Reviews

Comparative Assessment of Technologies for Extraction of Artemisinin

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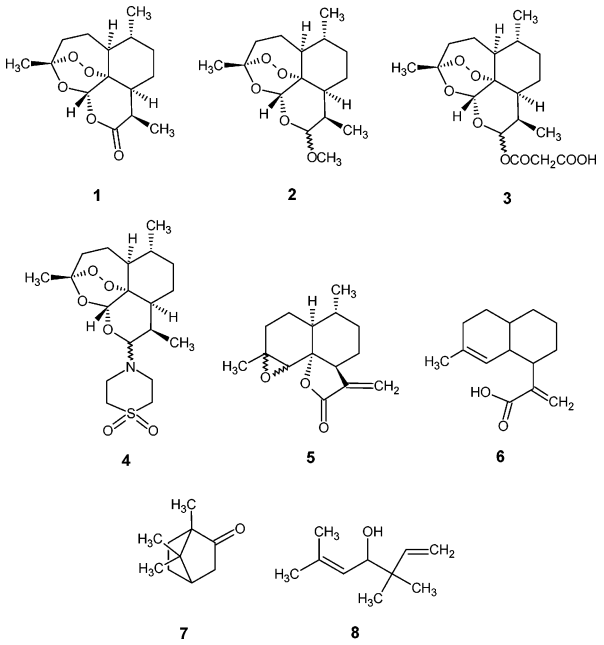
This paper describes results of a multiobjective comparative assessment of several established and emerging technologies for extraction of a natural antimalarial substance, artemisinin. Extractions by hexane, supercritical carbon dioxide, hydrofluorocarbon HFC-134a, ionic liquids, and ethanol were considered. Hexane extraction is an established technology and appears to be the most cost-effective. However, it is characterized by lower rates and efficiency of extraction than all other considered techniques and is also worse in terms of safety and environmental impact. Similarly, EtOH extraction was found to be worse than hexane in all assessment parameters. The new technologies ($scCO_2$, HFC, and ILs) are based on nonflammable solvents and are characterized by faster extraction cycles and more complete extraction of the useful substances and enable continuous extraction processes with reduced solvent inventory. Ionic liquid and HFC-134a technologies show considerable promise and should be able to compete with hexane extraction in terms of cost-effectiveness following due process optimization. New technologies are also considerably safer (no risk of explosions, low toxicity) and greener (having a lower environmental impact in use, potential for biodegradability after use). The methodology of comparative assessment of established and emerging technologies is discussed.

Introduction

Artemisinin **1**, itself an antimalarial compound, is the precursor to more potent substances such as artemether **2**, artesunate **3**, and several others,[1−3] including the most recent addition to the family of compounds, the artemisone **4**.[4] The importance of this class of compounds in malaria treatment stems from their very rapid action against most widespread *Plasmodium falciparum* malaria and its cerebral complications.[3] Although artemisinin-based monotherapies and combination therapies (ACTs) with other antimalarial drugs have been used for over 10 years, there is still no reliable evidence of parasite resistance to artemisinin,[5] hence the increasing importance and demand for the artemisinin-based compounds and ACTs.

The main source of artemisinin is *Artemisia annua*, which grows in temperate climates and is most widespread in China and Vietnam, although also found and/or grown in East Africa, the United States, Russia, India, Brazil, and some other countries.[2,6,7] Total synthesis and biochemical synthesis of artemisinin have also been demonstrated, but these routes are expensive and, therefore, are presently not viable as the mainstream source of artemisinin.

The main artemisinin-related compounds found in the plant are artemisinin, arteannuin B (**5**), and artemisinic acid **6** contained in the plant leaf, with ca. 42% of the total artemisinin content found in upper leaves.[2,8,9] The total amount of artemisinin found in different varieties of *A. annua* is between 0.01 and 1.4 wt % based on dry leaf mass. In some plant varieties the acid **6**, which is accepted to be the precursor to artemisinin in the biotransformation pathway, is found in significant concentrations, up to an order of magnitude higher than that of artemisinin.[9] Chemical conversion of artemisinic acid is, therefore, an option of developing a semisynthetic route to artemisinin, increasing its total production from biomass.[9−13] An alternative strategy, currently pursued by a number of research groups, is to develop a new plant variety with



an increased concentration of artemisinin. Apart from sesquiterpenes, which represent the main medicinal value, the plant's essential oil contains a large number of components of potential commercial value, such as camphor **7**, artemisia alcohol **8**, and ketone, borneol, etc.[9] Recently, the lipid content of *A. annua* was analyzed and the medicinal effect of lipophilic extracts on the skin investigated.[14] Although there is already a limited commercial use of other than artemisinin compounds of the plant to produce cosmetics and flavorings, there is a significant scope to increase the commercial value of *A. annua* cultivation by more complete utilization of different biomolecules, i.e., the biorefinery concept.[15]

The World Health Organization (WHO) estimated that they will require 120 million courses (adult equivalent) of artemisinin-based

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combination therapies (ACTs) in 2006,[16] although up to 500 million cases of malaria are reported to occur annually worldwide. At the time when this paper was written the only ACT approved by WHO was Coartem, manufactured by Novartis. One treatment course of Coartem consists of between 16 and 24 tablets, each containing 20 mg of artemether **2**.[17] On the basis of this information it is possible to estimate the required area of *A. annua* plantations to satisfy current annual demand. Given that the chemical yield of artemether **2** from artemisinin **1** is approximately 60%,[4] the global requirement for artemisinin in 2006 is projected to be ca. 96 000 kg. The content of artemisinin in the leaf varies widely, but a reasonable assumption is that most commercial plantations would be based on the higher yielding crops with an average content of ca. 1 wt %. The amount of leaf harvested also varies greatly and depends not only on the plant variety but also on the climate, growth density, and use of fertilizers and irrigation. Thus, a study based on the field trial in Vietnam reported up to 7000 kg·ha^{-1} dry mass yield and ca. 30 kg·ha^{-1} artemisinin yield at a plant density of 20 plants·m^{-2}.[9] A more recent report describes a multiharvest approach in trial in India, exploiting the fact that artemisinin content is higher in young leaves.[18] This technique allows increasing the yield of artemisinin by harvesting young leaves from the same plants up to four times, with the maximum reported yield of 77.5 kg·ha^{-1}. The potential least efficient option is based on the yield of dry mass of *A. annua* of ca. 1000 kg·ha^{-1}.[19] More generally, it is believed that large commercial farms using best agricultural practice and irrigation are achieving between 4000 and 5000 kg·ha^{-1} dry mass yield, whereas small holders on the fields without irrigation are managing only ca. 1000 kg·ha^{-1}.[20] Based on these values, the annual worldwide area of *A. annua* plantations should be between 3000 and 14 000 hectares (7413−34 595 acres) to provide enough artemisinin for manufacturing of 120 million adult treatment courses of ACTs. Such an area of land is fairly small, and given the uncertainty over the future demand for artemisinin due to potential (i) development of vaccines, (ii) decrease in price of the synthetic alternatives, and (iii) development of resistance to artemisinin in parasites, it is prudent to consider the potential new extraction facilities as a multicrop versatile plant, rather than focusing solely on *A. annua*.

The main commercial extraction process is based on the use of hot petroleum ether (single-component solvents such as heptane, hexane, and toluene are also used and are almost identical in performance to petroleum ether) as extracting agent, sometimes modified with EtOAc to increase solubility of artemisinin.[21−25] The initial extraction is performed in several steps to increase efficiency with respect to artemisinin content in the leaf. Separation of artemisinin-related compounds from the raw extract, containing also waxes, essential oils, and chlorophylls, is performed by crystallization from EtOH and also requires several stages. The product of crystallization is off-white crystals and is further purified by chromatography. A variation of this method, involving partitioning of the hexane extract with acetonitrile, was proposed.[26] The advantages of this method are (i) a significant reduction in the volume of solution after partitioning with CH_3CN (the ratio of hexane raw extract to CH_3CN is 3:1) and (ii) the ability to cleanly separate the three main "artemisinin" components, namely, artemisinin **1**, artemisinic acid **6**, and arteannuin B **5**.

The use of hexane/petroleum ether as extracting agent is widely practiced in China and Vietnam, who are currently the main growers and processing centers of *A. annua*. This is mainly due to low capital cost, technical simplicity, and wide applicability of hexane extraction, despite fairly low overall efficiencies of extraction, as low as 62−70% to crude extract, in large-scale processes. This process is hazardous due to high flammability of hydrocarbon solvents and the potential for generation of explosive hydrocarbon vapor−oxygen (air) mixtures. The use of large volumes of a volatile hydrocarbon solvent results in significant environmental impact of the process via several routes: contribution toward global warming through emission of greenhouse gases, photochemical smog generation, the use of nonrenewable materials, and its human and biotoxicity. There is also sufficient concern over the entrapment of hexane in extracted products and residual biomass that is being used as fertilizer or animal feed. It is therefore desirable to develop an alternative method of extraction of artemisinin, which would compare favorably with hexane extraction in terms of environmental impact, hazard, and also extraction efficiency, cost, universality, i.e., ability to extract other materials in different seasons, and scaleability. Hexane extraction is also used in the production of vegetable oils for biodiesel and other uses, and the demand for these products is destined to increase dramatically.

