european pharmaceutical review

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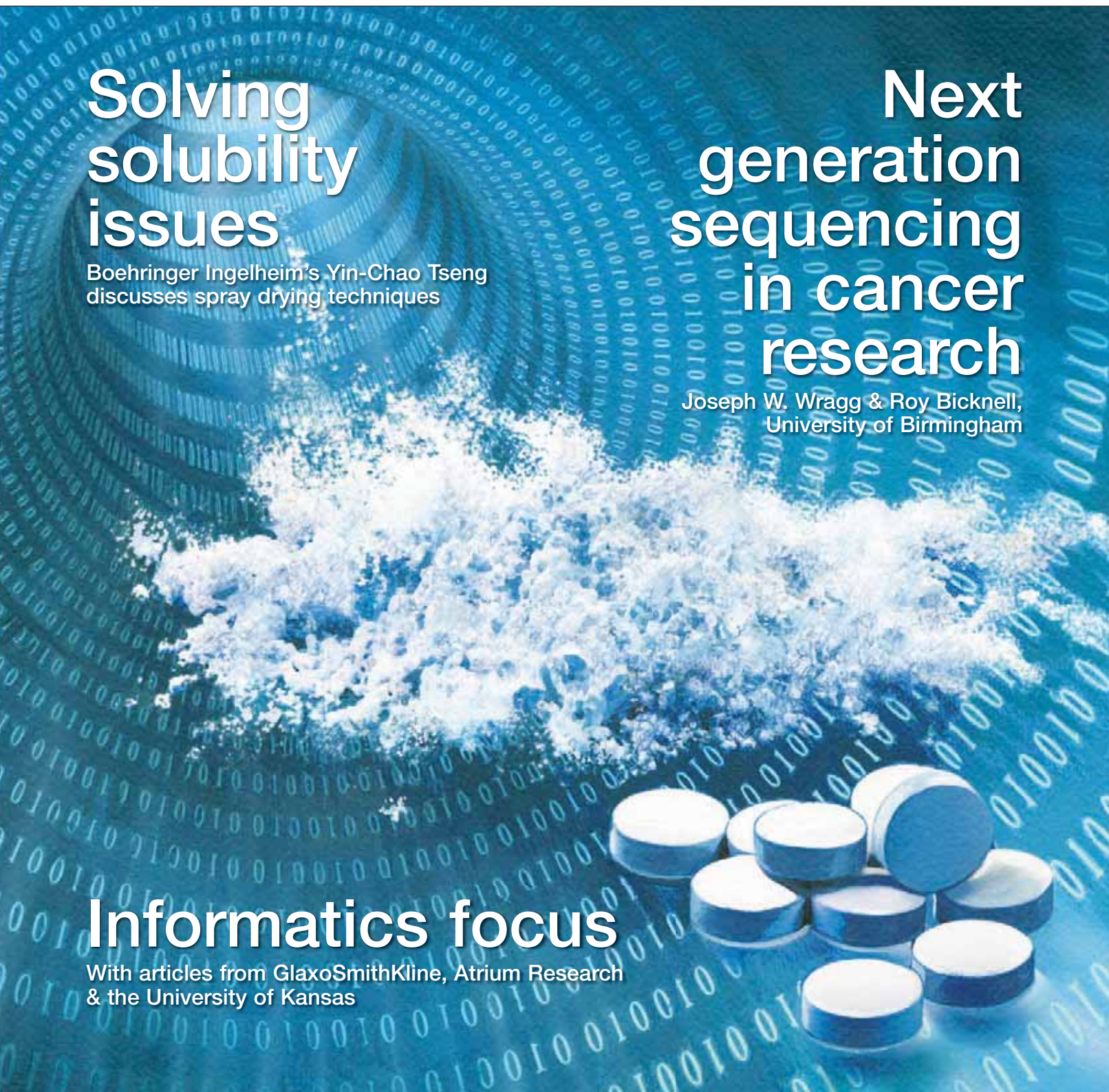
Issue 4 · 2015



Solving solubility issues

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Next generation sequencing in cancer research

Joseph W. Wragg & Roy Bicknell, University of Birmingham

Informatics focus

With articles from GlaxoSmithKline, Atrium Research & the University of Kansas

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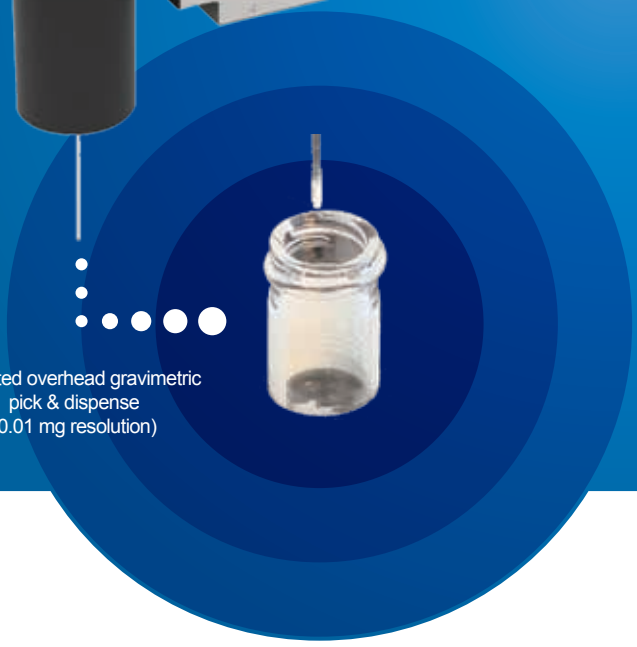

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The big deal about big data

The amount of data the pharmaceutical and healthcare industries generate is enormous, and businesses must increasingly face up to the importance of finding sophisticated methods to analyse, integrate, visualise and manage this wealth of data, as they eye the potential for improved efficiencies throughout the pharmaceutical chain, from drug discovery to post-regulatory approval and beyond.

But will these gains be fully achieved in a decade's time? Michael. H. Elliott, CEO of Atrium Research and Editorial Board Member at *European Pharmaceutical Review* poses this question in the opening article of our Informatics In Depth Focus starting on page 15. Of course, without a crystal ball it is impossible to predict the future with complete accuracy, but trends over the past few years can certainly give us a lot of insight, and it appears informatics will continue to, as Elliott puts it, "play a major role in helping organisations solve strategic challenges".

Also contributing to our supplement is Brittany L. Melton, Assistant Professor of Pharmacy Practice at the University of Kansas School of Pharmacy (page 20). She delves into the topic of mining text, such as patient records, for identification of novel drug-drug interactions, in her article. Informatics is increasingly being applied to pharmacovigilance, and as these disciplines merge, new methods that meet the needs of healthcare systems, providers and patients are needed. Another interesting slant on informatics is taken by David Elder (GlaxoSmithKline and JPAG) in his feature (page 23) on *in silico*, computational methods for the screening of impurities.

Other topics you'll come across in this issue of *European Pharmaceutical Review* are next-generation sequencing (page 10), spray drying (page 28) and our usual Microbiology Series (page 38). The Regulatory Insight feature this month concerns pharmaceutical co-payment reforms in Spain. No European country was immune to the effects of the global economic downturn that started in 2008, as Spain's situation demonstrates. But have global healthcare system reforms been successful? In Spain at least, Economist Jaume Puig-Junoy and colleagues can see the shortcomings.

As always, you can contact me by email on crichards@russellpublishing.com if you would be interested in contributing a feature article to a future issue of *European Pharmaceutical Review*. And to ensure you receive every issue of the magazine, you can subscribe at (www.europeanpharmaceuticalreview.com/subscribe); it's free to do so. On our website, you can also find details of current and future issues, sector news and event details. And find us on LinkedIn and Twitter – details are opposite.

Caroline Richards
Editor, *European Pharmaceutical Review*

 Published August 2015

Pharma&Biotech

Lonza

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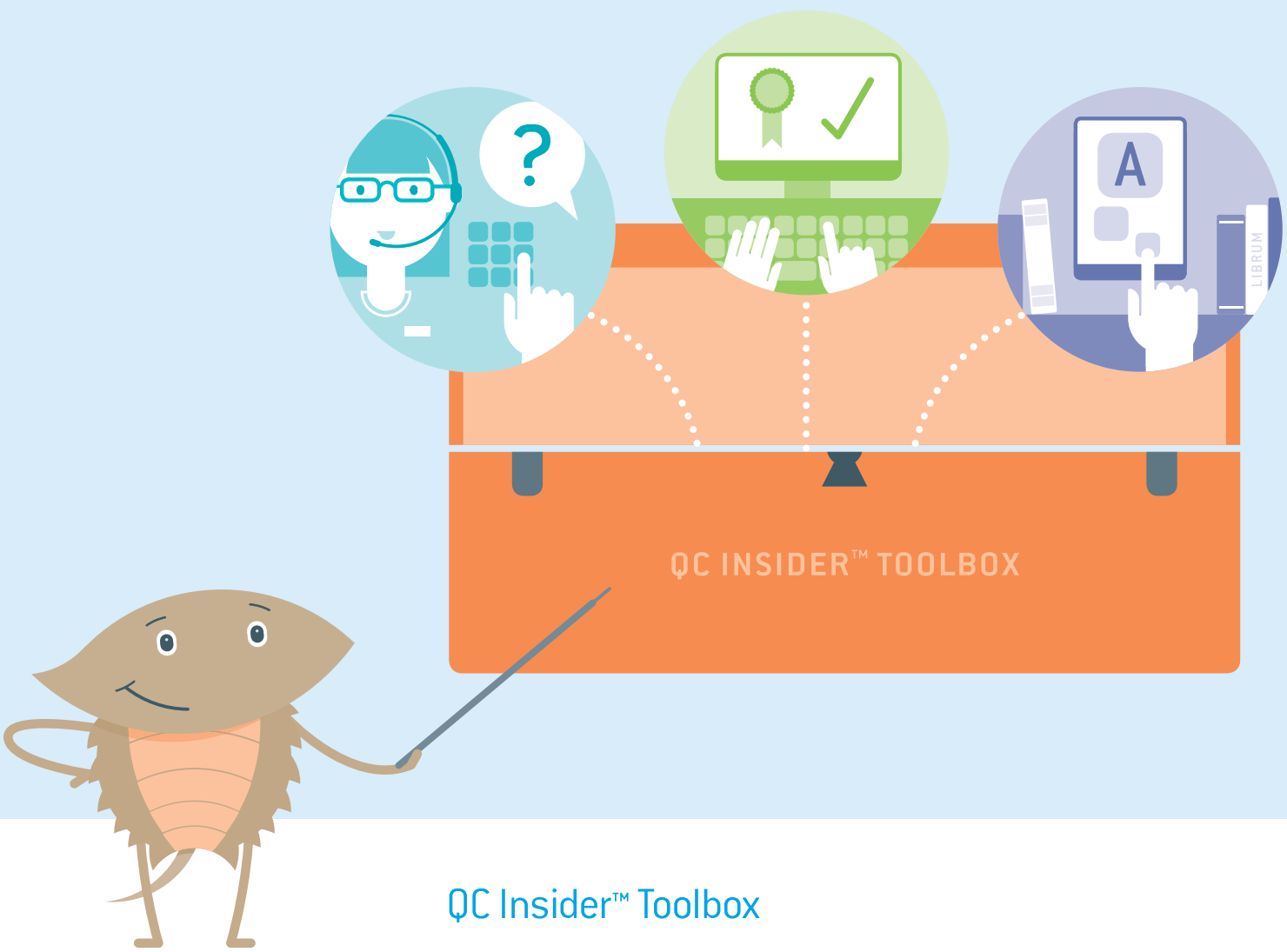

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COMING UP IN THE NEXT ISSUE:

- **Rapid Methods In-Depth Focus**
- **Raman In-Depth Focus**
- **Polymorphs**
- **Emerging Markets**

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Dave Elder
GlaxoSmithKline and JPAG

FOREWORD

The importance of good distribution practice

Historically, the regulation and control of medicinal products has relied on national and supranational guidelines covering good manufacturing practice (GMP). However, the quality of these medicinal products can be adversely affected by a lack of adequate control over the myriad activities that occur during the distribution process[1]. In addition, the necessity for developing, establishing and maintaining an adequate control system has not generally been well understood. This may result in differences in documentation practices and handling requirements, as well as complex communication between the various organisations, companies, groups or entities that comprise the supply chain.

This is where good distribution practice (GDP) comes in. The US Pharmacopeial Convention believes that GDP should "facilitate the movements of drug products throughout a supply chain"[2], while the European Medicines Agency has stated that GDP ensures "the level of quality determined by GMP is maintained throughout the distribution network, so that authorised medicines are distributed to retail pharmacists and others selling medicines to the general public without any alteration of their properties"[3].

Like GMP, GDP is regulated and controlled by numerous national and supranational guidelines. It is reliant on a series of inter-connecting quality systems operated by wholesalers or distributors of pharmaceutical drug products. The systems ensure the following: distributed products are authorised in accordance with the relevant legislation; appropriate storage conditions are maintained at all times, including movement of goods between various parts of the distribution network; contamination by other products is avoided; an appropriate turnover of stock takes place; and that products throughout the distribution chain are stored in safe and secure areas[4]. In addition, to help combat counterfeiting, there should be a system(s) to enable faulty products to be rapidly found and recalled. In parallel, an effective quality system is required to ensure that the right product is delivered to the right location within a designated time period[4].

Those GDP issues that affect product quality are similar to those bedevilling GMP, that is, errors (mix-ups), contamination and cross-contamination. However, inadequate or inappropriate storage conditions, as well as supply chain security considerations (counterfeiting or adulteration) are also important[1]. Therefore, all organisations that are part of the supply chain have a shared responsibility for ensuring that they transport/store drug products under appropriately controlled conditions that will not affect drug product quality, efficacy or safety and that they pass the drug product onto the next part of the supply chain in an appropriate manner[2].

One of the most important considerations of GDP is therefore storage and shipping. Each organisation is required to define and control appropriate facilities, which include those which receive, hold and transfer the product. It is thus important that drug products should be continuously monitored and verified by appropriately calibrated monitoring systems or that the supply chain is appropriately qualified based on historical data.

However, tt may be acceptable to use a combination of drug product stability data and supply chain risk assessments to justify shipping products without continuous verification of supply chain qualification[2]. One of the most important considerations in the latter approach is to assess the impact of temperature excursions, i.e., those parts of the supply chain where temperature is not effectively controlled within pre-defined ranges. This can be accomplished using mean kinetic temperature (MKT). MKT can be considered as 'an isothermal temperature that stimulates the non-isothermal effects of storage temperature deviation'[5]. The MKT calculation is therefore deemed to be an appropriate approach to justify temperature excursions that occur during transit, but the MKT calculation must be justified by ensuring that the drug product follows first order kinetics over the temperature range encountered1. That is, that product exposed to temperatures of 80°C for 1 week will follow the same degradation pathway as product exposed to temperatures of 40°C for 6 months.

In summary, complex multinational supply chains for pharmaceutical products are increasingly becoming the global norm. Against this background GDP is an essential practice to ensure the continuing quality of medicinal products. However, the resultant supply chain is only as strong as the weakest part and it necessitates the combined efforts of all parties to ensure the continuing success of GDP and thereby the continued assurance of product quality.

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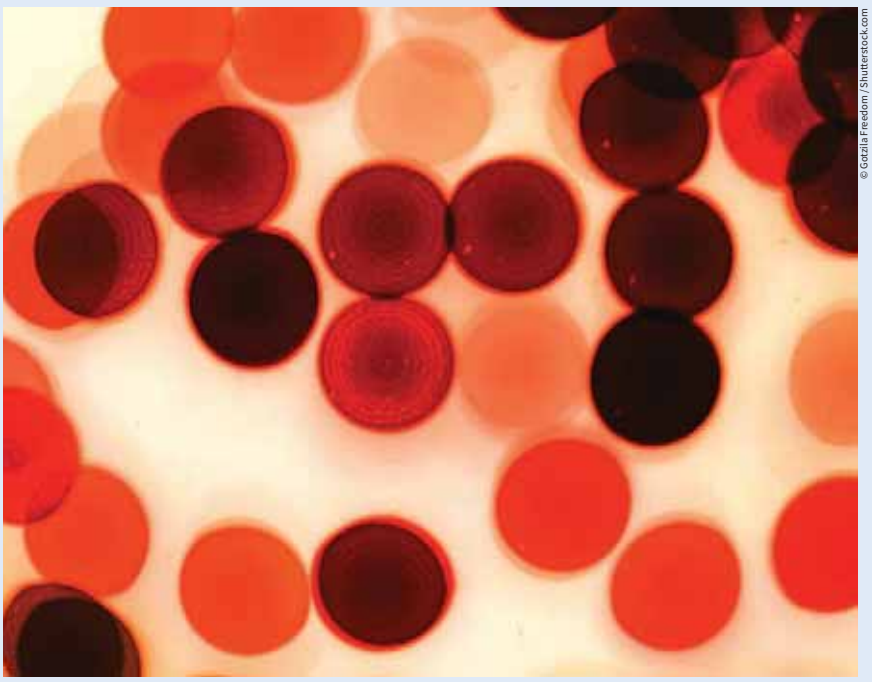

TAKEDA

EMA accepts MAA for multiple myeloma drug ixazomib

The European Medicines Agency (EMA) has accepted the marketing authorisation application (MAA) for Takeda's ixazomib, an investigational oral proteasome inhibitor for the treatment of patients with relapsed and/or refractory multiple myeloma.

On 23 July, ixazomib was granted accelerated assessment by the Committee for Medicinal Products for Human Use of the EMA. Takeda has filed a new drug application for ixazomib in the US.

The MAA submission was primarily based on the results of the first pre-specified interim analysis of the pivotal Phase 3 trial TOURMALINE-MM1, an international, randomised, double-blind, placebo-controlled clinical trial of 722 patients designed to evaluate the superiority of ixazomib plus lenalidomide and dexamethasone over placebo plus lenalidomide and dexamethasone in adult patients with relapsed and/or refractory multiple myeloma. Patients continue to be treated to progression in this trial and will be evaluated for long-term outcomes. Additional filings in other countries are planned to begin later this fiscal year.

JANSSEN

Janssen submits extension MAA for new paliperidone palmitate formulation

Janssen has submitted an extension marketing authorisation application to the European Medicines Agency (EMA) for a paliperidone palmitate once-every-three-months formulation for the treatment of schizophrenia. If approved, it will be the first antipsychotic schizophrenia medication to be administered four times a year, and the new formulation will be marketed as Trevicta in Europe.

Paliperidone palmitate once-monthly (marketed as Xeplion® in the European Union) is an atypical long-acting injection to treat schizophrenia, and is approved in more than 80 countries.

"This treatment has the potential to offer patients a new dosing schedule, which may result in improved care for many people with schizophrenia," said Dr Andreas Schreiner, European Therapeutic Area Leader, Neuroscience and Pain, Janssen.

The European filing is based on two Phase 3 studies. The first, which was the basis for the US Food and Drug Administration (FDA) submission, is a randomised, multi-centre, double-blind, placebo-controlled relapse prevention study in more than 500 patients with schizophrenia. The second is a randomised, double-blind non-inferiority clinical trial of paliperidone palmitate in once-every-three-month and once-monthly formulations. The results will be presented at a scientific congress later this year.

