Derivatisation of artemisinin

Alexei Lapkin, Xiaolei Fan

School of Engineering, University of Warwick

Dedicated to the memory of Michaela von Freihold and Ian Bathurst

Previous studies on artemisinin in the group

2006 Review of artemisinin extraction technologies

A. Lapkin, M. Cutler, P. Plucinski, Comparative assessment of technologies for extraction of artemisinin, *J.Natural Products, 69, 2006, 1653-1664.*

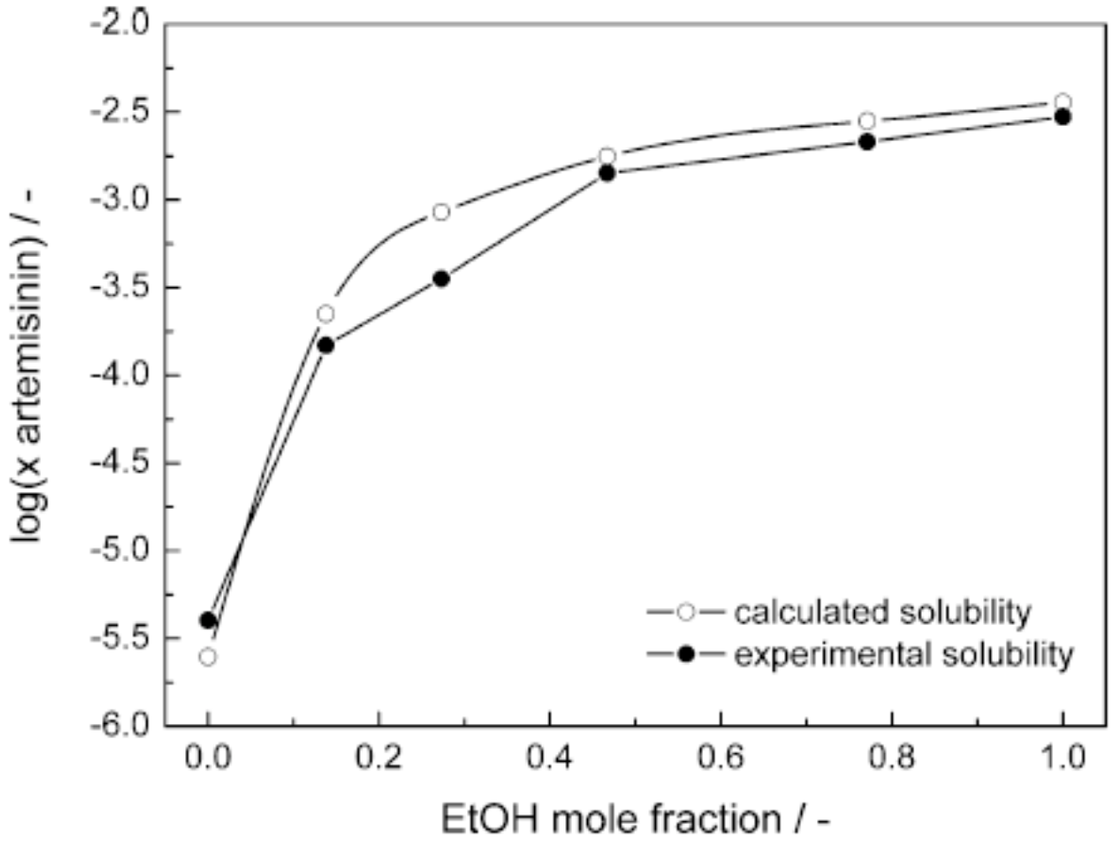
2009 HPLC protocol for artemisinin and co-metabolites

A.A. Lapkin, A. Walker, N. Sullivan, B. Khambay, B. Mlambo, S. Chemat, Development of HPLC analytical protocols for quantification of artemisinin in biomass and extracts, *J. Pharmaceutical and Biomedical Analysis, 49, 2009, 908-915.*

2010 Computational method for screening solvents for extraction; new solvents for extraction or purification of artemisinin

