EXTRACTION OF THE ANTIMALARIAL ARTEMISININ FROM *ARTEMISIA ANNUA L.* LEAVES WITH SUPERCRITICAL CO_2

D. Sánchez[1], S. Quispe[2], P. Rosa[2], M. A. A. Meireles[2*], C. Zetzl[1], G. Brunner[1],
[1] Techn. Univ. Hamburg-Harburg, Eißendorfer Str. 38, 21073 Hamburg, Germany.
[2] Univ. Est. de Campinas, LASEFI, DEA-FEA, Campinas(SP) Brazil.
Fax: 55 19 3788 4027. E-mail: meireles@fea.unicamp.br
Project integrated in the German-Brazil program "SUPERNAT" (CNPq- BMBF 98/078)

Abstract :

The antimalarial artemisinin, the active principle of the plant *Artemisia annua L.*, was extracted by Supercritical Fluid Extraction (SFE). Soxhlet extraction was studied as comparison processes. The extracts were analyzed by the indirect method GC-FID. The maximal extraction yield (1.82%DW) was obtained by SFE at T=40°C, P=200bar, CO_2flow=4.5g/min, and 20% ethanol as modifier. The artemisinin extraction can be enhanced by adding of non-polar modifier, this fact is applicable for the Soxhlet extraction also. Further was studied the kinetic of the extraction and fitting the experimental data with several mass transfer models.

1 INTRODUCTION

Malaria is a major disease in many countries. The rapid development of drug-resistant Malaria parasite strains leaves the need for new effective antimalarial drugs. *Artemisia annua L*. also known as *Qinghao*, *sweet* or *annual wormwood* has been used in China against fever and malaria for over 100 years Actually is this herb used for the crafting of aromatic wreaths, as a source of essential oils used in the beverages industry and also as a source of artemisinin, a potent antimalarial drug. Today grows the plant in many regions as Australia, North- and South America, Southern Europe[1]**.** The main active principle, artemisinin, was isolated and had its structure through elemental analysis and high-resolution mass spectrometry correctly defined in 1972 in China as a sesquiterpene lactone with an endoperoxide bridge[2]. The empirical formula of artemisinin, depicted in Fig. 1, is $C_{15}H_{22}O_5$.

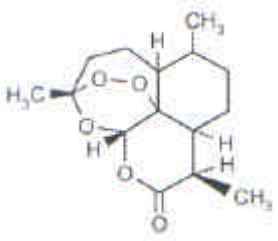

Fig 1. : Artemisinin

Several methods have been used for the extraction of artemisinin from the plant structure. The most frequently used extraction methods are decoction, liquid solvent extraction, Pressurized Solvent Extraction (PSE), Microwave-assisted Extraction (MAE) and Supercritical Fluid Extraction (SFE)[3,4]. In this work was Soxhlet extraction and Supercritical Solvent Extraction (SFE) as extraction methods tested. It is generally known that supercritical conditions enhance the dissolution from the solid bulk. In comparison with liquid solvents, supercritical fluids have a high diffusivity, a low density and a low viscosity, thus allowing rapid extraction and phase separation[5].

2 MATERIALS AND METHODS

2.1 Substrate

The influence of operating parameters on the artemisinin extraction process was studied in the laboratories of the Department Thermische Verfahrenstechnik at the Technical University Hamburg-Harburg. The Kenyan *A. annua* L. used for these experiments was supplied by ANAMED-Germany (Action for Natural Medicine). The humidity of this material was 5.4% (w/w), the Sauter-diameter of the employed fraction was d_S=0.7516 mm.

In the Physical Separation Laboratory (LASEFI), Department of Food Engineering at the Universidade Estaduale de Campinas, Campinas, SP Brazil was studied the mass transfer phenomena during the artemisinin extraction. The dried *A. Annua L.* leaves for the latest experiments were kindly supplied by Dr. Mary Ann Foglio from the Centro de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA), Campinas, Brazil. The humidity of this material was 8.2% [w/w]. The Sauter-diameter of the employed fraction was d_S= 0.4915 mm.

2.2 Soxhlet Extraction

The extractor consists in a heating device, solvent reservoir and extract flask (round bottom flask), an extraction chamber containing a porous paper extraction thimble and a condenser. 10g of *A. annua* be placed in the thimble and covered with glass wool. It is placed in the extraction chamber. The extract flask is weighed and about 200 mL of solvent poured into it. The system is then and the heating started. After above 6 hours, the solvent and extract in the extract flask be vaporized using a rotavapour. Certain volume of solvent is added to the extract in order to prepare it for de analysis.

