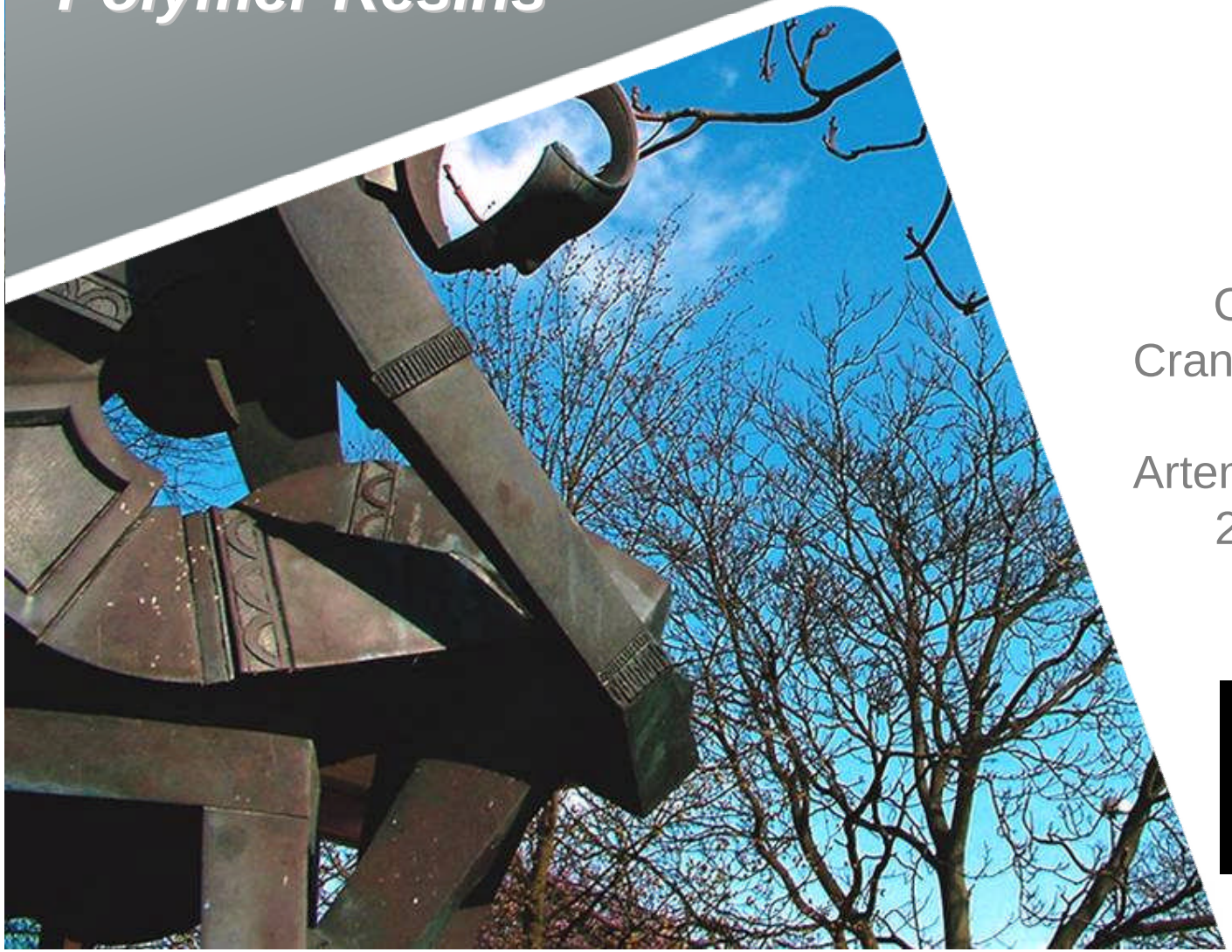
Artemisinin Extraction Using Designed Polymer Resins

World Health Organization

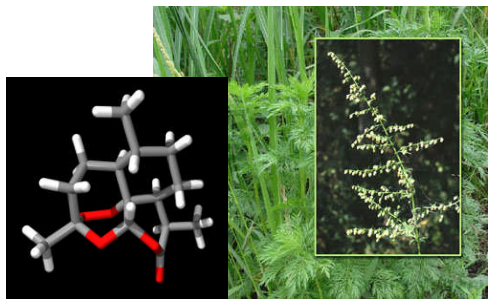
Cranfield UNIVERSITY

Medicines for Malaria Venture



Dr Yi Ge
Cranfield Health
Cranfield University, UK

Artemisinin Forum 2008
24-26/Nov/2008



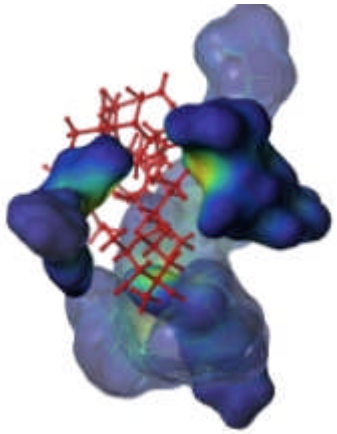
www.cranfield.ac.uk

Cranfield University & Cranfield Health

- Postgraduate-only University
- Emphasis on Business, Science and Technology
- On two sites in southern England
- Cranfield main campus adjacent to Cranfield village
- The Ministry of Defense college at Shrivenham