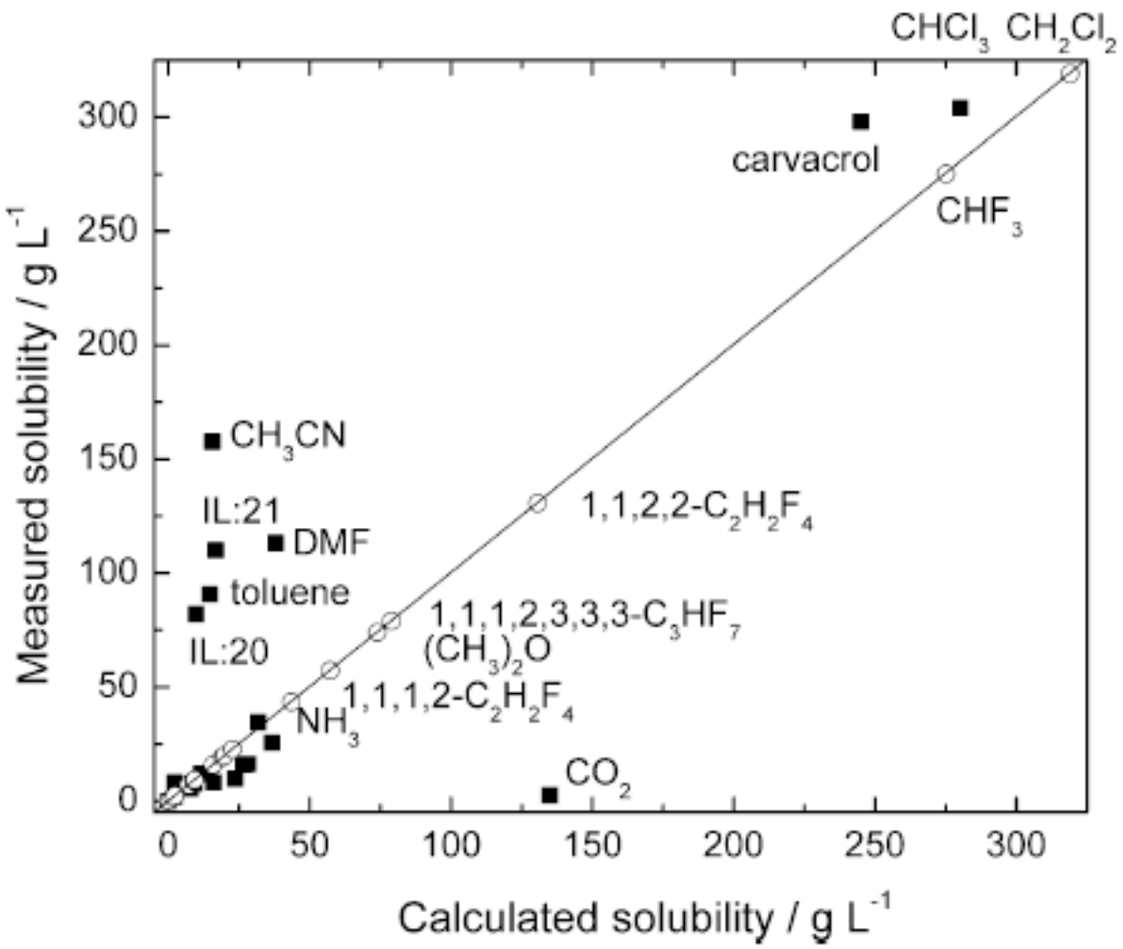
A.A. Lapkin, M. Peters, L. Greiner, S. Chemat, K. Leonhard, M.A. Liauw, W. Leitner, Screening of new solvents for artemisinin extraction process using *ab initio* methodology, *Green Chem., 12, 2010, 241–251.*

Computational screening of solvents



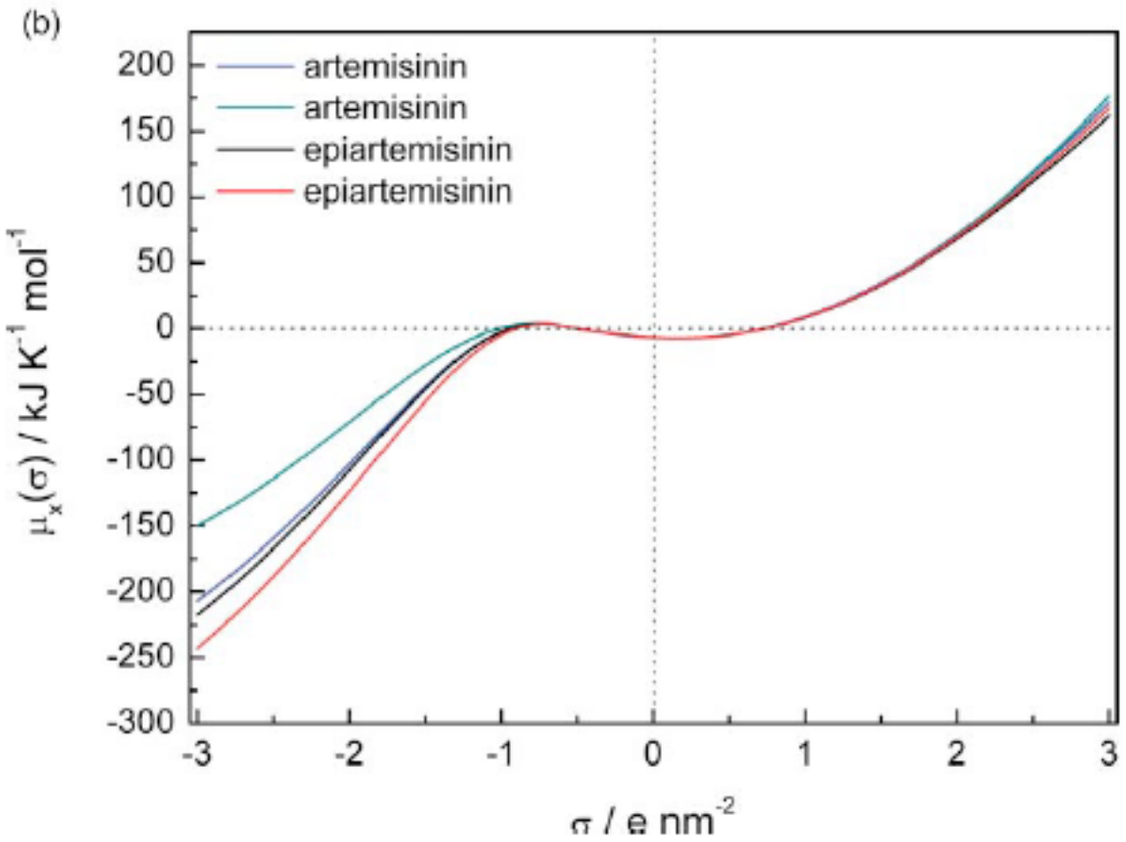
For many solvent systems the method based on COSMO-RS is very accurate.

