

Happy Birthday BTL!

Celebrating our 10th birthday



Biopharma Technology Ltd (BTL) was set up in 1997. BTL's sister company, Biopharma Process Systems, had already been successfully selling freeze drying equipment for eight years when the founder, Tony Gaster, identified a need in the market for qualified specialist assistance and advice. Freeze drying has always been seen as something of an art form and handled with "hit-or-miss" tactics and results. So BTL's aim was to approach the field with rational, scientific methodology to provide reliable information pertinent to the freeze drying process.

Our Analytical Instruments

BTL has always been involved in research and development and part of this has been in developing analytical instruments for freeze-drying investigation. Our custom-designed and purpose-built instruments *Lyotherm2* and *Lyostat2* are used in our laboratory to provide key information that can be fed back into formulation and development.

Lyotherm2 is a unique instrument incorporating DTA and impedance technology to determine a range of critical events taking place within formulations. The original *Lyotherm* was built in 1998 but new Zsin ϕ impedance technology, completed in 2005, now allows finer pinpointing of the onset of events taking place within frozen formulations.

Lyostat2 is our fully integrated freeze-drying microscope that enables critical events such as collapse and skin or crust formation to be observed *in situ* and in real

time. The original *Lyostat* was completed in 1998 but has also undergone continuous improvement and refining since then, with *Lyostat2* being released in 2002 and 21 CFR 11- compliant software becoming available in 2004.

Training Courses

Our specialist training courses in freeze drying technology have been running successfully worldwide since our very beginning. Courses in Winchester, Amsterdam and California have become annual fixtures, while 2006 saw our very first Indian course, held in Mumbai. The success of this event means we will be back again this October.

Extensive Knowledge

BTL has worked on a wide range of products over the years. We have freeze dried everything from pharmaceuticals to fish slurry. We've carried out analysis work on everything from small drug molecules to large complex biomolecules. We have built up an extensive knowledge of the freeze-drying process which enables us to select excipients compatible with the process and the formulations. Today we have customers on every continent who rely on us for this expertise and in-depth knowledge.

Birthday Promotion!

To celebrate BTL's 10th birthday we are giving away one *Lyotherm* analysis with a value of up to £650 with every *Lyostat* analysis purchased!

The offer is open to orders placed up until the end of 2007. One free analysis per customer. Please contact us for more details.

Research Poster Presented to Parliament

A poster has been presented at the House of Commons describing some of the interesting and novel findings from a joint research project between BTL and Dr Claire Martin of Wolverhampton University, funded by the university's ERAS (Early Researchers Award Scheme).

The aim of this research is to determine process conditions for enhancing product shelf life and stability, as well as identifying potential formulation strategies to minimise denaturation of biomolecules in nutraceuticals. The project employs dielectric spectroscopy to examine the stability of therapeutically relevant excipients at reduced temperatures.

Optimising a formulation is a critical stage in the development of suitably stable frozen or freeze dried products. Bulking and protective agents are commonly added to formulations to aid recovery as well as preserve the activity of the biological compounds.

In this study, dielectric spectroscopy in the form of fixed frequency electrical impedance analysis was performed with the use of Lyotherm2 (designed, made and sold by BTL). This instrument measures electrical impedance during the cooling and warming of materials to give an indication of molecular mobility changes within frozen structure. This can provide evidence of critical behaviour patterns of frozen solutes/ mixtures which has implications for low temperature operations such as freeze drying, cold storage and transport.

Initial work has been carried out with tea tree oil and a series of therapeutically relevant excipients and other novel formulations. Results show potential stability advantages for processing, storage and transport of lipophilic nutraceuticals when formulated with low concentrations of emulsifying polymers such as PEG. Data also suggest that it may be possible to maintain oil based products at temperatures between 0 and -5.0 degrees Celsius thus conserving their bioactive properties.



Claire Martin, and the poster her team worked on.