Apart from conventional solid−liquid extraction with nonpolar hydrocarbon solvents, extraction of artemisinin with EtOH[27] and supercritical CO_2[28−32] was reported. Extraction of oils and fragrances by hydrofluorocarbon solvents, such as 1,1,1,2-tetrafluoroethane (HFC-134a)[33] and iodotrifluoromethane (ITFM),[34] was reported, and test trials of artemisinin extraction were performed. Another potentially promising new class of extraction solvents is ionic liquids.[35] It is not yet clear how these methods of extraction compare with the conventional hydrocarbon extraction, especially in the absence of direct experimental pilot-scale trials.

There are several comparative studies of artemisinin extraction available in the public domain. Earlier results on EtOH, 1,2-dichloroethane, $CHCl_3$, Et_2O, acetone, and petroleum ether using conventional solid−liquid extractors were reported by Klayman et al.[21] No differences were found between different hydrocarbon solvents, i.e., toluene, hexane, and petroleum ether.[24] Supercritical CO_2, pressurized H_2O and EtOH, and microwave-assisted hydrocarbon solvent extraction methods were experimentally compared by Christen and Veuthey on the lab scale.[36] $scCO_2$, hexane, and EtOH were compared on the basis of efficiency, cost, and environmental impact in the study aimed at potential development of *A. annua* plantations in Tanzania and Kenya.[23] However, there is significant discrepancy between some reports, and more recent trials of HFCs, EtOH, and ionic liquids have not been benchmarked against the more conventional techniques.

The aim of the current study is to provide a comparative assessment of performance of different technologies of artemisinin extraction, which would enable the potential end-users to make a rational decision on the suitability of these methods of extraction to specific applications: the scale and location of the extraction facility, the potential for extraction of more than one plant material, and the potential for developing a small-scale mobile extraction facility. The study compares mature technologies, such as extraction with hexane and $scCO_2$, with developing technologies, extraction by HFCs and EtOH, and an emerging technology of extraction by ionic liquids. Hence, it is also the aim of this study to identify the areas of future development that may improve the performance of the developing and emerging technologies. Comparison of mature and emerging technologies is not straightforward, and there are no well-developed methodologies. Therefore, the paper also demonstrates an approach to such an analysis in the area of Green Chemical Technologies, i.e., technologies aimed at elimination of waste, risk, hazard, and dependence on nonrenewable feedstocks through the design of materials and processes.

Comparison of technologies on the basis of cost is the normal approach to comparative assessment of processes, even when environmental performance is taken into account, i.e., environmental cost accounting approach.[37] However, the monetary approach is not ideal for comparison between mature and emerging technologies, because of the uncertainties and, hence, low accuracy of cost estimates, necessarily associated with emerging technologies, as well as the lack of optimized performance data for emerging processes. Furthermore, environmental assessment in monetary terms requires assignment of arbitrary monetary values, a value judgment, to the inherently nonmonetary concepts, such as clean

Table 1. Artemisinin Properties[41]

	artemisinin content	
	97.0−102.0 (by IR)	98.0−102.0 (by TLC)
TM/°C	151−154	
$[\alpha]_D^{20}$	+75 to +78	10 mg·mL^{-1} solution in dehydrated EtOH
loss on drying	<5 mg·g^{-1}	at 80 °C
sulfated ash	<1 mg·g^{-1}	

air or biodiversity. Another approach is to compare the environmental impact of alternative processes over the life-cycle, and these approaches have been developed for biofeedstocks.[38] However, traditional life-cycle assessment is done at a fixed point in time and also does not deal explicitly with the issue of emerging breakthrough technologies.[39] It is argued, that a multiobjective hierarchical metric, which is based on the philosophy of environmental impact of the product/process over its life-cycle, but stops short of indicator aggregation and maps indicators onto stakeholder interests analysis, should be more adept at dealing with problems involving a high degree of uncertainty.[40] This paper attempts to present a comprehensive comparison of alternative artemisinin extraction technologies using the multiobjective metric approach, explicitly tackling the issue of comparing established and potentially breakthrough technologies.

The Method of Assessment

The viability of each technology has been assessed on the basis of the potential to achieve the required feed throughputs, their energy efficiency and global warming potential, running and capital costs, toxicity, and risk, as well as several qualitative parameters, such as potential for using other crops, existence of undesirable hazards, and potential further use of spent biomass. Other important criteria that were necessary taken into account are the operating conditions under which to compare technologies, the scales of production, and the purity of the final product. These factors define the *system boundary* and the *initial conditions* for comparative assessment.

System boundary was selected as gate-to-gate: each process was evaluated under its optimal or best known conditions, and environmental impacts were considered for the process of extraction itself, without including life-cycle impact of synthesis and disposal of the solvents. It was also necessary to specify the desired purity of the obtained artemisinin extraction and whether extraction should be done under GMP conditions (GMP ≡ good manufacturing practice). The purity of pharmacological products is defined within their monographs; for example, there is a publicly available monograph of Coartem, published by Novartis.[17] There is some uncertainty over the monograph for artemisinin. However, some data are publicly available[41] and reproduced in Table 1. At present only final purification of artemisinin and conversion of artemisinin **1** into artemether **2** or artesunate **3** are being done under GMP conditions. The extension of GMP regulation to extraction, which is by its nature more variable, would significantly increase the cost of biomass processing and was considered unnecessary. Some aspects of GMP relating to phytopharmaceuticals have been discussed recently.[42] It is, therefore, considered sufficient if an extraction process is capable of producing crude artemisinin extract (crystalline or powder compound with "off-white" color) that can attain monograph standard following chromatographic purification.

The main initial condition of comparative assessment is the condition of comparison of technologies under their optimal operating conditions. Further requirements are to satisfy European environmental legislation and have potential for multicrop operation. Three sizes of extraction facilities were analyzed: (i) a mobile "back-of-a-truck" plant capable of servicing a number of small holder farmers, thus reducing the need for transportation of biomass and increasing the income of farmers, (ii) a large-scale facility capable of processing up to 2500 tonnes of dry biomass per annum, and (iii) a very large extraction plant with the annual throughput of 6000 tonnes. The largest scale of extraction facilities was considered due to the anecdotal evidence of the planned areas of *A. annua* plantations.

The environmental performance of the extraction technologies was assessed using the reported multiobjective approach.[39,40] This approach requires identifying the relevant stakeholders and to agree to the set of the most appropriate indicators/criteria; results of this analysis are shown in Table 2. It appears that only a small number of quantitative indicators can be assembled that are relevant to the majority of stakeholders and which are feasible to estimate on the basis of the available data. Hence, the analysis will not be focused solely on numerical indicators, but also on qualitative factors. This is especially important, since several technologies assessed in this study are "emerging technologies" and therefore cannot be directly compared in terms of raw performance and economics with the well-established hydrocarbon solvent extraction.

General Aspects of Artemisinin Extraction

Artemisinin compounds (**1**, **5**, **6**) have been predominately found in the upper parts of the *A. annua* plant, with the concentration of artemisinin said to peak just before or during full flowering, the difference being attributed to climatic conditions, plant variety, or other, yet undetermined, factors.[9] More specifically, artemisinin **1** and its precursor artemisinic acid **6** have been shown to be localized in the glandular trichomes on the leaf surface.[43,44] The main consequences of this are that (i) it may not be necessary to mechanically crush the plants prior to extraction for reasons other than to increase the packing density, and (ii) the artemisinin content depends on the age of the leaf, since in older leaves the glands were often found to be ruptured. The latter has been exploited in developing a multiharvest approach from the same plants, allowing a considerable increase in the amount of artemisinin produced per area.[18] Due to the physicochemical properties of artemisinin (low thermal and chemical stability of the endoperoxide function, low polarity and, hence, poor solubility in H_2O, and good solubility in organic solvents, see Table 3), its extraction with nonpolar solvents is necessarily complicated by simultaneous extraction of essential oils, chlorophylls, and waxes. Therefore, the extraction step must be followed by separation of artemisinin from the initial liquor, which is generally achieved by sequential crystallization from an EtOH solution.