PFIZER

EMA validates MAA for Pfizer's Ibrance

The European Medicines Agency (EMA) has validated for review Pfizer's marketing authorisation application for Ibrance® (palbociclib) in combination with endocrine therapy for the treatment of hormone receptor-positive, human epidermal growth factor receptor 2-negative (HR+/HER2-) advanced or metastatic breast cancer. With this validation, the EMA will now begin the review procedure.

Ibrance is an oral, first-in-class inhibitor of cyclin-dependent kinases (CDKs) 4 and 6. CDKs 4 and 6 are key regulators of the cell cycle that trigger cellular progression.

The submission is based on the final results of the PALOMA-1 and PALOMA-3 trials in metastatic breast cancer. Both trials demonstrated that Ibrance® in combination with an endocrine therapy improved progression-free survival (PFS) compared to endocrine therapy alone. Ibrance was approved by the US FDA in February for use in combination with letrozole as a treatment for postmenopausal women with ER+/HER2- advanced breast cancer as initial endocrine-based therapy for their metastatic disease.

DIABETES UK

Diabetes cases rocket in the UK

The number of people living with diabetes in the UK has soared by nearly 60% in a decade, according to a new analysis by Diabetes UK. The new figures show that there are now 3,333,069 people diagnosed with diabetes, which is an increase of more than 1.2 million adults compared with 10 years ago.

Diabetes UK is warning that this exponential growth in numbers reflects an urgent need for the UK government to take action to ensure effective care for people living with diabetes, as well as highlighting the importance of prevention and that failure to act on this threatens to bring down the NHS. Diabetes currently costs the NHS nearly £10 billion a year, and 80% of this is spent on managing avoidable complications, Diabetes UK points out.

At present only six in ten people with diabetes in England and Wales receive the eight care processes recommended by the National Institute for Health and Care Excellence. These are the checks identified as essential in high quality care for people with diabetes and include getting blood pressure and blood glucose levels measured, as well as the kidney function monitored. Otherwise, poorly managed diabetes can lead to devastating and expensive health complications such as kidney disease, stroke and amputation.

BOEHRINGER INGELHEIM

Jardiance demonstrates CV risk reduction in type 2 diabetes

Boehringer Ingelheim and Lilly have announced positive top-line results from EMPA-REG OUTCOME®, a long-term clinical trial investigating cardiovascular (CV) outcomes for Jardiance® (empagliflozin) in more than 7,000 adults with type 2 diabetes at high risk for CV events. The study met its primary endpoint and demonstrated superiority of Jardiance®, when added to standard of care, in CV risk reduction. The primary endpoint was defined as time to first occurrence of either CV death, or non-fatal myocardial infarction or non-fatal stroke.

Jardiance® is the only glucose-lowering agent to have demonstrated CV risk reduction in a dedicated cardiovascular outcomes trial. The safety profile of Jardiance was consistent with previous studies.

NOVARTIS

EC gives go-ahead to non-invasive basel cell carcinoma drug Odomzo

The European Commission has approved Novartis' Odomzo® (sonidegib, formerly LDE225) 200mg capsules for the treatment of adult patients with locally advanced basal cell carcinoma who are not amenable to curative surgery or radiation therapy.

Odomzo was recently approved in the US and is also approved in Australia and Switzerland. Additional regulatory submissions are being reviewed by health authorities worldwide.

Basal cell carcinoma (BCC) consists of abnormal, uncontrolled growths or lesions that arise in the skin's basal cells, which line the deepest layer of the epidermis (the outermost layer of the skin) and accounts for more than 80% of non-melanoma skin cancers. Advanced BCC is thought to represent roughly 1–10% of all cases of BCC and there are few treatment options at this stage of the disease.

The EU approval was based on data from the Phase II randomised, double-blind, multi-centre BOLT (Basal cell carcinoma Outcomes in LDE225 Trial) study in patients with laBCC not amenable to local therapy or metastatic basal cell carcinoma (mBCC). In patients with laBCC treated with Odomzo 200mg, the objective response rate (ORR) was 56% per central review and 71% per investigator review. The median duration of response per central review has not been reached. The median progression-free survival was 22 months per central review and 19 months per investigator review.

CMA

CMA accuses Pfizer and Flynn Pharma of overcharging

The UK Competition and Markets Authority (CMA) recently issued a statement of objections to the pharmaceutical suppliers Pfizer and Flynn Pharma alleging that they have breached competition law by charging excessive and unfair prices in the UK for phenytoin sodium capsules, an anti-epilepsy drug.

Phenytoin sodium capsules are used in the treatment of epilepsy in order to prevent and control seizures and are an important drug for over 50,000 patients in the UK. Pfizer manufactures phenytoin sodium capsules and supplies them to Flynn Pharma, which then distributes them to UK wholesalers and pharmacies. The statement of objections concerns both the prices that Pfizer has charged to Flynn Pharma and the prices that Flynn Pharma has charged to its customers, since September 2012.

Prior to September 2012, Pfizer manufactured and sold phenytoin sodium capsules to UK wholesalers and pharmacies under the brand name Epanutin®. Pfizer sold the UK distribution rights for Epanutin to Flynn Pharma, which de-branded the drug and started selling its version in September 2012. Pfizer continued to manufacture the drug, which it sold to Flynn at prices that were significantly higher than those at which it had previously sold Epanutin® in the UK – between eight and 17 times Pfizer's historic prices. Flynn then sold the drug on to customers at prices which were between 25 and 27 times higher than those historically charged by Pfizer.

Prior to September 2012, the NHS spent approximately £2.3 million on phenytoin sodium capsules annually. This spend (paid to Flynn and other suppliers of phenytoin sodium capsules) was just over £50 million in 2013 and over £40 million in 2014. Ann Pope, CMA Senior Director of Antitrust Enforcement, said: "While businesses are generally free to set prices as they see fit, those that hold a dominant position have a special responsibility to ensure that their conduct does not impair genuine competition and that their prices are not excessive and unfair."

NICE

NICE tackles overuse of antibiotics

UK health watchdog, the National Institute for Health and Care Excellence, has published guidance to help doctors, nurses and pharmacists promote and monitor the sensible use of antimicrobials.

Antibiotics have been the mainstay of treating infections for over 60 years. Although a new infectious disease has been discovered nearly every year over the past 30 years, very few new antibiotics have been developed. This means existing antibiotics are used to treat an ever greater variety of infections and infectious diseases.

Overall, antibiotic prescribing in England has been steadily increasing over several years. Nationally, 41.6 million antibiotic prescriptions were issued in 2013-14 at a cost to the NHS of £192 million. Despite considerable guidance that prescribing rates of antibiotics should be reduced, nine out of ten GPs say they feel pressured to prescribe antibiotics, and 97% of patients who ask for antibiotics are prescribed them.

VERNALIS

Failed pain drug endpoint prompts Vernalis to stem cash flow

Vernalis has announced disappointing results from its Phase II proof-of-concept study of neuropathic pain drug V158866, and will no longer invest in its development.

V158866 is a fatty acid amide hydrolase inhibitor, which is being investigated for spinal cord injury-induced pain. In the randomised, double-blind, placebo-controlled, two-period cross-over study, although dosing of V158866 resulted in elevated endocannabinoid levels, on an intent-to-treat basis the study failed to meet its pain reduction primary endpoint. Treatment with V158866 did, however, show a trend towards efficacy on a per protocol basis and was generally well tolerated.

Consistent with its strategy of becoming a commercial business, Vernalis said it is not planning to make any further investment in the programme, seeking to realise its potential value through partnering instead.

Ian Garland, CEO of Vernalis commented, "The goal of this study was to identify a therapeutic setting for this programme. Its completion ends the investment in our NCE pipeline and we aim to partner the remaining unpartnered programmes to realise value where possible. The key focus of the organisation remains the transition of Vernalis to a commercial company, with the forthcoming launch of Tuzistra™ XR, our extended release cough cold product for the US prescription market, which is progressing as planned."

NOVARTIS

Novartis pays $1 billion to bolster its pipeline with GSK's ofatumumab

Novartis is to acquire all remaining rights to experimental multiple sclerosis drug ofatumumab from GSK for up to $1 billion plus royalties.

Ofatumumab, a fully human monoclonal antibody which targets CD20, is being developed for relapsing remitting multiple sclerosis (RRMS) and other autoimmune indications. Novartis previously acquired the rights to ofatumumab for oncology indications, marketing it under the brand name Arzerra®.

Gilenya is Novartis's other MS treatment, which reached sales of $2.5 billion in 2014. However, it is due to lose patent protection in the next few years, leading analysts to believe that Novartis is hoping that ofatumumab will help alleviate the loss in revenues it incurs when generic forms of Gilenya® become available.

RRMS is thought to be associated with activation of the immune system's B cells. Ofatumumab works by binding to the CD20 molecule on the surface of B cells and depleting them in lymphatic tissues. Positive Phase IIa results for subcutaneous ofatumumab demonstrated a significant reduction of up to 90% in the cumulative number of new brain lesions in patients with MS between weeks 4-12 in the study. No unexpected safety findings were reported.

VALEANT

Valeant jumps on Sprout following approval of Addyi

Valeant Pharmaceuticals has entered into a definitive agreement to acquire Sprout for approximately $1 billion in cash, plus a share of future profits based upon the achievement of certain milestones.

This comes closely after the US Food and Drug Administration approval of Sprout's new drug application for flibanserin, which will be marketed as Addyi in the US. Addyi has demonstrated improvements in desire for sex, reducing distress from the loss of sexual desire and increasing the number of satisfying sexual events. Sprout also has global rights for flibanserin. Valeant will leverage its global scale to register flibanserin internationally.

Valeant expects Addyi to be available in the US in the fourth quarter of 2015. Following the closing of the acquisition, Sprout will remain headquartered in Raleigh, N.C. and become a division of Valeant. Cindy Whitehead, Chief Executive Officer of Sprout, will join Valeant to lead this division dedicated to the introduction and global commercialisation of Addyi, reporting to Anne Whitaker, Executive Vice President and Company Group Chairman.

GLOBALDATA

Brazil's pharmaceutical market value will approach $48 Billion by 2020, says GlobalData

The Brazilian pharmaceutical market will expand in value from $29.4 billion in 2014 to reach approximately $47.9 billion by 2020, representing a strong Compound Annual Growth Rate of 8.5%, according to research and consulting firm GlobalData.

The company's latest report, CountryFocus: Healthcare, Regulatory and Reimbursement Landscape – Brazil, states that Brazil's increasingly elderly population, which will lead to a rising incidence of chronic and lifestyle-associated diseases, as well as the country's robust investment in healthcare, will be key drivers of market growth during the forecast period.

Joshua Owide, GlobalData's Director of Healthcare Industry Dynamics, says that the Brazilian pharmaceutical industry continues to prosper, primarily thanks to the country's economic policies and reforms.

Owide commented, "Brazil has emerged as a global manufacturing hub for pharmaceutical and biotechnology companies, with countries such as India investing heavily in the manufacturing sector after former Brazilian health minister, José Serra, invited investment from generic companies. As a consequence, Brazil is now one of the most attractive and promising pharmaceutical markets in the world. Indeed, its pharmaceutical market value has increased considerably over the past six years, having more than doubled from $14.1 billion in 2008."

FORBES

Pharma companies rank high in Forbes' most innovative list

Alexion Pharmaceuticals, Regeneron Pharmaceuticals, Incyte and BioMarin Pharmaceutical have all been ranked in the top 10 on Forbes magazine's 2015 list of the 'World's Most Innovative Companies'. The companies ranked third, fourth, seventh and tenth, respectively.

Forbes list identifies companies expected to be innovative now and in the future.

Rankings are based on what Forbes calls the companies' "innovation premium," the difference between their market capitalisation and a net present value of cash flows from current businesses. Forbes' method relies on investors' ability to identify companies they expect to be innovative now and in the future. The recognition only includes businesses that have at least seven years of financial data and a market value greater than $10 billion, and have measurable investment in research and development.

This is the second consecutive year that BioMarin has received the recognition. The company was previously ranked seventh.

APRECIA

FDA approves first ever 3D printed drug product: Spritam

The US Food and Drug Administration (FDA) has approved the first ever three dimensional (3D) printed drug product: epilepsy treatment Spritam (levetiracetam).

Spritam is approved for oral use as a prescription adjunctive therapy for the treatment of partial onset seizures, myoclonic seizures and primary generalised tonic-clonic seizures in adults and children.

It utilises Aprecia's proprietary ZipDose® Technology platform, a ground breaking advance that uses 3D printing to produce a porous formulation that rapidly disintegrates with a sip of liquid. While 3D printing has been used previously to manufacture medical devices, this approval marks the first time a drug product manufactured with this technology has been approved by the FDA.

ZipDose Technology enables the delivery of a high drug load, up to 1,000mg in a single dose. As a result, Spritam enhances the patient experience: administration of even the largest strengths of levetiracetam with just a sip of liquid. In addition, with Spritam there is no measuring required as each dose is individually packaged, making it easy to carry this treatment on the go. Spritam is expected to be available in the first quarter of 2016.

SEPTEMBER 2015

Basel Life Science Week – MipTec 2015
Date: 21 – 24 September
Location: Basel, Switzerland
e: registration.blsw-miptec@congrex-switzerland.com
w: http://www.basel-life-science-week.eu

4th World Conference on Physico Chemical Methods In Drug Discovery and Development
Date: 21 – 24 September
Location: Red Island, Croatia
e: office@iapchem.org
w: http: //www.iapchem.org/page.php?page_id=56

2nd Annual Cell Culture & Bioprocessing Forum
Date: 23 – 25 September
Location: Berlin, Germany
e: andrea.weber@flemingurope.com
w: http://pharma/flemingeirope.com

HUPO 2015
Date: 26 – 30 September
Location: Vancouver, Canada
e: office@hupo.org
w: www.hup.org/events

FIP 2015
Date: 29 September – 3 October
Location: Dusseldorf, Germany
e: congress@fip.org
w: http://www.fip.org/dusseldorf2015/

3rd Annual Clinical Trials Supply Chain
Date: 29 – 30 September
Location: Berlin, Germany
e: andrea.weber@flemingeurope.com
w: http://pharma.flemingeurope.com/clinical-trials-supply-chain-conference/request-agenda?utm_source=listing_europeanpharmaceuticalreview&utm_medium=listing&utm_campaign=BALS170_agenda

Cell & Gene Therapy Europe
Date: 29 – 30 September
Location: Barcelona, Spain
e: carina@phacilitate.co.uk
w: www.cgteurope.com

OCTOBER 2015

7th Global Summit on Cancer Therapy
Date: 5 – 7 October
Location: Dubai, UAE
e: cancermiddleeast@conferenceseries.net
w: http://cancer.global-summit.com/middleeast/

Biotechnica 2015
Date: 6 – 8 October
Location: Hannover, Germany
e: info@messe.de
w: www.biotechnica.de/home

What's new in the approval and conduct of clinical trials in Europe?
Date: 8 October
Location: Royal Society of Chemistry, London
e: events@jpag.org
w: http://www.jpag.org/?p=meetiungs&r=66

5th Annual Next Generation Sequencing Asia Congress
Date: 13 – 14 October
Location: Singapore
e: s.punfield@oxfordglobal.co.uk
w: www.ngsasia-congress.com

Stem Cell Asia
Date: 15 – 16 October
Location: Singapore
e: g.alonso@oxfordglobal.co.uk
w: www.stemcellasia-congress.com


Basel, Switzerland, location of MipTec 2015
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3rd Annual International Conference on Neuroscience and Neurobiology Research (CNN 2015)
Date: 26 – 27 October
Location: Bangkok, Thailand
e: secretariat@neuro-conf.org
w: http://neuro-conf.org/index.html

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Date: 26 – 28 October
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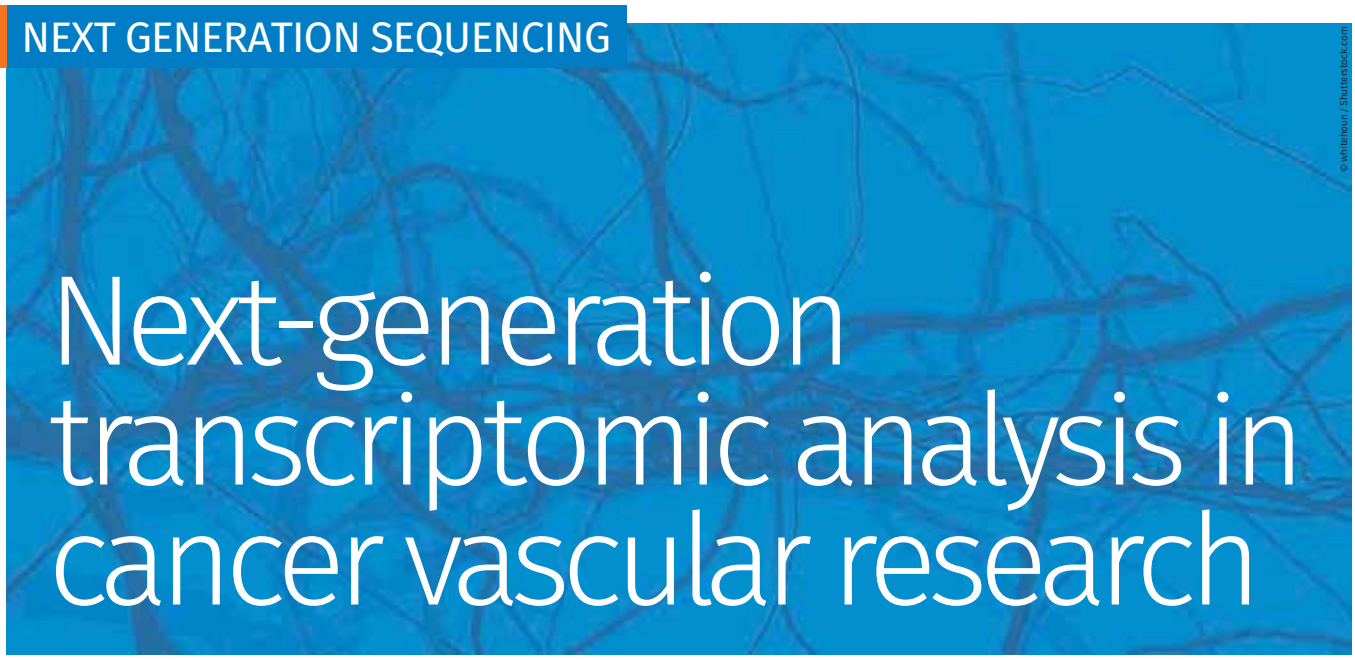

Next-generation transcriptomic analysis in cancer vascular research

Joseph W. Wragg and Roy Bicknell
University of Birmingham

Over the past decade significant advances have been made in the fields of genomic and transcriptomic profiling, inspired by the advent of next-generation sequencing (NGS). Yet despite the considerable promise of these new technologies, uptake has been slow. The focus of this review is the use of next-generation transcriptomic analysis in the field of cancer endothelial biology, highlighting its advantages and a few of the disadvantages compared with current-generation technologies.