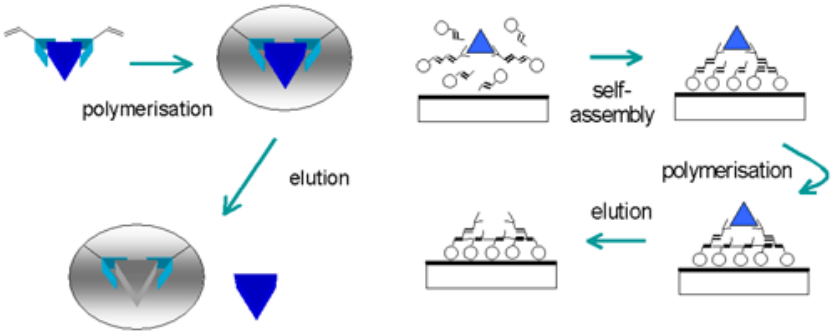
- Cranfield Health is a school of the University offering "Total solutions for health and wellbeing"

Our Cranfield Team



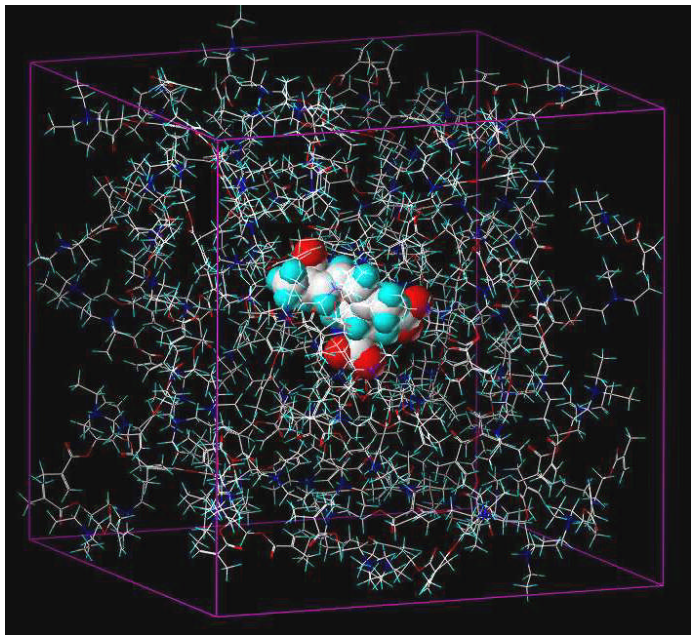
Cranfield UNIVERSITY

- Development of world class material/polymer science for solving practical problems in separation and sensing
- Turnover of £ 1 million by 2009
- World Leaders in Molecular Imprinting, National Leaders in Synthetic receptors and Smart Materials



Area of Expertise

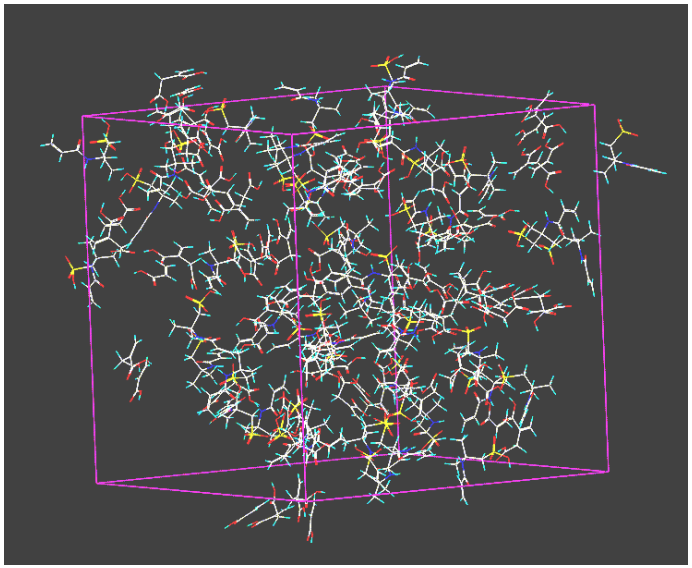
- Synthetic receptors
- Molecularly imprinted and conjugated polymers
- Computational design
- Nanotechnology
- Molecular recognition
- Drug development
- Membrane technology
- Sensor technology



Design of Imprinted Polymers

Cranfield UNIVERSITY

For the design of imprinted polymers, Cranfield pioneered a new computational approach which allows fast and effective optimisation of polymer composition.

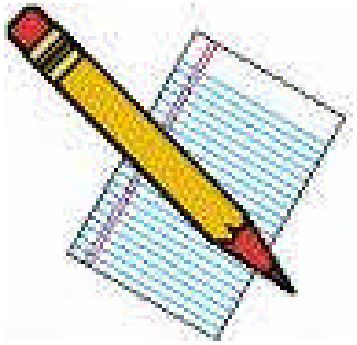


State-of-the-art Facilities

Cranfield UNIVERSITY

Equipped with modern instruments for the polymer design, preparation and characterisation: NMR, HPLC-MS-MS system, IR-spectrophotometer, HPLCs, UV spectrophotometer, porosimeter, particle size analyser, electron scanning microscope, atomic force microscope and confocal microscope.