Based on *ab initio* calculations, which are very fast, we can screen a large number of solvents and then target experiments for validation. However, experimental know-how and insights cannot be underestimated also!

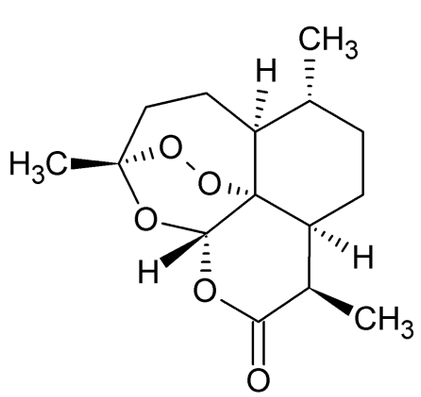


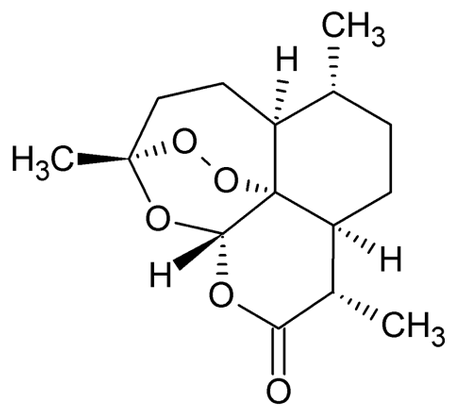
Green Chem., 12, 2010, 241–251

Computational screening of solvents



Calculations pick up minor differences in electronic structure of artemisinin epomers, which do influence their interactions with solvents.





Green Chem., 12, 2010, 241–251

Computational screening of solvents

Table 3 Solubility of artemisinin in n-hexane–ethyl acetate solvent in the presence of co-metabolites at 293 K. Solvent comprises n-hexane–ethyl acetate (95 : 5% v/v) unless stated otherwise

Co-metabolite	Molar fraction co-metabolite in extract, $\times 10^{-4}$	Molar fraction artemisinin in extract, $\times 10^{-4}$	% Increase
—	—	8.6[a]	—
—	—	12.4	0
Camphor	19	12.6	2.2
	1.9	12.6	1.6
Dodecanoic acid	2.6	12.4	0.6
1,8-Cineol	25	12.4	0.5
	2.5	12.6	2.0
aArtemisinin ketone	68	12.7	2.7
Casticin	12	12.7	2.7
Casticin monoglycoside	12	13.3	7.5
C_{30} paraffin	6.7	12.5	0.8
Deoxyartemisinin	12	12.5	1.1
	24	12.6	2.2
	48	12.9	4.4

[a] Solvent: pure n-hexane without EtOAc additive

Calculations of mixed systems allows us to start identifying key interactions that may influence bio-availability and crystallisation efficiency.

Green Chem., 12, 2010, 241–251

Current project on artemisinin in the group

2009-2012 PhD project on synergistic effects in bio-pharmaceuticals
Sponsored by Sensapharm Ltd, and
Engineering and Physical Sciences Research Council (EPSRC).