NGS is an emerging technology for genomic, epigenomic and transcriptomic profiling, which can be performed more rapidly than traditional Sanger sequencing and in far greater depth than microarray technologies[1]. NGS technologies have been used in a wide variety of fields from sequencing of bacterial and viral genomes[2,3], searching for patient-specific genetic variations[4] and profiling of DNA-binding proteins by ChIP-Seq[5], to characterising the transcriptomes of cells, tissues and organisms by RNA-Seq[6]. NGS covers a wide variety of technologies but primarily operates by sequencing DNA or RNA samples in a massively multiplexed manner, generating images, from which short sequence reads can be determined, aligned to a reference genome and converted to genomic data[7].

Tumours are highly dependant on their vasculature to support their growth, being unable to expand beyond 2mm in size or metastasise in the absence of vessels to supply nutrients, remove waste products and propagate tumour cells around the body[8,9]. Considerable effort has been expended to investigate the mechanisms by which tumour vasculature develops, as well as examining its distinctiveness from the vessels of healthy tissues, with the aim of depriving the tumour of its blood supply. The vast majority of transcriptomic analysis involved in this work has been performed using microarray technologies, though SAGE and cDNA library analysis have also been used (reviewed in[10]). The advent of high-throughput next-generation analysis offers the opportunity for new discoveries to be made in this field, discoveries which are not possible with older technologies, and this will contribute to refinement and improvement of vessel-targeted anti-cancer therapies.

RNA-Seq to study antiangiogenic therapies

Cancer vessel-targeted therapies broadly divide into two categories: antiangiogenic therapies, which aim to block the processes by which cancers vascularise[11] and vascular disrupting agents, which aim to destroy existing vessels in the tumour[12]. Antiangiogenics are the better-established therapeutic intervention, contributing several therapies to the clinic, including Sutent® (sunitinib), Nexavar® (sorafenib), cediranib (in development by AstraZeneca) and Avastin® (bevacizumab), which have contributed considerably to survival in several cancer types (reviewed in[10]).

However, the efficacy of this approach has been curtailed by difficulties of acquired and innate resistance (reviewed in[10,13]). Transcriptomic analysis techniques have been utilised in the search for the molecular mechanisms behind resistance and for markers of response to these therapies, to better tailor the use of these drugs to the most profitable indication (reviewed in [14]).

The majority of this work has been performed using present-generation technologies. Next-generation technologies do offer some advantages, however. For example, Illumina RNA-Seq NGS was used to genetically profile the tumour from a patient exhibiting an unusually positive response to sunitinib in a clinical trial of testicular cancer. This study identified the amplification of several candidate markers of response to sunitinib including *RET, EGFR* and *KRAS*[15]. The study was part of a wider program at the MD Anderson Cancer Center in Texas, profiling abnormal responders to therapeutics using next-generation genomic technologies with the aim of identifying novel markers of drug response or resistance[16].

RNA-Seq differs from traditional transcriptomic analyses such as probe-based microarray systems in that rather than providing a relative expression level of a candidate gene via comparative fluorescence signal analysis, NGS provides an absolute expression level. This is

undertaken by sequencing the prepared genetic material from the sample and determining the number of copies of transcripts corresponding to candidate genes. In the example given, the use of NGS technologies allowed the identification of genes that were not just differentially expressed but also present in sufficient quantities to be clinically relevant for further investigation[15]. Thus RNA-Seq has overcome one of the weaknesses of microarray-based analysis, which identify a huge number of differentially expressed genes, most of which are present in such minuscule quantities that they are irrelevant for further investigation.

RNA-Seq based analyses offer considerable power in investigating tumour and stromal responses to anti-angiogenic therapies with human tumour xenograph models, improving understanding of the effect these therapies have on the tumour environment. Widely used array-based systems profile the transcriptome expression through the use of probes complementary to transcripts from any known gene in the genome. Thus being a semi-targeted approach it suffers from biased transcript coverage[17], but also is often unable to differentiate between transcripts derived from homologous genes in different species. Bradford *et al.* (2013)[17] demonstrated how RNA-Seq can be used to dissect transcripts derived from the host (mouse) tissue and the tumour (human) tissue through a species-specific mapping approach, which allowed them to independently investigate tumour and stromal responses to AstraZeneca's cediranib (Figure 1).

Vascular-targeted therapies

Vascular disrupting therapies target differences in the vasculature supplying the tumour and healthy tissues. These are either structural

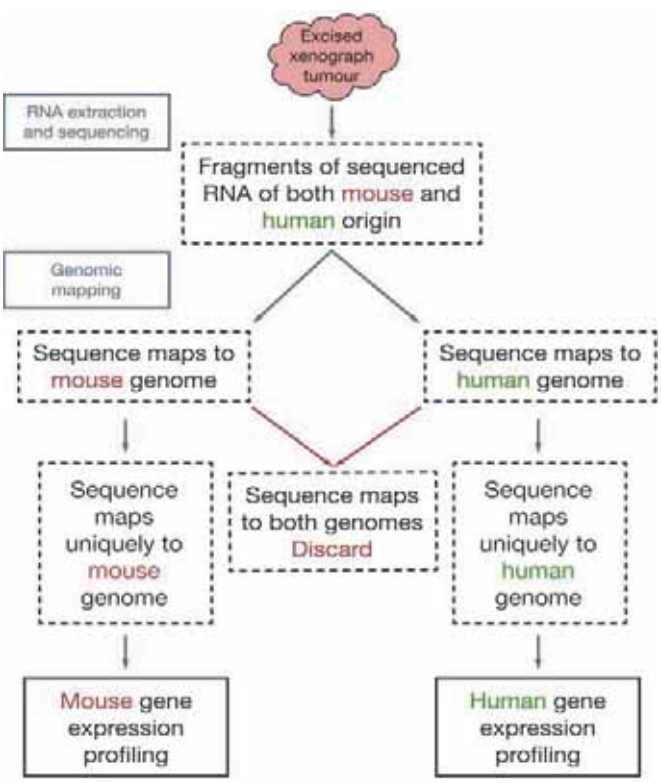

Figure 1: Dissection of human tumour and mouse stromal responses in a xenograph model by RNA-Seq. The human Calu-6 non-small cell lung cancer cell line was grown in mouse. The tumour was excised, the RNA extracted and subjected to RNA-Seq. The sequence read fragments were aligned to a human and mouse reference genome and reads that mapped uniquely to either genome were used for gene expression profiling of both the mouse- and human-derived tumour compartments

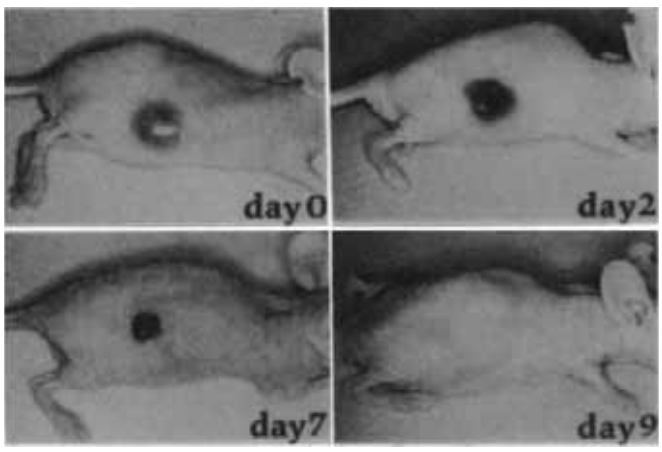

Figure 2: Vascular-targeted therapies induce tumour necrosis. Gross appearance of a subcutaneous neuroblastoma tumour treated with a MHC class II targeted immunotoxin. At day 0 the tumours appear highly vascular. Two days after treatment, the tumour is blackened (indicating massive intratumoural haemorrhage). At day 7 the tumour has collapsed into a scab-like plug and by day 10 there is no visible living tumour (figure adapted from[19])

differences, such as combretastatin-based therapies, which destabilise the vessels[18], or molecular changes that have occurred in the endothelial cells lining the vessels, such as vascular-targeted therapies (VTAs), which aim to specifically kill these cells in a targeted manner[12]. For VTAs to be effective, targets must be found that are uniquely over expressed on tumour vessels, so that therapeutics can be engineered to target these molecules, localise to the tumour and deliver a toxic payload, killing the vessels. Burrows and Thorpe 1993[19] were the first to demonstrate the power of this approach. They engineered a neuroblastoma tumour to express interferon gamma and this induced the expression of MHC-class II on the surface of the vessels supplying the tumour. They treated the tumour with the intravenous inoculation of an antibody targeted against MHC class II and conjugated to ricin. This resulted in rapid haemorrhagic necrosis within the tumour, which became completely necrotic over a period of nine days[19] (Figure 2).

The search for specific tumour vascular targets is an active area of investigation with many groups using transcriptomic analysis to probe for molecular differences between the vessels supplying the tumour and healthy tissues[20-23] (reviewed in[10]). Again, the majority of this work has been performed using current-generation technologies, however NGS is starting to be used to provide a more in-depth analysis of the tumour vessel transcriptome.

Zhuang *et al.* (2013)[24] used a combined approach of both microarray and RNA-Seq NGS transcriptomic profiling of isolated endothelial cells from lung cancer. It has been reported that there is only moderate concordance between differentially expressed genes identified by RNA-Seq and by microarray, probably due to the considerable inherent differences between the approaches[1]. Both approaches suffer from high false positive rates[25,26]. By using the current- and next-generation technologies concurrently, the selection process for candidate genes in lung cancer was streamlined, since the true differences between the healthy and cancer vessels should be identified by both approaches[24].

One approach used to identify novel markers of tumour endothelium is to model the environment to which the tumour vessels are exposed. The tumour often grows at a rate exceeding the capacity of the vessel bed to expand and supply it sufficiently[27]. Because of this, the tumour microenvironment is generally highly hypoxic,

hypoglycaemic and acidic with poor blood flow. The vessels are additionally often disordered and poorly structured, leading to breakages within the vessel wall and opening a thrombogenic surface on which clots can form[28]. This environment has a considerable impact on the expression profile of tumour vessels, creating distinct changes that can be targeted therapeutically.

Zhang *et al.* (2012)[29] modelled the impact that exposure to thrombin has on gene expression in pulmonary microvessels, in cancer and other diseases by RNA-Seq transcriptomic profiling. This analysis identified 150 known genes and 480 known isoforms which were upregulated and 2,190 known genes and 3,574 known isoforms downregulated by exposure to thrombin by at least two-fold. Of note however, is that 1.775 previously unknown isoforms were found to be upregulated and 12.202 downregulated[29], highlighting another considerable advantage provided by the next-generation approach. As discussed, microarray-based systems rely on a finite number of predesigned probes to assess the transcript content of a sample, meaning that it is only ever possible to compare the expression of those genes and isoforms for which probes have been generated. On the other hand, by sequencing the entire sample and providing a readout for each transcript encountered, RNA-Seq provides virtually unlimited genomic analysis capacity, allowing the identification of previously unknown genes, isoforms and splice variants[1]. NGS therefore offers a huge potential for the identification of previously unknown candidates for tumour vascular-specific targeting.

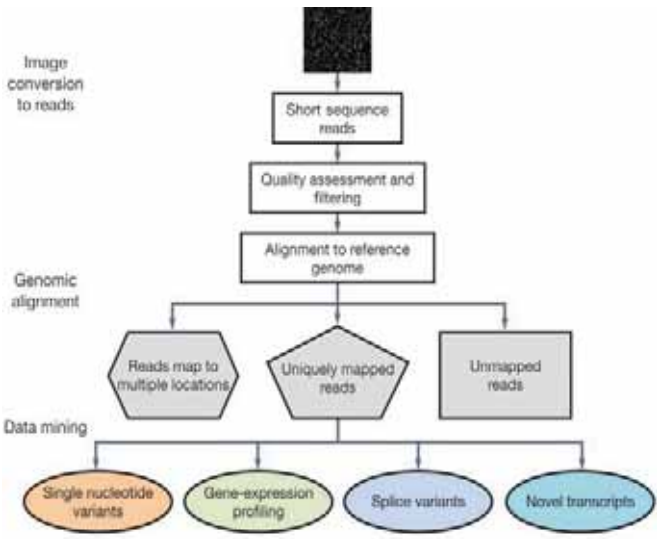

Figure 3: A typical workflow for the analysis of RNA-Seq data. Consists of the conversion of raw data images to short reads, the quality assessment of the reads, alignment to a reference genome (if available) and production of useful data from uniquely mapped reads

Complexity of analysis

The generation of such in-depth genomic data is not without its disadvantages. Gigabytes of data are produced for each sample analysed, making the handling of large data sets from multiple samples a considerable and costly challenge. Additionally, bioinformatic tools to assure sequence quality, conduct sequence alignment and assembly and biologically interpret the data are in their infancy, and require considerable development in order to allow NGS to fully achieve its potential for genomic and transcriptomic discovery. The use of these tools to analyse NGS data is costly due to its requirement for considerable investment in infrastructure, but also due to its need

for specialist bioinformaticians. The analysis process is also labour- and time-intensive. Firstly, the images from the NGS sequencers must be analysed and converted into sequence reads. The reads are then quality assessed and aligned to a reference genome, if one exists. Finally, mapped and unmapped reads are assessed through a process of gene expression profiling[1] (Figure 3; page 12). This process of data analysis is considerably more complex and costly than for array-based systems[30].

Therefore, despite the rapidly falling costs of performing NGS, the difficulties of analysing the data make it an unattractive option for all but the best-funded research groups, compared with cheap and easily analysed array-based systems. This issue must be addressed with institutional investment in infrastructure, improvement in the quality and usability of analysis software and education in how to use it. The most intelligently designed experiments and the best analysis is achieved when researchers themselves can perform it rather than relying on specialist bioinformaticians unfamiliar with the research area for which NGS is being used. Until the usability of NGS analysis software is improved to the level at which microarray analysis operates, the utility of NGS to enable scientific discovery will be curtailed.

Conclusion

In conclusion, NGS is an extremely promising approach to enable scientific discovery in the field of tumour vascular biology and in the future could transform our ability to both research and treat cancer. The MD Anderson's genetic profiling of abnormal responders is an early example of the potential of NGS to permit personalised medical care. With time, databases for disease-specific mutations and alterations will become more comprehensive and better characterised, allowing NGS of patient material to become a viable option for clinical as well as scientific evaluation, superseding traditional diagnostic methods, which are limited by their narrow focus and usually only useful for one intended purpose.

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Joseph W. Wragg carried out his undergraduate studies at The University of York and is currently studying for his PhD at the University of Birmingham. He has published three peer-reviewed articles in the fields of cancer microenvironment and cancer vascular targeting. He has a particular interest in the use of high-throughput gene expression profiling techniques to identify specific genes for cancer targeting.

Roy Bicknell carried out his undergraduate and doctoral studies at Oxford University and his postdoctoral training at Harvard Medical School. He was formerly Professor of Cancer Cell Biology at Oxford and is currently Professor of Cancer Biology and Genomics at the University of Birmingham. He has published over 200 peer reviewed articles in the fields of endothelium and angiogenesis and a particular interest is the difference between tumours and healthy tissue endothelium.

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BIO-Europe® 2015 is regarded by the international life science industry as its most productive business development event. The main draw for executive-level decision makers from pharma, biotech, finance and service partners is what is known in the industry as 'partnering.' These are one-to-one, private business development and discovery meetings that are the backbone of the event, and which have led to some of the biggest merger and acquisition and collaboration deals over the past two decades.

Now in its 21st edition, BIO-Europe is a key strategy for global biotech, pharma and investors who attend in search of new collaboration partners and deal opportunities. Munich is a favourite central European location and a hotbed of drug development activity. The event is expected to draw a high caliber of companies, not only from Munich and Bavaria, but from across the globe to fuel the insatiable drug development industry.

BIO-Europe is also highly regarded for the variety and high caliber of presenting companies, ranging from academic innovators and next-generation companies to mid-size pharma and biotech, who bring their innovative technologies, therapies and solutions to the event with the goal of securing development and commercialisation partners. If the partnering can be considered the engine, the presenting companies are the fuel that drives the event. BIO-Europe also features a diverse list of industry leaders speaking on panels and workshops.

Leading pharmaceutical companies sponsoring the event send teams of scouts to BIO-Europe to engage with new and innovative products, ideas and companies. Past sponsors include big names in pharma such as AbbVie, Amgen, AstraZeneca, Bayer HealthCare, Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Johnson & Johnson, Lilly, Merck, Merck Serono, Novartis, Novo Nordisk, Pfizer, Roche, Sanofi, Takeda and many others.

"There is tangible excitement around the arrival of BIO-Europe every year," said Anna Chrisman, Group Managing Director of EBD Group. "And Munich is an ideal central European location that is expected to draw a compelling list of dealmakers from biotech, pharma and finance. Partnering continues to be the engine that drives the industry's growing demand for transformational breakthroughs."

The perfect complement to the productive one-to-one partnering meetings are the programme sessions at BIO-Europe. Panels and workshops led by CEO-level speakers are designed to facilitate discussions ranging from disease research, financing models, and external innovation models to trending research areas and commercialisation advice from drug development partners. Evening events are ideal for networking and take place in historic and exclusive venues.

The 2014 event boasted an all-time record of 17,902 scheduled one-to-one meetings with 4,032 licensing opportunities posted from among 1,772 companies that attended.

Event Profile

BIO-Europe is the preeminent partnering conference for the life sciences, bringing together international decision makers from the biotechnology, pharmaceutical and financial sectors, offering networking opportunities, workshop and panel participation, a high profile exhibition, and private, prescheduled one-to-one meetings.

Delegates from all parts of the biotechnology value chain come to BIO-Europe to quickly identify, engage and enter into strategic relationships that drive their businesses successfully forward. Investment and collaboration opportunities developed in prior BIO-Europe conferences have produced many highly successful business partnerships.

The BIO-Europe 2015 partnering event is expected to draw over 3,200 industry attendees for three days of high level networking, representing upwards of 1,850 companies from over 50 countries.

Partnering as an overall business strategy has transformed drug development in bringing together innovators and visionaries with seasoned experts from every corner of the globe. Partnering at BIO-Europe is powered by EBD Group's partneringONE®, the industry's most advanced networking system, which enables participants to efficiently mine a large pool of potential partners and pre-arrange private one-to-one meetings with dozens of company targets. The system – industry-specific, web-based and interactive – is uniquely suited for the needs of life science companies.

In addition to the program, two key elements at our partnering events are the presenting companies and the exhibition. At BIO-Europe, presenting companies are grouped into several tracks, including Biotech, Midsize Pharma, Next Generation, and Academic Innovators™. The high-exposure Exhibition is the heart of the conference, and presents a prime opportunity to showcase products and services in a high-traffic location.

