

## TCS TCS project with Queen's University Belfast

Since February 2002, BTL has been involved in a TCS project with the School of Pharmacy at Queen's University Belfast. TCS is a UK government scheme that enables small businesses and universities to take advantage of the wide range of knowledge and specialist expertise that each other offers. From October 2003, projects will be called Knowledge Transfer Programmes (KTP). These programmes focus on innovation projects that are central to the strategic development of the company and are funded by government grants together with financial contributions from the company. These funds support the employment of a Research Associate who works on a two year project under the supervision of both company and university staff.

Our project centres on the development of rational freeze-drying strategies for the freeze-drying of proteins. The project involves using BTL's in-house techniques (freeze-drying microscopy using the *BTL Lyostat2*, freezing resistance analysis and differential thermal analysis using the *BTL Lyotherm*, to determine critical characteristics of formulations to be lyophilised. Methods used at QUB include Modulated Differential Scanning Calorimetry and Thermo Gravimetric Analysis. Our partners at QUB are Prof. Duncan Craig and Dr Vicky Kett. Our Associate, Debra McMahon, has registered for an M.Phil. at QUB as part of the programme, which has given us some interesting data. So far, we have had the opportunity to present posters showing some of our results at international conferences, including TAC2003 in the UK and the Protein Stability Conference, held in Colorado, USA, organised by protein expert Dr John Carpenter of the University of Colorado.

### We will be presenting at:

- ◆ Society for Low Temperature Biology Meeting 2003, 4-5 Sept, NIBSC, UK
- ◆ British Pharmaceutical Conference 2003, 15-17 Sept, Harrogate, UK
- ◆ International Society for Lyophilization Conference 2003, 16-19 Sept, Chicago, Illinois, USA
- ◆ The Parenteral Society "Advances in Freeze Drying" Seminar, 21<sup>st</sup>-22<sup>nd</sup> Oct, in Manchester, UK
- ◆ American Association of Pharmaceutical Sciences Conference 2003, 26-30<sup>th</sup> Oct, Salt Lake City, Utah, USA

**Correlation of Pre-lyophilized Characteristics of a Model Protein Formulation with Process Behavior of Final Product Qualities in a Single Comprehensive Study**

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**INTRODUCTION**  
Freeze-drying (lyophilization) is a unit operation in which a solvent, usually water, is frozen and then sublimed in a vacuum. It is commonly used in the pharmaceutical industry where there are stability issues with the active ingredient in solution, as a route to protect proteins. In order to prevent processing defects during freeze-drying, active ingredients are often tested with excipients, which may have specific functions, such as providing bulk properties, thermal stability and activity preservation to the product. Many groups of molecules have been shown to perform these functions, including disaccharides, amino acids, polymers and non-toxic surfactants. The aim of this project was to complete a characterization of a large series of excipient combinations in model solutions prior to freeze-drying, and to compare these characteristics to a number of product qualities after freeze-drying.

**MATERIALS AND METHODS**  
Dextran and sucrose (D5000) glucose, mannitol, sorbitol and trehalose were obtained as analytical grade from Sigma. Lyophilized dextran (LDH), also Sigma was obtained as an amorphous stable suspension and diluted prior to formulation. The LDH stability study was based on the spectrophotometric method of Healy, et al.<sup>3</sup> was obtained from ThermoTrace (Winchester, UK) and mannitol were used as protectant. Sucrose was used in a combination with Mannitol, a source of mannitol. The solutions were analyzed using MTDSC, DTA and freeze-drying microscopy (FD/DM).

**RESULTS**  
The sub-ambient T<sub>g</sub> of dextran was clearly observed using MTDSC (Figure 1) and in solution the onset of collapse as observed by freeze-drying microscopy (Figure 2a & b). Also shown is the effect of adding glucose to the collapse temperature and image (Figure 3a & b).