Mr John Suberu
Dr Guy Barker and Prof Alexei Lapkin
Schools of Life Science and Engineering @ Warwick

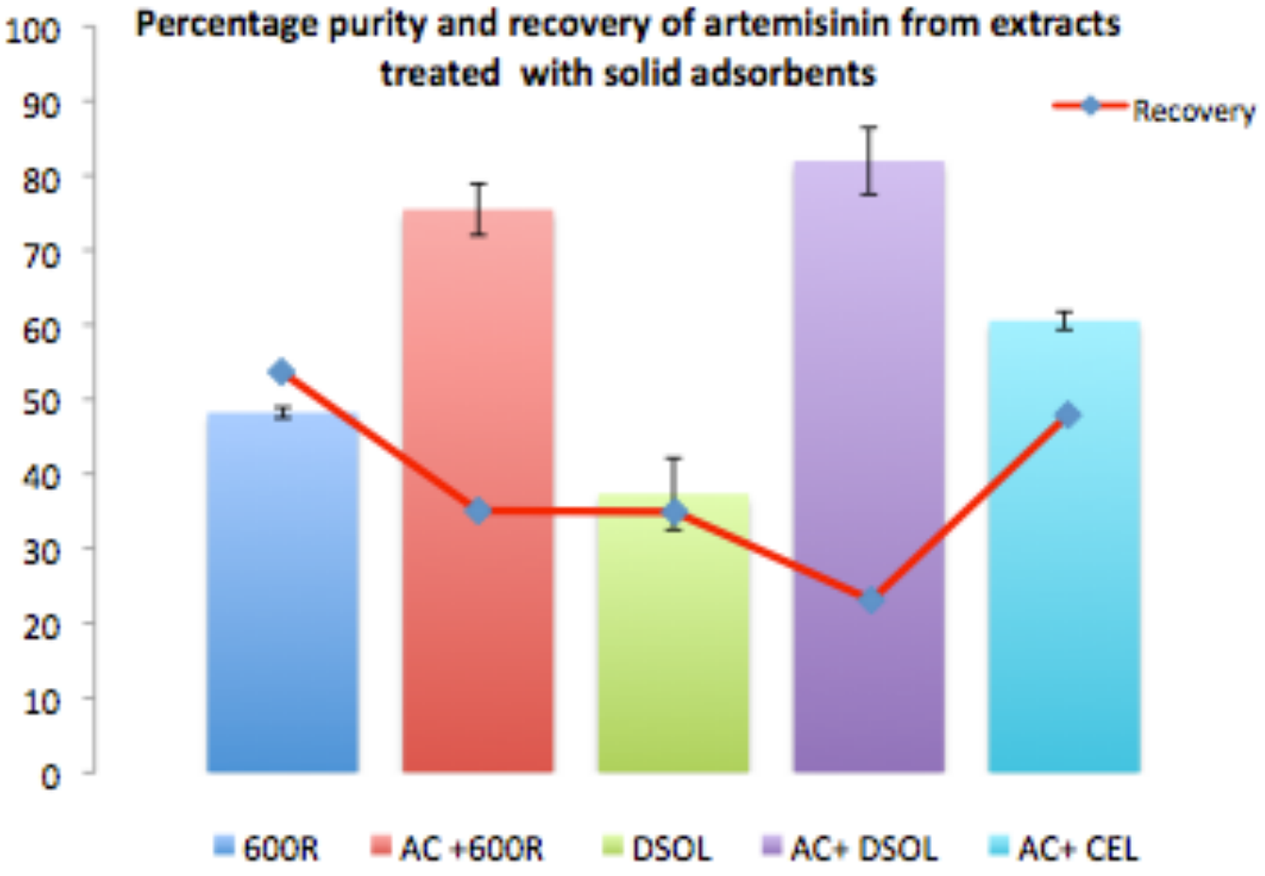
Dr Neil Sullivan
Sensapharm Ltd

Objectives:

- enhance separation of artemisinin from raw extracts
- investigate synergistic mechanisms of bio-activity of metabolites of three plant varieties (including *A. annua*)

Current project on artemisinin in the group

Treatment of ART raw extracts by different adsorbents

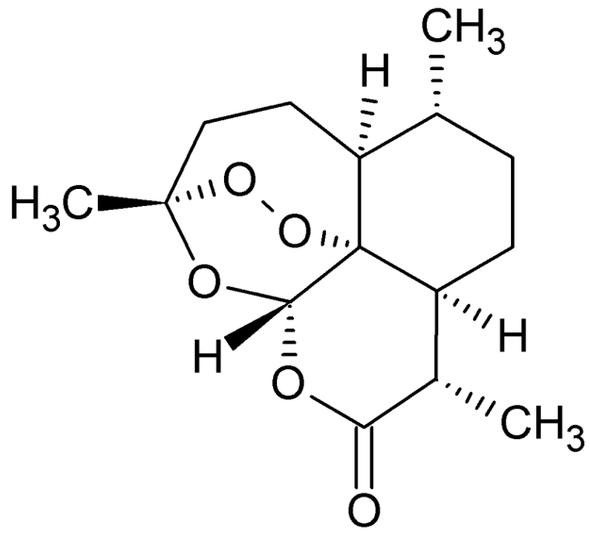


Our aim was to identify the co-metabolites that reduce the yield and purity of crystals of artemisinin produced in some regions of the World. Several classes of compounds were eliminated as having no influence. Most significant influence is associated with components of waxes.