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Informatics

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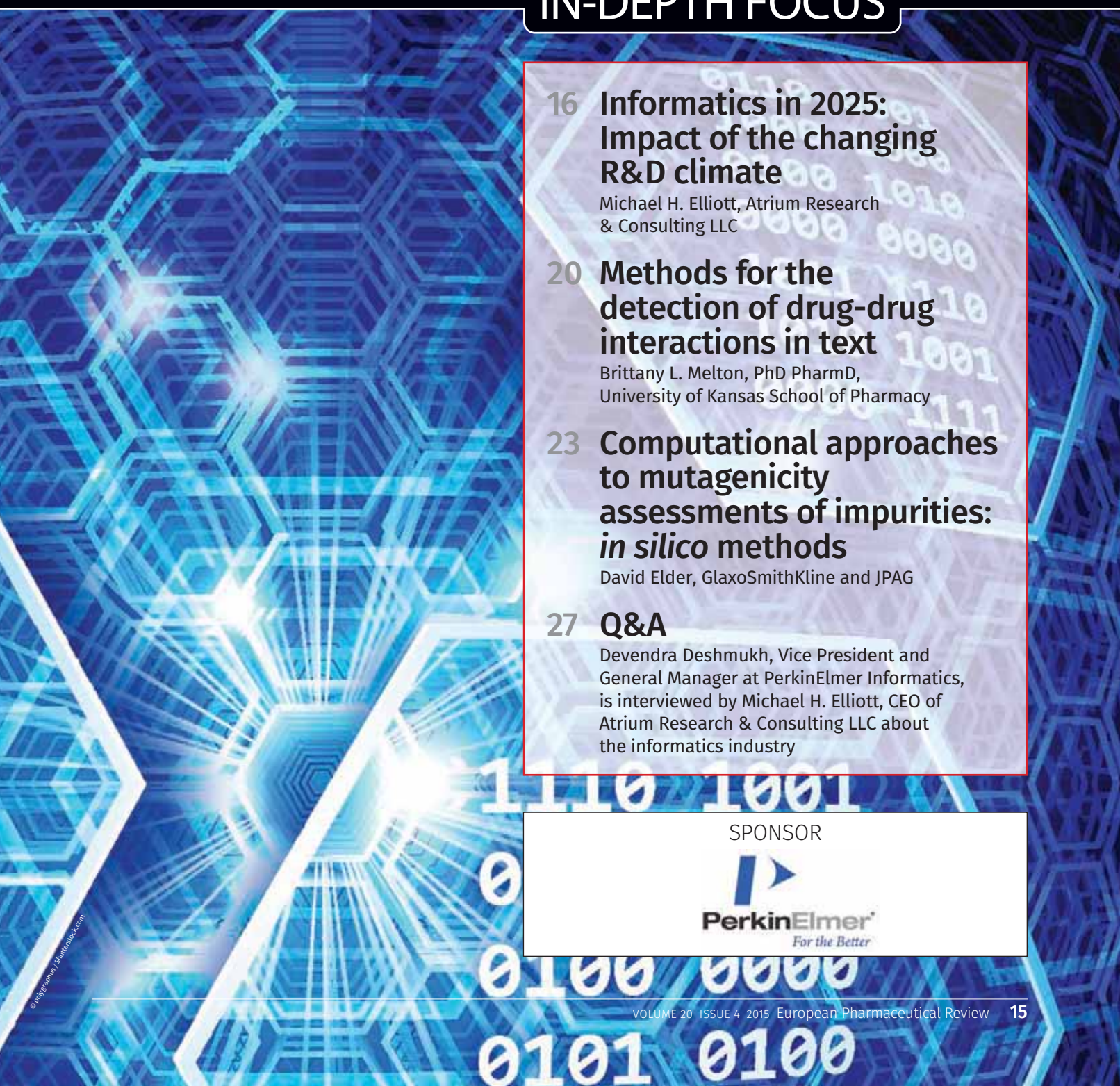

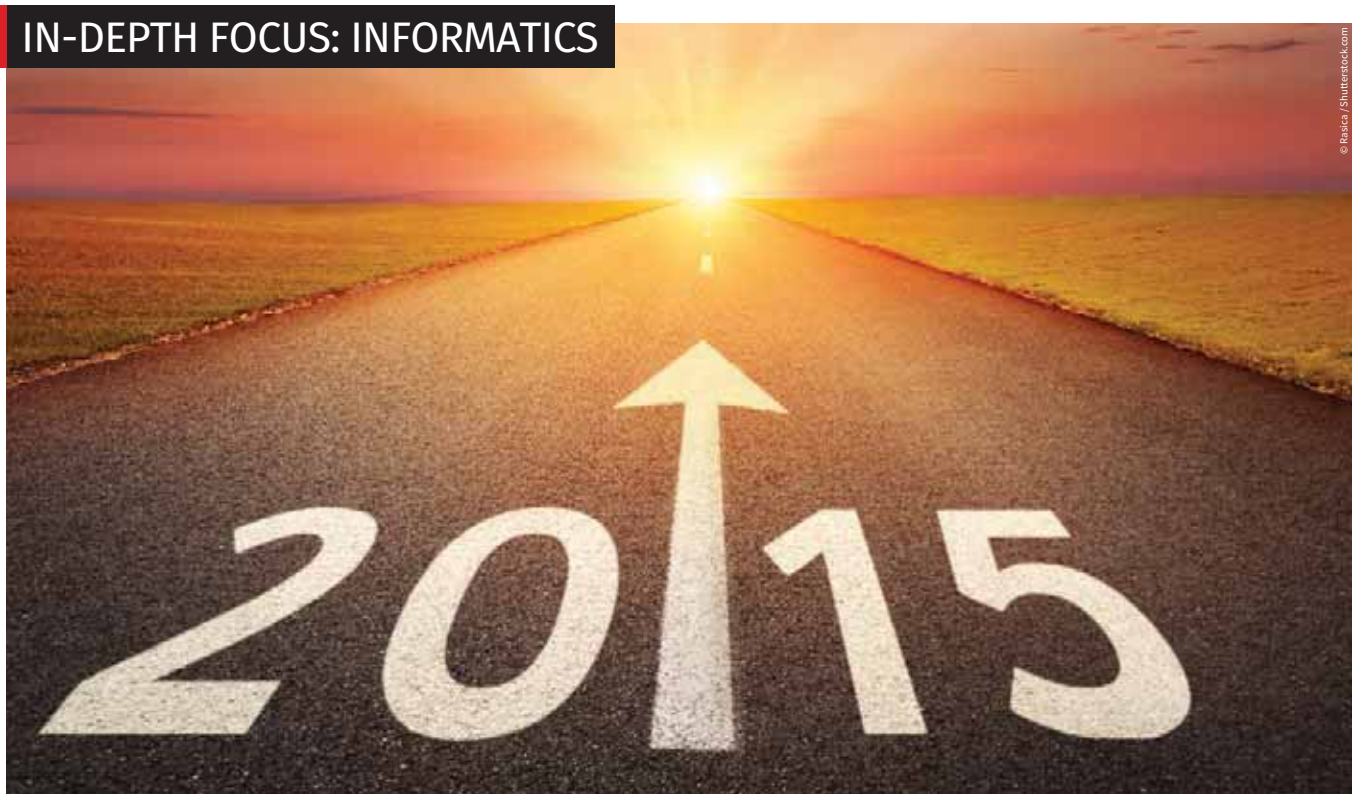

Informatics in 2025: Impact of the changing R&D climate

Michael H. Elliott
Atrium Research & Consulting LLC

As a market research and strategic consulting firm, Atrium Research is often asked to project future trends in research and development (R&D) within the field of informatics. This is not an easy task; there are breathtakingly rapid changes in technology and the pharmaceutical industry is undergoing considerable transformation. Peter Drucker said it best: "Trying to predict the future is like trying to drive down a country road at night with no lights while looking out the back window." An analysis of historical trends does, however, provide a streetlight for envisioning the technology necessary to buttress future state R&D operating models.

A look out the back window

Since 2005, biotechnology and pharmaceutical companies have spent over $20 billion on informatics to support their R&D efforts. There are well over a hundred suppliers developing a range of solutions from chemical/bioinformatical and electronic laboratory notebook to data analytics. Reading vendor marketing material would lead one to believe that investing in these systems is absolutely necessary for an efficient and innovative R&D environment.

But is this true? Has this spending enabled 'better science' or 'accelerated R&D' as is claimed? When examining the data, the answer is probably not. The hard reality is that there is only a 10% chance a clinical drug candidate will make it to market for all indications; lower than the 20% in 2005[1]. Half of all tested compounds fail due to issues with efficacy and 30% to safety. Where was informatics when researchers sought to better understand targets, pathways and biomarkers? Why did informatics not create more predictive *in vivo* models?

If informatics has not supported improved outcomes, then surely it has played a role in enhancing operational effectiveness. Suppliers say there have been big gains in terms of efficiency, reduced cycle times and improved time to market. However, time to market has not actually improved; development time is around 10 years today, which has remained unchanged over the past decade. In terms of measuring on total cost, there is a steady decline in the number of drugs per billion

dollars of R&D spending[2]. And of drugs that make it through clinical trials and are approved, only two in 10 recoup their R&D investment[3].

Scientists today are armed with technical capabilities they never had back in 2005, so it would be easy to assume that their work lives have improved. However, this does not seem to be the case. A survey we conducted in 2005 indicated that their number one data challenge is 'finding data when I need it'. Fast forward to a study we performed in 2014 and the answer was the same. 80% of R&D data are unstructured data files (reports, spreadsheets, presentations, images, etc.), yet these are the most poorly managed data assets. The ad hoc file shares, paper and eRooms of 2005 have been replaced with 2015's ad hoc file shares and poorly managed and ineffectual SharePoint sites.

Has informatics been treating the symptoms rather than curing the disease? The vast majority of informatics projects are initiated to address department-level workflow needs or corporate requirements for record, intellectual property and/or compliance management. These annual budget-based projects give scant attention to addressing the strategic, macro-level challenges organisations face. In our consulting practice, time and time again we find that efficiency gains in one group are wasted due to a lack of a systemic optimisation across departmental barriers.

> "The hard reality is that there is only a 10% chance a clinical drug candidate will make it to market for all indications"

Information technology should be an enabler of transformative process improvement and innovation across the biopharmaceutical R&D continuum just as it is in electronics, aerospace and other industries. By 2025, organisations must take a hard look at their R&D business models. Informatics must be the engine to enable its evolution rather than the trailer pulled from behind.

Driving to the future

As we look to 2025, one must consider the internal and external environmental factors effecting pharmaceutical R&D if one is to project where suppliers will direct their future development. Of the many influences shaping the future state, three will be impactful: shifts in the types of organisations served by suppliers, disruption of outmoded R&D operational models and changes in science.

Traditionally, big pharma has led the way in purchasing multi-million dollar software licenses. Vendors have targeted these companies to maximum revenue with the smallest level of sales

Table 1: Evolution of R&D Informatics

2005-2015	2015-2025
Support existing R&D operations	Enable flexible R&D business models
Optimise internal workflows with a focus on productivity	Connect organisations with a focus on innovation
Departmental focus	Strategic challenge focus
Small molecule emphasis	Biologics and precision medicine emphasis
Analytics of structured data	Analytics of unstructured and structured data
Defined vocabularies	Natural language processing
Fixed system integration, defined schemas	Machine learning, probabilistic and semantic data integration
Eliminate paper	Knowledge management
Data management	Predictive modelling

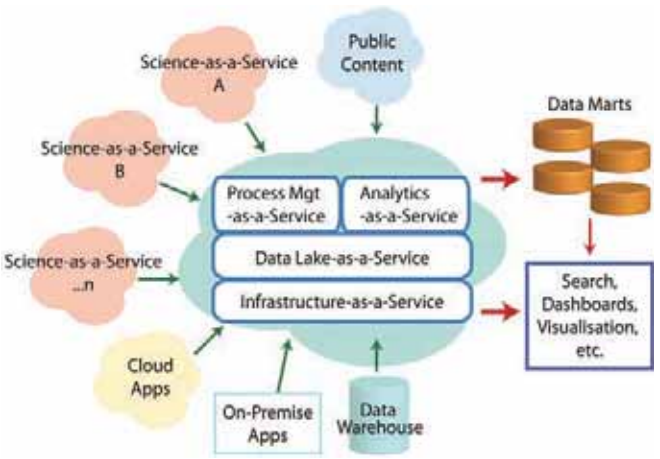

Figure 1: Data Lake-as-a-Service

involvement. Accelerated mergers and acquisition activity, site closures and reductions in work force are unraveling this dedication to large companies and the continuous revenue they provide. In the past year alone, seven of the top 10 pharmaceutical companies announced large R&D employment reductions, leaving thousands of previously purchased software licenses unused. There is no need to pay annual support contracts on shelved software seats.

Despite the reductions by large pharma, the number of scientists employed by private pharmaceutical manufacturers has actually risen slightly over the last 10 years in the US[4]. Hiring by small and mid-sized companies counterbalance these reductions. There has also been a sharp increase in employment in scientific services. Globally, there are now over 6,000 corporate entities with a majority of their R&D directed to biotechnology/life sciences (inclusive of contract research, agriculture, reagent suppliers, etc.)[5]. The majority of these are quite small: 60% of firms have fewer than 50 employees and 95% have fewer than 250.

The number of companies with an active drug pipeline has actually doubled to over 3,200 from the 1,600 in 2005[6]. 56% of these are small with only one to two drugs. This expanding landscape of smaller organisations without the large budgets or personnel to implement software will force changes to pricing structures, simplification of the user experience and hasten the movement to the cloud.

In addition, in 2025, disruption of existing R&D structures will impact on how software products are positioned. Many people have projected the future of pharma R&D as being a complete embrace of the 'Hollywood model'. Back in 2005, large pharmaceutical companies were primarily vertically integrated, performing all required functions from target validation to manufacturing. There was externalisation of some functions, but these activities were primarily outsourcing prevailing work practices to areas of lower cost. The Hollywood model is a transition from a fully vertically integrated organisation to one of distributed innovation and virtualised research. It is so named due to the changes made by the big movie studios. Motion picture companies historically controlled the entire filmmaking process from concept through to distribution, burdening them with an enormous fixed cost. They directly employed directors, cameramen, actors, stagehands and other personnel requiring a large number of films to be made to cover

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overhead. Studios came to realise their strength was in distribution and financing; new movies could come from independent producers, costs could be variable and talent could be on-boarded on a film-by-film basis.

There are signs the movement down the path toward the Hollywood model has already begun for pharma. There are mounting efforts to in-license, open innovation models are being tried, partnerships have formed and therapeutic areas are starting to act like independent producers. However, this transition is still in the early phases, since many companies appear to have a difficult time fully embracing the model; innovators are acquired for their assets with a subsequent shutting of the operation. The trend is inevitable for most; some form of distributed innovation and production model will prevail. In 2025, informatics will be less about supporting internal workflows and more about enabling collaboration, stimulating innovation and the transference of data and information between partners.

The rapid scientific advancement in areas such as next-generation sequencing, precision medicine and next-generation biologics increases the volume, variety and the complexity of datasets. IT teams are having a difficult time keeping up with these changes. In translational research, only a handful of major companies have an integrated strategy for the management and analysis of genomic, phenotypic, clinical and image data. The shift in investment from small molecule to monoclonal antibodies is forcing IT to modify – or replace – legacy infrastructures.

> “By 2025, organisations must take a hard look at their R&D business models. Informatics must be the engine to enable its evolution rather than the trailer pulled from behind”

The relatively recent rise of antibody drug conjugates and data dimensions beyond naked antibodies is having a compound effect. Data warehouses, registration systems, analytics and biologics engineering platforms all need to adapt. At the same time, initiatives in other next-generation biologics (e.g., fusion proteins) further complicate designs. Unlike with small molecule informatics that undergoes little change over years, an era of chronic change is upon us. Conventional methodologies will not be adaptable to the variations in the types of data generated. Architectures in 2025 will have to be malleable, open and modular. There will be little use for fixed data models and authoritarian master data management.

2025-as-a-service

To tackle the increasing number of small entities served, the disruption of historical R&D models and address the growing complexity, there will be an evolution in informatics over the next 10 years (**Table 1,** page 16).

Many, if not most, of the capabilities in 2025 will be delivered via cloud-based services rather than by on-premise software. This will not happen overnight, but the hurried alteration of the R&D landscape will force a lowering of the historically high level of resistance to the cloud. Small companies with little IT support already embrace the cloud. The Hollywood model will force larger companies to work with these organisations via cloud services.

There is presently a strong trend in genomics with the increasing number of *Science-as-a-Service* firms. These companies combine *Analytics-as-a-Service* (sequence analysis) with *Data-as-a-Service* (data management, collaboration and security), connecting corporate, government and academic entities. Other Science-as-a-Service providers are emerging to address needs as diverse as imaging, protein characterisation and *in silico* drug design. Differentiation is not from providing assay results at the lowest cost, but through the bridge of scientific expertise and data services that can be rapidly exploited on a per project basis.

The growing network of Science-as-a-Service providers, each with their own informatics platforms, will create new challenges. Collaborators will be tasked with connecting the various data formats and types across the entire Virtual Research Ecosystem (VRE). Since experts can come and go on a project-by-project basis, IT will not be able to adapt systems quickly enough. The concept of *Scientific Process Management-as-a-Service* will be necessary to manage the workflow of projects, connect organisations into virtual collaboration spaces and integrate data across the network. Due to the high variability in formats and terminologies, machine learning and semantic technologies will be essential for data interoperability.

The ad hoc manner of managing unstructured data will no longer be acceptable in the future state, particularly when collaborating across the VRE. The concept of a *Data Lake-as-a-Service* (**Figure 1**, page 16) enables small organisations to have a flexible object-based repository combining internal, partner and public data without restriction to volume or variety. Content in the lake is stored in its native format, combining both structured and unstructured content. Today, data scientists are needed to extract the maximum value out of the lake. By 2025, there will be a plethora of tools for self-service and automated analytics.

As we look to 2025, informatics must play a major role in helping organisations solve strategic challenges rather than just address tactical short-term problems. Pharma must embrace information technology not as a line-item cost, but as an enabler of R&D transformation. To do this, informatics architectures must be more adaptive than they are today. Interweaving the growing data diversity generated by virtual research bodies will be essential.

Michael H. Elliott founded Atrium Research & Consulting in 2003 to provide scientific informatics market research and strategic consulting. His career began as a bioanalytical chemist developing mainframe data management software on the side. In 1983, he joined Perkin-Elmer Corporation as a LIMS specialist. His PE career progressed to sales, Division Director, VP of instrument marketing, and GM of the pharmaceutical analysis division. In 2000, he joined Scientific Software as SVP responsible for commercial and technical operations. Mr. Elliott has authored multiple research studies on laboratory informatics, has over 30 published articles and has presented in over 25 countries.

