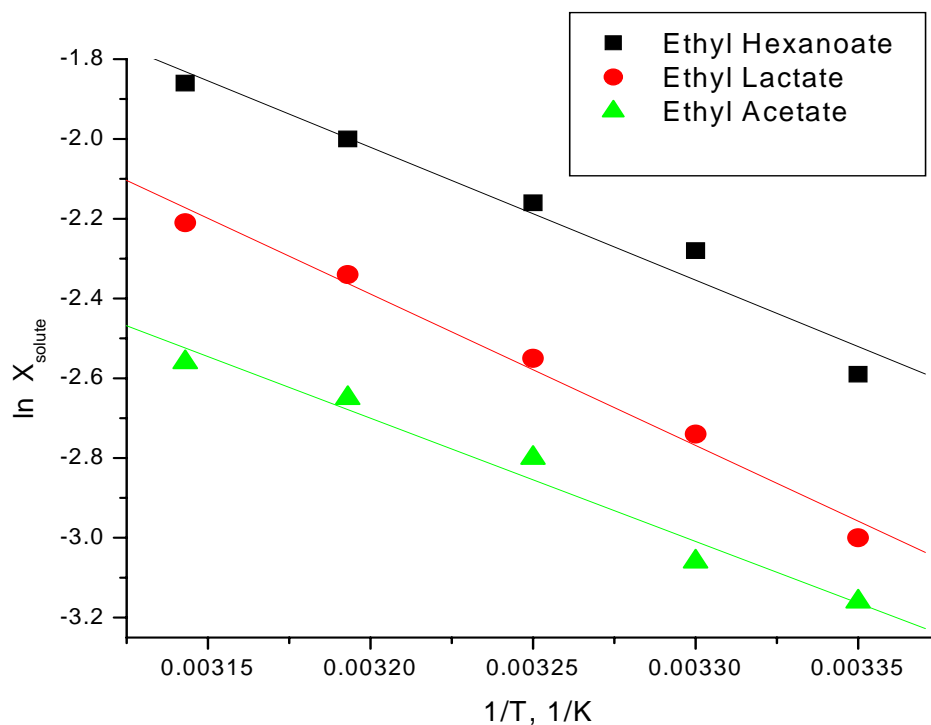


Figure 4.8 Van't Hoff plot for solubility of sclareol in ethyl ester solvents

The extended Hansen solubility parameter equation is used to predict the solubility of sclareol over the range of temperatures.

$$\left(\frac{RT}{V_2\phi_1^2}\right)\left\{\ln\frac{x_2^{ideal}}{x_2}\right\}-\left[C_1(\delta_{d2}-\delta_{d1})^2+C_2(\delta_{p2}-\delta_{p1})^2+C_3(\delta_{h2}-\delta_{h1})^2+C_o\right]=0 \quad (7)$$

The Hansen solubility parameters (HSPs) at different temperatures were calculated from the molar volumes of the solvents using the equations proposed by Williams and coworkers [12]

$$\delta_d = \frac{\delta_d ref}{\left(\frac{Vref}{V}\right)^{-1.25}} \quad (12)$$

$$\delta_p = \frac{\delta_p ref}{\left(\frac{Vref}{V}\right)^{-0.5}} \quad (13)$$

$$\delta_h = \frac{\delta_h ref}{\exp\left[-1.32 \times 10^{-3}(Tref - T) - \ln\left(\frac{Vref}{V}\right)^{0.5}\right]} \quad (14)$$

where V_{ref} is the reference molar volume; $\delta_d ref$, $\delta_h ref$ and $\delta_p ref$ are the reference Hansen solubility parameters at $T_{ref} = 25^\circ \text{C}$.

The density values and molar volume of the ethyl ester solvents are gathered from one source or reference, because density values differ significantly from one reference to another, depending on how they were determined. The Rackett equation [2] was used to calculate for the densities at other temperatures. The saturated liquid density were gathered from the DIPPR database, product label information or published data in the literature [13;14]. The Rackett parameters of ethyl acetate, ethyl lactate and ethyl hexanoate are backcalculated from the critical temperature, critical pressure and the saturated liquid density.