Application of Electrical Impedance Analysis for Investigation of Nutraceutical Formulation Stability in the Frozen State

Claire Martin, Clare Ross*, Thomas Peacock* & Kevin R. Ward*

Pharmacy and Natural Products Research Group, Department of Pharmacy, School of Applied Sciences, University of Wolverhampton, Wulfruna Street, Wolverhampton, WV1 1SB, United Kingdom

*Biopharma Technology Limited, Biopharma House, Winnall Valley Road, Winchester, SO23 0LD, United Kingdom

Aims
Dielectric spectroscopy was used to examine the polarisation-relaxation response of a model nutraceutical, tea tree oil (TTO), alone and formulated with traditional excipients, when exposed to an electromagnetic field. The response provides a measure of mobility within a material, which is dependent on both structural and molecular properties, as well as the ratio of components in binary and ternary systems (Craig, 1995).

Tea Tree Oil (TTO)
TTO is a natural product derived from steam distillation of leaves and twigs of the *Melaleuca alternifolia* tree. Pharmaceutical grade TTO contains 45% cineole and 35% terpinen-4-ol, in addition to more than one hundred other constituent materials. (European Commission, SCOP, Opinion on Tea Tree Oil). Traditionally TTO is applied topically for localized treatment of bacterial, fungal, viral infections, etc and is available commercially as essential oil, cream, ointment and lotion formulations.

Thermal Analysis
Analysis of pharmaceutical grade pure essential oil and polymer-stabilised oil-in-water emulsion formulations was conducted with Lyotherm2 (Biopharma Technology Limited) which assesses electrical impedance and differential thermal analysis (DTA) during cooling and warming of materials to locate molecular mobility changes (possibly resulting from softening, relaxation, crystallisation, rearrangement of melting) which may be applicable to reduced temperature operations for those materials (i.e. freeze drying, cold storage and transport).

Figure 1: Arrangement of Lyotherm2 Equipment
(Biopharma Technology Limited, Winchester).

Table 1: Summary of thermal events accompanying the reduction in impedance values approaching 0 °C in organically diluted TTO and aqueous emulsions of TTO.

Sample	Thermal Event	Change	Impedance Event (°C)
1% v/v TTO in O/W	T_{max}	-	42.2 - 58.0
	T_{end}	-	-
	T_{onset}	-	-
TTO: 1% w/v PEG to KCl	T_{max}	-	-3.0
	T_{end}	-	-7.0
	T_{onset}	-	-7.0
TTO: 1% w/v PEG to KCl	T_{max}	-	-3.5
	T_{end}	-	-3.0
	T_{onset}	-	-3.0
TTO: 1% w/v PEG to KCl	T_{max}	-	-1.0
	T_{end}	-	+0
	T_{onset}	-	+0

KEY: T_{max} = temperature point of deviation from the maximum impedance (5025 AD) under an applied field of frequency 1.000 Hz; T_{end} = onset point of an elevated mobility region; T_{onset} = end temperature point of elevated mobility region; and T_{onset} = point of sudden onset of mobility associated a thermal melting event.

Figure 2: Sample electrical impedance data showing T_{onset} , T_{max} and T_{end} events with increasing temperature.

Methods
Emulsions were prepared by vigorously mixing aqueous solutions of two different molecular weights (1 kDa and 10 kDa) of poly(ethylene glycol) (PEG) at three different concentrations (10.0, 1.0 and 0.1 % w/v) with TTO in a 1:1 volumetric ratio. Cooling to $-40\text{ }^{\circ}\text{C}$ below the maximum impedance temperature was provided within the liquid nitrogen chamber and the samples were reheated to 20 °C (at 1.5 °C/min). The data was exported directly into MS Excel for analysis of the warming profile to determine the temperature of significant events, which may be due to increases in molecular mobility and/or relaxation.