2.3 Supercritical Fluid Extraction (SFE), CO_2 as supercritical solvent.

The apparatus used was Spe-ed SFE unit (Applied Separations, Allentown, PA). CO_2 is withdrawn from the local supply network, condensed and pressurized up to the operating pressure by means of a pneumatic pump driven with pressurized air. The 1.5 g samples were filled into the 10mL column, and glass wool was placed at both ends of the solid bed. First, the CO_2 valve and the pressurized air valves are opened, and the condenser is started. The SFE oven is also taken into operation and desired working temperature and desired valve temperature are set. After the cooler has cooled the condenser, and desired operating temperature is achieved inside the SFE-oven and the valves, the column is pressurized using the air-driven pump. After the system becomes stable with the operating pressure (100 - 400 bar), and operating temperature (30 - 60 °C), and the equilibrium time of 15 min is reached, the micro-regulating valve is set to maintain a CO_2 flow rate from 2 to 4 NL/min (solvent ratio equivalent to 2.4 – 5.4 min^{-1} respectively) and the experiment is started. The extraction time was defined 3h for experiments without modifier and 1.5 h for experiments with modifier. Samples are collected inside pre-weighed glass tubes, which were kept in a cold tramp weighed and preserved in a refrigerator at 4°C until being analyzed.

The experiments related to the extraction mass transfer phenomena developed in Brazil were carried out with the same apparatus, Spe-ed SFE unit (Applied Separations, Allentown, PA), and the same procedure. The extraction cell used was the model CL1373 supplied by Thar Designs Inc. with an internal volume of 300mL. 50 g of air-dried Artemisia annua leaves were disposed into the Thar Designs extraction cell.

2.4 Analysis:

The employed analysis method for the artemisinin detection in Artemisia annua extracts was Gas Chromatography (GC) with Flame Ionization Detection (FID). Artemisinin is a thermally unstable compound that cannot be determined by GC without degradation[6]. Thermal stability studies have demonstrated that artemisinin is stable up to 150°C but degrades into number of products when heated at 180-200°C[7]. GC analyses measure artemisinin indirectly by detecting its degradation products. The standard curve was obtained with the linearity between concentration and total area of the artemisinin peaks.

3. RESULTS AND DISCUSSION

3.1 Supercritical Fluid Extraction

Kohler et al. [8**]** did not find a significant influence of pressure on the artemisinin extraction yield with supercritical CO2. Nevertheless, the solvent capacity of gases generally increases with pressure at constant temperature[5**]**. Figure 2 shows that the artemisinin extraction yield does not appear to depend on the pressure, at these operating conditions. It varies from 0,972% at 100bar to 0,735% (DW) at 200 bar.

The solubility of substances in supercritical fluids is strongly influenced by the temperature, since the solvent power of a fluid increases with increasing density, The other reason for increase amount of extract per unit of time is increasing mass transfer rates with temperature[5].

The solvating power of CO_2 declines with increasing temperature because the density decreases. Parallel increases the vapor pressure of the solute exponentially with temperature and the mass transfer is positively influenced. The last two factors appear to be dominating in the extraction of artemisinin with supercritical carbon dioxide. Figure 3 shows the behavior of the artemisinin yield with the temperature. The maximal artemisinin extraction yield was given at 60°C, 1.127 % (DW).

Solvent ratio is the most important parameter for gas extraction, once approximate values of pressure and temperature are selected. With increasing solvent ratio the extraction can be enhanced more than with changing process parameters in a relatively narrow limit [5].

As shown in Figure 4, it is possible to increase the artemisinin extraction yield up to certain value, 0.676% DW at 4.2 min^{-1}. Solvent ratios higher than this conduce to decreasing of the extraction yield.

The influence of several modifiers on the extraction process was also tested. Hexane, ethanol and ethanol have different polarity since the Snyder's polarity index.