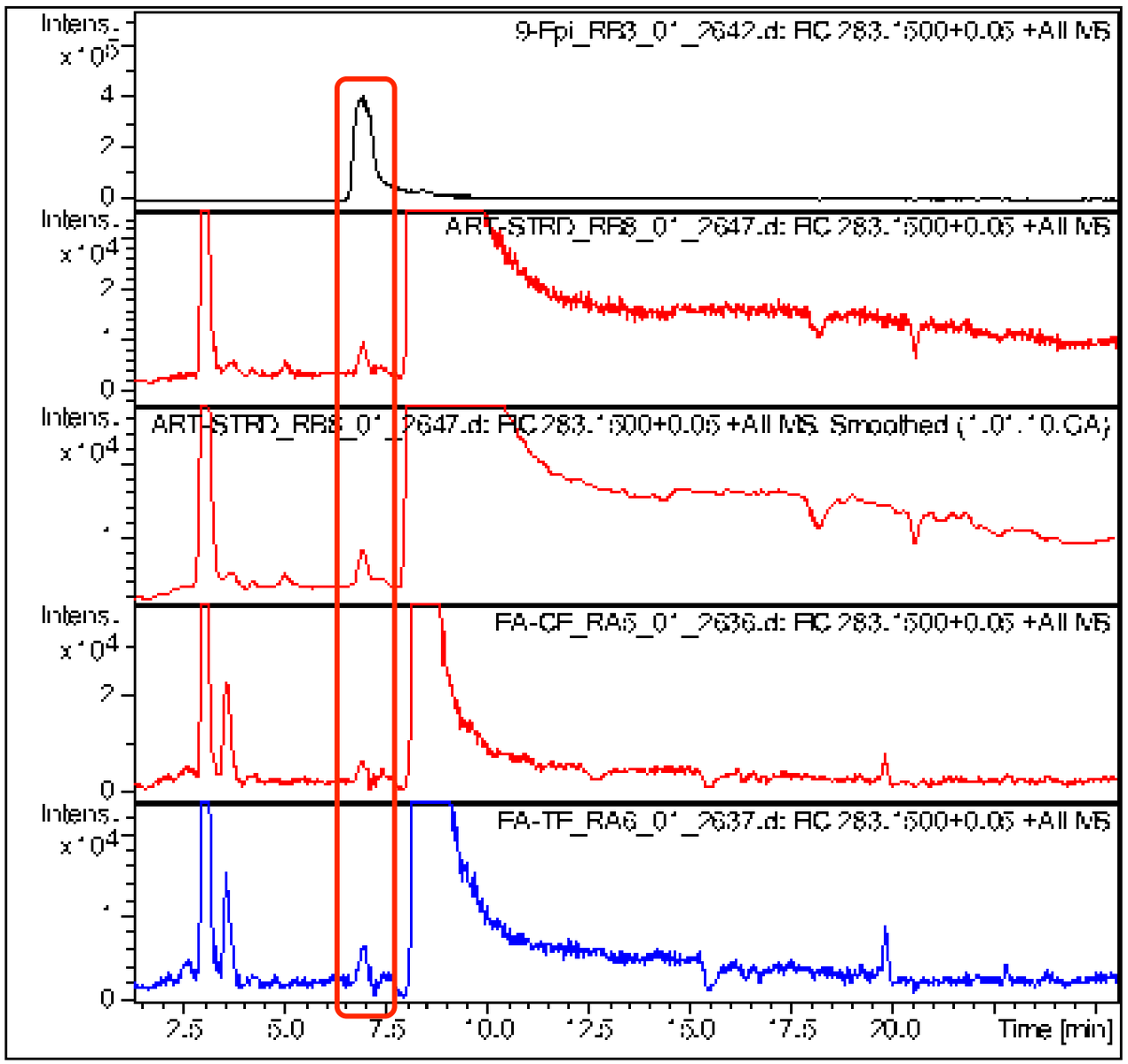
Current work is on identification of specific main compounds that reduce the yield and purity of artemisinin crystals.