Table 4.11 Physical properties of the ethyl ester solvents

Solvent	Saturated Liquid Density at 25 ° C , ρ_L g/L	Critical Pressure, P_c MPa	Critical Temperature, T_c K	Rackett Parameter ^b
Ethyl lactate	1033 ^a	3.86	588	0.2663
Ethyl acetate	894	3.78	524	0.2524
Ethyl hexanoate	869 ^c	2.55 ^d	612 ^d	0.2562

Note: Physical properties are gathered from the DIPPR Database (www.dippr.byu.edu) otherwise specified. (a) from Fluid Phase Equilibria (2005), 230, 1-2, 197-203 , (b) backcalculated from the Rackett equation using the saturated liquid density, critical temperature and critical pressure, (c) from Sigma Aldrich product information sheet (Sigma Aldrich, 2005), (d) from J. of Supercritical Fluids, 28 (2004), 1, 1-9.

Table 4.12 Density of ethyl esters from 25 to 45 ° C

Solvent	Temperature ° C	Density of solvent g/L	Source
Ethyl lactate MM = 118.13 g/mol	25	1033	Fluid Phase Equilibria (2005), 230, 1-2, 197-203
	30	1027	using Rackett equation
	35	1022	using Rackett equation
	40	1016	using Rackett equation
	45	1010	using Rackett equation
Ethyl acetate MM= 88.11 g/mol	25	894	DIPPR database
	30	888	DIPPR database
	35	882	using Rackett equation
	40	875	DIPPR database
	45	870	using Rackett equation
Ethyl hexanoate MM= 144.21 g/mol	25	869	Sigma Aldrich product label information
	30	864	using Rackett equation
	35	860	using Rackett equation
	40	855	using Rackett equation
	45	851	using Rackett equation

Table 4.13 Reference values of HSPs of the ethyl ester solvents at 25° C

Ester solvent	molar volume cm ³ /mol	Hansen solubility parameters, MPa ^{1/2}		
		δ_d dispersion	δ_p polar	δ_h hydrogen bonding
Ethyl lactate	114.4	16.0	7.6	12.5
Ethyl acetate	98.6	15.8	5.3	7.2
Ethyl hexanoate	165.9	15.6	2.9	5.6

The assumptions and the data in the calculations are the following:

1. Reference values for the HSPs of the ethyl lactate and ethyl acetate were taken from Hansen Solubility Parameter: A User's Handbook [3].
2. The HSPs of ethyl hexanoate are not available in the handbook, so the HSPs of butyl butyrate are assumed to be the same as HSPs of ethyl hexanoate. Both esters have the same hydrocarbon chain length.
3. Molar volume of sclareol is constant over the temperature range. Thus, the dispersion and polar parameter are constant. The hydrogen bonding parameter of sclareol at different temperatures are not much affected by temperature and molar volume changes. Thus, HSPs of sclareol are constant over this temperature range.
4. The ideal solubility is dependent on temperature.
5. The regression coefficients, C_1 , C_2 , C_3 , and C_0 , which were gathered from solubility data at 25 °C, can be applied over this range of temperature.
6. The calculation procedure involves iteration of equation (1) starting with the experimental data as the initial guess value.

Results of the calculation are in Table 4.14. An increase in the temperature results in lower HSPs. Upon heating, the density of the solvent decreases while the molar volume (the molar mass divided by the density) increases. An increase in the temperature increases the kinetic energy of the solvent molecules, weakening the interaction among the molecules. Thus, an increase in the temperature results in decreased intermolecular interactions, as represented by the decrease in HSPs.