**CONCLUSIONS**  
The results indicate that whilst dextran shows a more pronounced thermal properties to aid stable freeze-drying it does not protect LDH. The addition of low molecular weight excipients had the effect of maintaining the protein activity throughout the process, although they did not reduce the collapse temperature of the solution. The same effect was observed for mannitol, which by itself had desirable thermal qualities but retains little protein activity. However the formulations also containing the low molecular weight excipients exhibited good activity, thermal properties and stable co-lyophilization.

**REFERENCES**  
1. M.J. Pikal, *Biopharm*, 3(8), 18-27 (1990).  
2. J.F. Carpenter, M.J. Pikal, et al., *Pharm Res*, 14(9), 969-975 (1997).  
3. R.J. Healy et al. *Am J Clin Pharm*, 34(38), 333 (1993).

**Figure 1** MTDSC of 2% dextran solution showing T<sub>g</sub>

**Figure 2** DSC of Dextran at -12.4°C and -10.0°C respectively

**Figure 3** DSC of Dextran/Glucose at -12.0°C and -10.0°C respectively

The collapse temperature, product T<sub>g</sub> and product water content for each of the mixtures are shown in table below:

Mixture	Product T <sub>g</sub> (°C)	Water Content (%)	Collapse Temp (°C)
Dextran	-12.4	~10	-12.4
Dextran/Glucose	-12.0	~10	-12.0
Dextran/Sorbitol	-12.0	~10	-12.0
Dextran/Mannitol	-12.0	~10	-12.0
Dextran/Trehalose	-12.0	~10	-12.0
Dextran/Sucrose	-12.0	~10	-12.0

Poster Presented at the Protein Stability Conference in Colorado

### OFFICE HOURS

Please note our office hours are now

8.30am to 5.00 pm Monday to Thursdays 8.30am to 2.30pm on Fridays

## Two Courses Happening Soon!!

During October and November two Freeze-Drying courses will take place in San Francisco, CA, USA and Winchester, UK. Both these courses will offer the participants the opportunity to review Freeze-Drying Technology related to both the equipment and the process. Both courses will cover:

- ◆ Principles of Freeze-Drying: The Design, Specification & Operation of Freeze-Dryers
- ◆ Product Formulation and Analysis Specifically for Freeze-Drying
- ◆ Steps of the Freeze-Drying Process, how they work and how they affect formulations
- ◆ Industrial freeze-drying, Validation Issues and regulatory issues (e.g. sterilisation, 21CFR11)

### San Francisco, CA, USA. 8<sup>th</sup> – 10<sup>th</sup> Oct 2003

This is a 3-Day Comprehensive Course.

For further details or to book your place, please contact Thomas Petersen at:

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### Winchester, UK. 10<sup>th</sup> – 13<sup>th</sup> November 2003

This is a 4-Day Comprehensive Course.

For further details or to book your place, please contact us at:

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## Lyostat Into India

We are pleased to announce that Biopharma have recently sold our first Lyostat Freeze-drying Microscope system into India. The system was purchased by the Neon Group, manufacturer of a broad range of cardiology, gynaecology and other speciality drugs.

Mohan Jain and Mahesh Hariharan of the Neon Group visited our laboratory here at Biopharma to have a demonstration of the system. Many of Neon's products are freeze-dried so they were very interested to see how the system could help them in developing their freeze-drying cycles. They were so impressed with what they saw that they purchased the system there and then! The system has now been installed and is in use at their site in Mumbai.



Following on from this sale, we have taken steps to set up representation in India. Our Managing Director, Tony Gaster has recently travelled to India to meet with Empire Instrumentation based in Mumbai. We hope that this will lead to a successful partnership.

## 'Alternative' Freeze-Drying Formats Becoming More Popular!

The range of freeze-drying formats BTL is working with is increasing – in addition to the more traditional vials, ampoules and bulk trays; we have noted a significant increase in alternative formats, particularly purpose-designed containers. The starting materials don't have to be solutions either...some 'alternative' starting materials we have worked on to date include polymer gels, sols, slurries, suspensions, ceramics, paper and food!