Current project on artemisinin in the group

Identification of 9-epi artemisinin in raw extract



Low abundance Epi-ART



HPLC protocols for 9-epi gave erroneous data, since 9-epi peak is masked by the elution of flavones.

MS analysis does allow to positively identify 9-epi in raw extracts.

Quantification by MS is underway: raw extracts, treated extracts, purified artemisinin...

ART derivatisation to DHA

Determining a Viable Protocol for the Derivatisation of Artemisinin into Dihydroartemisinin

A study commissioned through

Medicines for Malaria Venture (MMV)

Silke Buzzi
Institute of Pharmaceutical Sciences
Dept. of Pharmaceutical Chemistry
University of Graz

Armin Presser
Institute of Pharmaceutical Sciences
Dept. of Pharmaceutical Chemistry
University of Graz

Michaela von Freyhold
Department for Development Policy
Institute for Social Science
University of Bremen

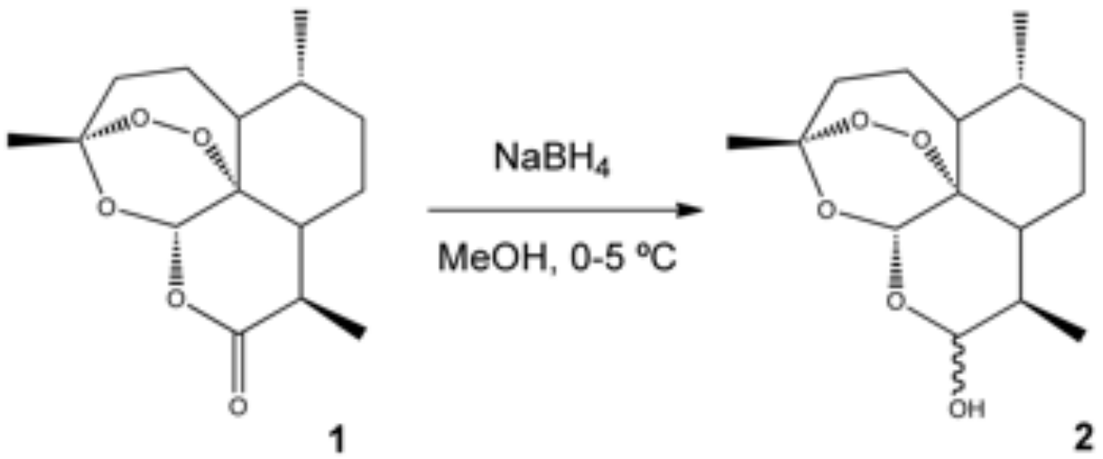
April 2007

Analysis and validation of previously reported in open literature protocols.

Main driver is the cost of raw materials to reduce the cost of the final product.

ART derivatisation to DHA

Starting protocol

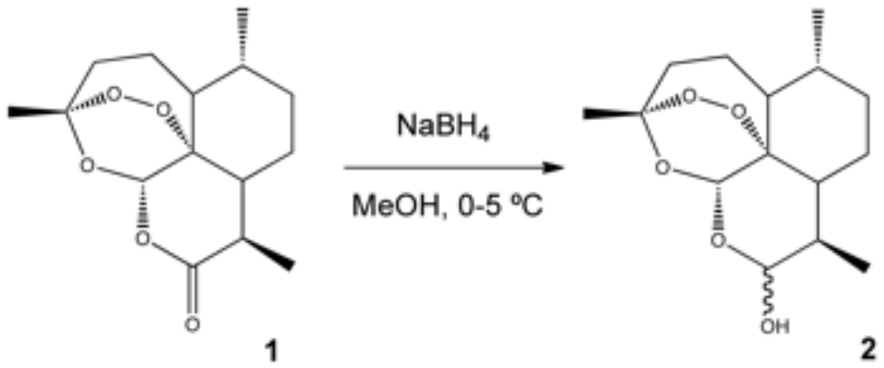


ART (200 mg, 0.71 mmol) was suspended in methanol (10 mL) under moderate stirring speed and cooled in an ice-water bath to *ca.* 4 °C. Sodium borohydride (67 mg, 1.77 mmol, 2.5 *equiv.*) was added in portions to the suspension over a period of 5 minutes. The reaction mixture was stirred vigorously under N_2 until TLC showed no ART left in the reaction mixture (*ca.* 90 min).

Reaction mixture was neutralised (pH 5-6) with 50 % v/v of a mixture of acetic acid/methanol (added by portion, 50 µL each time). The reaction mixture was evaporated to dryness under vacuum (at 40 °C). Dry residue was extracted using ethyl acetate 2 − 3 times (10 mL each time) for transferring the product completely (monitored by TLC) into ethyl acetate. The combined ethyl acetate extracts were dried with Na_2SO_4 (for 6 hours), filtered, and evaporated to dryness to give a white flake-like product.