Table 4.14 Hansen solubility parameters of the ester solvents

Solvent	Temperature ° C	Molar volume cm ³ /mol	Hansen solubility parameters, MPa ^{1/2}		
			δ_d dispersion	δ_p polar	δ_h hydrogen bonding
Ethyl lactate	25	114.4	16.0	7.6	12.5
	30	115.0	15.9	7.6	12.4
	35	115.6	15.8	7.6	12.3
	40	116.3	15.7	7.5	12.2
	45	117.0	15.6	7.5	12.0
Ethyl acetate	25	98.6	15.8	5.3	7.2
	30	99.2	15.7	5.3	7.1
	35	99.9	15.5	5.3	7.1
	40	100.7	15.4	5.2	7.0
	45	101.3	15.3	5.2	6.9
Ethyl hexanoate	25	165.9	15.6	2.9	5.6
	30	166.9	15.5	2.9	5.5
	35	167.7	15.4	2.9	5.5
	40	168.7	15.3	2.9	5.4
	45	169.5	15.2	2.9	5.4

The following tables and graphs show the data from experiment and the HSP model.

The overall average % error is 42.8 %.

Table 4.15 Experimental and calculated solubility of sclareol in ethyl esters from 25 to 45 °C

Solvent	Temperature ° C	from experiment	Solubility, mole fraction from HSP model			
			unconstrained	% error	constrained	% error
Ethyl lactate	25	0.0496	0.0323	34.9	0.0317	36.2
	30	0.0647	0.0384	40.6	0.0387	40.2
	35	0.0780	0.0463	40.6	0.0478	38.7
	40	0.0965	0.0550	43.0	0.0588	39.0
	45	0.1090	0.0656	39.8	0.0730	33.1
Ethyl acetate	25	0.0423	0.0339	19.8	0.0327	22.7
	30	0.0469	0.0395	15.7	0.0396	15.6
	35	0.0582	0.0459	21.2	0.0481	17.4
	40	0.0706	0.0519	26.6	0.0579	18.0
	45	0.0746	0.0620	16.9	0.0728	2.4
Ethyl hexanoate	25	0.0751	0.0216	71.2	0.0229	69.5
	30	0.1030	0.0247	76.0	0.0272	73.6
	35	0.1060	0.0287	73.0	0.0327	69.1
	40	0.1350	0.0323	76.1	0.0388	71.3
	45	0.1430	0.0372	74.0	0.0467	67.3