Earlier literature suggests that extraction with hot H_2O is inefficient, extraction with EtOH results in rapid decomposition of artemisinin (isopropanol appears to have no deleterious effect on the stability of artemisinin), and other conventional low boiling point solvents, such as Et_2O, acetone, $CHCl_3$, and 1,2-di- and trichloroethane, are also suitable, albeit not as selective as petroleum ether.[21] However, more recent literature demonstrates efficient extraction with EtOH.[27]

Specific Aspects of Different Extraction Technologies; Comparison of Extraction Efficiency

Solid−Liquid Low-Pressure Extractions. Conventional solid−liquid extraction is performed in the "soak" (batch) regime or the percolation regime, with the solvent pumped through the biomass to increase the efficiency of intraparticle mass transfer; see first two schemes in Figure 1. Such processes are limited by the equilibrium solubility of the solute in the solvent and, therefore, almost always require more than one extraction stage with fresh solvent portions. Pressurized liquid solvents could also be used, especially in the case of percolation extraction. Elevated pressure allows an increased liquid flow rate and, hence, further improves mass transfer efficiency.

Three different solvents considered in this study are used in these types of processes: hexane, EtOH, and ionic liquids. The common

Table 2. Stakeholder Analysis for Artemisinin Extraction Technologies

stakeholders	drivers	indicators
patients	price, efficiency, side effects, availability of medicines	(euro)·kg^{-1} (artemisinin) or $·kg^{-1} (artemisinin)
growers of *Artemisia* annua	price of fresh/dry leaf *Artemisia* annua, availability of processing facilities, potential to switch to other crops	kg (artemisinin) · ha^{-1} (euro)·kg^{-1} (artemisinin)
owners of extraction facilities	capital and running cost of processing technology, quality/price of final product, safety, other crops, environmental impact	(euro)·kg^{-1} (artemisinin) kg (CO_2)·kg^{-1} (artemisinin) (euro)m capital costs kg (artemisinin)·ha^{-1}
technology developers	quality of produced extract, compliance with regulations enabling implementation, safety, environmental impact, cost, other crops	(euro)·kg^{-1} (artemisinin) (euro)m capital costs kg (artemisinin)·ha^{-1} other markets safety, hazard to environment and human health
pharmaceutical industry	availability of artemisinin at the correct price and purity	(euro)·kg^{-1} (artemisinin)
WHO and NGOs	availability and price of artemisinin, environmental and social impact of technologies in local areas	(euro)·kg^{-1} (artemisinin)

Table 3. Physicochemical Properties of Artemisinin

parameter	value	ref
molecular mass/g·mol^{-1}	282.3	
melting point/°C	156−157	3
thermal stability in nonpolar solvents/°C	150	3
solubility in H_2O @ pH 7/g·L^{-1}	0.063	4
solubility in H_2O @ pH 7, 37 °C/g·L^{-1}	0.048[a]	66
solubility in EtOH @ 21 °C/g·L^{-1}	12	67
solubility in EtOAc @ 20 °C/g·L^{-1}	100	67
solubility in hexane @ 40 °C/g·L^{-1}	0.46	23
solubility in hexane/EtOAc (5 vol %)/g·L^{-1}	33	23
solubility in **9**/g·L^{-1}	82	50
solubility in **10**/g·L^{-1}	110	50
octanol/H_2O partitioning coefficient/log *P*	2.94	4

[a] Value for triclinic crystals obtained by recrystallization from cyclohexane; recrystallization from EtOH (50 vol %) solution yielded orthorhombic crystals with lower and slower solubility in H_2O.

feature of the three systems is the relatively simple scale-up due to low pressure: the large-scale facilities were assumed to be based on 10 m^3 extractors, which is believed to be close to the size of existing hexane extractors.

Hexane Extraction. Although solid−liquid extraction with hexane is a well-established method, for example in extraction of flavors from partially dried whole hops[45] and extraction of seed oils, detailed information on the extraction of artemisinin is difficult to access. Available open literature on extraction with hexane, toluene, cyclohexane, or petroleum ether give widely variable numbers of extraction efficiencies, times for extraction, ratio of solvent to biomass, and the type of the secondary purification method employed to separate artemisinin from the primary extract.[8,21,23−26,36,46,47] Two schemes of hydrocarbon solid−liquid extraction of artemisinin are shown in Figure 2. Scheme A is what is believed to be the most commonly used method with some variations in the actual solvent, i.e., petroleum ether, hexane, or hexane/EtOAc mixture, and the type of extraction plant used, i.e., batch or semibatch percolation.

In the simplest hexane batch extraction, dried, crushed leaf is soaked 3 or 4 times in fresh portions of warm (30−45 °C) hexane or petroleum ether, each extraction cycle taking between 10 and 48 h.[26,47] Under the flow conditions (solvent percolation through packed biomass bed) at the same temperature the duration of each cycle can be reduced to 90−120 min,[48] whereas under microwave irradiation the duration of extraction can be further reduced to about 12 min.[49] However, scale-up of the microwave process to industrial-size extractors is problematic due to the short depth of penetration of microwave irradiation. Therefore, it is not considered as a viable option. The effect of the size of leaf particles was explicitly addressed in the lab-scale microwave-assisted extraction study, showing that at a fixed extraction time the smallest studied particle sizes (<0.125 mm) gave the highest extraction efficiency.[49] The effect of particle size might be due to the improved mass transfer. However, due to the location of artemisinin external to the leaf glands, the main effect of mass transfer should be expected due to convection, which is controlled by the solvent flow rate through the biomass bed, rather than diffusion, which is controlled by the particle size. One additional aspect of the particle size effect is the packing density of dry mass: fine particles allow higher packing density, which increases pressure drop and decreases percolation efficiency. There is not enough systematic data on the effect of particle size to provide a qualified recommendation on the optimum size. In order to improve the efficiency of extraction, a small amount of cosolvent EtOAc can be added to the main nonpolar hydrocarbon solvent. This increases the solubility of artemisinin in the solvent mixture by about 2 orders of magnitude.[23]

Following extraction, the solvent is drained and spent biomass must be stripped of the residual solvent. Biomass is said to absorb solvent in the ratio of 1 L·kg^{-1}.[46] Solvent can be removed by pressing the biomass removed from the reactor. This allows recovery of the artemisinin contained in the residual solvent. Stripping of the solvent can also be achieved by evaporation in air under natural convection. This is potentially hazardous and leads to the release of significant quantities of environmentally harmful volatile hydrocarbon, as well as reduces the amount of recovered artemisinin. Steam stripping followed by condensation is a more efficient method of recovery of the solvent, but also does not increase the yield of artemisinin.[48] The recovery and reuse of the solvent reduces the environmental impact and improves the cost-effectiveness of the process. Vacuum stripping may also be used to avoid potential biomass decomposition under steam and to avoid downstream water−solvent separation.

The obtained initial crude extract is flash-evaporated to 10% of its initial volume, and the remaining liquor is left to stand at ambient temperature over ca. 48 h to crystallize crude artemisinin, allowing decanting of the remaining liquor. Crude artemisinin is washed with warm hexane to remove the waxes and other precipitated impurities. In order to remove the waxes, artemisinin is recrystallized several times from EtOH/H_2O azeotrope (95 wt % EtOH) in the presence of activated carbon adsorbent, followed by vacuum evaporation.[46] Further purification is achieved by chromatography. An alternative method of separating artemisinin from the initial hexane extraction involves liquid−liquid extraction of artemisinin-related compounds from hexane into acetonitrile;[25,26] see Figure 2, part B. This allows a reduction in the volume of extract and also the capability to

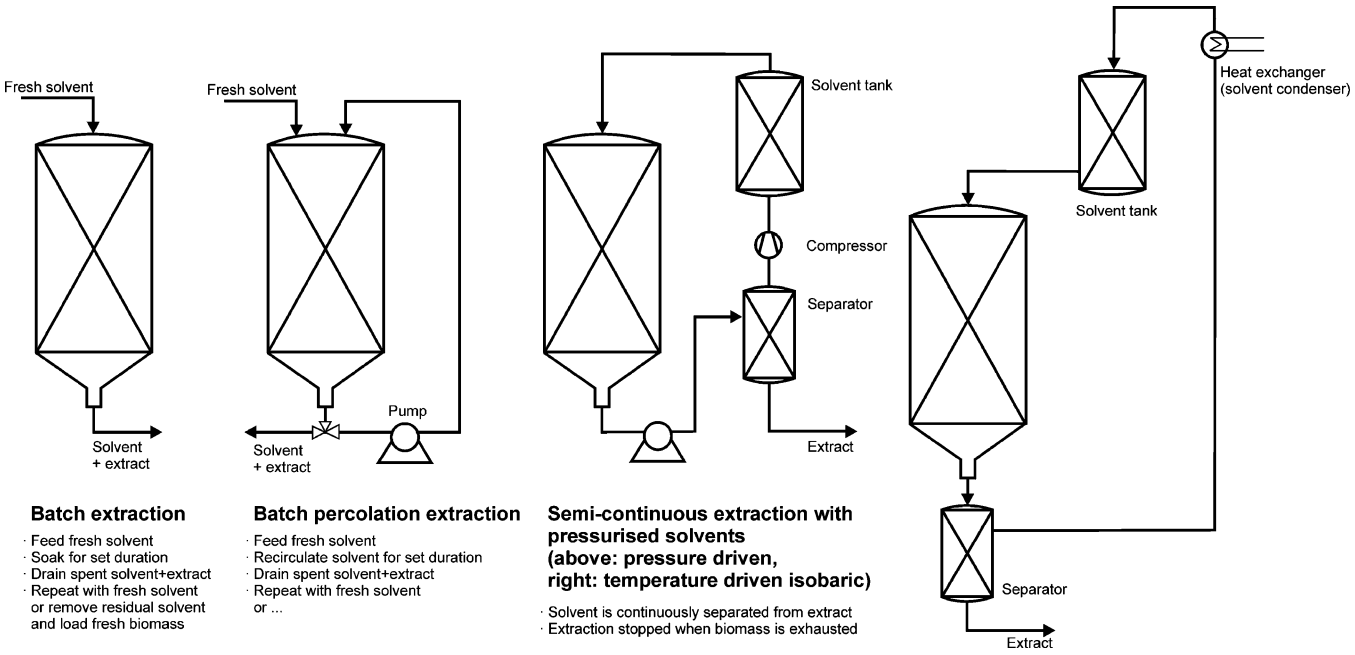


Figure 1. Schematic diagrams of extraction plant options.