ART derivatisation to DHA

Starting protocol

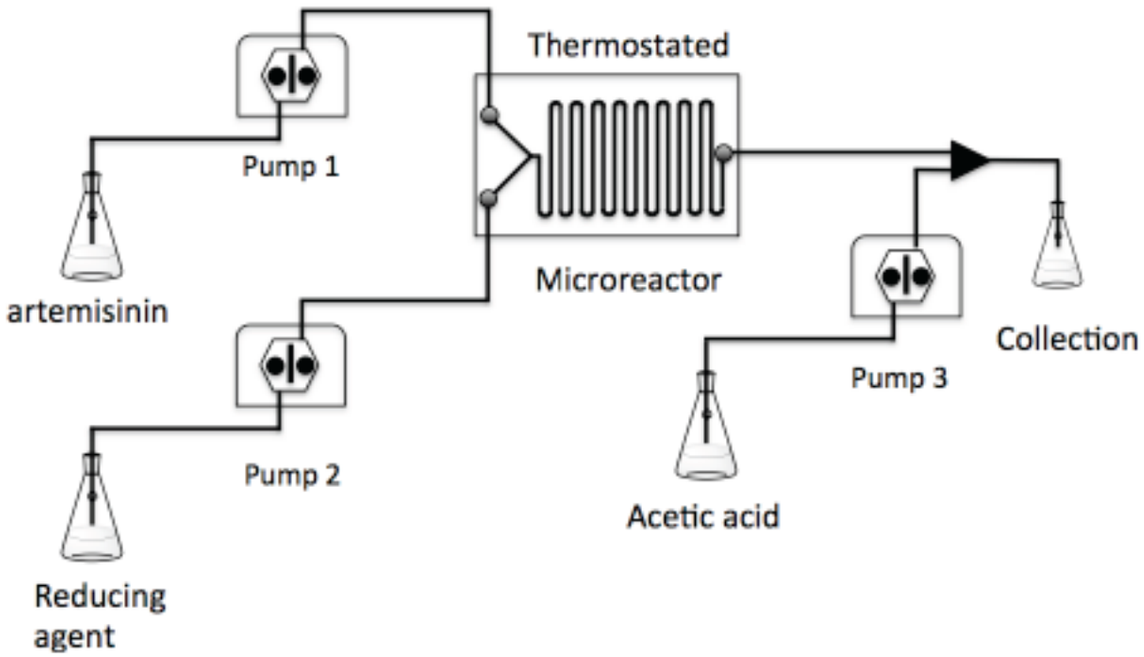


Entry	Reductant	Mole eq / -	T / ºC	t / min	Solvent	X / %[a]	Y / %[b]
1	$NaBH_4$	2.5	4	90	MeOH	96	87
2		3	4	100		98	90
3[c]		3	4	100		97	89

[a] x, corresponds to conversion of artemisinin, determined by HPLC
[b] y, corresponds to yield of DHA, determined by HPLC
[c] 1 g of substrate

ART derivatisation to DHA

Flow protocol

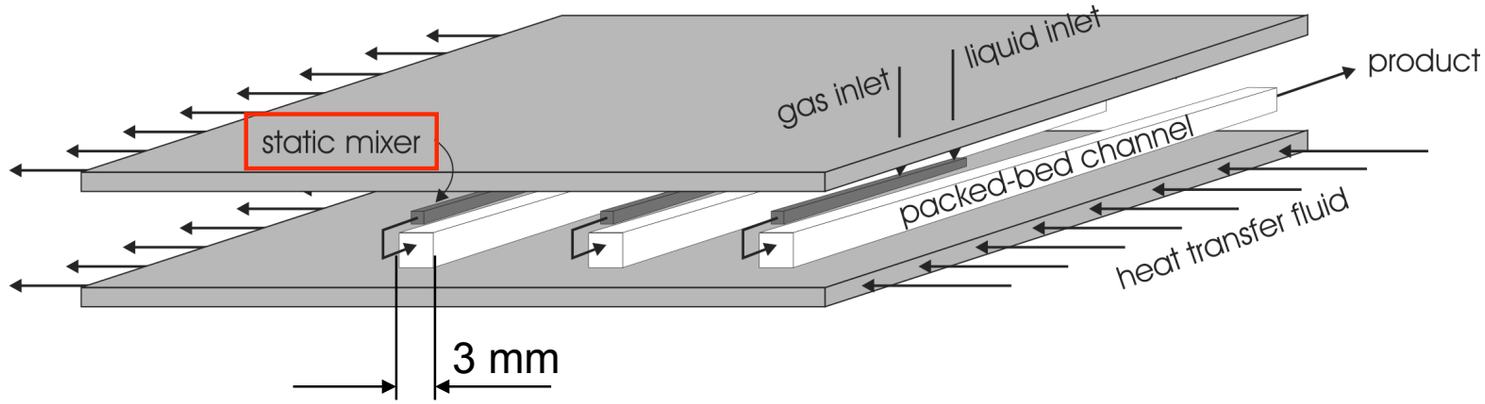


Reduction of artemisinin is a fast exothermic reaction, complicated by limited solubilities of reagents and products.