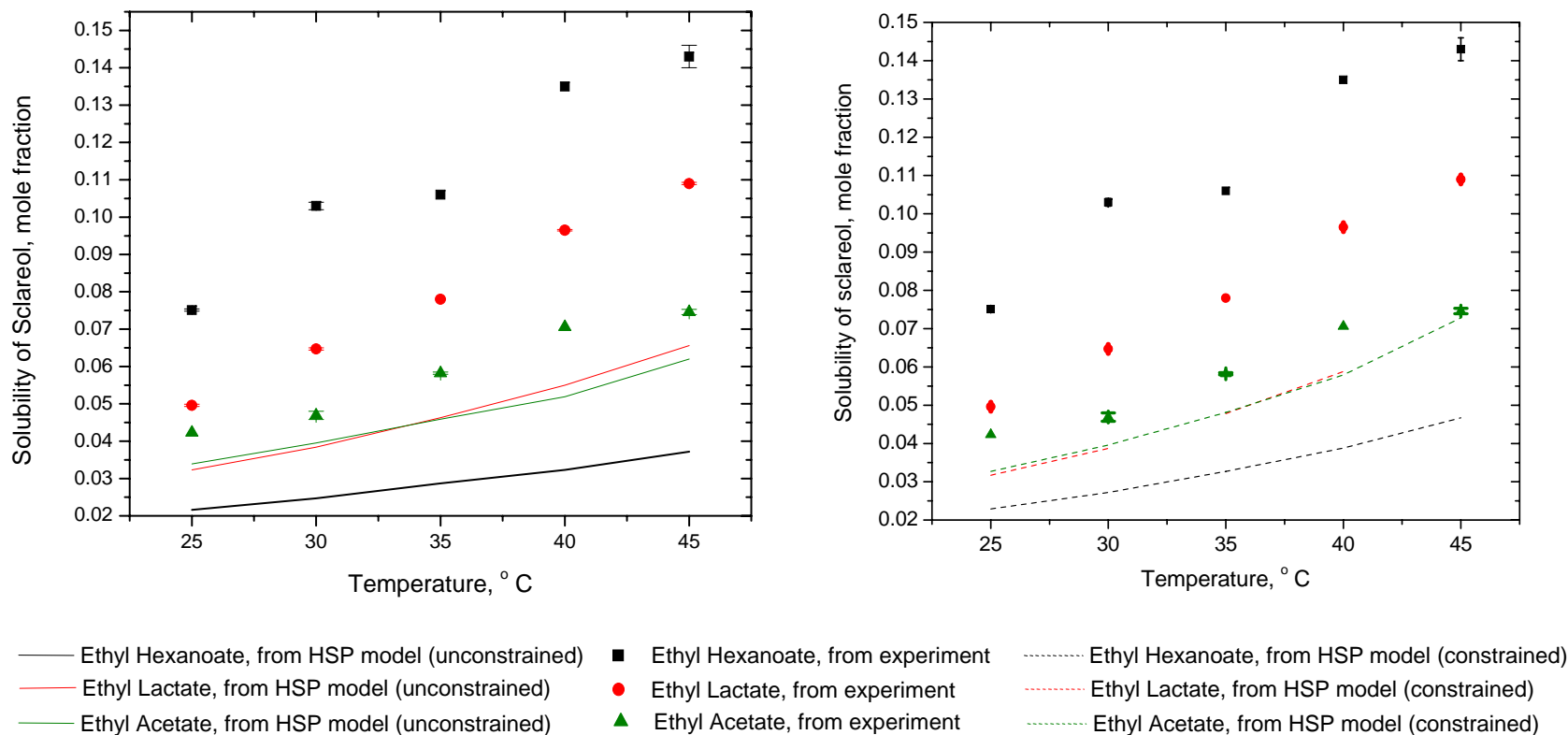


Figure 4.9 Solubility of sclareol in 3 ethyl ester solvents from 25 to 45 °C from experiment and HSP model. Data points represent the solubility from experiment. The lines represent the calculated solubility using unconstrained (solid lines) and constrained (dashed lines) coefficients in the extended HSP model. The lines were obtained by connecting the backcalculated solubility data points at each temperature.