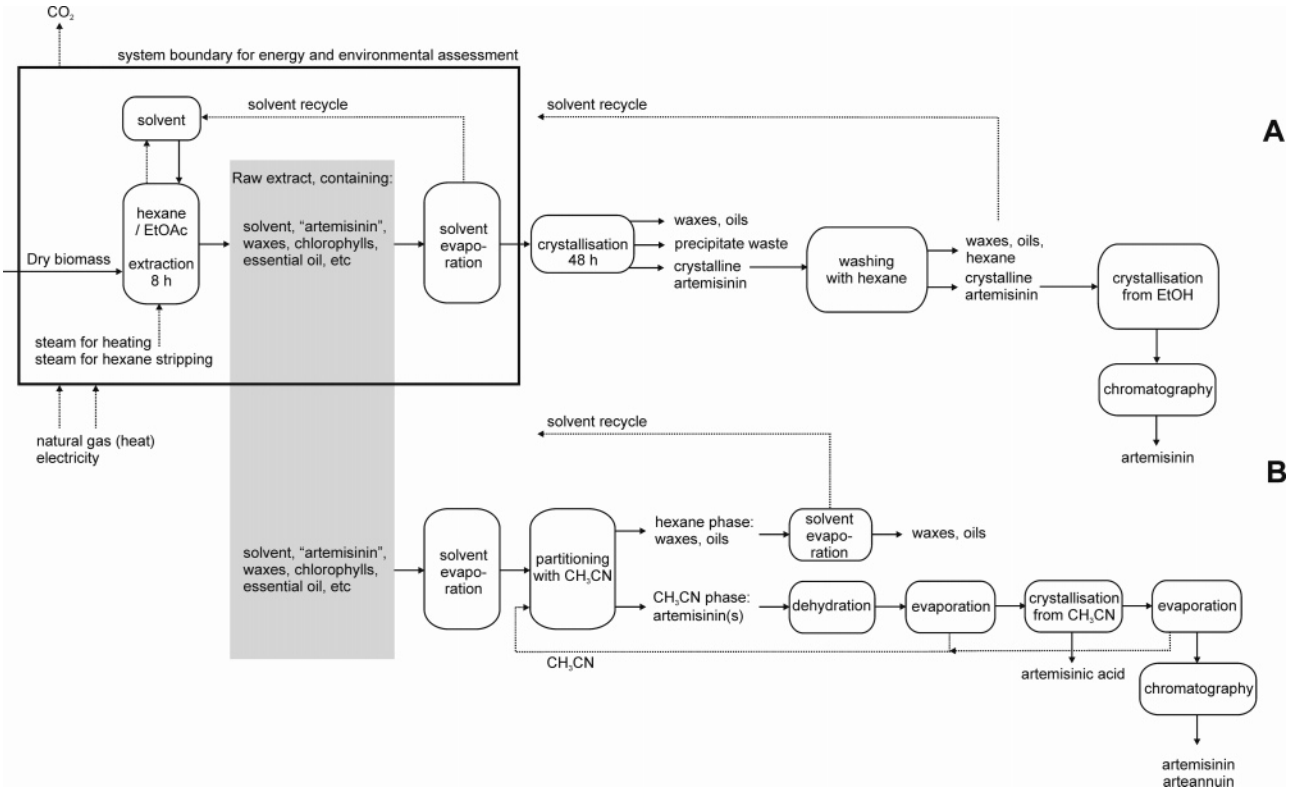


Figure 2. Schematic diagrams of artemisinin extraction with hexane; solid bold line corresponds to gate-to-gate system boundary for energy and environmental assessment.

selectively isolate artemisinic acid **6** and arteannuin B **5**, as well as artemisinin **1**. This method will not be considered in this study due to the hazardous nature of acetonitrile to the environment and human health (lowest LD_{50} 50 mg·kg^{-1}, MSDS data), rendering its large-scale use unacceptable.

Based on the reviewed information on hexane/hydrocarbon solvents extraction, the process summary and performance figures are shown in Table 4. Hexane/EtOAc (95:5 molar ratio) mixed solvent was assumed. The efficiency of extraction, i.e., the amount of artemisinin in the primary extract relative to the amount of artemisinin in starting dry biomass even after several extractions with fresh solvent portions, is believed to be about 70%.[46] A much lower efficiency of extraction with hexane was also reported (ca. 39%); however, this value was obtained using unoptimized conditions.[49] The efficiency of artemisinin recovery from the primary extract is not known. On the basis of the data for other extraction methods, which also require recovery of artemisinin from the primary extract, the overall efficiency of extraction with hexane is estimated to be about 60%. This value was used to estimate the two figures of the overall artemisinin yield that could be obtained by a large commercial farm and a small holder, which are reported in Table 4.

Table 4. Direct Comparison of Extraction Performance of Different Methods

method of extraction	operating temp/°C	operating pressure/ MPa	ratio of solvent to dry leaf/ kg:kg	no. of extractions with fresh solvent[a]	duration of a complete extraction cycle[b]/h	solvent residue in biomass/ wt %	extraction efficiency[c]/ %	yield of primary extract/wt % (dry basis)	conc of artemisinin in primary extract/wt %	overall artemisinin yield[d]/ kg·ha^{-1}
hexane	30−40	0.1	4:1	3−4	8−10	66[f]	70/60	7.3	9	6/30
EtOH	ambient	0.1	5:1	3	7	unknown	91/73	unknown	unknown	7.3/36
$scCO_2$	30−50	15−30	N/A	N/A	3−6	0	−/82	∼6	unknown	8/41
HFC-134a	15−40	0.4−1.2	N/A	N/A	6	19[f]	77/62	>2.7	∼40	6.2/31
IL, **9**	ambient	0.1	6.3:1	1	2.5	unknown	∼79/64		0.08−0.6[g]	6.5/32
IL, **10**			0.9:1[e]		6		∼96/79			7.9/39

[a] Number of extraction steps with fresh solvent in the given ratio of solvent to biomass to achieve close to complete recovery of artemisinin. [b] Includes changeover and heating time estimated to be 2 h and excludes downstream separation of artemisinin from the primary extract. [c] With respect to biomass content of artemisinin: first number corresponds to artemisinin content in the primary extract; second number corresponds to the overall efficiency after separation of artemisinin from the primary extract. [d] Assuming initial artemisinin content in dry leaf of 1 wt % and biomass yield of 1000/5000 kg·ha^{-1} (the cases of small holder and an efficient commercial farm). [e] Best case estimate. [f] Solvent residue before stripping. In the case of HFC-134a stripping of solvent reduces the residual amount to below 300 ppm. [g] In the case of IL primary extract is not concentrated. The range is given by the estimated best and the experimental[50] volumes of solvent used.

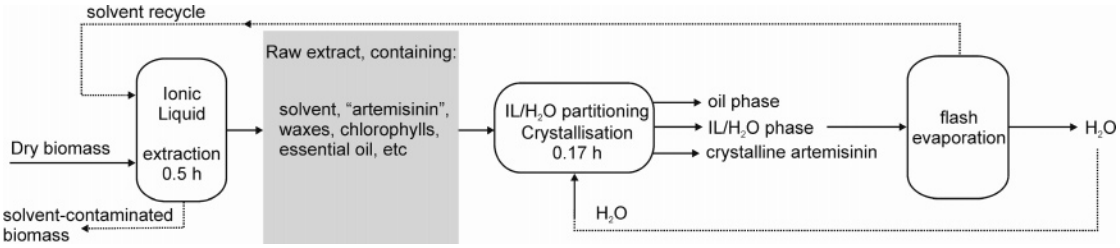
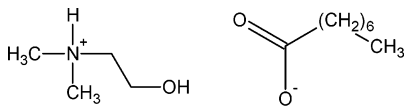


Figure 3. Schematic diagram of artemisinin extraction with an ionic liquid solvent.