This reaction is well-suited to be run in novel intensified reactors, such as compact structured reactors-heat exchangers or microreactors.

Paten application filed:
A. Lapkin, X. Fan, V. Sans, J.M.J. Williams, Method of continuous flow stoichiometric reductions, 2011, GBP 1115988.6

Exothermic reactions in intensive flow reactors



- Selective oxidation of aromatic alcohols
- Selective reduction of aromatic ketones
- Tandem Heck coupling-hydrogenation
- Integration of compact reactors with nanofluids

Appl. Catal. A: Gen., 2005, **288**, 165-174

Catal. Today 147S (2009) S313-S318

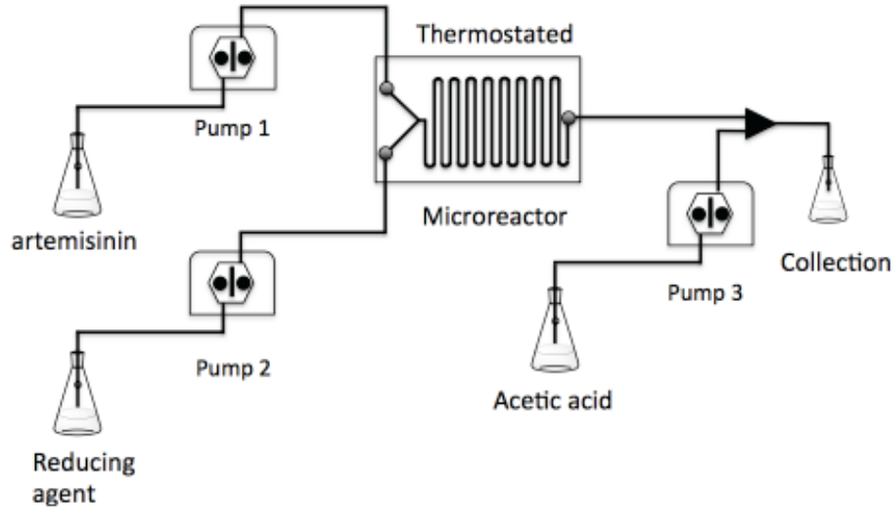
Journal of Catalysis 267 (2009) 114–120

Green Chemistry 10:6 (2008) 670-677

ART derivatisation to DHA

Entry	Solvent	Residence time / min	T / °C	X / %[a]	Y / %[b]
1	THF	2	5	99	98
2		1	5	99	98
3		2	25	99	98
4		0.5	25	99	98
5		1	25	99	98
6		1	15	99	97
7		1	0	98	95
8	2-	0.5	25	97	93
9	MeTHF	0.5	5	96	95
10		0.33	25	97	94
11		0.33	5	97	94

ART 0.033 M, $LiBHEt_3$ 0.1 M, acetic acid 20%.



Space time yield: 0.55 kg h^{-1} mL^{-1}

Paten application filed:
A. Lapkin, X. Fan, V. Sans, J.M.J. Williams, Method of continuous flow stoichiometric reductions, 2011, GBP 1115988.6

Conclusions

- Synthesis of DHA was shown to be highly efficient under flow conditions in intensive integrated reactor.
- Currently economic and environmental assessments (short-cut methods and full Life Cycle Assessment [LCA]) are being undertaken.
- Current demonstration project (funded by U.Warwick) will demonstrate 100 g artemisinin conversion, including product purity and isolated yield.
- Current demonstration project will demonstrate tandem conversion of artemisinin into artesunate and arteether in an integrated flow process (covered by patent application).

Invitation

- We are looking for an industrial partner to develop production scale artemisinin derivatisation process under flow conditions.
- Joint development would proceed *via* a shared mini plant facility for continuation of R&D programmes (optimisation of integrated tandem synthesis, further development of new reducing agents [catalysts] and better solvents) and a company-housed pilot miniplant (process robustness, scale-up issues, staff and operators training).
- Current available funding includes travel for discussion at company site and a research seminar on flow processes to be delivered at a company site.

a.lapkin@warwick.ac.uk

Acknowledgements

- HPLC instrument was funded by MMV
- Attendance of Hanoi conference was funded by Engineering and Physical Sciences Research Council's (EPSRC) Impact programme