Results
The following cooling to $-100\text{ }^{\circ}\text{C}$ pure TTO (and TTO diluted (1% v/v) in dichloromethane) remains mobile and displays no discernible T onset temperature point, indicating a melt-like system; this implies that the pure essential oil would remain in a liquid state and during reduced temperature processing and storage. Conversely, TTO-PEG emulsions display distinct T onset values between -1 and -5 °C (depending on molecular weight and concentration of PEG used), which implies the potential stability of these formulations at $-5\text{ }^{\circ}\text{C}$ by virtue of their reduced molecular mobility. Interestingly, decreasing the concentration of PEG in TTO emulsions does not lead to a concurrent reduction in impedance and hence the effective electric field, permittivity and capacitance remain unaltered.

Conclusions
In conclusion, these results show the potential stability advantages for processing, storage and transport of highly lipophilic nutraceuticals, when formulated with low concentrations of inert, surface active polymers.

References
Craig, D. (1995). Dielectric Analysis of Pharmaceutical Systems. Taylor and Francis, Bristol and London, UK. ISBN: 0-7503-0202-2, 200pp.

Acknowledgements
The authors wish to gratefully acknowledge The Financial support provided by the Early Researcher Award Scheme (ERAS) through The Graduate School, University of Wolverhampton.

New Appointment



Isobel Cook - Principal Research Scientist

Isobel joined BTL in January 2007 having worked as an Analytical Chemist in pharmaceutical method development and environmental marine research. Isobel has been involved in various projects including method validation, site cleaning validation, investigating and implementing laboratory procedures. Isobel has also participated in a number of marine research projects e.g. NERC Consortium funded AMT research on photo-protective pigments. Isobel has a BSc in Environmental Chemistry and is responsible for BTL's internal and joint research projects in freeze drying technology.

12 Months Ahead of Schedule

BTL was thrilled to receive a letter of thanks and recommendation from another happy customer recently.

BTL completed a series of work for UGM Engineering earlier in the year. Based in Canada, UGM Engineering's work applies the principles of chemical engineering to a range of industries in the categories of process design, plant troubleshooting and loss control. When they needed some expert assistance with their lyophilization, they turned to us – and were very pleased with the results.

UGM Engineering's Director of Pharmaceutical Technology writes:

“You and your colleagues have provided us with exceptional service. The results of your lyophilization studies have gone beyond our most optimistic expectations. Based on your work, our project is about 12 months in advance of where we expected to be.

“We look forward to working with you again and would gladly recommend Biopharma.”

4th International Conference on Freeze Drying

BTL's Dr Kevin Ward will be speaking at the 4th International Conference on Freeze Drying in Dublin, Ireland.

The conference is being held jointly by the International Society of Lyophilization (ISL) and the Pharmaceutical and Healthcare Science Society (PHSS) meeting at the Carlton Dublin Airport Hotel in Dublin, Ireland, this October. Speakers include delegates from such recognised companies as Bayer, West Pharmaceutical Services and AstraZeneca, and organisations such as the UK's Oxford University, Brazil's Sao Paulo University and Cuba's Centre for Molecular Immunology. They will be sharing advanced and novel papers on different applications of lyophilization.

Dr Ward's presentation is entitled “Advanced Uses of Freeze-Drying Microscopy (FDM) for Product and Lyo-Cycle Development”. It will review the current state of freeze-drying microscopy, and outline the applications of the technique to product development.

As well as the conference itself, the 5-day program features a seminar, providing a broad overview of the process, a workshop focusing on cGMP for lyophilized products, and a tabletop exhibition.

The meeting runs from the 14th-19th of October, with the conference running from 17-19th.

For booking information go to:

www.islyophilization.org.



Upcoming Courses



September 17th-19th 2007, San Francisco, USA—

3-day comprehensive course

October 8th-10th 2007, Amsterdam, the Netherlands—

3-day comprehensive course

October 22nd-23rd 2007, Mumbai India—

2-day introductory course

October 29th-30th 2007, Mumbai India—

2-day advanced course

November 12th-14th 2007, Winchester, UK—

3-day comprehensive course



Contact us at:

Biopharma House, Winnall Valley Road, Winchester SO23 0LD, UK

Tel: +44 1962 841092 Fax: +44 1962 841147

btl@biopharma.co.uk

www.lyophilizationtechnology.com