Institution: University of Birmingham

Unit of Assessment: UoA 5 – Biological Sciences

Title of case study: Development and commercialisation of a stopped-flow cuvette for fast reaction studies of proteins and enzyme reactions by FTIR spectroscopy

1. Summary of the impact (indicative maximum 100 words)

The impact of this research has been of commercial benefit for TgK Scientific Ltd, a Wiltshire-based SME, who have successfully commercialised a FT-IR Stopped-Flow instrument. This has achieved market share as a result of incorporating an innovative cuvette designed and fabricated by the University of Birmingham's School of Biosciences. The company has sold nine of these instruments since they were first marketed in 2008, generating ~£200,000 in sales. This has made a substantial contribution to the company's total sales, most obviously in 2012 where sales of four instruments accounted for around 10% of their ~£800,000 turnover. The instrument allows the study of fast biological reactions by rapid scanning Fourier Transform Infrared Spectroscopy. The Birmingham contribution is a cuvette of a unique design that enables biological materials to be mixed and observed after 2-3 ms, allowing enzyme-catalysed reactions which have non-chromophoric substrates to be studied in physiological conditions. TgK have combined the cuvette with their stopped-flow drive system and a spectrometer produced by Bruker to make a complete apparatus; it is believed that this gives the instrument a unique functionality valued by a significant niche market.

2. Underpinning research (indicative maximum 500 words)

The underpinning research for this impact was the development led by Professor C. W. Wharton (Professor of Biochemistry to Sept 2007; subsequently retired to Emeritus status) of a fast reaction system for monitoring changes in the infra-red signature of substrates and intermediates in enzyme catalysed reactions.

The initial motivation for developing this was research into the mechanisms of alpha-chymotrypsin. This had hitherto relied on the use of non-physiological conditions to slow down reaction speeds in order to observe the changes occurring. In particular, the mechanism involves an acyl-enzyme ester intermediate which has a unique infrared signature distinct from the un-reacted enzyme background spectrum.

Ultimately, the aim of Wharton's group was to develop a time-resolved method that could be used to monitor a wide range of enzyme-catalysed reactions in working conditions that were as close to physiological as possible.

Initial work, starting in the late 1980's, led to the adaptation of a traditional IR cuvette with large flat CaF_2 plates and the addition, exterior to the cuvette, of a complex tangential mixer. The resulting device, which mixed and delivered solutions to the observation window in ca. 15ms worked well with FT instruments of the time. The construction and evaluation of this cuvette was described in detail in 1995 (ref. 1) and an example of the use of this cuvette is described in George et al 1997 (ref. 2). Although it worked well, this cuvette had a number of disadvantages. The most serious of which was the overall fragility of the device. The large flat CaF2 plates were very brittle and the high pressures required to push liquids through the 50 micron path length could often fracture the (expensive) plates. The device was also difficult to assemble, often requiring several attempts. Finally the mixing time was rather slow for modern FTIR spectrometers, which can scan at 100 s^{-1}, owing to the remote location of the mixing jets relative to the observation point.

In the early 2000's the group thus set out to devise and develop a device which would be both more robust and faster. This took a long time and involved around 10 intermediate designs, the later designs produced in stainless steel (ref. 3).

It was obviously of great importance to integrate the mixer very close to the observation point and to ensure that the CaF_2 windows were more robust. The newest design, used in the commercial product since 2008, therefore used thick stainless steel plates with much smaller 1 cm dia. CaF2 windows set into the stainless steel (IR beam diameter is 8 mm). It was found that a simple micro-channel (1 mm channels) T-mixer, cut into the steel immediately adjacent to the window provided

very effective mixing. There was a difficult problem with uneven propagation of the newly mixed material across the observation window. As it displaced 'old' mixed material when the 'new' mixed material was delivered to the observation channel by a series of parallel 1 mm channels, it 'drove' through' the 'old' reaction mix in the observation chamber as a series of discrete lines. This was solved in what was believed to be a unique way by machining a special fan-shaped weir of constant cross sectional area after the mixer. This changes in depth from 1mm at the mixer to 0.1 mm at the observation window and spreads in width from 1 mm to 10 mm and ensures that the in-rushing liquid is uniformly spread across the observation window and displaces it evenly. In this way it was possible to achieve a uniform propagation and displacement front through the observation chamber. This latter machining fully exploited the capabilities of the numerical milling system. The final product is very robust and reliable and mixes in 2-3 ms.

By 2006, the researchers were able to publish a study of the inhibition of the MRSA transpeptidase by penicillin in PNAS, based upon use of the cuvette (reference 3).

A parallel line of research on the effectiveness of micro-T-mixers was conducted in collaboration (2000-2004) with the School of Engineering at Birmingham. The micro-mixers were fabricated in silicon chips using masking & etching techniques. These studies showed that T-mixers were effective even on a small (micron) scale where turbulent flow is not possible; the diffusion length was however quite small. This work led to a number of highly cited publications, including one published in Sensors and Actuators B (ref. 4).

Key researchers in the development of the cuvette: Prof. C.W. Wharton; C. Hardman (Head of Workshops (deceased 2002); Derek Green, present Head of Workshops; Kanjana Thumanu PhD student and first author for ref. 3 (graduated 2006).