Ionic Liquids (ILs). Organic ionic liquids (organic equivalent of molten salts) is a new class of solvents, characterized by negligible vapor pressure, nonflammability, and the possibility to tune solvation properties over a very broad range. Since ILs lack two major drawbacks of the hydrocarbon solvents, vapor pressure and flammability, these solvents are often cited as a "green alternative". However, life-cycle impact of their manufacturing and disposal and the reported toxicity of some ionic liquids are just two concerns. Ionic liquids have been reported as a very good reaction medium for many organic reactions catalyzed by chemical as well as biocatalysts. Despite a fairly recent development of this field of research, several chemical processes based on ionic liquids have already been commercialized. However, there are very few publications on the application of ionic liquids in extraction of biomolecules.[35] The assessment presented in this study is based on the preliminary study undertaken by Bioniqs Ltd (U.K.) and funded by MMV.[50]



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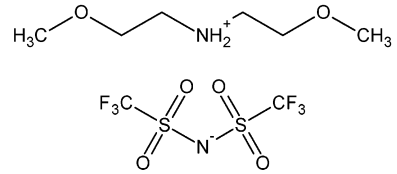

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Initial tests were performed using five ionic liquids of which *N*,*N*-dimethylethanolammonium octanoate (DMEA oct, **9**) and bis(2-methoxyethyl)ammonium bis(trifluoromethylsulfonyl)imide (BMOEA bst, **10**) have shown the best performance. The extraction process is similar to a standard liquid−solid extraction and was performed in a batch regime. Extraction was performed using a solvent to biomass ratio between 6.3:1 and 9:1 (w/w) at 25 °C. With DMEA oct the maximum artemisinin concentration in solution (0.79 g·L^{-1}) was reached after 30 min of extraction, after which a slight decrease in concentration of dissolved artemisinin was observed, perhaps due to either the decomposition of artemisinin or its migration back to the spent biomass. The problem of stability of artemisinin in some solvents was reported for conventional systems as well.[21] The observed concentration of artemisinin in solution was similar to that obtained in the benchmark experiment with hexane at the same temperature (0.78 g·L^{-1}).

In the case of the solvent **10** the rate of extraction was considerably slower than that with **9**. However, the maximum concentration of artemisinin in solution was higher (by 23%) and no apparent loss of artemisinin was observed. Furthermore, the obtained rate of extraction with **10** was similar to the rate of extraction with *n*-hexane at the same temperature. Thus, in comparison with hexane, ionic liquid **9** gave a similar efficiency of extraction at a considerably faster rate, whereas ionic liquid **10** gave a higher extraction efficiency at the same rate.

The second step is the separation of artemisinin from the solvent. The proposed process involves partitioning−recrystallization with H_2O at ambient temperature, which causes simultaneous separation of the oil fraction and crystallization of artemisinin. Crystallization allows a separation of 82% of the total extracted amount of artemisinin; the remainder is assumed to be lost with the oil phase. The crystals are 95% artemisinin (by NMR) and are essentially free of solvent (not detectable by NMR). The amount of H_2O used in the initial experiments was 3:1 (v/v) with respect to the IL solvent. Separation was achieved in about 10 min. The potential scheme of extraction with ionic liquid solvent is shown in Figure 3. The final process is likely to be considerably different, since there are many alternative options for recovery of artemisinin from the primary extract. Furthermore, the regeneration of ionic liquid solvents (periodic removal of accumulated nonvolatile impurities) has not been studied, and it is therefore impossible to comment on the long-term stability of the solvent and its impact on the process economics. These aspects require further development.

The estimated performance data obtained with the two ionic liquids are shown in Table 4. The time of extraction in the case of IL **10** was estimated from the kinetic data based on 95% of the

maximum solution concentration, since further extraction required too long process times. Using the same calculation method the overall efficiency of extraction with respect to the area of cultivated land was estimated. It should be emphasized that the obtained initial results are not optimized and the overall yield of artemisinin, as well as the ratio of solvent to biomass, is likely to be significantly improved. More specifically, it is likely that under optimal conditions the required amount of solvent would be equivalent to the void fraction in the packed extraction vessel, thus resulting in the ratio of solvent to biomass of ca. 0.9:1 (w/w). The amount of residual solvent in the biomass was not measured and is a matter of ongoing further study, since this would significantly impact on the process economics and environmental impact, as well as on the potential further use of the spent biomass. Some ionic liquids (e.g., DMEA oct) are biodegradable, which opens a new process option without solvent recovery from spent biomass. This requires further study. Finally, extraction from fresh leaf was also tested and was shown to be feasible, although considerably lower efficiencies of extraction were achieved.

Ethanol Extraction. Despite earlier reports on the poor stability of artemisinin in EtOH solutions,[21] a recent study of extraction by EtOH aqueous azeotrope at room temperature claims high efficiency.[27] EtOH is an attractive solvent due to its benign nature and widespread availability from renewable feedstocks. The latter fact is especially important for processes that are predominantly focusing on locally sourced materials to improve the overall process sustainability. A potential constraint on the use of EtOH as a process solvent is its use as a spirit. This can be resolved by using a spiked solvent, which is the customary practice in the EU. However, there are similar concerns with the use of EtOH, as in the case of hexane: it is a flammable solvent, with high toxicity (based on MSDS data, see discussion below) and high risk in use.

The process based on EtOH extraction involves three sequential extractions with fresh solvent portions followed by flash evaporation of solvent to reduce the volume of the primary extract.[27] Some process optimization is possible to reduce the ratio of solvent to biomass, as described in the original publication. The described process uses mechanical stirring, which is impractical and can be replaced by solvent percolation. The efficiency of extraction with respect to the biomass content is reported to be about 91%, whereas overall efficiency after taking into account recovery of artemisinin from the primary extract is about 73%.[27] A very recent study of EtOH extraction of artemisinin in a pressurized percolator extractor was unable to replicate such high extraction efficiencies.[51]

Semicontinuous Extraction with Condensable or Supercritical Solvents. A very significant drawback of batch and batch-percolation extraction processes is the equilibrium limitation. This drawback is avoided in the semicontinuous extraction processes, where a solute is continuously separated from the solvent outside the extractor vessel, thus regenerating the solvent as shown schematically in Figure 1. A fully continuous extraction is established when the solids bed is moving through the extractor; such processes are less common in natural products extraction and are not considered in this study. A semicontinuous process is not equilibrium limited, and solvents with relatively poor partitioning coefficients but high selectivity could be used very effectively. Extraction processes with continuous solvent regeneration are also significantly more flexible than batch extraction in terms of optimizing for different biomasses.

In order to achieve semicontinuous extraction, it is necessary to establish an effective separation of solute from solvent. This is achieved, for example, by changing the solubility when liquid or supercritical solvent is transformed into a gas by depressurization. Temperature-dependent solubility has also been demonstrated (e.g., in higher molecular weight fluorinated solvents). Continuous extraction with conventional solvents may also be feasible, e.g., via evaporation/condensation of hexane. However, this is impractical due to very high energy costs and due to low selectivity of hexane extraction. Two solvent systems considered in this study allow continuous extraction processes, hydrofluorocarbons and supercritical carbon dioxide.

Both solvent systems allow separation of extracted solute by depressurization of the solvent. The pure solvent gas is then compressed or condensed back to the supercritical or liquid state. Thus, isothermal pressure driven and isobaric temperature driven processes are possible, as shown in Figure 1. There are several specific aspects that are unique to such extraction processes.

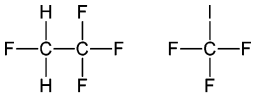
Since expansion of $scCO_2$ in the separator results in a decrease in temperature due to Joule−Thomson effect, the presence of excess H_2O may result in freezing of the expansion valve and of the biomass bed at the end of the extraction cycle. Thus, $scCO_2$ extraction requires <4 wt % water content of biomass, whereas all other extraction methods typically use biomass with 10−15 wt % H_2O content, which is dictated by the optimal biomass storage conditions, rather than the solvent. Additional drying of biomass to such low H_2O content is hugely time consuming and energy intensive. However, in this study the drying step was outside the system boundary for all processes. Depressurization of the extractor, or recovery of residual solvent by vacuum evaporation required in the case of HFC-134a, could also result in freezing of the biomass bed. However, due to much lower pressures, there is no specific requirement on the lower H_2O content of the biomass. A more careful design of the extractor heating is required to avoid problems with biomass changeover. Finally, in the case of continuous extraction processes, the ratio of solvent to biomass is dependent on the size of the equipment and desired recirculation flow rate, rather than the amount of biomass, as in the case of the batch extraction. The extraction cycle time is then governed by the solubility and recirculation flow rate.