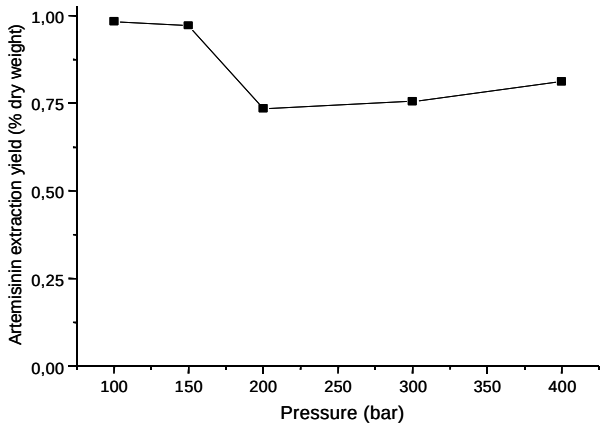

Fig. 2: Influence of the pressure on the artemisinin yield (solvent ratio=3.0min^{-1}, T=40°C, CO_2flow=4.5g/min, time=3h)

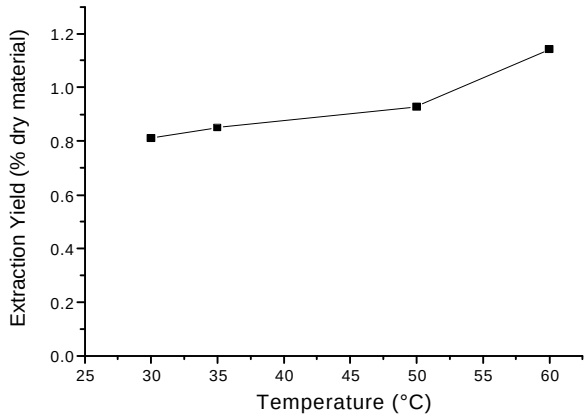

Fig. 3: Influence of the temperature on the artemisinin yield (solvent ratio=3.0 min^{-1}, P=200bar, CO_2flow=4.5g/min, time=3h)

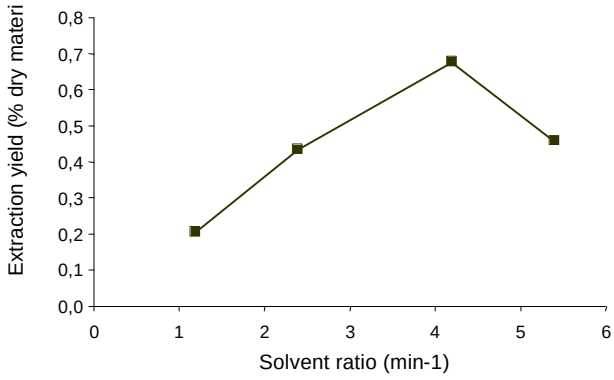

Fig. 4: Influence of solvent ratio on the artemisinin yield (T=40°C, P=200bar, extraction time=3h)

The extraction selectivity can be manipulated by using of modifiers. Sometimes can modifiers also influence process phases, which are not directly related with the diffusion or transport of analytes out of the plant cell: high solubility enhances the transport through the capillary tubes in the extraction apparatus. Kohler et al. [8] found necessary to add a small proportion of the modifier mainly for increasing solubility and therefore avoiding the adsorption of the analyte on the capillary wall and certainly not to enhance its diffusion trough the plant material. Figure 5 shows the artemisinin yield can be considerably enhanced by using non-polar modifiers. The maximal achieved value was 1.86% DW for hexane and 1,12% DW for ethanol.

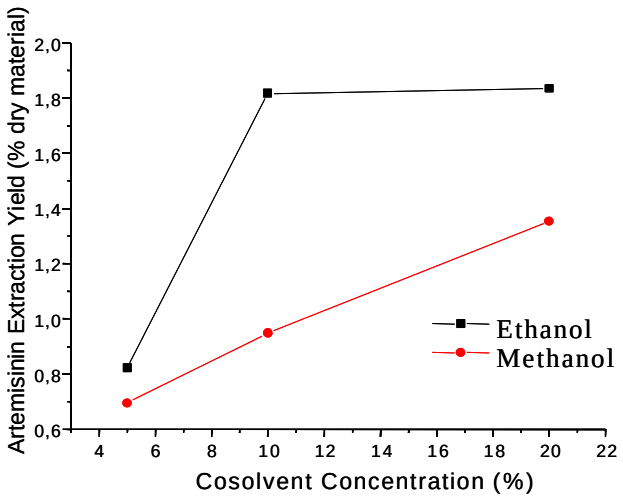

Fig. 5: Influence of the modifier (solvent ratio=3.0 min^{-1}, T=40°C, P=200bar, CO_2flow=4.5g/min, extraction time=3h)

3.2 The kinetic of the SFE

An experimental design (factorial design 2^3) was carried out with the tree factors of pressure, temperature and solvent (CO_2) flow. The inferior and superior values for pressure, temperature and CO_2 flow were 200 and 400bar, 30 and 50C; and 2 and 4NL/min (0.144min^{-1} and 0.072min^{-1} solvent ratio) respectively.