3. References to the research (indicative maximum of six references)

1. The design and testing of the 'original' Cuvette.
A.J.White, K. Drabble & C. W. Wharton, A stopped-flow apparatus for infrared spectroscopy of aqueous solutions. 1995, Biochemical Journal 306, 843-849. [can be supplied by the University]

2. The first collaborative application of the original cuvette.

S. J. George, G. A. Ashby,C. W. Wharton & R. N. F. Thorneley, Time-resolved Binding of Carbon Monoxide to Nitrogenase by stopped-flow IR Spectroscopy. 1997 J. Amer. Chem. Soc. 119, 6450-6451. DOI: 10.1021/ja971088s

3 Application of the 'new' Cuvette to MRSA Antibiotic Resistance.

K.Thumanu, J.Cha, J.F.Fisher, S. Mobashery & C.W.Wharton., Discrete steps in the sensing of beta-lactam antibiotics by the BlaR1 protein of the methicillin resistant *Staph. aureus* bacterium. 2006, Proc. Natl. Acad. Sci. (U.S.A.) 103, 10630-10635. DOI: 10.1073/pnas.0601971103

4 The collaborative programme with the School of Engineering on Micro-T-Mixers.

S.H.Wong, M.C.L. Ward & C.W.Wharton. 2004, Micro-T-Mixer as a Rapid Mixing Device Sensors & Actuators B Chemical. 359-379. DOI: 10.1016/j.snb.2004.02.008

4. Details of the impact (indicative maximum 750 words)

TgK Scientific Ltd is a Wiltshire-based SME that employs six people and produces instrumentation for transient kinetics and spectroscopy. This company has commercialised a new instrument that allows the study of fast biological reactions by rapid scanning Fourier Transform Infrared Spectroscopy. This FT-IR Stopped-Flow instrument is the only instrument on the market to fulfil this role. It is able to meet customer needs by incorporating the cuvette designed and fabricated at the University of Birmingham. "*The innovative cuvette design has made it possible for TgK to commercialise an IR stopped flow instrument, responding to customer demand*" (*s1)*.

The company specialise in products used to characterise fast reactions in solution:- stopped-flow, temperature-jump, rapid quench-flow and other more specialised variants of these such as high pressure stopped-flow, low temperature stopped-flow and stopped-flow for FT-IR. They had an initial request from researchers at the University of Manchester (Professor Nigel Scrutton, Faculty of Life Sciences) for an instrument to study specific enzyme reactions by IR spectroscopy. TgK

were aware of Wharton's work, and were able to combine his cuvette with their sample delivery technology and the Bruker VERTEX 80 FT-IR spectrometer to produce an innovative Stopped-Flow instrument that has been successful in the market.

Since 2008, there have been nine sales of the instrument: two in the UK, two in South Africa, one in the USA, three in Germany and one in China; hence seven of the nine have been exported. This has produced sales income of around ~£200,000 over the four-year period. Four of the sales were in 2012, with these generating around 10% of the company's £800,000 turnover in that year. The product has enabled TgK to extend their product portfolio and boost sales performance at a time when the instrument market was under pressure.

The Birmingham contribution to instrument is the cuvette; the unique design enables biological materials to be mixed and observed after 2-3 ms. Two incoming liquids are rapidly mixed and uniformly spread across the observation chamber within the cuvette whilst displacing evenly the liquid that is already present. This allows enzyme-catalysed reactions which have non-chromophoric substrates to be studied in physiological conditions.

The flow cuvette which is central to this product was designed by Wharton's group with the support of engineering colleagues at Birmingham. The cuvettes continue to be fabricated in the workshop in the School of Bioscience, and have generated an income of £47,811 for the University since 2008.

TgK believe that this collaboration has allowed a novel product to be commercialised. IR as a technique is particularly useful for studying molecules that have no chromophore. It is also a very complementary technique to CD in studying protein structure. Applications have varied from protein folding and enzyme kinetics to inorganic chemistry including studies in transition metal catalysis and novel energy production.

Feedback from TgK customers demonstrates the value of this product to them and its unique nature (*s2, s3, s4*). A customer at the University of East Anglia said "*We make very regular use of the stopped-flow system, which allows us to follow organometallic reactions in a sub-second timeframe. Only stopped-flow methods offer the possibility to examine reactions in this way, and for the systems we are most interested in only stopped-flow IR offers the detail we need. To the best of my knowledge, the Tgk Scientific system featuring the University of Birmingham cell is the only commercial set up available to carry out this work.*" (*s3*)

Another customer at the Manchester Institute of Biotechnology has confirmed that they do use the cell in their stopped-flow IR instrument.to analyse any intermediates and products that are formed during reactions catalysed by enzymes. He said "*In some cases it is only possible to study these species by using IR light…..As far as I know this is the only instrument that is capable of these measurements.*" (*s4*)

5. Sources to corroborate the impact (indicative maximum of 10 references)

s1. Director, TgK Scientific Limited, 7 Long's Yard, St Margaret's Street Bradford-on-Avon, BA15 1DH, United Kingdom. website: www.hi-techsci.com

s2. Professor, Fachbereich Chemie, Universität Konstanz, Universitätsstraße 1078464 Konstanz, Germany.

s3. Lecturer in Energy Materials, School of Chemistry, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, United Kingdom.

s4. Senior Experimental Officer, MIB Biophysics Facility, Manchester Institute of Biotechnology, Faculty of Life Sciences, University of Manchester, 131 Princess Street, Manchester, M1 7DN, United Kingdom.