$scCO_2$ Extraction. Extraction of artemisinin by $scCO_2$ or subcritical liquid CO_2 has been described,[28−32] and large-scale trials are currently being undertaken. The efficiency of extraction of artemisinin from biomass is reported to be quantitative, rapid, and with higher selectivity compared with the hydrocarbon solvent extractions, based on the gram-scale laboratory tests.[28,52] However, there is wide variability in the efficiencies of extraction with $scCO_2$ cited in the open literature, dependent on the scale of extraction, use of cosolvents, temperature and pressure of extraction, and superficial velocity of the solvent in the extractor. Thus, a lower efficiency than that obtained with a hydrocarbon solvent was reported in the absence of a hydrophilic cosolvent,[30] whereas an earlier patent[29] gives a wide range of extraction efficiencies, between 25 and 100%, depending on the operating conditions. Such a wide variation can be attributed to four factors: (i) accuracy of the analytical methods of determining artemisinin concentrations, (ii) variability in the operating conditions (pressure and temperature, duration of extraction, concentration of cosolvent), (iii) variability in the water content of dry biomass, and (iv) variability between and within biomass samples. A typical value of overall extraction efficiency, including the secondary purification by crystallization, of ca. 80%[53] was used to estimate the overall yield of artemisinin from the area of land, shown in Table 4. The duration of the extraction cycle depends greatly on the scale of extraction and use of cosolvents, as well as more specific aspects of extractor design that influence optimal solvent mass flow rate. Thus, a 20 min extraction cycle was quoted for ca. 1 L scale in the case of the $scCO_2$−ethanol system,[53] whereas detailed kinetic study of artemisinin extraction with $scCO_2$ without cosolvents showed extraction times up to 2 h on a 0.2 L scale.[30] Further analysis of costs and energy efficiency was based on the assumption that the extraction times should be similar to these obtained in the case of hydrofluorocarbon solvent and that it is impractical to run more than a single extraction per working day in a large-scale facility equipped with 10 m^3 extraction vessels. Smaller scale units with short cycle

time allow scheduling of three extractor vessels, which increases the throughput of the system while minimizing the size of an individual extractor vessel.[53]

Hydrofluorocarbons (HFCs). Following the withdrawal of chlorinated hydrocarbons as refrigerants and propellants, they were replaced by hydrofluorocarbons (HFCs), many of which are nonflammable, have low toxicity, and are not ozone-depleting substances. A number of HFCs have been developed and found widespread commercial applications as refrigerants, propellants, fire-retardants, etc. HFC-134a (1,1,1,2-tetrafluoroethane) (**11**) specifically is classed as nonflammable and has zero ozone-depleting potential. HFC-134a is among the most studied and utilized materials for which there is a life-cycle impact study.[54] Additionally in Europe, Japan, and the United States, HFC-134a is accepted by regulatory bodies for use as a solvent in the extraction of food flavorings. One drawback of the HFCs is their very high global warming potency factor: 1300 times larger than that of carbon dioxide for HFC-134a. Therefore, complete recycle and capture of the solvent within a process is of significant importance.

Hydrofluorocarbons are gases under normal conditions and are liquefied at relatively low pressures (see operating conditions in Table 4). Therefore, these solvents are ideally suited for continuous extraction processes, when depressurization of the solvent results in a rapid separation of the extracted material; see Figure 2. Because of the modest pressures and low operating temperatures, the energy required for continuous depressurization/pressurization cycle is not high, resulting in low energy costs, low operating costs, and low greenhouse gas emission due to energy duty. Furthermore, recirculation of solvent can be achieved without pumps, by establishing an isobaric condensation−evaporation cycle, thus avoiding the need for expensive capital investment in the pump and the compressor. In this case the flow rate of solvent depends on the efficiencies of the condenser and evaporator, as well as percolation properties of the packed biomass; see process scheme in Figure 1. A commercial extraction plant (Phurua Natural Oils Limited) based on this principle has been operating in Thailand since 2004.[55]



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Extraction of natural compounds by HFC-134a (**11**) and iodotrifluoromethane (ITFM, **12**) have been reported,[33,34,56] although not specifically of artemisinin. Physical properties of ITFM and HFC-134a are quite similar. However, because of the presence of a weaker C−halogen bond with iodine, there are potential toxicity issues with the use of ITFM. More specifically, acute toxicity of ITFM itself was found only in the conditions of exposure to very high concentrations (>25 vol %). However, there is a significant risk of cardiac sensitization at levels of exposure of 0.2 vol % (2000 ppm), and there are also suspicions of a potential carcinogenic effect.[57,58] ITFM has poor stability in sunlight, in the presence of artificial UV light, and at temperatures above 100 °C; its decomposition is facilitated in the presence of copper and moisture.[58] The products of ITFM decomposition are HF, HI, and COF_2, which are highly toxic themselves and can react further with organic matter, leading to acute toxicity. Relatively poor stability of ITFM requires specific safety measures during storage and use. HFC-134a is a considerably more stable compound and has been subject to long-term (5 yr) stability trials for its pharmaceutical applications.

There are also significant differences in the extraction efficiency and prices. For comparison, 100 g of HFC-134a and ITFM were quoted at 111 and 365 euro (U.S. $142 and $467), respectively, by the SigmaAldrich catalog in September 2006. Note that catalog prices cannot be used for scaling, would differ in other countries, and are only given for comparison purposes. On the basis of the data reported in the patent literature,[33,34] ITFM is a much stronger solvent than HFC-134a: HFC-134a is more selective toward mobile oils containing fragrant compounds and extracts little waxes and heavier oils, which are effectively extracted with ITFM. Due to this difference in solvation properties, HFC-134a is expected to be more selective toward artemisinin than ITFM. By combining ITFM with cosolvents, including HFC-134a, it is possible to regulate the extraction efficiency. Thus, a patent[34] claims that the amount of extracted waxes decreases proportionally with an increase in the concentration of HFC-134a cosolvent in ITFM. Similarly, it is possible to increase the extraction power of HFC-134a by addition of nonpolar hydrocarbon cosolvents.

The data reported in this study are based on the information kindly provided by Ineos Fluor Ltd.[59] These data are supported by similar results obtained by Peter Wilde.[55] Values reported in Table 4 were calculated using the efficiency of artemisinin recovery from the primary extract of 80%. This is a conservative value based on the efficiency of artemisinin extraction from hexane and EtOH extractions. Because HFC-134a extract has a much higher concentration of artemisinin and lower amount of tars, it is likely that a more efficient secondary extraction can be developed. The amount of residual solvent in the biomass reflects experimental values prior to any attempt to strip the solvent. This value is not especially meaningful since residual solvent must be recovered to reduce the environmental impact and recover expensive solvent. Solvent recovery by vacuum stripping has been included in the energy and cost estimates.

Other Solvent Systems. The solvent systems described in detail above are either already commercially used for artemisinin extraction or hold great promise for this specific application. Extraction by steam or pressurized H_2O, which can be used for oil extraction from seeds, is not applicable to artemisinin, due to its poor solubility and stability in aqueous solutions. Higher molecular weight dearomatized hydrocarbon solvents were reported as efficient solvents for artemisinin extraction both in the standard and in the microwave-assisted processes.[49] The microwave-assisted extraction is potentially a highly efficient method, allowing significant reduction in extraction times. However, its technical complexity, high cost, and requirement for skilled operators render this technology impractical for the process of artemisinin extraction. The data on extraction by reviewed solvent systems indicate that the higher content of essential oil in the plant is beneficial for efficient extraction, acting as a cosolvent. It may be possible to use natural oils as extraction medium, especially if such industry is available locally. There are industrial precedents of such extractions. Thus, sea-buckthorn (*Hippophae rhamnoides*) oil is being commercially extracted from seeds by sunflower oil. There are no available data on such extraction of artemisinin.

Table 4 summarizes the best available data on the extraction efficiency and potential artemisinin yields from two types of commercial farms, the small holders and large, efficient commercial operators, achievable by each extraction method. On the basis of these data, hexane extraction shows the worst artemisinin yield. This is due to poor solubility of artemisinin in hexane and poor selectivity of hexane toward artemisinin. It is now necessary to compare these processes in terms of environmental impact, energy efficiency, capital and running costs, and the potential for multicrop extraction, before a recommendation could be made on the most promising alternative to hexane extraction technology.