The extraction rate clearly depends on conditions of state for the extraction, which determines the solvent power of the supercritical solvent.

As shown in Figure 6 the behavior of the extraction rate curve is not singular and widely known in the extraction processes. The shape of this curve is typical for a by convection leaded extraction process Tree factors are for this behavior responsible:

1. Artemisinin is content in a very small amount in Artemisia annua leaves: in this work were founded maximal artemisinin concentrations of 1.2% related to dry weight (DW) of the plant. Furthermore, artemisinin is located just in the glandular trichomes[9]; this no uniform distribution of artemisinin in the plant raw material can have difficult accessibility for the solvent during the extraction.
2. *Artemisia annua* extracts have a viscose nature, which means that transport resistance through the solid bulk to the interface solid fluid-substrate solvent is dominating the mass transport from the beginning.

The length of the fixed bed (here 70mm for 50g raw material) with the initial content of artemisinin is not long enough: the residential time of solvent in the column does not allow the maximal loading of CO_2 with artemisinin. The extraction of substances from solid substrates with supercritical solvents can be analyzed and modeled in a simple way by considering only medium values and determination of unknown coefficients by fitting to the extraction curve and a mass balance. The results are simple equations, which can represent parts of the extraction curve sufficiently, but fail for others, especially during the first part of the extraction. If the process is to be modeled accurately, the analysis is a far more complex. The extraction kinetic curves were fitted with the models of Goto et al[10], Tan & Liu[11], Crank[12] and Empirical[13]. For the interpretation of the extraction kinetic curves were employed the software TECANALYSIS[14].

For the better appreciation of the experimental data fitting with these models the method of the square deviation was applied.

There are two factors to be considered: at first, at high solvent flow rate decreases the thickness of the mass transfer film and the concentration gradient, responsible for the convective mass transfer between fixed bed and solvent flow. At second, with increasing solvent ratio, the extraction rate increases, but loading of the supercritical solvent declines at high solvent ratios, due to short residence times of the solvent. The last factor appears to influence the artemisinin extraction more than the first one at these operating conditions. Table 1 shows the mean square deviation of the experimental data fitting.

Table 1 : Mean square deviation of Fitting experimental data with mass transfer models (Goto et al[10], Tan & Liu[11], Crank[12] and Empirical[13])

N	Cond. (bar,°C,NL/min)	Models			
		Crank	Tan&Liu	Empir.	Goto
1	400,50,4	0.4998	0.2775	0.4356	0.5646
2	400,50,2	0.2983	0.0787	0.0390	0.0881
3	200,50,4	0.2861	0.2083	0.1638	0.1915
4	200,30,4	0.1040	0.0909	0.0154	0.0891
5	400,30,4	0.1390	0.9265	0.0297	0.0690
6	400,30,2	0.2412	0.9751	0.0893	0.1005
7	200,30,4	0.0922	1.1111	0.1085	0.0991
8	200,30,2	0.1834	0.0111	0.0228	0.0017

As shown in Table 1 the fitting of experimental data was successful with the models Empirical[13] and Goto et al[10]. The Empirical[13] model predicts successfully the kinetic studied here, its mathematical form is the same for the description of other phenomena that occur in the nature like micro organism growth and adsorption kinetic, on the other hand this model contains only the adjustable parameter k and does not consider the existence of system parameters like solubility, components density, porosity etc., for this reason this model can be employed for the prediction of extraction kinetic with more or less effectiveness but not for the description of the mass transfer phenomena that occur inside of the extraction vessel. The model of Crank[12] is for diffusion leaded processes and the artemisinin extraction is convection leaded one. The Tan&Liu[11] model cannot describe the extraction because some mathematical disappointments : according to this model extract amounts superior than the maximal extractible amount of extract X_0 are possible after a certain time, this is a considerable limitation of this model and perhaps the reason for the unsuccessful description

of the extraction process. A further disadvantage of this model is given by the desorption constant *k*, which depends only on the temperature.