Structure of Presentation



- Project Outline
- Methodology & Outcomes
- Conclusions
- Future Work

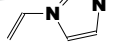
Project Outline

- Computational design of polymers with specificity for artemisinin
- Optimisation of the artemisinin quantification methods
- Polymers synthesis and testing
- Evaluation of artemisinin purification from the plant extracts

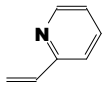
Computational Design of Polymers

- Design of functional monomer database
- Design of molecular model of artemisinin
- Screening using a LEAPFROG™ algorithm

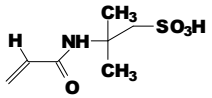
Library of Functional Monomers



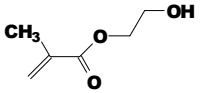
F1 1-VINYLIMIDAZOLE



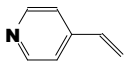
F2 2-VINYLPYRIDINE



F3 ACRYLAMIDO-2-METHYL-1-PROPANESULFONIC ACID (AMPSA)

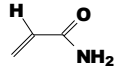


F4 2-HYDROXYETHYL METHACRYLATE

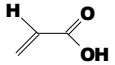


F5 4-VINYLPYRIDINE

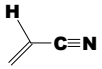
F6 ACROLEIN



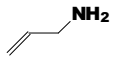
F7 ACRYLAMIDE



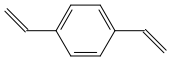
F8 ACRYLIC ACID



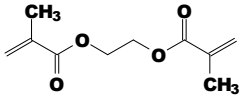
F9 ACRYLONITRILE



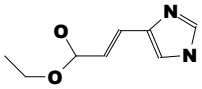
F10 ALLYAMINE



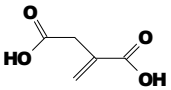
F11 p-DIVINYLBENZENE



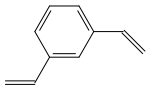
F12 ETHYLENE GLYCOL DIMETHACRYLATE (EGDMA)



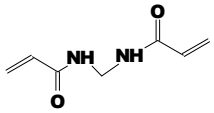
F13 UROCANIC ACID ETHYL ESTER



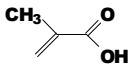
F14 ITACONIC ACID



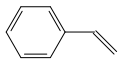
F15 m-DIVINYLBENZENE



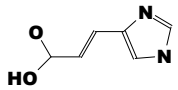
F16 N,N-METHYLENE BIS ACRYLAMIDE



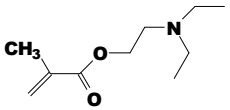
F17 METHACRYLIC ACID



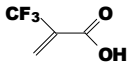
F18 STYRENE



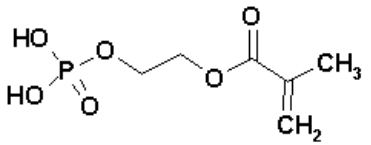
F19 UROCANIC ACID



F20 N,N-DIETHYLAMINO ETHYL METHACRYLATE (DEAEM)