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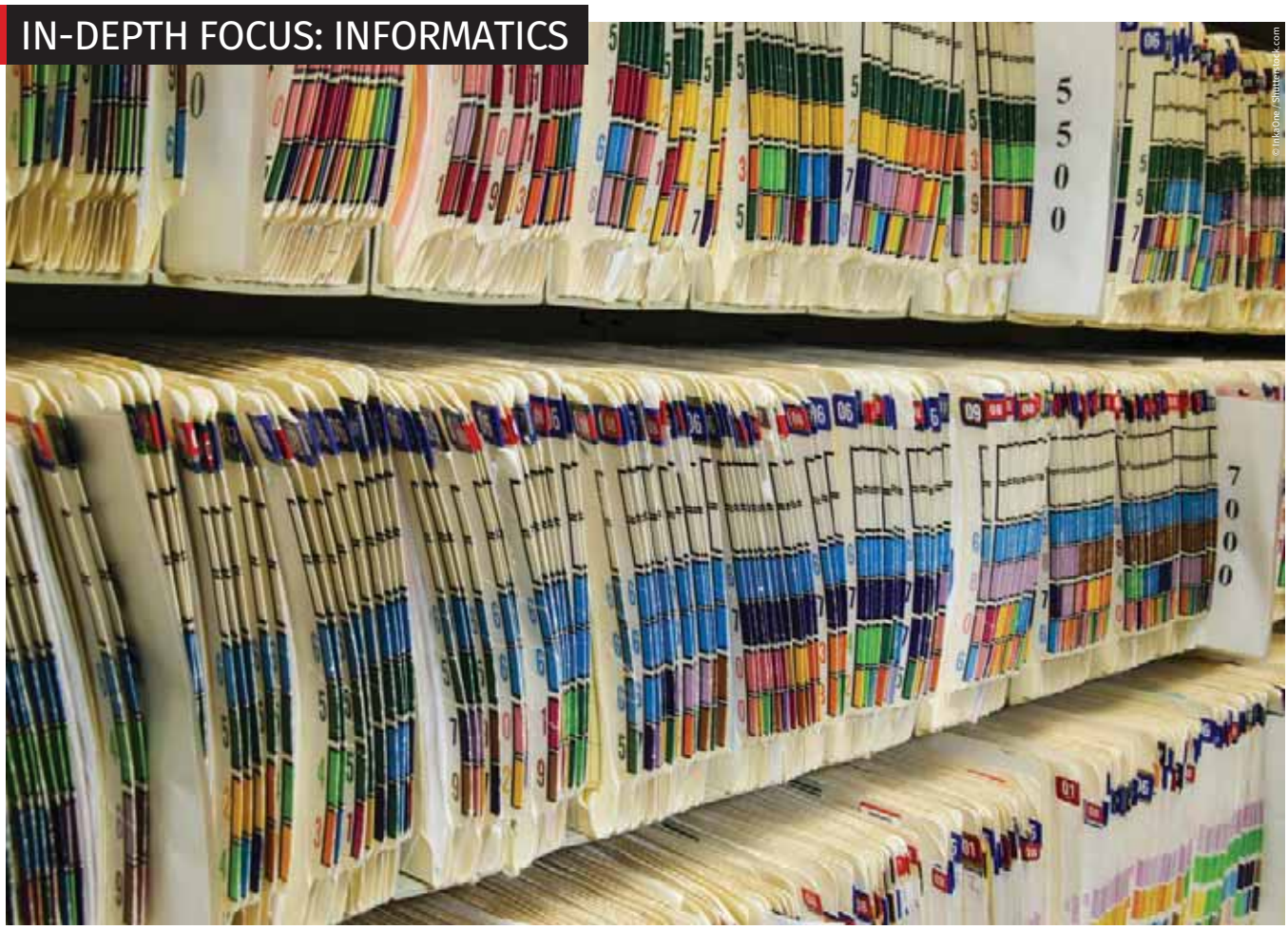

Methods for the detection of drug-drug interactions in text

Brittany L. Melton, PhD
University of Kansas School of Pharmacy

Pharmacovigilance is important in monitoring for drug safety, but it also serves as an opportunity to detect new drug-drug interactions. While some interactions can potentially be identified by chemical structures or action sites, not all are identifiable by this method, necessitating additional ways of detection. An enormous amount of data is collected daily in healthcare and it is often the first time drug-drug interactions may be detected due to the larger number of patients using medications in practice. While some of the data are in easily extractable and analysable formats, information regarding drug-drug interactions is more likely to be found embedded in text, such as physician and therapy notes within the patient's medical records. In order to identify novel drug-drug interactions, methods are being developed to mine information within text.

There is a large corpus of work on the topic of literature mining. The concept has taken on a variety of forms, and sometimes utilises a combination of methods in an attempt to maximise the benefit of literature mining. Medline abstracts may be mined for sentences which contain specific drugs and genes. Once those statements are pulled from the literature, the relationship between the drug and gene can be established and normalised to identify drugs which have an effect on gene expression and thereby drug metabolism. This can be taken a step further with a machine learning classifier, which in one study was able to correctly classify about 80% of such interactions.[1]

Another method of literature mining uses integrated text mining and automated reasoning to identify potential drug-drug interactions.

Text mining often involves natural language processing and may include parse trees which allow for the linking of specific words within a sentence. Through both syntactic and semantic information extraction in the parse tree, the identified information can be compared to a database to facilitate discovery of drug-drug interactions. This process can be used for any clinical text, but one specific study used the parse tree method to identify novel drug-drug interactions through Medline abstracts. In the study by Tari *et al.*, parse trees were used to extract information from Medline abstracts that suggested the use of one drug modified gene expression to induce or inhibit the effect of another drug. Once the potential drug-drug interactions were extracted from the text, automated reasoning was applied to the statements in an attempt to represent the pharmacokinetics of the drugs in question and assign ordering for the potential interaction. This process had a better-than-80% agreement with the gold standard of drug interactions, DrugBank, and identified new drug-drug interactions which were supported by the evidence but were not documented in DrugBank.[2]

An alternative means for extracting drug-drug interactions from text involves the use of a feature-based approach. Similar to other data mining processes, this method takes extracted sentences and converts them into a structured format to allow for the mapping of the potential drug-drug interaction into a syntactic structure. Once that is done, features are used to create vectors which are then able to be classified as either a drug-drug interaction or not. This may be completed through machine learning, similar to other methods. When Bui *et al* developed new features, which included the phrasing around a drug-drug pair and their relations, they were able to precisely identify drug-drug interactions to a significantly higher extent than with previous feature-based approaches.[3] Additionally, the feature-based approach can be taken a step further when logistic regression is also applied to the extracted text. The inclusion of logistic regression with a feature-based approach allows for the building of a predictive model, which can facilitate discovery of true patterns hidden within the text. In one study, the inclusion of features and logistic regression resulted in greater sensitivity and specificity in the detection of drug-drug interactions when compared with a feature-only approach.[4]

While text mining the published literature is undoubtedly beneficial, there is a delay between the observation of a drug-drug interaction and the publication of the article documenting it. Adverse drug-drug interactions can be costly and early identification is of great benefit, not only in terms of cost to the healthcare system itself, but also to the patients who may be harmed. Mining electronic health records and clinical notes provides another valuable opportunity to identify potential drug-drug interactions prior to their publication in the literature. When ontologies are applied to clinical notes, the drugs and event concepts can be annotated and a timeline of events can be constructed. This creates a timeline of drug-drug-events, which can then be evaluated for the identification of a novel drug-drug interaction. Using disproportionality ratios, the drug-drug-event sequences can be compared to those patients who had taken one of the drugs or neither drug to determine if the event occurred more frequently when both drugs were used. This comparison can then be used to ascertain if a drug-drug interaction may have occurred. When this technique was compared to the gold standard of DrugBank and Medi-Span combined databases, one study found high sensitivity and specificity in identifying drug-drug interactions through the text annotation and disproportionality ratios.[5]

> “Medline abstracts may be mined for sentences which contain specific drugs and genes”

As informatics becomes more involved in pharmacovigilance, more methods will be developed to meet the needs of healthcare systems, providers and patients. While a number of text mining options exist, they vary in effectiveness and text source. Some of these methods rely upon published literature, and that can mean significant delays in drug-drug interaction identification. Conversely, methods which utilise electronic health record data can suffer from too few documented incidences of an interaction to allow for identification, despite being closer to the actual event. Methods will continue to evolve to answer these issues.

Brittany Melton is an Assistant Professor of Pharmacy Practice at the University of Kansas School of Pharmacy. Her research interests include the design of health care interfaces and the development of CPOE alerts to improve patient safety and reduce user fatigue. She can be contacted at: bmelton2@kumc.edu

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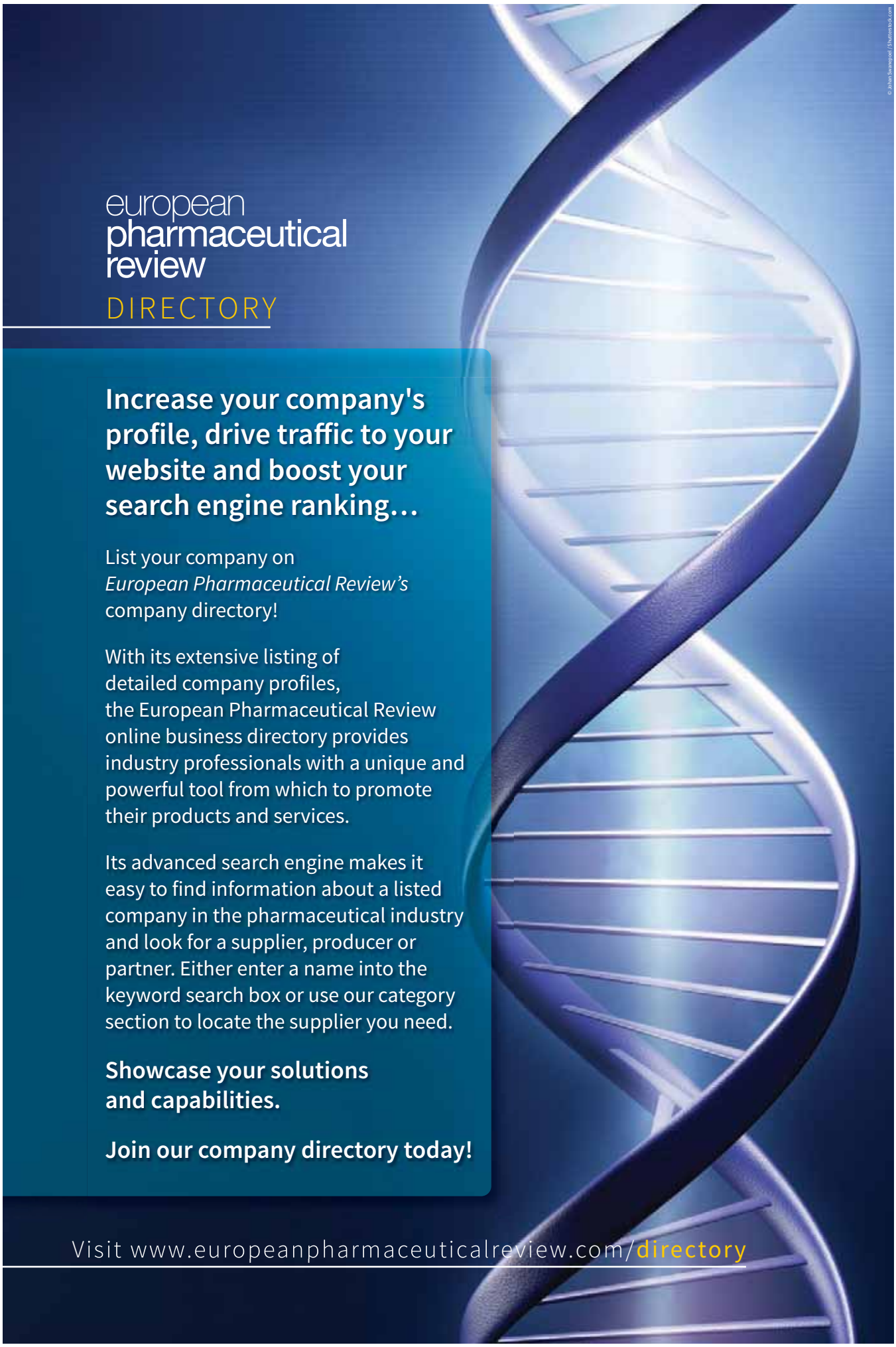

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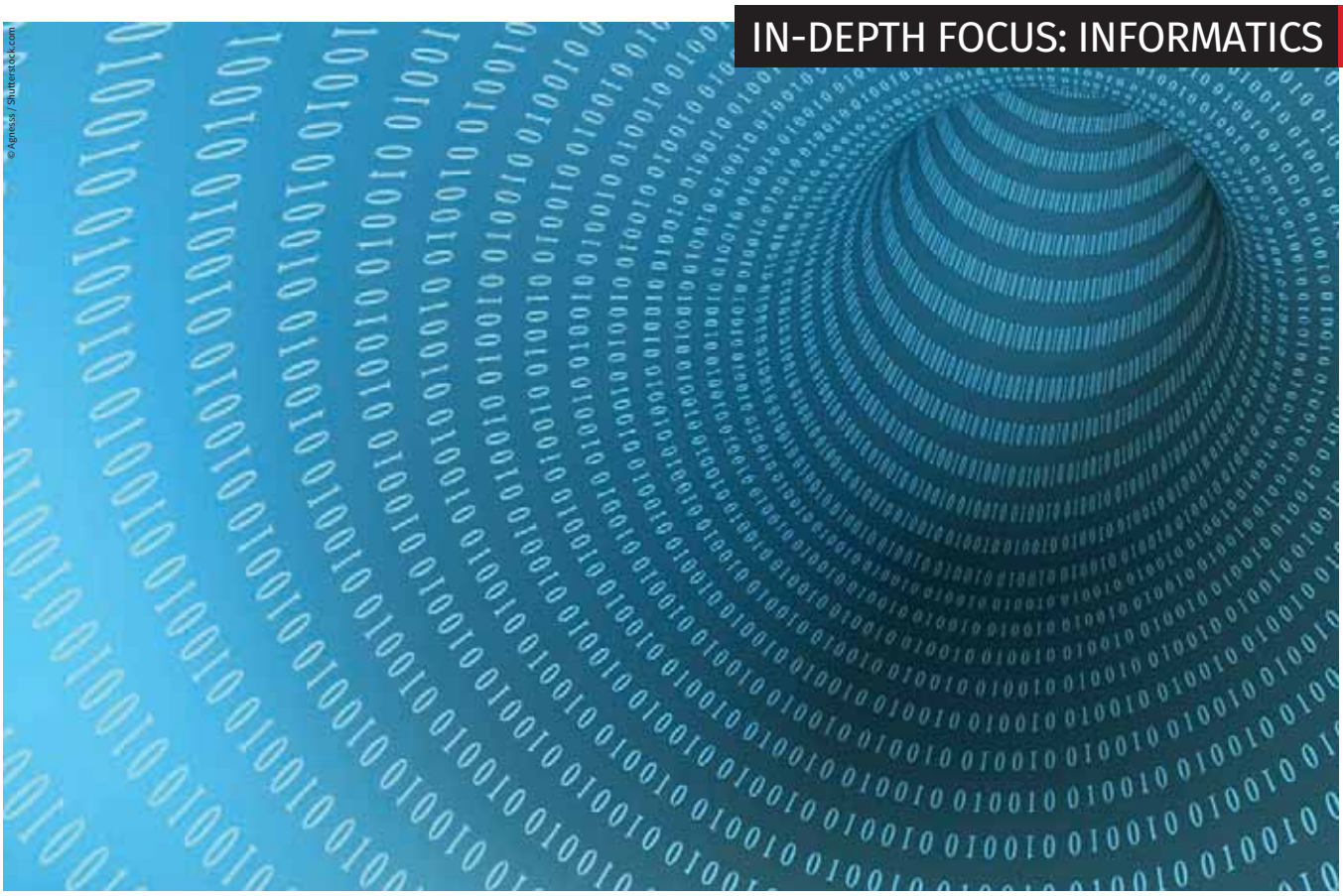


Computational approaches to mutagenicity assessments of impurities: *in silico* methods

David Elder
GlaxoSmithKline and JPAG

***In silico*, computational methods provide a cost effective, rapid and predictive means for screening impurities and putative metabolites for mutagenicity. Obviously, *in silico* methods need to be used in concert with *in vitro* mutagenicity assays, e.g., the Ames test[1]. The prediction of mutagenicity based on alerting structures was initiated by Ashby and Tennant[2,3], who established that there were significant correlations between certain chemical structures, *Salmonella* mutagenicity and carcinogenicity. The original Ashby and Tennant alerting structures were based on industrial chemicals and these are typically electrophilic and reactive in nature. However, reactive intermediates are also used in synthetic chemistry and to a lesser extent also reflect the type of breakdown products, e.g., degradants of drug substances and drug products.**

The ICH M7[4] Guideline highlights that both safety and quality risk management are required to establish suitable levels of mutagenic impurities (MI) that will be expected to pose negligible carcinogenic risk in man. It recommends assessment and subsequent control strategies for MIs that may be carried over into the final active pharmaceutical ingredient (API) or drug product, taking into consideration the intended human use. The risk assessment involves the use of literature searches for carcinogenicity or mutagenicity in order to classify potential MIs (PMIs) or real MIs as class one, two or three or *in silico* predictions of mutagenicity in order to classify PMIs or MIs as class three, four or five (see **Table 1**; page 24).

Therefore, the major challenge facing Industry was how to perform genotoxic risk assessments (GRAs) and how to use quantitative structure analysis (QSAR) tools to predict PMIs or MIs. ICH M7[4] highlights that computational toxicology assessments should be undertaken using QSAR methodologies that will be highly predictive of the outcome

of a bacterial mutagenicity assay. The guidance directs that two QSAR methodologies that are complementary in nature should be used. One approach uses an expert rule-based system (i.e., Derek Nexus[5], Leadscope Genetox Expert Alerts[6]) and the second (Q)SAR approach is statistically-based (i.e., Sarah Nexus[7], Leadscope Genetox Statistical QSAR[8], Case ULTRA[9], etc.). It is worth looking in depth at two representative computational tools from both of these classes: Derek Nexus and Leadscope Genetox Statistical QSAR system.

Expert rule-based system

Historically, Derek Nexus[4] has played a fundamental role in structure-based assessments of mutagenicity[10]. Derek Nexus uses expert knowledge rules generated using both public and proprietary mutagenicity data, and applies those rules to facilitate *in silico* predictions about the mutagenicity of synthetic intermediates, impurities, degradants, as well as potential metabolites.

Proprietary information provided by Lhasa consortium members has been utilised in approximately one quarter of the bacterial *in vitro* mutagenicity alerts found in Derek Nexus[4]. Additionally, proprietary information is used to validate these alerts to demonstrate the predictive outcome of this tool within the chemical space of most interest to users (often proprietary in nature). These data donations can take several different forms:

1) Consortium members make data sets designed to either improve or validate existing alerts available to the rest of the consortium. This is then further analysed to identify lead compounds for alert development. Additionally, data sharing collaborations, such as Vitic Nexus[11] intermediates and Consortium for the Investigation of Genotoxicity of Aromatic Amines (CIGAA)[12] have also been used for alert development[13].
2) Consortium members contact Lhasa to highlight that an existing alert is not sufficiently predictive within their proprietary chemical space, and they make available the data to support an alert modification. In some instances, this includes dialogues with Lhasa QSAR scientists that can lead to additional focussed testing within a specific class to either fill those data gaps or resolve contradictory outcomes within a data set.

In both these cases, the objective is to translate proprietary data held by a specific member into generic, publically-available structure-activity relationships. The level of disclosure is entirely negotiable. Some consortium members prefer to remain unidentified; for example, alert 760 for 2-halopyridines states that: 'four compounds donated by a Lhasa member which have all been reported non-mutagenic' whilst other consortium members disclose their identity, for example alert 475 for 3-aminomethyl-1,2,4-oxadiazoles states that 'This alert is based on data from Hoffmann-La Roche AG and describes the Ames test activity observed for a series of 3-aminomethyl-1,2,4-oxadiazole compounds'.

This collaborative effort within the consortium improves the predictivity within the chemical space that is most important to member companies. As a result, Derek Nexus has been shown to exhibit higher sensitivity within the proprietary chemical space[14].