Comparison of Energy Efficiency, Environmental Impact, and Economics of Artemisinin Extraction Processes

Calculations of energy and environmental impact indicators were performed within a gate-to-gate system boundary (see boundary shown in Figure 2 for hexane extraction), in order to achieve the most appropriate comparison across all considered solvent systems. It should be emphasized that separation of artemisinin from primary

Table 5. Comparison of Environmental and Economic Performance of Different Extraction Methods

extraction method	energy efficiency/ GJ·kg (artemisinin)$^{-1}$ best case	energy efficiency/ GJ·kg (artemisinin)$^{-1}$ worst case	GHG emissions/ CO_2 kg·kg (artemisinin)$^{-1}$ best case	GHG emissions/ CO_2 kg·kg (artemisinin)$^{-1}$ worst case	running costs/ (euro)·kg (artemisinin)$^{-1}$	no. of extractors in large-scale facility: plant throughput/kg (biomass)·annum^{-1} 2.5×10^6	no. of extractors in large-scale facility: plant throughput/kg (biomass)·annum^{-1} 6×10^6	capital cost[a]/M(euro): plant throughput/kg (biomass)·annum^{-1} 2×10^4	capital cost[a]/M(euro): plant throughput/kg (biomass)·annum^{-1} 2.5×10^6	capital cost[a]/M(euro): plant throughput/kg (biomass)·annum^{-1} 6×10^6
hexane	1.3		87		28	3	8	0.06	0.7	1.6
EtOH	2.3		148		47	3	8	0.06	1.0	2.1
$scCO_2$	3.5		221		42	3	8	1.2	4.1	7.5
HFC-134a	0.9		131		19	3	8	0.3	1.0	1.5
IL, **9**	1.1	6.3	68	407	22	2	4	0.2	0.3	0.9
IL, **10**	0.9	5.2	56	335	21	3	8	0.2	1.0	2.8

[a] Capital cost includes equipment directly related to extraction process (see system boundary in Figure 2) and the cost of solvent inventory for the specified size of a plant.

extract was excluded from these calculations. This is due to the lack of systematic data on this processing step for most of the technologies considered, and consequently experimental work on the purification steps to convert the crude extract to acceptable artemisinin would be required to make a final evaluation of the relative merits of these extraction technologies. The monetary data are not intended as an indication of actual process costs, but as a relative comparison between the methods.

In the case of hexane, EtOH, and HFC-134a it was assumed that solvent is recovered completely or to a given residual content. The energy "cost" of recovery was included in the calculation. The energy requirement for all processes was split into two categories: energy demand for process heating, including steam generation, and electricity to drive pumps and compressors. Efficient condensing evaporators/heat exchangers were used in the heating duty calculations. Energy requirements were then converted into total global warming potential, as emissions of equivalent mass of CO_2 (greenhouse gas) using conversion coefficients for natural gas (64.2 kg CO_2·GJ^{-1}) and electricity (51.7 kg CO_2·GJ^{-1}). Global warming potential is, therefore, the only numerical environmental index considered at this stage.

Several assumptions were made; most significantly, heat integration was not included and heat loss from insulated vessels was ignored. These two contributions have opposite effects on the energy efficiency; the two assumptions taken together minimize the error. In the absence of exact data, the pumping energy requirement was calculated assuming the same linear liquid velocity inside percolator reactors, using the conditions experimentally tested on a 0.5 m^3 pilot scale with the HFC-134a solvent as a basis.

The energy efficiency was normalized to the mass of artemisinin produced and was based on the large-scale extraction facilities. Normalization to value added, often used in process industries,[60] is not convenient in the case of artemisinin, due to its rather volatile and unpredictable price. Cost indicators were calculated for the extraction process only: capital cost includes only primary extraction and the required solvent stock and excludes separation of artemisinin from the extract. Capital costs were estimated using step count methodology and cost curves for the year 2000, and recalculated including 3% annual inflation to the year 2006.[61] The size of large-scale extraction vessels was assumed to be 10 m^3, which is practical for all considered technologies. Running costs include only the cost of natural gas (as an equivalent of heating demand) and electricity.

Results of these calculations are shown in Table 5. In the case of hexane extraction, the main demands on energy are pumping of solvent in the percolator-type extractor, heating of the extractor over prolonged extraction periods, distillation of large volumes of hexane, and steam stripping of hexane from spent biomass. It is understood that the latter is not always performed in the existing commercial facilities, but is required to satisfy the European emissions legislation and, hence, included in the calculation. It is, therefore, assumed that a negligible amount of hexane vapor is released to the environment. The main environmental impact is due to greenhouse gases generated in the production of heat and electricity required by the process.

Hexane extraction is the least expensive of all technology options. However, other factors, such as risk of explosion, reliance on nonrenewable feedstock, and persistence of solvent in the product and spent biomass, should be taken into account. Capital and running costs do not include costs associated with recovery of artemisinin from the primary extract: there is too little data available for this process. The cost of solvent is included in the capital cost, since the process was calculated as zero loss of solvent, similar to all other systems.

EtOH extraction is performed at ambient temperature. Therefore, the main energy requirements are due to pumping in the percolator reactor and evaporation of EtOH aqueous azeotrope following extraction. Due to considerably higher latent heat of evaporation of EtOH−H_2O azeotrope, the amount of energy required for EtOH extraction is considerably higher than that in the case of hexane. Hence the energy efficiency and greenhouse gas emissions are worse than in the case of hexane.

The higher capital and running costs are due to the higher price of EtOH and the higher requirements for heat to evaporate large amounts of EtOH−H_2O azeotrope after each extraction cycle. This is only slightly offset by having one less extractor in the large-scale facility due to a shorter extraction cycle, since the cost of equipment required to produce sufficient heating power is by far the biggest contribution to the overall capital cost.

Extraction of artemisinin with ionic liquids has been tested only very recently, and no attempt to optimize extraction efficiency or operating conditions was made. Since extraction is performed at ambient temperature and pressure, the main energy demand is for the process of separation of ionic liquid from H_2O (see process description above). Due to very high heat capacity and latent heat of H_2O, its evaporation is hugely energy consuming. The amount of H_2O to be distilled depends on the ratio of H_2O to ionic liquid extract and the ratio of ionic liquid to biomass. Two limiting cases were estimated: the larger energy demand corresponds to the experimentally tested ratio of ionic liquid to biomass, whereas the smaller value corresponds to the minimal amount of ionic liquid that was assumed feasible for the process based on high solubility of artemisinin and the amount required to wet the packed biomass bed. The values clearly show that the step of water distillation is hugely expensive in terms of energy demand and should be minimized or avoided during further process development.

Cost estimates were done for two ionic liquids that have different cycle times, but assuming the lower ratio of ionic liquid to biomass. The strong effect of the cycle time is due to the increase in the heat duty (water distillation), but also due to the higher price of the second ionic liquid, since the inventory of solvent is tripled when more extractors are required to provide the necessary biomass throughput. These calculations were performed within the uncer-

tainty limitations over the data on heat capacities of these specific ionic liquids. Only limited data are available on heat capacity of ILs.[62]

In summary, of the three low-pressure batch/batch-percolation extraction processes, the inventory of solvent required on site due to high solvent:biomass ratio in the case of EtOH and hexane extraction, as well as the requirement of elevated extraction temperature, significantly increases the capital and operating cost. Although ionic liquids are comparatively more expensive than hexane and EtOH, in the case of ionic liquid **9** the cost is offset by the much shorter extraction cycle time, which reduces the inventory of solvent and other capital investment.

In the case of semicontinuous extraction processes based on HFC-134a and $scCO_2$, additional equipment is required, i.e., compressors and heat exchangers. A fairly detailed calculation of energy efficiency of hydrofluorocarbon HFC-134a-based artemisinin extraction was possible due to the availability of thermophysical data. The obtained values of energy requirement for specific items of the flowsheet corresponded well with the power ratings for a 1 m^3 scale reactor, provided by Ineos Fluor. This validates employed calculation methodology. Solvent recovery from spent biomass was assumed to take place at 60 °C under evacuation over 2 h. This should be sufficient to reduce the level of residual solvent to below 300 ppm (=0.03 wt %; this equates to the annual loss of <5% of solvent inventory). The temperature of extraction was 40 °C to avoid potential degradation of temperature-sensitive compounds. The duration of the extraction cycle was based on the actual performance data in a 1 m^3 reactor.[55]

Although extraction with HFC-134a is performed under elevated pressure and requires either a compressor or a heat exchanger, the energy efficiency, capital, and running costs are very low. The main contribution to global warming potential is due to the estimated loss of solvent from residual amounts in the spent biomass at ca. 300 ppm.

Calculation of the energy requirement of supercritical carbon dioxide artemisinin extraction was based on the earlier detailed study for rapeseed oil.[63] The process of extraction is similar in principle and would differ in exact operating conditions and cycle time. Thus, a typical cycle for a large-scale extraction plant used in the original calculation is about 6.5 h, with 4 h of actual extraction.[64] In the case of artemisinin the total cycle time is assumed to be shorter (4 h) and the pressure of extraction is also lower than in the example calculation used for rapeseed oil. However, the mechanical energy component of calculation, which takes into account operating pressure and recompression of CO_2, is low in comparison with the heating and cooling requirement.[63] Therefore, it is reasonable to use conditions similar to those in the original work to recalculate the energy requirement of artemisinin extraction. Although the best results were reported with addition of about 3−5% of cosolvent,[36] the contribution of cosolvent toward overall cost is insignificant and was ignored. The values shown in Table 5 are not exceptionally high in comparison with other processes, as it may be expected. The energy requirement values are most likely to be significantly overestimated, since it was shown that an optimized extraction cycle could be considerably more efficient.[63] The capital and operating costs were estimated using the same methodology as in the case of other solvents, to allow comparison across all technologies.