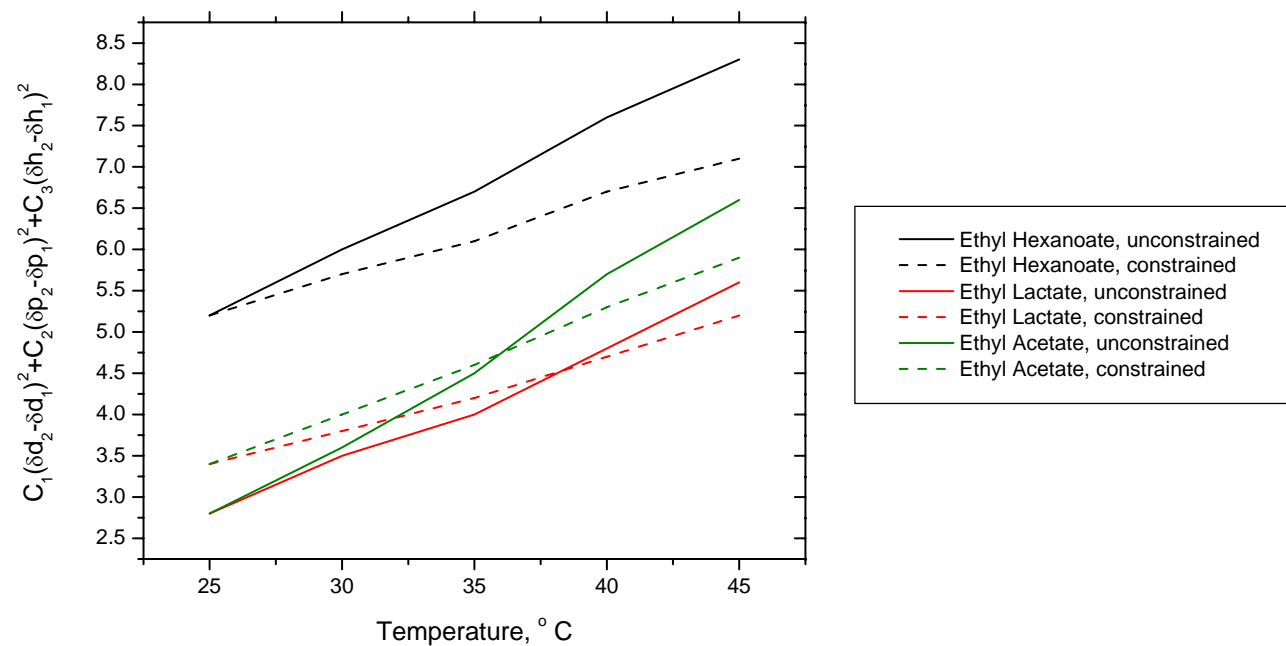


Figure 4.10 Weighted sum of the differences in the solubility parameters for the ester solvents as a function of temperature. The trend in the weighted sum is the same as the trend in the prediction by the HSP model. As the HSPs of the solvent (1) deviates from the HSPs of sclareol (2), the predictive capability of the model decreases.

As shown in Figure 4.9 and 4.10, the calculated solubility of ethyl hexanoate has the lowest set of values but the experimental solubility of ethyl hexanoate has the highest set of values. For ethyl lactate and ethyl acetate, both experimental and calculated solubility values give the same trend.

The HSP model predicted the sclareol solubility in the following solvents with decreasing order: ethyl lactate, ethyl acetate and ethyl hexanoate. It can be noted that the decrease in the solubility prediction can be attributed to the increase in the difference in the solubility parameters of the solute and solvent. Figure 4.10 shows that ethyl hexanoate has the largest value for $C_1(\delta_{d2}-\delta_{d1})^2 + C_2(\delta_{p2}-\delta_{p1})^2 + C_3(\delta_{h2}-\delta_{h1})^2$, which is the weighted sum of differences of the sclareol and solvent in equation 7. Therefore, the predictive capability of the HSP model decreases (resulting in a larger % error from the experimental data) as the δ_d , δ_p and δ_h of the solvent deviates from the δ_d , δ_p and δ_h of sclareol. This is also observed in a study on the HSPs of polyaromatic compounds in mixed solvents [15]. For the solubility of 9-anthracenecarboxylic acid and phenanthridine, the predictive capability of the HSP model decreased significantly when the polar and/or hydrogen bonding contributions to the solvent HSPs deviated significantly from the HSPs of the solute.

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5 Summary and Conclusion

The experimental solubility of sclareol at 25 ° C was determined in various organic solvents. These solvents have a wide range of polarities, as shown in their wide range of Hansen solubility parameters (HSPs). The solubility is highest in butyl butyrate and lowest in formamide. The solubility of sclareol in several GRAS ester and non-ester solvents can be a starting point in designing an environmentally benign extraction process for sclareol.

The HSPs of sclareol were gathered from multiple linear regression (by the method of least squares). The regressed Hansen solubility parameters depend on the HSPs and number of the solvents used. The HSPs of sclareol, regressed from 17 solvents, are similar to the alcohols, which is attributed to the presence of hydroxyl groups in their chemical structures. The HSPs from group contribution method did not agree with the experimental HSPs.

The extended Hansen solubility parameter model showed average % error between the experimental and calculated solubility up to 110 %. These results are comparable with the % error in solubility studies for polar solutes in organic solvents in the literature. The model accounts for the dispersion (non-polar), polar and hydrogen bonding interactions between the solute and the solvent. However, the spatial arrangement of the functional groups of the solute and solvents in the mixture are not considered in this model.

The regression coefficients and HSPs of sclareol at 25 ° C were used for modeling the temperature dependence of solubility experimental solubility in selected GRAS ester solvents. The extended HSP model did not provide a good fit to the experimental solubility from 25 to 45 ° C. As the HSPs of the solvent deviates from the HSPs of sclareol, the predictive capability of the model decreases.