Statistically-based (Q)SAR systems

Leadscope uses a feature-based QSAR approach utilising molecular descriptors which include structural features and calculated properties (molecular weight, aLogP, polar surface area, hydrogen bond acceptors, hydrogen bond donors, number of rotational bonds and Lipinski score). The models use partial logistic regressions to encode the relationship between these descriptors and the bacterial mutagenesis endpoint. When making a prediction, the same structural features and properties held in the model are calculated for the query compound. These descriptors are then weighted and used to calculate the probability of a positive result. The applicability domain is also evaluated by measuring

Table 1: Impurity classification, based on mutagenicity or carcinogenicity and proposed control strategy (as per ICH M7[4])

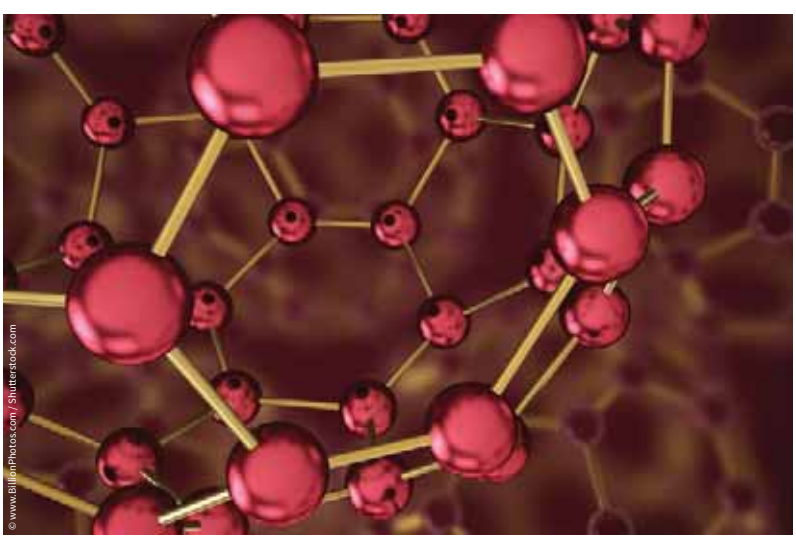
Classification	Classification Definition	Control Strategy
1	Known mutagenic carcinogen	Control at or below acceptable limit (TTC *or LTL** threshold)
2	Known mutagens with unknown carcinogenicity potential	Control at or below acceptable limit (TTC *or LTL** threshold)
3	Alerting structure that is unrelated to the structure of the API in the absence of mutagenicity data	Either control at or below acceptable limit (TTC *or LTL** threshold) or conduct Ames test; if positive classify as Class 2; if negative classify as Class 5
4	Alerting structure that is related to the structure of the API and where the API is non-mutagenic	Treat as an ICH Q3A or Q3B non-mutagenic impurity
5	No alerting structures or an alerting structure with adequate data to demonstrate a lack of mutagenicity or carcinogenicity	Treat as an ICH Q3A or Q3B non-mutagenic impurity

*TTC Threshold of Toxicological Concern, **LTL Less than Lifetime

the distance between the query compound and similar compounds in the training set. Compounds that are found to be outside the predefined applicability domain will not return a prediction as they are considered out of domain (OOD)[15].

Leadscope[8] has introduced a knowledge-sharing collaboration to address any specific QSAR regulatory issues that have been identified via discussions with interested parties or regulatory agencies. Knowledge sharing can either be with the aim of improving the model's predictivity for structurally similar compounds or more usually to improve the performance of an entire alert and/or the QSAR model; e.g., to improve the applicability domain and prediction accuracy of specific classes of compounds that are not well represented in the public domain, such as boronic acids. The extent of data disclosure is assessed on a case by case basis that is dependent on the sensitivity of the proprietary information provided. Full data disclosure, where both the structure and supporting data are incorporated into the QSAR statistical models, helps to maintain the model's transparency. However, where required, compound/company confidentiality can be maintained.

For example, the specificity for the predictivity of primary aromatic amines was increased by 14% with no commensurate decrease in sensitivity, as a result of such data sharing with a single pharmaceutical company[16]. This was achieved by the use of structural fingerprints for different compound classes. Nearly 600 chemical fingerprints based on the Leadscope fragment hierarchy were used. Subsequent data analysis of a reference set and existing external knowledge containing a variety of primary aromatic amines allowed an updated alert to be derived. This list of sub-structures included meta-, para-, ortho- and hetero-substituted, as well as more complex substitution patterns. Only those results for the pre-defined sub-structures in the fingerprint are summarised and it is therefore possible to apply these fingerprints to a proprietary database without revealing sensitive information for individual compounds or supporting data. This collaboration now includes 13 different pharmaceutical companies and regulatory agencies and has led to a continued improvement in the predictivity of this class of compounds. This fingerprint methodology is also being applied to various other chemical classes.



The use of two (Q)SAR systems

A transnational inter-company survey of eight companies showed an increased usage of (Q)SAR-based evaluations[17]. The authors indicated that many publications have historically focused on the potential of these QSAR models either individually or in combination, to predict accurately the mutagenic effects of certain proprietary chemicals in the Ames assay. Typically, these assessments evaluate significant numbers of these compounds together with QSAR-based approaches to try and predict their likely mutagenic liability. However, these publications did not address the associated expert assessments that are also necessary to interpret any alert(s). The authors were concerned that structural assessments alone were being used to conclude that a given impurity was non-mutagenic. This perspective was assessed using an inter-company survey to understand companies' individual success rates for accurately predicting mutagenicity. This survey established that the Negative Predictive Value (NPV) of these QSAR *in silico* approaches were 94%. However, when 'expert knowledge' was added to this process, the NPV was increased to 99%. The authors commented on the significance of expert interpretation of *in silico* predictions and they indicated that the use of multiple *in silico* models (as required by ICH M74) is not a significant factor in the outcome of these evaluations, with respect to NPV.

Another trans-national survey from 14 different companies focussed on the different QSAR methodologies used within the pharmaceutical industry, together with the predictive value of these different QSAR approaches[18]. The authors indicated that most pharmaceutical companies used an expert rule-based system, e.g., Derek Nexus[5] as their standard default methodology[5,6], and these approaches yielded negative predictivity values of ≥78% across all participating companies. A further augmentation in predictive power

(>90%) was typically achieved by the use of a second usually statistically-based[7,8,9] QSAR methodology, or an additional expert review. However, when two QSAR methods were used in tandem, an additional expert review was still required, since conflicting outcomes were often obtained.

There are five potential outcomes from any assessment using two *in silico* systems[19]:

1) Positive/Positive. It would require very strong evidence during the expert review to dismiss both positive predictions (likely positive).
2) Positive/Equivocal or OOD. The lack of a prediction (equivocal or OOD) from the second system implies insufficient evidence to derive any other conclusion during the expert review (likely positive).
3) Positive/Negative. Very strong evidence from the expert review will be required to overturn the positive prediction (likely positive).
4) Negative/Equivocal or Out of Domain (OOD). The expert review may conclude this is a negative outcome where there is strong evidence showing that the structural motif responsible for the no prediction is also present in known negative examples (assuming there are no deactivating features). Some companies may assign this a positive outcome as negative is non-proven (likely uncertain).
5) Negative/Negative. The expert review needs to assess any concerning features, i.e. unclassified, misclassified, etc. (likely negative)

Based on the available data, it was concluded that an expert rule-based system augmented by either a second statistically based QSAR system or expert knowledge is an acceptable strategy[18]. Transparency of the 'expert review' process, i.e., methodologies, results, weight-of-evidence approaches, etc., were achieved by collaborative initiatives involving full data sharing. It was hoped that in the future, the use of standard reporting strategies would enable regulators to more fully understand the submitted data. The authors concluded that this approach was appropriate for regulatory submissions aligned with ICH M7[4].

Conclusion

Computational, *in silico* tools that correlate chemical structure with bacterial mutagenicity play an important role in the risk assessment of reactive intermediates, impurities and breakdown products of drug substances and products. These methods are either expert rule-based systems or are statistically derived. However, irrespective of whether these QSAR methodologies are used singly or in tandem, an expert review is still required. Indeed, it has been reported[17,18,19] that if these 'complimentary' (Q)SAR systems are used in tandem (as required by ICH M7) the expert review is more involved and takes significantly longer, because in addition to positive and negative outcomes, there are uncertain results where the two systems give conflicting conclusions. In addition, it has been reported that the use of multiple *in silico* models is not a significant factor in the outcome of these evaluations, with respect to negative predictive power[17].

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Dr Elder has BSc and MSc degrees in chemistry from Newcastle upon Tyne, before he moved to Edinburgh to study for a PhD in Crystallography. He is a visiting professor (King's College, London). Dr Elder has 37 years' experience at a variety of different pharmaceutical companies (Sterling, Syntex and GSK). He is currently a Director within the product development group in GSK R&D. Dr Elder is a member of the British Pharmacapoeia (Expert Advisory Group PCY: Pharmacy), a council member of the Analytical Division, Royal Society of Chemistry (RSC), UK and a council member of the Joint Pharmaceutical Analysis Group, UK. He is a fellow of the RSC (FRSC) and a member of the Royal Pharmaceutical Society (SRPharmS). He has co-edited one book on the Analytical Characterisation and Separation of Oligonucleotides and their Impurities.

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Q&A

Michael H. Elliott, CEO of Atrium Research & Consulting LLC, interviews Devendra Deshmukh, Vice President and General Manager at PerkinElmer Informatics, on trends within the informatics industry.

The pharmaceutical industry is evolving on a number of fronts. What do you see as the most significant trends that are impacting on laboratory informatics? How are these trends affecting your future software development?

Customers expect integrated solutions, not just for traditional R&D needs, but to support partnerships with contract research organisations (CROs) and academic collaborations that are integral to discovery. Cost is always a factor, since customers want more capabilities for their technology dollars. Beyond results, they want access to information that helps them make better decisions, and quickly. PerkinElmer has responded by embarking on a major product and technology transformation initiative to offer an integrated cloud-based, mobile-enabled platform which customers can use to capture, collaborate and analyse data from a variety of disparate sources and manage the metadata around it, exposing the functionality through workflow and analytics-focused solutions.

While numbers of biopharmaceutical and CROs have been increasing, big pharma has been streamlining R&D staff. What are you doing to address the growing number of small companies? Do you see their requirements differing from those of large organisations?

We view small organisations as having similar needs to their larger counterparts, with generally more limited resources. Both are interested in lowering total operational costs and need a more horizontal, broader solution stack that is seamless to use across organisations. Using SaaS models and leveraging the cloud, which companies have historically shied away from, helps bring high performance solutions and capabilities within reach. We are adding capabilities to our E-Notebook™ software for greater CRO collaboration, so the sponsor can maintain data integrity and organisation while supporting CROs with a lightweight framework that lowers cost. Simultaneously, we are introducing Elements®, a cloud-based collaboration platform.

Many IT departments are under increasing pressure to deliver value in an era of constrained costs and resources. How do you view the importance of informatics services to assist IT and what are you doing to help organisations provide the maximum return on their investment while helping them to keep costs low?

Laboratory support is essential, so we offer OneSource® Laboratory Services, a comprehensive, multivendor managed services portfolio that leverages PerkinElmer's global scale, application knowledge and scientific depth. We offer support models for multivendor R&D software technologies, including instrument software support, enterprise application management services for LIMS, ELNs, SDMS, etc., and validation services. This range of R&D software support capabilities allows us to combine these competencies into an integrated support model and provide a high value service to scientists, while achieving time and cost productivity, as we break down traditional support silos and measure results. Our project methodology focuses on continuous improvement, ensuring that enhancing value remains our priority.

Across R&D, companies are looking to minimise the number of technologies they use. However, users' requirements versus meeting corporate needs must be balanced. Some suppliers are providing a broad suite of capabilities while others are focusing on a narrow range with deep functionality. What is your approach and why is this beneficial to users?

We have been successful at offering single-stack solutions that also serve broad-based analytics needs. The TIBCO Spotfire® platform, for example, is a business intelligence and visual analytics offering that meets corporate needs and IT objectives. Add-on modules, such as Lead Discovery™ software powered by TIBCO® Spotfire for SAR analysis, OmicsOffice® software for analysing omics data, and High Content Profiler™ software for screening data, provide scientific depth and expertise for targeted workflows. An extensible API allows the platform to be extended across research, clinical trial monitoring, operations, and safety analytics, as well as at the corporate level to manage product portfolios among other applications.

The 'consumerisation' of technology is changing users' perceptions of system interaction and mobility. What are you doing with your products to enhance the user experience?

PerkinElmer has been leading the transition to mobile platforms with the introduction of mobile versions of our 30-year-old market-leading chemical structure drawing tools, ChemDraw® software and its 3D counterpart Chem3D® software, for use on iPad® devices. Users want scientific software to be as simple to use as consumer products and we are taking that to heart. We are working to provide users with powerful tools and functionality which are still streamlined and easy to use. Therefore, a large majority of our solutions are designed by scientists, for scientists. The Elements® software is a great example – it offers an app-like environment that will be very familiar to users of consumer technology.

Solving solubility issues with amorphous solid dispersions

Yin-Chao Tseng
Boehringer Ingelheim

Amorphous solid dispersions (ASDs) are increasingly being used as a means of improving bioavailability of poorly water-soluble compounds in research and development, and spray drying technology has been recognised as one of the useful methods to generate ASDs. Although the application of spray drying for the production of ASDs in the drug discovery stage is still limited, ASDs prepared with a small-scale spray drying process can be an effective approach to deliver high doses of poorly water-soluble compounds and to enhance their plasma exposure in *in vivo* studies for early drug discovery efforts. This article reviews the application of this technology for the production of ASDs in a single process, and the use of small-scale spray dryers to produce ASDs as early preclinical formulations using only milligram quantities of drug substances.

Early preclinical formulations

It is estimated that 40 to 60% of drug compounds in today's drug discovery phases exhibit poor aqueous solubility, and these compounds frequently have certain delivery limitations. Early preclinical formulations are developed for *in vivo* pharmacology, safety pharmacology and toxicology studies in drug discovery. The main purpose of these formulations is to obtain sufficient plasma exposure in *in vivo* studies for optimising, selecting and advancing compounds to the clinic. However, inadequate plasma exposure does not sufficiently provide safety margins surrounding the predicted efficacious dose in animal models.

Oral gavage is the most common route in animal dosing, and in general, solution formulations are preferred due to their simplicity, dose accuracy and dose flexibility. For poorly water-soluble compounds, enabling solution formulations with solubility enhancement using a co-solvent, cyclodextrin, surfactant, lipid-based product, or the combination formulations, are employed to yield improved plasma exposures. However, these enabling solution formulations often have

Figure 1: Büchi Nano Spray Dryer B-90 (adapted from Nano Spray Dryer B-90[3])

limitations, such as poorly soluble compounds failing to reach target drug concentrations. Drug precipitation and vehicle-related side effects can also occur. These issues limit the amount of the poorly soluble compounds that can be administered to animals as a solution.

Conventional drug suspensions are capable of providing high drug concentrations, but suspensions of poorly water-soluble compounds often exhibit dissolution rate-limited drug absorption. In addition, conventional suspensions of different batches produced in the drug discovery stage often have batch-to-batch inconsistency, which can cause significant variability of dissolution and bioavailability. Crystalline nanosuspensions are frequently introduced as enabling suspensions to solve the dissolution rate issues. Meanwhile, nanosuspensions have shown significant improvement in enhancing drug dissolution and bioavailability. However, the batch-to-batch inconsistency of drug substances supplied in the drug discovery stage and solubility-limited absorption for high doses of poorly soluble compounds are the limitations in the application of crystalline nanosuspensions in drug discovery. That is where ASDs come to the fore – they offer an alternative and effective way to deliver high doses and enhance *in vivo* exposure of poorly soluble compounds.

Benefits of amorphous solid dispersions

A solid dispersion (SD) is defined as the dispersion of a drug compound in a carrier matrix (often polymers) at solid state. In the case of an ASD, a crystalline form of the compound is converted to an amorphous form, which is dispersed in, and stabilised by, a carrier. By eliminating the inherent solubility limitations associated with the crystalline form, the ASD offers the solubility benefits of a solution formulation without the need for non-aqueous vehicles, which can limit dosing volumes. Additionally, the dissolved carrier excipient(s) in solution delays precipitation and maintains supersaturation of the compound in the gastrointestinal tract. When supersaturation is sustained, the absorption of the compound can be maximised to be greater than that of a saturated solution.

While ASDs have been increasingly used to enhance bioavailability of poorly soluble compounds at a later stage of the drug development process, this approach has had limited utility at the stage of drug discovery, i.e., it has tended to be used only as a last resort. This is primarily because preparing reliable ASDs at the milligram scale is technically challenging, and therefore would require considerable development time and amount of drug substance.

Among the several different manufacturing methods explored to produce ASDs, hot melt extrusion (HME) and solvent evaporation are the two lead approaches to enable poorly soluble compounds. HME is not suitable in the drug discovery stage due to its requiring a relatively

Figure 2: ProCepT Spray Dryer/Chiller 4M8-TriX (adapted from ProCept Spray Dryer/Chiller[4])

large quantity of drug substance. For the small-scale preparation of an ASD, rotary solvent evaporation is commonly used. However, the stressed conditions (i.e., the gradual solvent evaporation process at an elevated temperature with a relatively long evaporation time) employed by this method often lead to the chemical instability of the drug compound and also drug–carrier phase separation of the ASD. In addition, the dried solid material will typically be in the form of a film, and thus post-processing techniques (isolating, milling and sieving) are necessary to obtain the desired properties of ASD powders. From a scalability and process perspective, rotary evaporation is not an ideal method in the drug discovery stage.

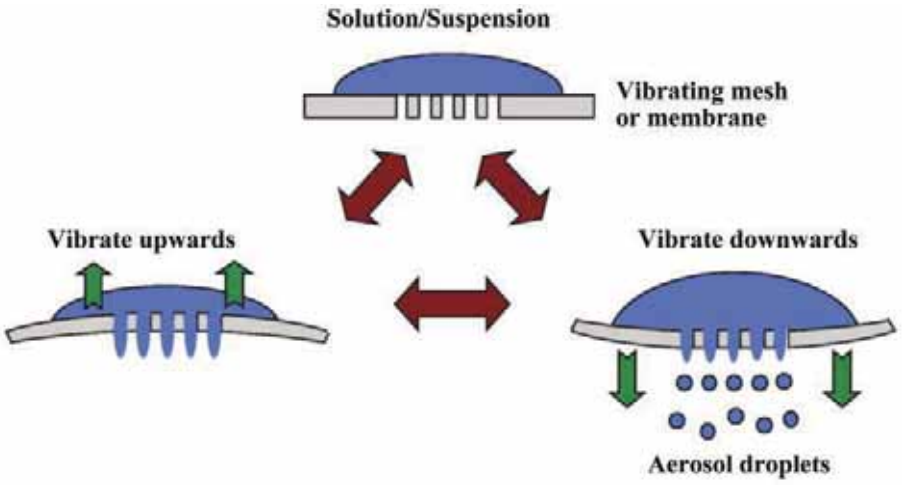

Figure 3: Mesh vibration at the piezoelectric driven spray head of the Nano Spray Dryer B-90 (adapted from Nano Spray Dryer B-90[3])

Application of the spray drying technology

Spray drying is a continuous and scalable solvent evaporation method. It converts solutions to powders in a single process, and it has been used for the preparation of ASDs of poorly soluble compounds. The spray drying process involves: 1) dissolving the drug and the carrier excipient(s) in a common solvent to prepare a feed solution; 2) pumping the feed solution into a spray nozzle; 3) atomising the solution stream into fine droplets via an appropriate device; 4) drying the fine droplets in a drying chamber; and 5) separating and collecting the dried powders via a suitable collector.