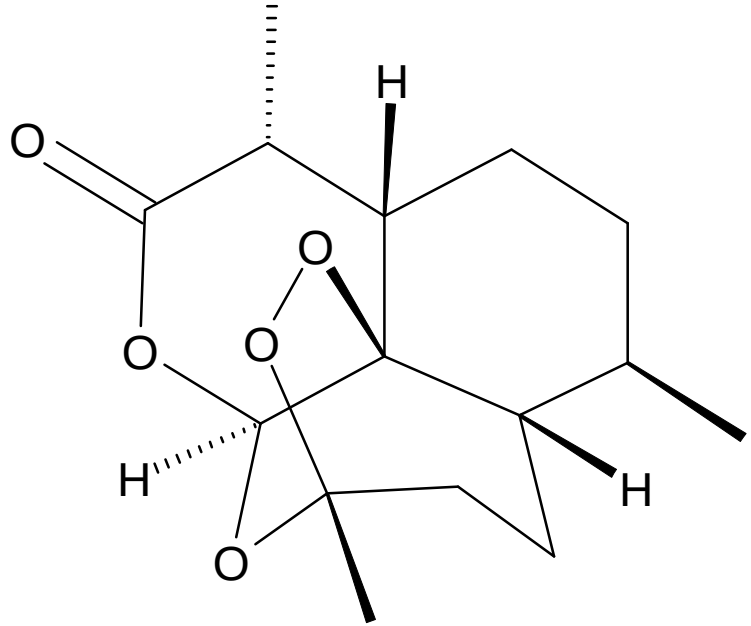


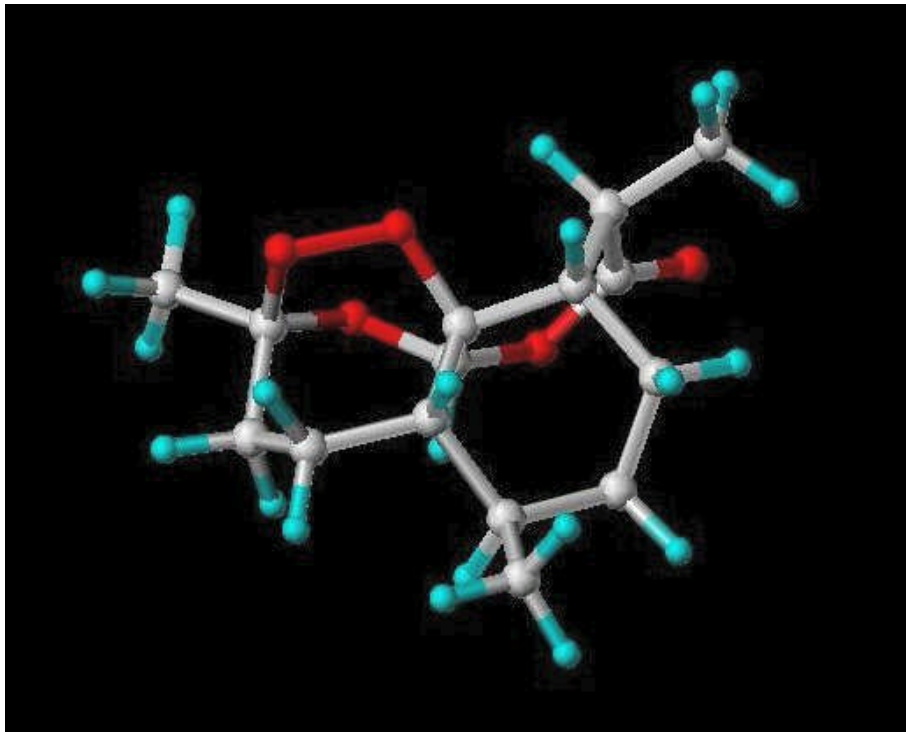
F21 2-(TRIFLUOROMETHYL)ACRYLIC ACID



F22 ETHYLENE GLYCOL METHACRYLATE PHOSPHATE (EGMP)