3.3 Soxhlet Extraction:

Figure 8 shows the results of the Soxhlet extraction of artemisinin with different solvents. Similar to the results obtained in the SFE with modifiers, the extraction yield can be enhanced considerable by adding of non-polar solvents. Artemisinin as pure substance has a slightly polar character[8]. Whatever, its possible that certain compounds present in the extract can enhance the solubility of artemisinin in polar compounds. The maximal extraction yield obtained with Soxhlet extraction was 1.39% DW with hexane, followed by ethanol and methanol with 0.74% DW and 0.36% DW respectively.

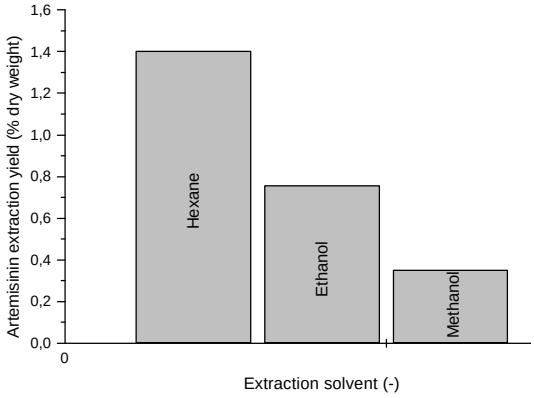

Fig. 8 : Soxhlet extraction: Influence of the solvent nature on yield.

4. CONCLUSION

It can be concluded that SFE at pressures about 400 bar and temperatures about 50 ^{o}C gives comparable extraction yields to ethanol or hexane. At moderate pressure, artemisinin yield increases with temperature, this leads to the thesis of overlaping effects of vapor pressure, compared to solvent density. No significat presssure influence has been found at T = 40 °C in the range of 100 to 200 bar. Modifiers may increase significantly artemisinin yield, it has to be taken into account that this measure strongly affects selectivity, with important drawbacks on the subsequent purification steps.

The support of BMBF – DLR fundss BRA 078/98 is gratefully acknowledged.

REFERENCES

[1]**Charles, D. J., Cebert, E., Simon, J. E**. J. Ess. Oil. Res. 3, p. 33-39, **1991**.

[2] **Coordinating Group for Research on the Structure of Qing Hao su**. K´o Hsueh T´ung Pao 22, p. 142, **1977**.

[3] **Christen, P., Veuthey, J. L.**. New trends in extraction, identification and quantification of artemisinin and its derivates. Current medical chemistry, 8, p. 1827-1839, **2001**.

[4] **Klayman, D. L**. Qinghaosu: An Antimalarial Drug from China. Science; 28, p.1049-1055, **1985**.

[5] **Brunner, G**. Gas Extraction. An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes, Springer-Verlag: New York, **1994**

[6] **Christen, P., Veuthey, J. L.**. New trends in extraction, identification and quantification of artemisinin and its derivates. Current medical chemistry, 8, 1827-1839, **2001.**

[7] **Klayman, D. L**. Qinghaosu: An Antimalarial Drug from China. Science; 28, p.1049-1055, **1985**

[8] **Kohler M., Haerdi W., Christen P., Veuthey J. L**., Extraction of artemisinin and artemisinic acid from Artemisia annua L. using supercritical carbon dioxide. J. of Chrom. A. 785, 353-360, **1997**

[9]**Ferreira J. F. S. and Janick J**. Distribution of artemisinin in *Artemisia annua.* J. Janick (ed), Progress in new crops. ASHS Press., 579 – 584,**1996**

[10] **Goto, M.; Sato, M.; Hirose, T**. Extraction of peppermint oil by supercritical carbon dioxide. Journal of Chemical Engineering of Japan, no. 26, p. 401-407, **1993**.

[11] **Tan, C.; Liou, D.** Modelling of desorption at supercritical conditions. AiChe Journal, vol 35, no 6, p. 1029-1031, **1989.**

[12] **Crank, J**. The mathematics of diffusion, Claredon Press, Oxford, p. 85, **1975**.

[13] **Martínez, J.; Rosa, P. T. V.; Meireles, M. A. A**. Modelagem matemática da extraçao de oleos essenciais com CO2 supercrítico. Proceding of the XIV Congresso Brasileiro de Engenharia Química (COBEQ 2002), Natal, Brazil, **2002.**

[14] **Rosa, P.V.T. & Meireles, M.A.A**. Influence of the operating time on the cost of manufacturing extracts from natural substratum by SFE**.** IV Encontro Brasileiro sobre Fluidos Supercríticos.CD-Rom, **2001**.