Compared to rotary evaporation and HME, the spray drying process gives us more diverse options for ASD carrier excipient(s). For instance, the cellulose-based polymers can be used as ASD carriers by the spray drying process, but it is difficult to use them with the rotary evaporation or HME processes. The solvent system used for the spray drying process should be volatile with good solubilising powder for both the drug and the carrier. The most common solvents are methanol, acetone, or a combination of the two. The solid load and solubility in the solvent

system determines the viscosity of the solution, which can have an adverse effect on the atomisation process. Therefore, the total solids load in the feed solution is generally dictated by the solubility of the drug and carrier as well as the viscosity of the solution.

The spray dried ASD powders are yielded from rapid solvent evaporation from droplet surfaces by the atomisation and drying processes. Because the solvent evaporation process is extremely fast (in the order of seconds), spray drying is particularly advantageous for preparing ASDs of compounds with poor thermal stability. Additionally, forming a single-phase mixture of drug-carrier through rapid solidification is particularly important to prevent drug–carrier phase separation. The phase separation can lead to physical instability (e.g., recrystallisation) of an ASD and consequently, poor dissolution and poor bioavailability.

Particle size and properties of ASD powders can be easily optimised through process parameter optimisation of spray drying and/or the type of spray nozzle. The typical properties of spray-dried powders with high surface area and low bulk density are useful to produce a homogenous suspension by constituting ASD powders in aqueous vehicles. Furthermore, the ASD powders made by the process have small particle sizes, so the drug can be easily and accurately dosed via a gavage needle of reasonable gauge by standard animal dosing practices. Accurate dosing in animals is prerequisite to establish dose-response relationship in *in vivo* studies.

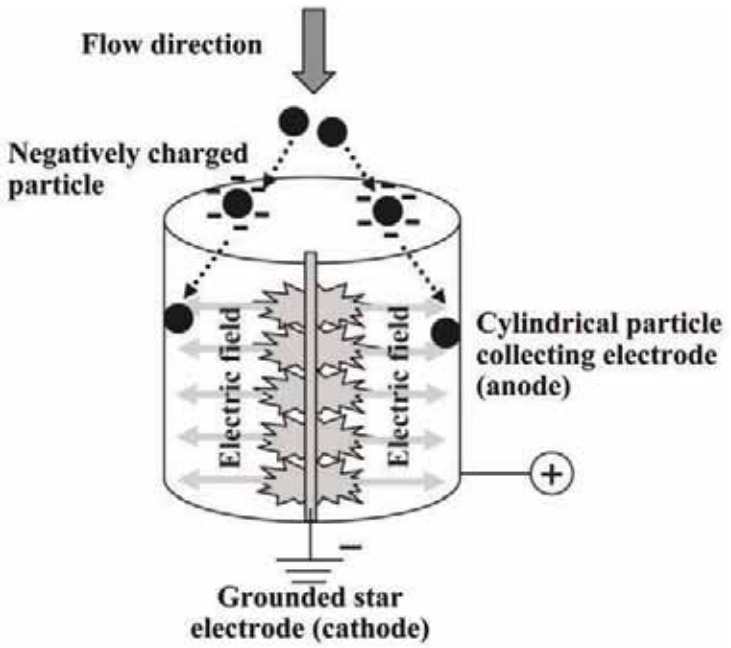

Figure 4: Figure 4: The electrostatic particle collector in the Nano Spray Dryer B-90 (adapted from [7])

Table 1: Comparison of Büchi nano-spray dryer B-90 and ProCept spray dryer 4M8-TriX

	Büchi Nano Spray Dryer B-90*	**ProCepT Spray Dryer/Chiller 4M8-TriX***
Spray nozzle	Piezoelectric (vibrating mesh)	Ultrasonic, two-fluid, mono-disperse
Heating system	Laminar flow	Laminar flow
Drying gas flow (kg/h)	12	36
Evaporation capacity (Liter H_2O/h)	0.2	1.0
Particle collector	Electrostatic	Cyclone
Particle size	300 nm – 5 µm	1 – 150 µm
Smallest sample	> 2 mL or > 200 mg	0.5 – 4000 mL
Yields	Up to 90%	> 90% (from 1 mL (10 mg) to 24 L)

*Information from [3,4]

For a more detailed discussion regarding the principles of spray drying and ASDs, the inquiring reader is referred to the 'Spray Drying Handbook'[1] and 'Amorphous Solid Dispersions: Theory and Practice' books.[2]

Small-scale spray dryers applicable to drug discovery

While the spray drying technique has been widely used to prepare ASDs in development, this technology has had limited utility in drug discovery, where bulk drug supply is extremely limited (in milligram quantity). Most of available lab-scale spray dryers have poor yield and require gram (vs. milligram) quantity of material. Recently, two small-scale spray dryers, Büchi Nano Spray Dryer B-90 (B-90, **Figure 1**; page 29)[3] and ProCepT spray dryer/chiller 4M8-TriX (4M8-TriX, **Figure 2**; page 29)[4], have become commercially available. Since they can generate particles in milligram sample quantities at high yields, they are suited for the use in drug discovery phases. However, there are only a few literature examples describing their use for the preparation of ASDs[5,6]. The two spray dryers are compared in **Table 1**.

As with other spray dryers, the two small spray dryers use three main steps: atomisation, drying and collection. Although pneumatic two-fluid nozzles are the most common atomisers used in the lab-scale spray dryers due to their simplicity and flexibility, the ultrasonic nozzles, which are also found in the 4M8-TriX unit, offer the advantage of spraying very small amounts of solution with high yields. Unlike other spray dryers, the B-90 unit has a unique piezoelectric driven spray head, which consists of 4, 5.5 or 7µm

spray mesh. Millions of precisely sized droplets are ejected from the holes by vibration of the spray mesh (**Figure 3**; page 30). The small-scale units have small dimensions of the drying chambers, which limit the residence time distribution of atomised droplets. Therefore, the atomised droplets must be small so that they can be easily dried in the drying process prior to exiting the chamber or impacting the chamber walls. The laminar drying gas flow pattern is introduced to the B-90 and 4M8-TriX units to reduce the collision of droplets/particles with the chamber walls and to increase the duration for drying of droplets/particles for obtaining high yields. Another unique feature of these systems is that the drying chamber is constructed of glass for visualisation of the drying process.

Cyclones are the most common collection systems in pharmaceutical spray drying. The 4M8-TriX unit is also equipped with a cyclone, which collects dried particles with particle sizes ranging from 1 to 150µm. The B-90 unit also has an electrostatic particle collector for collecting charged fine particles, which are negatively charged after atomisation (**Figure 4**; page 31).[7] These charged fine particles are discharged after they are deposited onto the inner wall of the cylindrical particle collecting electrode. The B-90 is the only unit capable of producing and collecting particles ranging from 300nm to 5µm at high yields of up to 90% even at small sample quantities in the milligram or milliliter range.

In summary, ASDs of poorly water-soluble compounds can enhance plasma drug exposure and deliver high doses in *in vivo* animal studies. Moreover, the adverse effect caused by the carriers of ASDs is much less than that of other enabling formulations. The simple spray-drying process is ideal for the production of ASDs with rapid solidification to avoid drug-carrier separation. The recently available small-scale spray dryers offer the opportunity to apply the spray drying technology for the production of ASDs in drug discovery with milligram drug quantities at high yields.

Dr. Yin-Chao Tseng is a Senior Research Fellow at Boehringer Ingelheim Pharmaceuticals, Inc. in the US. He is currently leading the Discovery Pharmaceutics group, and his key responsibilities include early preclinical formulation development and solid form evaluation for drug candidate nomination for development. Over the course of 23 years working in the pharmaceutical industry, he has been involved in all phases of the drug development process from drug discovery to full development at Wyeth, Bayer and Boehringer Ingelheim.

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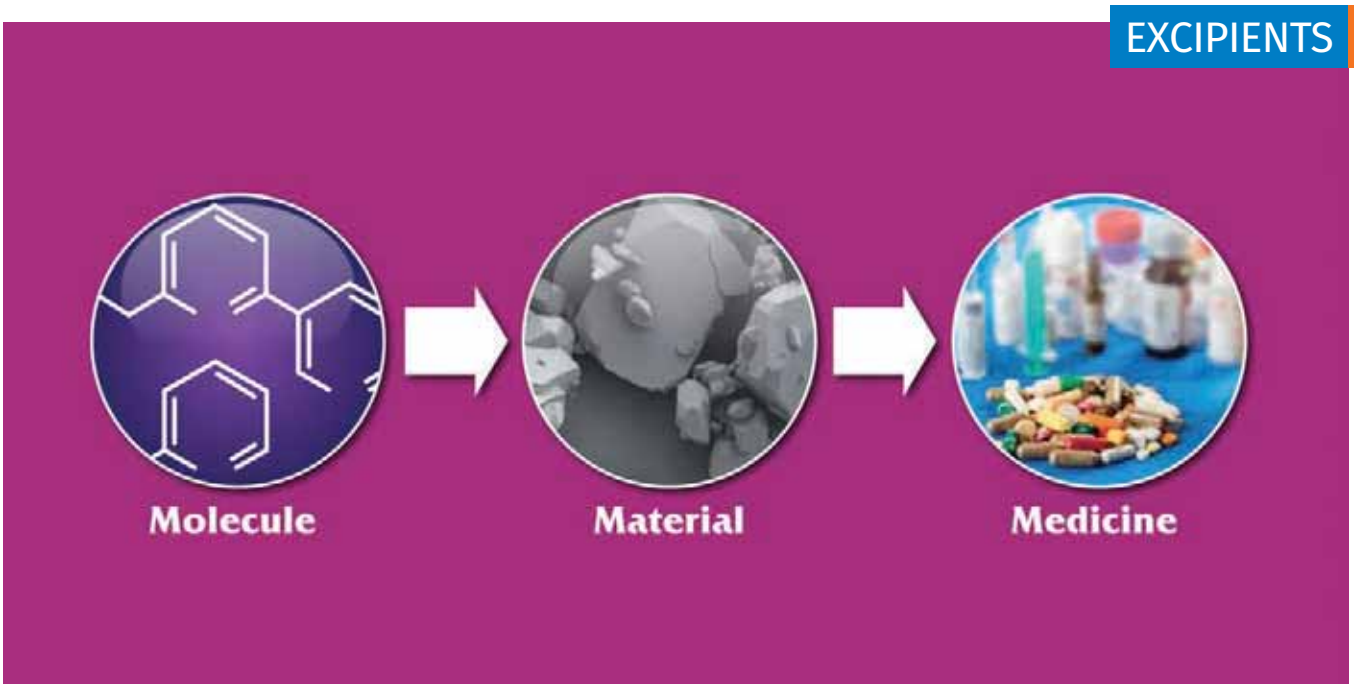


The SSPC: leading the way for next-generation medicines manufacture

Benjamin K. Hodnett, Anita R. Maguire, Pat J. Guiry, Ake C. Rasmuson, Brian Glennon and Abina M. Crean
SSPC

The Synthesis and Solid State Pharmaceutical Centre (SSPC), a global hub of pharmaceutical process innovation and advanced manufacturing, is funded by Science Foundation Ireland (SFI) and Industry, and represents a unique collaboration between 22 industry partners, nine research performing organisations and 12 international academic collaborators (*Figure 1*; page 34). It is a €42 million state-industry investment, which supports a globally-leading research team of 38 investigators, 34 post-doctoral researchers and 60 PhD candidates. As the largest research collaboration in Ireland and one of the largest globally within the pharmaceutical area, the SSPC transcends company and academic boundaries (*Figure 2*; page 34). Its role is to link experienced scientists and engineers in academia and the pharmaceutical industry, to address critical research challenges and to deliver industry-relevant solutions, which result in job growth and retention within the pharmaceutical industry.

From molecule to medicine

The SSPC leads the way for next-generation medicines manufacture. Its research programme spans the entire pharmaceutical production chain from synthesis of the molecule to the isolation of the material and the formulation of the medicine. The SSPC's globally-leading research programme is organised into three interconnecting strands (Molecule, Material, Medicine), which actively reflect the three distinct steps in the manufacture of modern medicines. The Centre supports 19 research projects across these three strands of research, which are divided into nine platform projects that aim to progress scientific state-of-the-art research, driven by scientific challenges of the area; eight targeted projects that are driven by scientific challenges of specific industrial needs; and two linker projects that are the interactions between the strands, which concentrate effort at interfaces where the most important developments need to take place. In addition to these 19 research projects, the SSPC has recently expanded its remit into the biopharmaceutical arena, through a new state-industry funded collaborative project, which will conduct cutting edge research in the area of extractable and leachable compounds.

Strand 1 – New frontiers in pharmaceutical synthesis (Molecule)

SSPC's strand 1 research programme centres on developing better and more environmentally sustainable ways to make active pharmaceutical

ingredients (APIs), by seeking to exploit the potential of biocatalysis in key synthetic transformations, utilising enzymes with improved stability, selectivity and reusability. SSPC investigators conduct unique research in areas such as:

- The development of new catalytic approaches, encompassing the discovery of novel biocatalysts via metagenomic analysis and random mutagenesis of recombinant enzymes.
- The exploitation of new synthetic methods for amides and esters, expanding this methodology to the synthesis of key APIs.
- The development of new methodologies to shorten enantioselective synthetic routes to existing APIs and future drug candidates.
- The use of flow technology and reaction telescoping of processes involving hazardous intermediates and incompatible conditions.

Figure 1: SSPC Partners

Strand 2 – Crystal growth and design (Material)

SSPC's strand 2 research programme focuses on developing optimal ways to crystallise APIs by developing crystallisation methods for controlling the size of product crystals, to directly meet the requirements of secondary manufacturing and drug performance. SSPC investigators conduct next-generation research in areas such as:

- The development of a more precise treatment of thermodynamic and kinetic data, and the development of models for crystallisations.
- The use of process design, modelling and control in order to develop new strategies from modelling for control of nucleation, growth, agglomeration and breakage, to deliver more reliable and predictable processes.
- The experimental investigation of impurity effects in crystallisation, along with molecular modelling studies focussing on parameters, which impact nucleation, growth and impurity incorporation.
- The investigation of a range of co-crystals and the examination of the mechanism of heterogeneous nucleation and crystallisation (or precipitation) of APIs into and onto excipient particles or pre-manufactured granules.

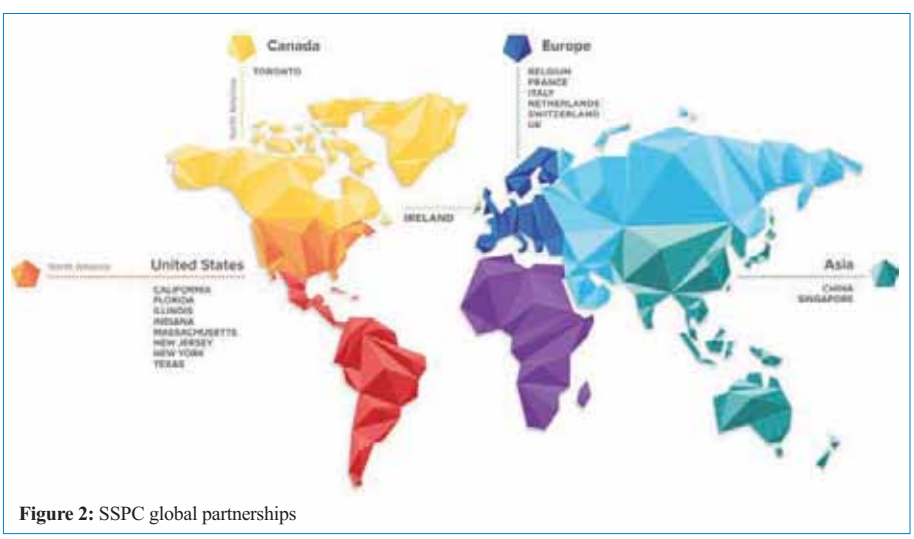
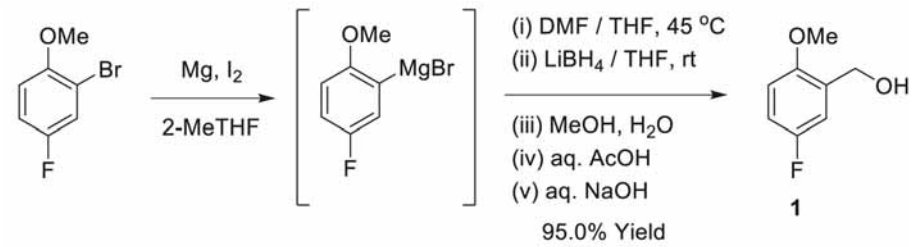


Figure 2: SSPC global partnerships

Strand 3 – Drug product formulation and manufacture (Medicine)

SSPC's strand 3 research centres on developing the dosage forms of the future, with particular focus on the generation and stabilisation of amorphous APIs in order to increase the fundamental understanding of the amorphous solid state and its stabilisation for new composite pharmaceutical materials. SSPC investigators conduct cutting-edge research in areas such as:

- The tracking of amorphous crystalline API, API/excipient and API/excipient/moisture interactions through a range of formulation processes.
- The identification and control of critical API characteristics and how such characteristics, in turn, impact on processability in drug product manufacture and drug product performance.



Scheme 1: Tandem Bouveault Formylation/Hydride Addition to produce Edivoxetine•HCl Intermediate

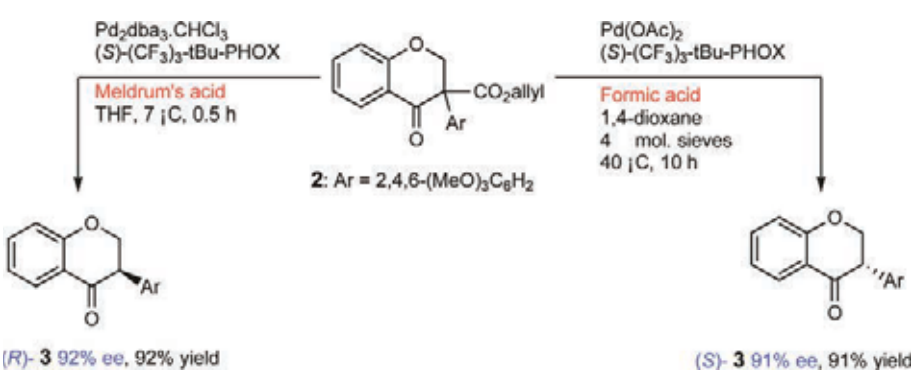
- The investigation of critical excipient characteristics, with an emphasis on understanding variability in composition, how this variability impacts on performance in formulation, and on developing a range of potential additional testing for a select range of excipients used in drug formulations.
- The development of novel technologies for the delivery of solid dispersions of BCS Class II drugs, so as to manipulate the performance of low solubility drugs in a predictive space via a Quality by Design (QbD) approach.
- The development of control strategies to deliver tuneable processes for API manufacturing, which are capable of producing specific API characteristics across a range of manufacturing scales, facilitating more robust and predictable design and optimisation.