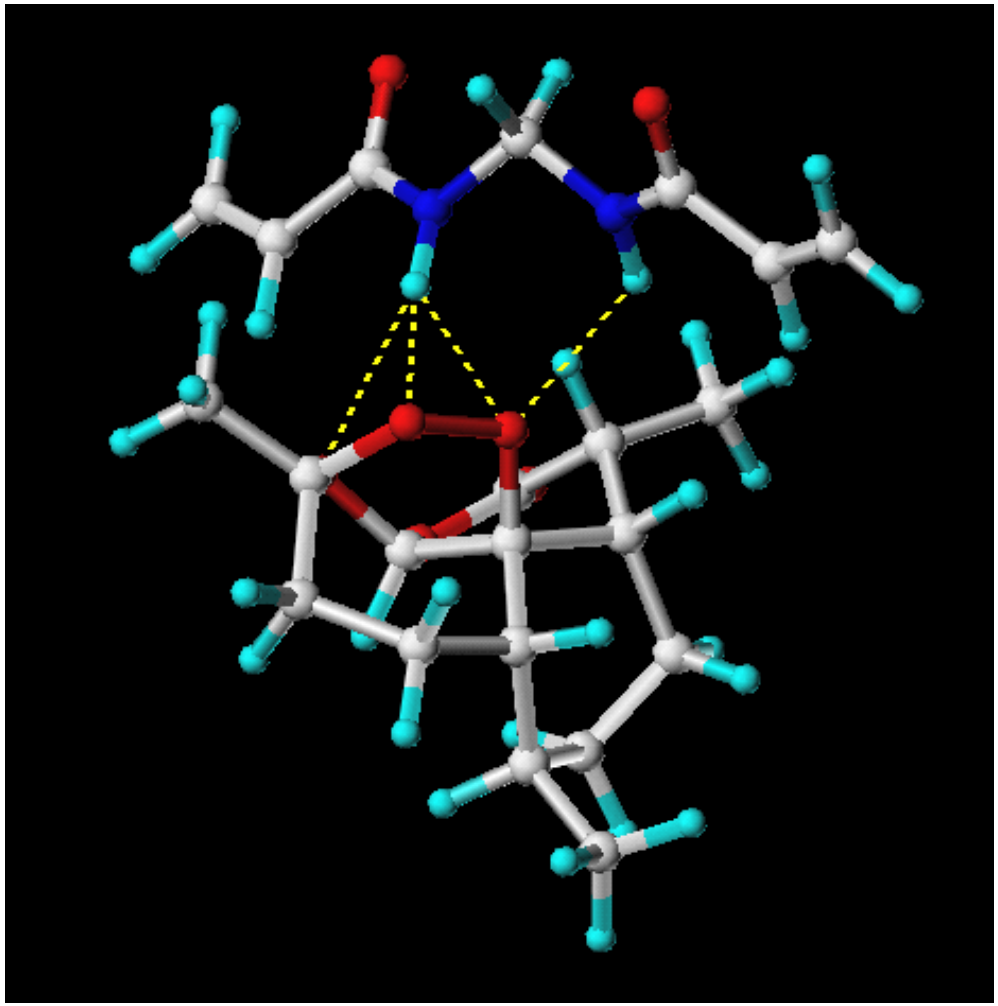
Molecular Structure of Artemisinin



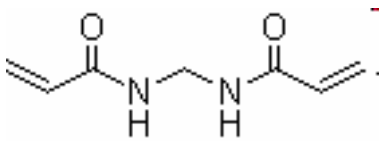


Artemisinin - MBAA Molecular Complex

Cranfield UNIVERSITY



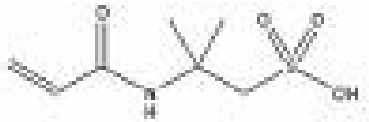
MBAA:
N,N'-methylenebisacrylamide

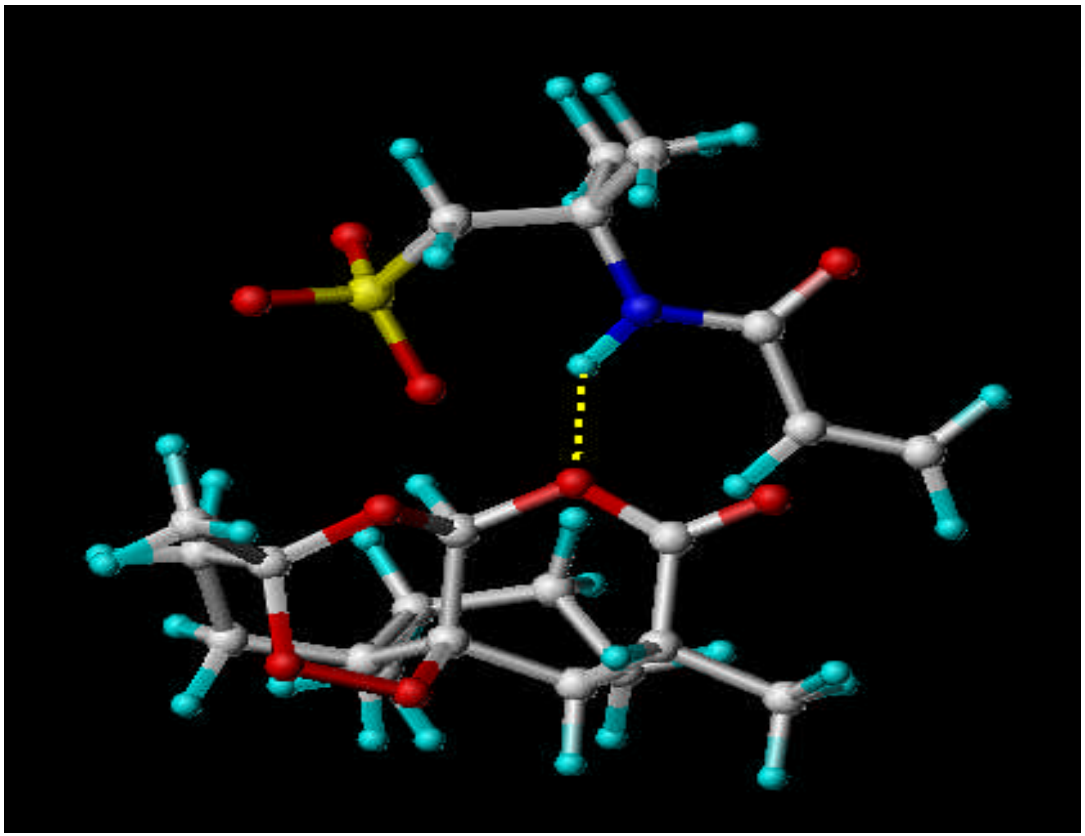


Artemisinin - AMPSA Molecular Complex