Feasibility of Developing a Small-Scale Mobile Extraction Unit

A mobile extraction unit is envisaged as a "back-of-a-truck" rig that would service a number of small holder farms and potentially extract more than a single crop. The initial criterion, which, if not satisfied, would make such a unit totally unfeasible, is the ability to process a given amount of dried biomass in the shortest possible time: if a unit requires several months to treat the biomass harvested from a single farm, it clearly is not a practical proposition. The following constraints were used in this calculation: the volume of reactor was limited to 1 m^3 and the maximum throughput was limited to 2×10^4 kg of biomass. In the case of continuous extraction processes the total reactor volume could be split into several smaller reactors, which would reduce the duration of each cycle and allow better process scheduling. However, the overall duration would remain the same.

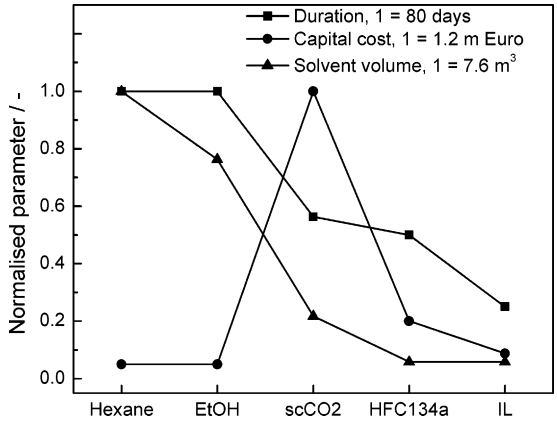

Figure 4. Feasibility criteria for a mobile "back-of-a-truck" extraction facility.

As shown in Figure 4, extraction with hexane and EtOH are unlikely to be feasible on such a scale due to the very long overall time required to process the given amount of biomass and very large inventory of solvent that must be available on site. A $scCO_2$-based mobile unit is feasible, but is the most expensive. The most promising solvent systems for mobile applications are based on HFC-134a and ionic liquids. The practical viability of a small, back-of-a-truck, extractor based on HFC-134a had already been demonstrated,[55] whereas development of a process based on ionic liquids requires a significant research effort to optimize solvent regeneration and solvent:biomass ratio.

Feasibility of Developing a Multipurpose Plant

The long-term viability of artemisinin as a commercial extraction product is affected by many factors that are difficult to predict: potential for parasites developing resistance to artemisinin-based drugs, availability of funds to buy ACT drugs, successful registration of new ACTs, development of an antimalarial vaccine or new drugs, and the development of synthetic artemisinins are the most obvious. It is therefore essential that any new extraction facilities are constructed with the view of potential switch to another feedstock. Apart from ionic liquids, the scope of applicability of other solvents has been tested and reported and depends mainly on the solvation power (partitioning coefficient) of the solvent to particular substances. Thus, $scCO_2$, HFC-134a, and hexane have very broad applicability, and little or no adjustment is required to equipment to switch between different biomasses. Extraction with EtOH is not as popular, due to high polarity of the solvent, which limits its efficiency. Ionic liquids are potentially highly tuneable solvents. However, each new biomass may require a different solvent for optimal process. This may lead to a more expensive changeover between biomasses.

Multiobjective Comparative Assessment of Extraction Processes

Based on the analysis of the stakeholder interests (Table 2) the main criteria for selection of the optimal extraction technology are the cost of artemisinin (i.e., running cost), capital cost of large-scale facilities, safety, and, to a lesser extent, environmental performance. This is due to the fact that extraction of highly functionalized biomolecules is a very small-scale process and is

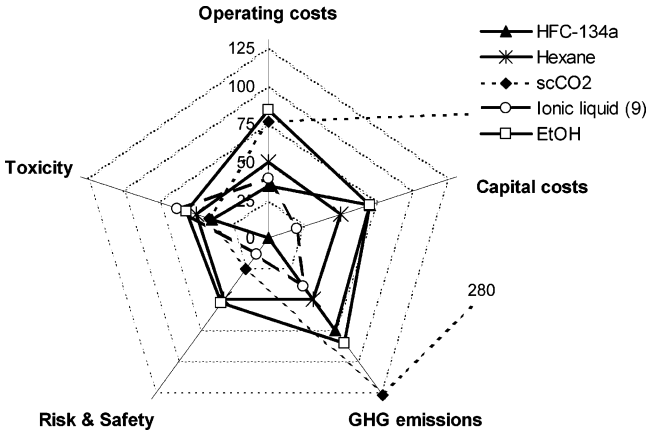

Figure 5. Multiobjective comparison of extraction technologies.

insignificant, in terms of its global environmental impact, e.g., petrochemicals or transport. However, environmental performance is a significant factor at the level of companies or owners of extraction facilities, due to the impact on the local environment and communities. Figure 5 shows selected criteria normalized to the base case of hexane, 50 on each axis; lower values correspond to improvement, higher values correspond to worse performance. Only best-case scenario data for the ionic liquid **9** were used. The values of risk were assigned on the basis of the number of specific risk and safety concerns based on materials safety data sheets (MSDS); risk for supercritical $scCO_2$ was assigned arbitrarily, due to perceived additional risk of high-pressure equipment. LD_{50} and LC_{50} data were used as measures of toxicity. These data were converted to log values and scaled assuming 50 for hexane. Different toxicity mechanisms are thus hidden inside these parameters: EtOH is most toxic on ingestion, whereas CO_2 is an asphyxiant. Unfortunately, no data on the specific ionic liquid's toxicity were available. There is limited data on ILs' toxicity. Solvent Innovation GmbH (www.solvent-innovation.com) provides a value of LD_{50} (rats) of 2000 mg·kg^{-1} for ECOENG 500 ionic liquid. Although this solvent is different structurally from the two ionic liquids used in this study, it is also based on a quaternary ammonium salt and does not contain imidazolium or pyridinium ions, which would result in considerably higher toxicity values.[65] Therefore, this LD_{50} value was used as a guide for toxicity of ionic liquids.

Figure 5 suggests that HFC-134a and ionic liquid solvents have the best comparison against the hexane base case: ionic liquids have the potential to outperform hexane extraction in all assessment criteria, except that the toxicity of specific ionic liquids must be assessed. HFC-134a is shown as less efficient in terms of greenhouse gas emissions, but has better indicators in risk, safety, toxicity, and operating costs. Performance of EtOH extraction is shown to be consistently slightly worse than hexane. It should be emphasized that hexane was assumed to be completely recovered, which is not attainable in practice. Therefore, the actual performance of hexane in terms of emissions, and therefore also risk, is likely to be worse than the presented base case.

The study was performed within narrow gate-to-gate boundaries, only considering the extraction process itself, apart from ionic liquids, where artemisinin was shown to be readily separated from the crude extract by partitioning with H_2O. It is apparent that since concentrations of artemisinin in the crude primary extracts obtained by different methods are different, the contribution of the secondary purification step toward environmental performance, energy efficiency, and cost will also be different. However, in the absence of systematic information on the secondary purification step this analysis is currently impossible to undertake.

Conclusions

This study provides a comparison of several technologies of extracting artemisinin based on their extraction efficiency, cost, energy efficiency, and global warming potential. Although there are no established methods of comparative assessment of mature and emerging technologies, in this study several key criteria were identified for process selection and as guidance for further process development and optimization of the emerging technologies. Review of available literature on different artemisinin extraction methods revealed several aspects that require systematic study or collection of information, namely, the effect of biomass particle size and, consequently, the optimal percolation conditions, and separation of artemisinin from crude extracts obtained by different extraction methods.

The study showed that new solvents, such as HFC-134a and ionic liquids, have higher extraction efficiencies than the optimized hexane extraction and have the potential to outperform hexane extraction in all assessment criteria. The HFC-134a solvent extraction has already been commercialized on the scale of 0.4 and 1 m^3. Further process development may be needed to optimize the cost versus greenhouse gas emissions and to reduce the capital cost. The ionic liquid solvents are the least studied and show the most promise. A considerable research effort is required (and is currently underway) to develop a more efficient separation of extracted compounds from the ionic liquid and also to develop the method of recovery of ionic liquids from the spent biomass. Since the latter step was not considered in this study due to lack of data, the actual operating cost and greenhouse gas emissions for ionic liquids could be somewhat worse. It is yet unclear whether the same ionic liquids will be effective in extracting other biomolecules of interest or a different solvent must be developed for each extraction. Supercritical CO_2 extraction is a mature technology, used on a very large scale for high-value products, such as decaffeination of coffee. It also has potential for artemisinin extraction, especially if equipment cost could be reduced significantly. EtOH extraction is worse than hexane extraction in all criteria. Furthermore, due to the current uncertainty over the existing performance data and very limited scope for a multiple crop facility based on EtOH, this solvent is considered less favorable.

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