Cranfield UNIVERSITY

AMPSA:
2-acrylamido-2-methylpropane sulfonic acid





Leapfrog Table

Cranfield UNIVERSITY

Rank	Monomer	Binding Energy (kcal mol^{-1})
1	DEAEM	-30.78
2	MBAA	-27.90
3	Acrylamide	-25.73
4	AMPSA	-20.53
5	TFMAA	-17.50

Polymer Synthesis

Cranfield UNIVERSITY

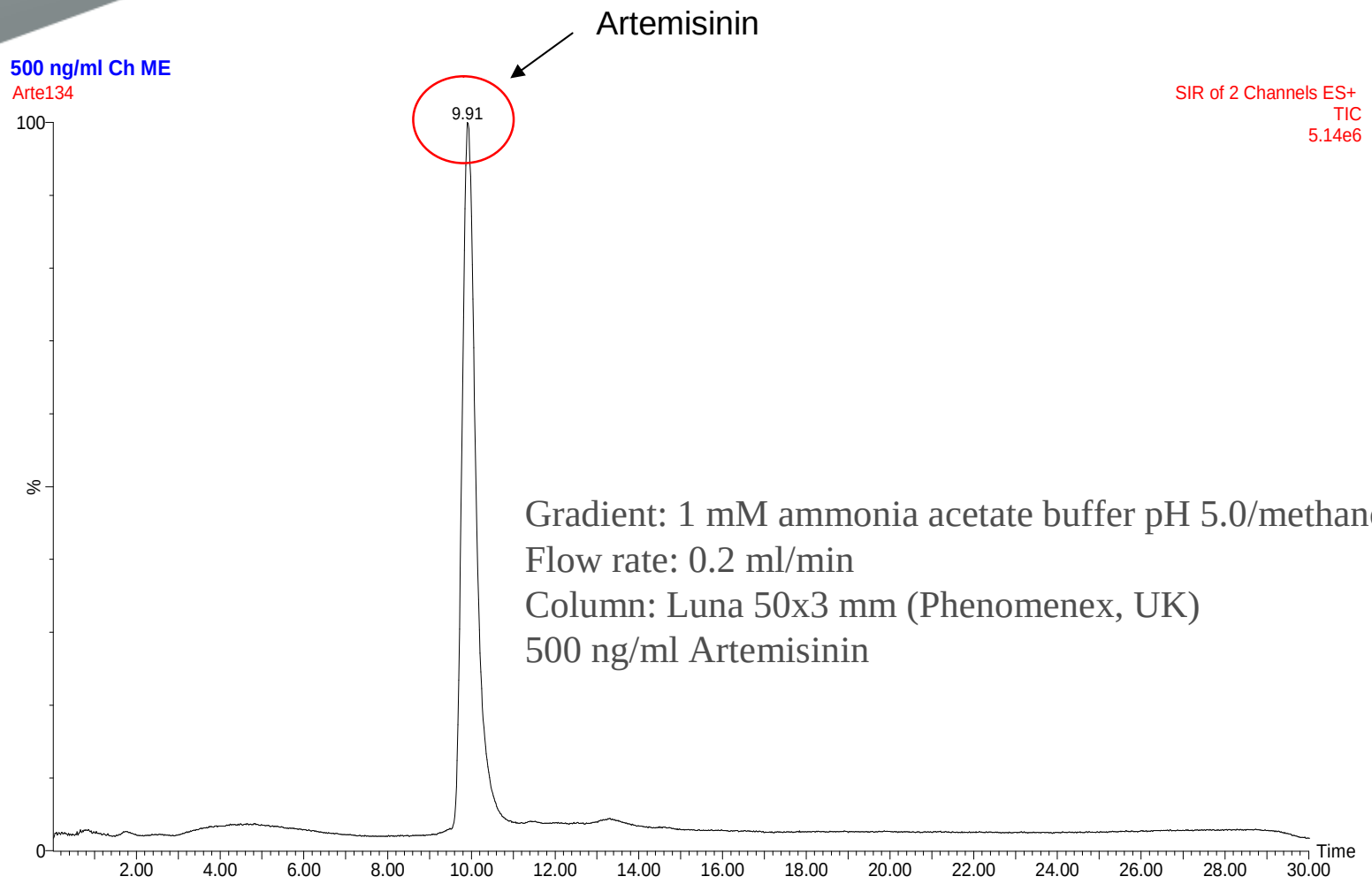
Polymerisation:

- 5 g of one functional monomer (DEAEM, MBAA, AMPSA, TFMAA)
- 20 g of cross-linker (ethyleneglycol dimethacrylate, EGDMA)
- 25 g of porogen (dimethylformamide, DMF),
- 500 mg of initiator (1,1-azobis(cyclohexanecarbonitrile))

- Thermo-polymerisation at 80 ºC

Quantification of Artemisinin Using HPLC-MS

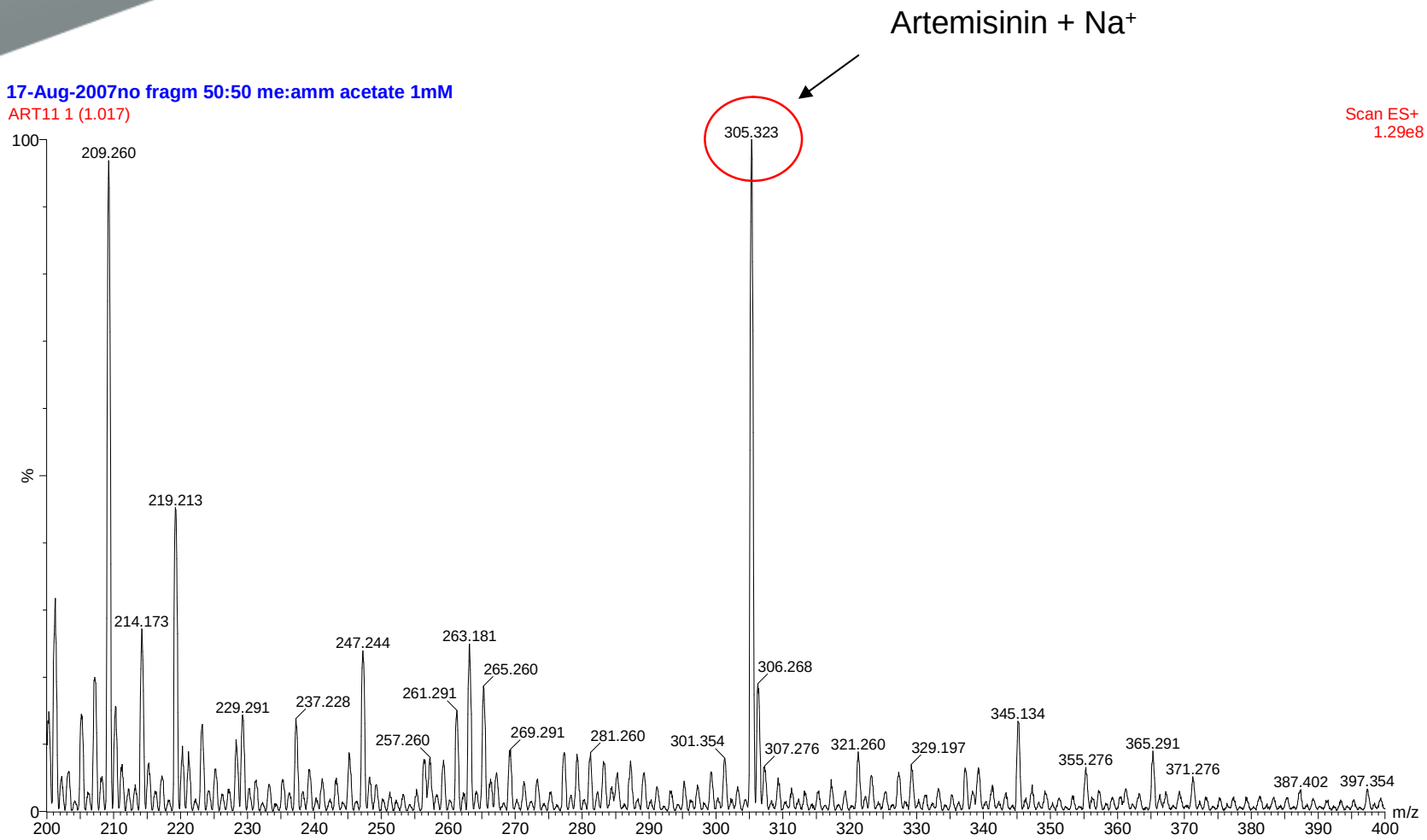
HPLC-MS protocol



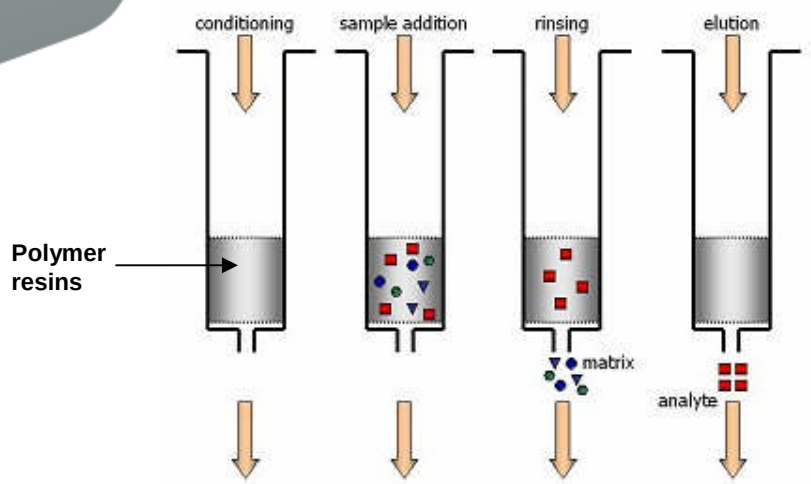
Gradient: 1 mM ammonia acetate buffer pH 5.0/methanol
Flow rate: 0.2 ml/min
Column: Luna 50x3 mm (Phenomenex, UK)
500 ng/ml Artemisinin

Quantification of Artemisinin Using HPLC-MS

Cranfield UNIVERSITY



Solid Phase Extraction (SPE)



1ml SPE cartridges are packed with 100 mg of pulverized and sieved polymers (30-125 mm ø)

- Conditioning: 1 ml of hexane
- Loading: 1-15 ml of hexane spiked with artemisinin
- Washing: 2 ml of hexane
- Elution: 3 x 1ml of the corresponding eluent

Primary Screening

Cranfield UNIVERSITY

Artemisinin binding from a model solution in hexane

- MBAA 85%
- TFMAA 83%
- DEAEM 79%
- AMPSA 76%

☺ MBAA-based polymer was selected for future investigation

Capacity of MBAA–Based Polymer

Cranfield UNIVERSITY

For the artemisinin in hexane:

Model solution: 1 mg/ml of artemisinin in hexane

- Capacity: 120 mg/g of the polymer resin
- Total adsorption: 80%
- *Effective bed volume: 150

* Ratio between volume of extract from which $\geqslant$ 50% could be adsorbed and volume of polymer

Capacity of MBAA–Based Polymer

For the artemisinin in plant extract:

Plant extract in hexane (0.7 mg/ml)

- Capacity: 70 mg/g of the polymer resin
- Total adsorption: 94%
- *Effective bed volume: 130

* Ratio between volume of extract from which ⩾ 50% could be adsorbed and volume of polymer

Elution Optimisation

Cranfield UNIVERSITY

keeping balance between elution of artemisinin and impurities using the plant extract

Eluent	Recovery, %	Degree of purification*
Acetonitrile	83.4	3
Methanol	88	2
Hexane/IPE (1/1)	55.6	4
Hexane/THF (5/1)	90	2.5

*Degree of purification: dry weight of the loaded sample divided by the dry weight of the eluted sample.

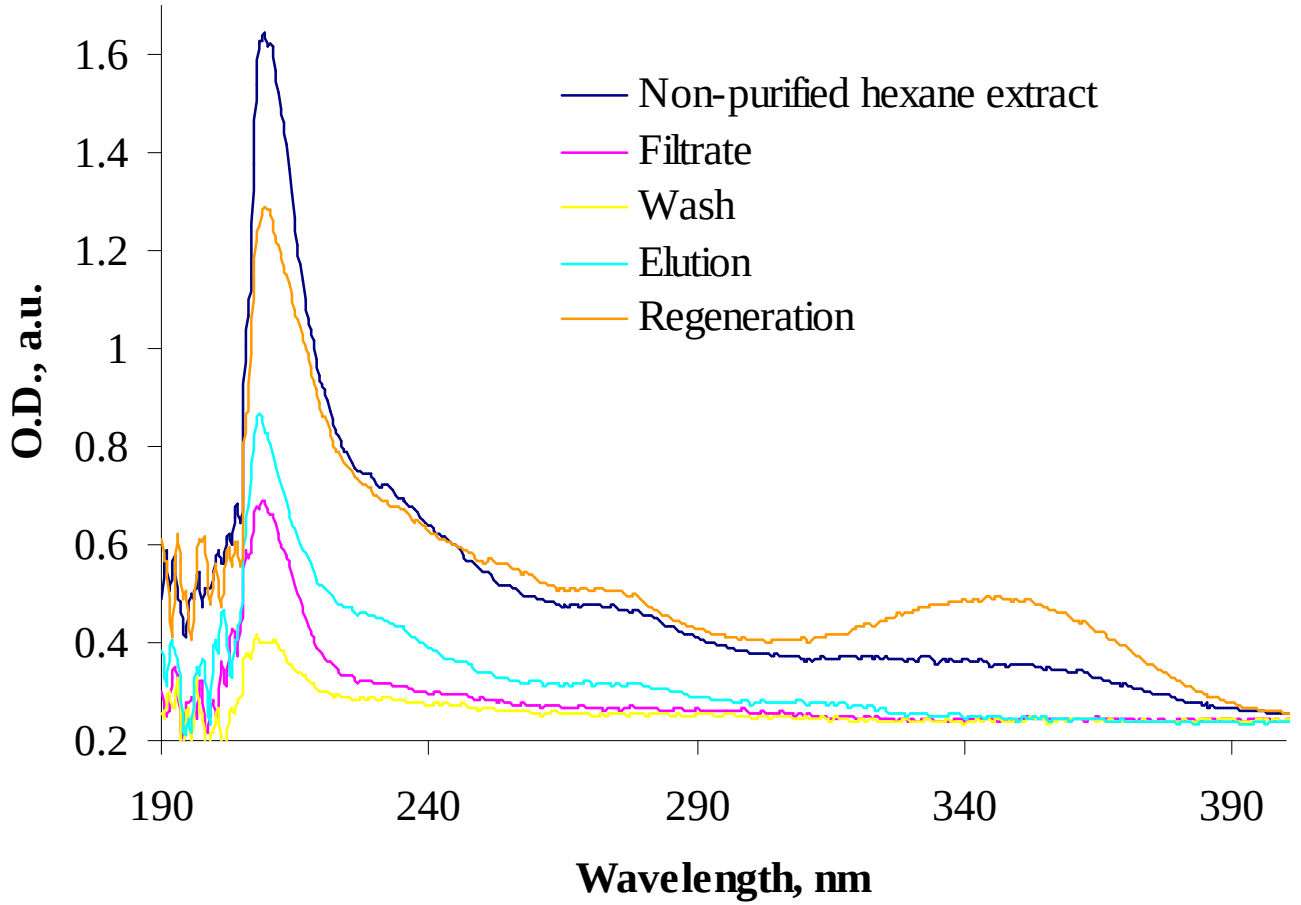
Regeneration Protocol

Cranfield UNIVERSITY

The protocol:

- Regeneration solvents: ethyl acetate or THF (2-3 ml)
- It was shown that polymer resins are stable and could be re-used at least for 10 regeneration cycles
- The regeneration should be conducted immediately after elution before resin is dried

UV-Vis Spectra of Different Steps in SPE



Not applicable for the evaluation of sample purity when working with the plant extract

Conclusions

- Monomer MBAA was selected for the polymer preparation.
- The HPLC-MS method for quantification of artemisinin was developed.
- The capacities of the MBAA-based polymer resins were obtained (120 mg/g for artemisinin in model hexane solution and 70 mg/g for artemisinin in plant extract, respectively).
- Methanol or hexane/THF (5/1) was selected as the optimised elution.
- Regeneration of the polymer resins was achieved using ethyl acetate or THF.
- The resulting polymer resins are stable and could be re-used for many times (>10).

Future Work

- Further optimisation of purification process for commercial samples using industrial volumes and larger size of columns is needed.

- It would be interesting to combine our polymeric materials with other techniques such as ionic liquids and HFC developed by other experts (Bhupinder Khambay, Rothamsted & Neil Sullivan, SensaPharm).

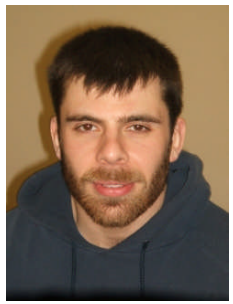
- There would be a need to optimise polymer purification stage with following crystallisation.

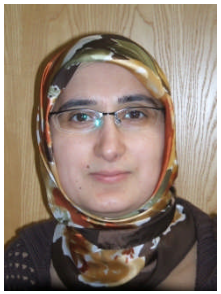
Special thanks to Prof. Sergey Piletsky & Dr. Elena Piletska !

Sergey Piletsky

Mike Whitcombe

Alessandra Bossi


Steve Fowler


Yildiz Uludag

Paula Pizarro De Sousa Brito

Tony Turner

Iva Chianella

Elena Piletska

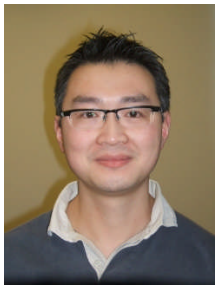
Sreenath Subrahmanyam

Anna Biela

Vasiliki Fragkou

Georgios Stavroulakis

Israel Sanchez


Yi Ge

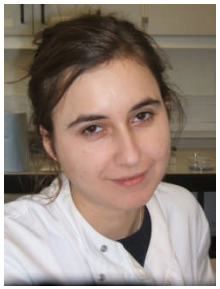
Dimitris Kyprianou

Antonio Guerreiro

Dhana Lakshmi

Andrew Spooner

Kal Karim


Ewa Moczko