SSPC API/excipient case studies

Case study 1: An industrial–academic partnership to optimise small molecule process development

One of the most successful of a range of projects carried out by a collaborative partnership between a synthetic chemistry research team led by Maguire and co-workers, University College Cork and Eli Lilly and Company, involved a telescoped approach to aryl hydroxymethylation employed in the synthesis of a key pharmaceutical intermediate using a rational, mechanistic-based approach, which has been successfully implemented by Lilly to produce in excess of 300kg of compound 1 for commercial validation of edivoxetine·HCl (**Scheme 1**).

The collaborative research enabled telescoping of two synthetic steps – a Bouveault formylation and hydride reduction – into a single efficient process, which is readily amenable to large-scale manufacture. This novel approach replaces highly hazardous chloromethylation chemistry that produces bischloromethyl ether as a byproduct, which is dangerous on all scales of operation. Telescoping of synthetic steps is particularly attractive to the pharmaceutical industry since this approach affords significant process safety advantages and reduces the overall process mass intensity. It is anticipated that this methodology could be readily extended for the synthesis of other useful pharmaceutical, fine chemical and agricultural product intermediates, where other hydroxymethylation processes are currently in operation[1].



Scheme 2: Investigation of original β-keto aryl ester 6 using formic acid as the H+ source was investigated

Case study 2: A stereoselective switch/enantiodivergent approach to the synthesis of isoflavanones

Guiry's research group at the University College Dublin has developed the first catalytic asymmetric synthesis of isoflavanones, an important class of natural products, with potential anti-cancer and anti-microbial therapeutic capabilities. This work includes a highly enantioselective synthesis of isoflavanones of type 3 in excellent enantioselectivities from 76-97% (**Scheme 2**).

A switch in the sense of stereoinduction was observed with substrate 2 when different H+ sources were employed as formic acid afforded the enantiomeric product to that obtained using Meldrum's acid, showing the first example of dual stereocontrol in an asymmetric protonation reaction[2].

Case study 3: The role of solvent–solute interaction in crystal nucleation

Rasmuson and co-workers at the University of Limerick are examining the role of solvents in crystallisations with a special emphasis on examining nucleation in pharmaceutical compounds (**Figure 3**; page 36). APIs examined in this way include risperidone, fenofibrate, tolbutamide, fenoxycarb, danthron and salicylic acid (SA). For example, crystal nucleation of SA in different solvents becomes increasingly more difficult in the order: chloroform, ethyl acetate, acetonitrile, acetone, methanol and acetic acid. Vibration spectroscopy, calorimetric measurements and density functional theory (DFT) calculations were used to reveal the underlying molecular mechanisms suggesting that SA exists predominately as dimers in chloroform, but in the other five solvents there is no clear evidence of dimerisation. An excellent insight into the role of the solvent in nucleation is provided by DFT calculated energy of binding the complete first solvation shell to the SA molecule. The different methods quantitatively reveal a consistent picture whereby the stronger the solvent binds to the API molecule in solution, the slower the nucleation becomes[3].

Case study 4: Automated self-seeding of crystallisations

Control of the final particle size from a crystallisation operation can be highly dependent

on the size of the initial seed particles used to initiate and control the crystal growth. A challenge is to generate seed of a specific size to deliver the required product specification, without recourse to additional costly size reduction operations. Glennon and co-workers, University College Dublin, demonstrated the use of a continuous crystallisation device to automatically generate seed crystals *in situ*. The size and amount of seed particles generated can be precisely controlled by varying the conditions and duration of operation of the device, which is a high-intensity Roughton vortex mixer. In the mixer, a portion of the batch is contacted with an anti-solvent under controlled mixing and flow conditions in steady state, until a sufficient seed bed has been generated. The batch process can then proceed to completion to deliver the required product[4].

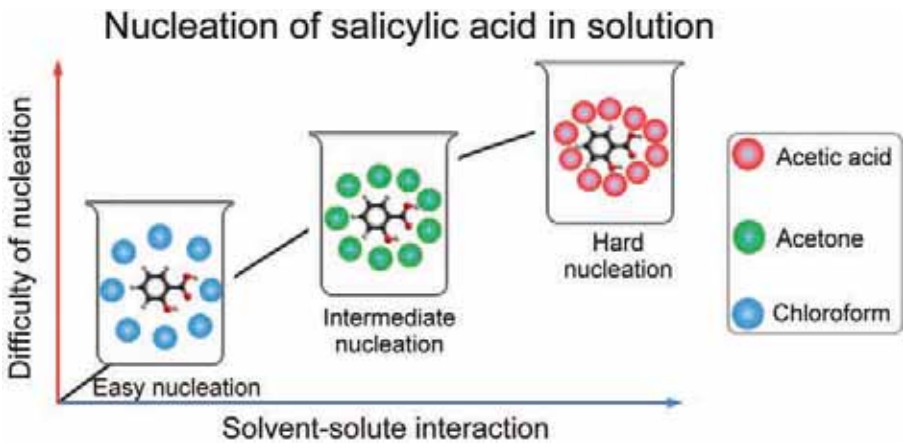


Figure 3: Solvent-solute interaction

Case study 5: PAT in solid oral dose manufacture

To assess the utility of a range of process analytical technology (PAT) tools for solid oral dose manufacture, a collaborative study was undertaken between SSPC academic groups led by Crean from University College Cork, and Walker, of the University of Limerick, in collaboration with SSPC industry partner Innopharma Labs in Dublin. The study applied a combination of novel in-line and off-line PAT tools and laboratory-based techniques to monitor a roller compaction granulation process. Ribbon percentage solid fraction was determined using a GeoPyc® envelope and Accupyc® true density analysers.

The percentage solid fraction measurements correlated with in-line NIR spectroscopy measurements for the Multieye® PAT tool, from Innopharma Labs. Granule particle size analysis was performed by traditional sieve analysis and two PAT tools; the Eyecon® (Innopharma Labs) and the Camsizer® (Retsch) off-line. Granule compressibility was determined using in-die Heckle analysis. Compacts analysed using off-line Raman spectroscopy showed that surface smoothness correlated with crushing strength. The study demonstrated a good relationship between in-line and off-line PAT measurement of key in-process material attributes. The findings support the exploitation of these new in-line PAT methods in monitoring and controlling continuous solid oral dosage form unit operations[5].

Figure 4: Metastable Form II Piracetam (rough dissolving crystal) undergoing a solution mediated polymorphic transformation to FIII (smooth growing crystal)[6]

Case study 6: solution-mediated polymorphic transformations

Hodnett and co-workers, from the University of Limerick, have studied the solution-mediated transformation of the metastable polymorphs of the drugs carbamazepine, piracetam, l-glutamic acid and sulphathiazole. A common feature of these transformations is that the stable form nucleates on the surface of the metastable form; as the stable form grows, the metastable dissolves roughening in the process whereas the growing stable crystal present smooth facets and clearly defined inter-facet angles (**Figure 4**).

The nature of the surface interactions between polymorphs are mostly unclear, but there are rare striking demonstrations of epitaxy between form as such as FII and FIV in sulphathiazole (**Figure 5**; page 37).

This transformation can occur on timescales ranging from seconds to years. A

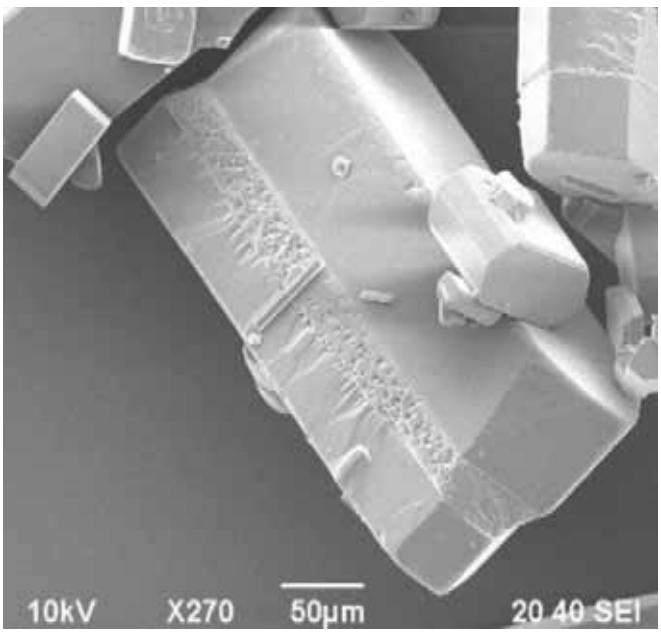


Figure 5: FIV Sulphathiazole epitaxially sandwiched between two layers of FII[7]

general trend was observed; the higher the solubility generated through choice of solvent or temperature, the faster the transformation to the stable form. From a practical perspective, pure metastable forms could be isolated from solvents in which the APIs were poorly soluble whereas the stable forms are readily isolated from solvents in which the APIs are readily soluble.

Conclusion

Trust, a culture of inclusivity and equity, clear goals and objectives, commitment and mutual benefit are just some of the factors that the SSPC fosters within its successful industry-academia and inter-industry collaborations. These successful collaborations enable the SSPC to conduct globally-leading research, which leads the way for next-generation medicines manufacture. The SSPC is continually seeking to develop new national and international academic and industry collaborations. To find out more about the SSPC please go to www.sspc.ie.

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Professor Benjamin K. Hodnett, SSPC's Scientific Director, is the former Dean of the Faculty of Science and Engineering (2007-2013), having previously served as Head of the Department of Chemical and Environmental Sciences (1995-1998), and Director of the Materials and Surface Science Institute (1999-2003) at the University of Limerick. Prof. Hodnett played a lead role in the Aughinish Alumina-UL five year research collaboration, which provided the foundations for the Solid State Pharmaceutical Cluster (2007-2013). Prof. Hodnett's research interests centre on polymorphism in pharmaceutical compounds and active pharmaceutical crystallisation into excipient matrices. Prof. Hodnett has published over 120 papers in refereed journals, which have over 2,800 ISI citations.

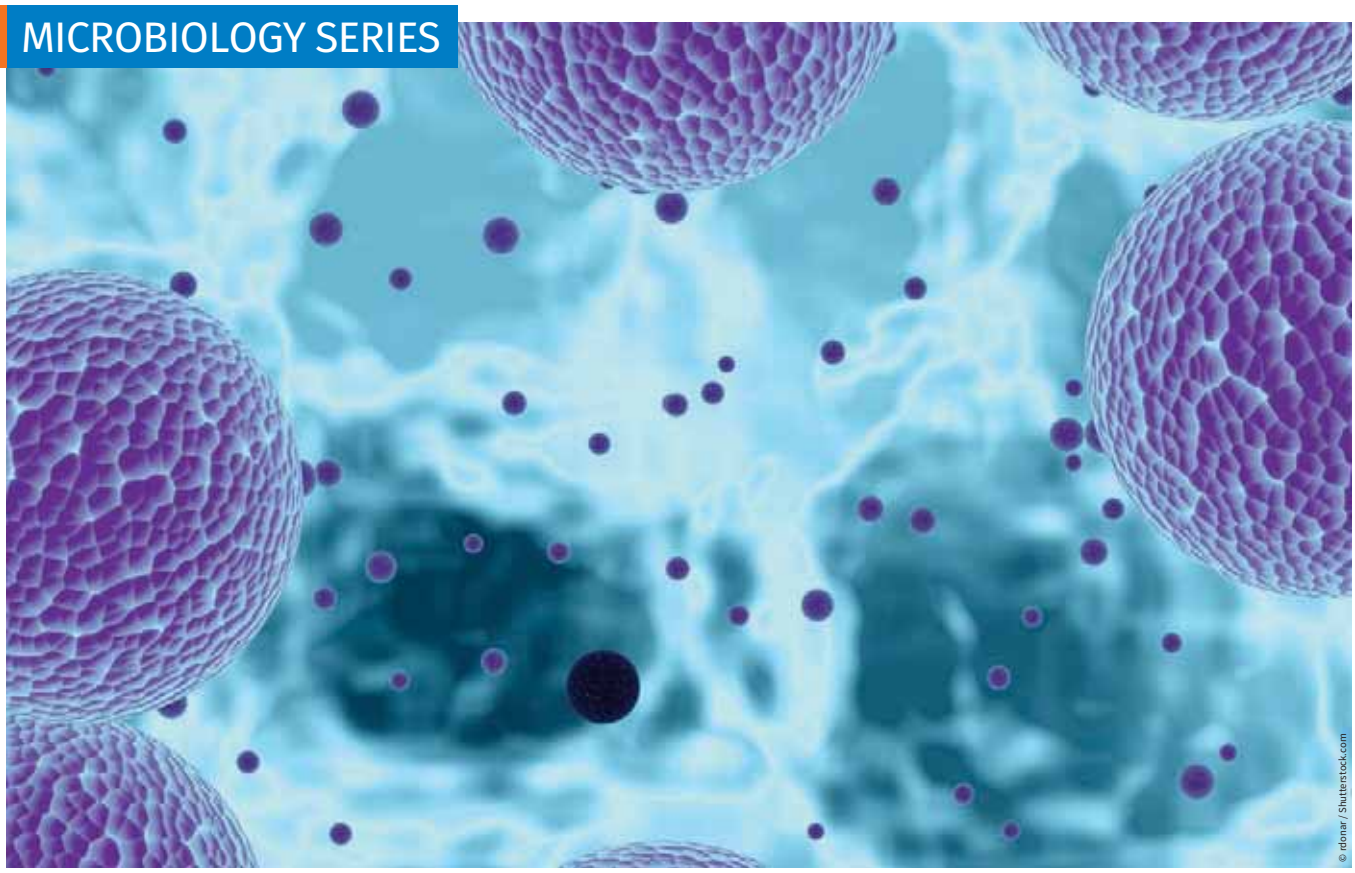
Professor Anita R. Maguire, SSPC Principal Investigator, is Vice President for Research and Innovation at University College Cork (UCC). Prof. Maguire has previously served as Head of the Department of Chemistry (2005-2007) and Head of the School of Pharmacy (2009-10) at UCC. Over the past 23 years at UCC, Prof. Maguire has led an active research team focusing on synthetic organic and pharmaceutical chemistry, which interacts extensively with the pharmaceutical sector in Ireland and internationally. Prof. Maguire is committed to postgraduate education, ensuring the research students gain the skills required to underpin their future careers. Since 1997, 37 PhD students have graduated from Prof. Maguire's research team, most of whom have developed careers within the pharmaceutical industry.

Professor Pat. J. Guiry, SSPC Principal Investigator, is Professor of Synthetic Organic Chemistry at University College Dublin (UCD), and holds a PhD and a BSc (Hons) in Chemistry from UCD. In 2013, Prof. Guiry was elected as a Member of the Royal Irish Academy and in 2014 he was appointed Royal Irish Academy Vice-Chair, Physical, Chemical and Mathematical Sciences, and was awarded the Boyle-Higgins Medal by the Institute of Chemistry of Ireland. Prof. Guiry's research specialises in synthetic organic chemistry, with interests in both the development of asymmetric synthetic methodology through the application of new chiral ligands in homogeneous metal-catalysed transformations, and in the total synthesis of compounds of biological interest.

Professor Ake C. Rasmuson, SSPC Principal Investigator, University of Limerick (UL), is Professor of Chemical Engineering and Industrial Chemistry and holds a MSc and a PhD in Chemical Engineering from the Royal Institute of Technology (KTH) in Stockholm, Sweden. Prof. Rasmuson received a chair at KTH before joining UL in 2008. His main field of research focuses on various aspects of crystallisation and crystallisation processes including: fundamentals of solubility, nucleation, growth and agglomeration; experimental determination of solution thermodynamics and crystallisation kinetics; crystallisation of organic molecules from solution; influence of hydrodynamics and mixing in crystallisation processes and batch crystallisation process design.

Professor Brian Glennon, SSPC Principal Investigator, is a Professor of Chemical and Bioprocess Engineering in University College Dublin (UCD), and is the Director of the Pharmaceutical Process Engineering Research Group, UCD. Prof. Glennon's research interests focus on the development and application of measurement, modelling and control strategies for the design and optimisation of pharmaceutical processes. Prof. Glennon is co-founder of APC Ltd, a process engineering technologies and solutions company which works with the majority of the world's leading pharmaceutical and biopharmaceutical companies.

Dr Abina M. Crean, SSPC Principal Investigator, graduated with a degree in Pharmacy from Robert Gordon's University, Aberdeen, Scotland in 1992, and in 1997 she completed a PhD in Pharmaceutics in the School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin. Since joining the newly-established School of Pharmacy in University College Cork (UCC), Dr Crean has been actively involved in the development of the new research facilities and curriculum. Dr Crean has established a pharmaceutical technology research group in UCC and has supervised a range research projects in the areas of pharmaceutical manufacturing and formulation science. Her research interests focus on the development of medicines through understanding the behaviour of constituent materials.



Rapid methods update: revisions to a United States Pharmacopeia chapter

Michael J. Miller, PhD
Microbiology Consultants, LLC

From 2010 to 2013, *European Pharmaceutical Review* published a very successful series on rapid microbiological methods (RMM) that included hot topics such as the European Medicines Agency's and US Food and Drug Administration's expectations, implementation strategies, scientific principles behind the technologies and validation. The final article of the 2012 series introduced the United States Pharmacopeia's (USP's) plan to revise informational chapter <1223>, *Validation of Alternative Microbiological Methods*.[1] On June 1, 2015, a substantially modified chapter <1223> was published in the second supplement to USP38/NF33 with an official date of 1st December 2015. Because the original USP chapter was published almost 10 years ago, this article will review the most notable changes and compare them with what is recommended in the Parenteral Drug Association (PDA) Technical Report Number 33 and the proposed revision to European Pharmacopoeia (Ph. Eur.) chapter 5.1.6.

A reason for change

In 2012, the scientific community learned of the USP Microbiology Expert Committee's desire to significantly revise the 2006 version of USP <1223>. The committee envisioned a chapter that would offer greater flexibility in accommodating future alternative microbiological methods and be less prescriptive for a wide range of stakeholders, especially those that require novel technologies for the rapid release of specialised products (e.g., cellular therapy and compounded medicines). For these reasons, the committee developed an improved chapter with enhanced guidance on equipment qualification, analytical method validation and suitability, user requirements and better explained how to demonstrate equivalence or non-inferiority to the compendial methods[2].

At about the same time the USP started its revision process, the European Directorate for the Quality of Medicines initiated a program to enhance Ph. Eur. chapter 5.1.6, *Alternative Methods for Control of*

Microbiological Quality. Earlier this year, a draft revision was published in Pharmeuropa for public review and comment[3]. The chapter was essentially completely rewritten to take into account new technological developments, the impact of process analytical technology (PAT) and real-life examples of how companies have validated alternative and rapid methods since the publication of the original chapter in 2006. Additionally, the proposed validation sections have been restructured to provide details on what are called primary validation and validation for the intended use associated with qualitative, quantitative and identification methods. And for those who recall the chapter's appendix of an example protocol based on bioluminescence, this has been removed in favour of three new case studies employing an adenosine triphosphate-based rapid sterility test using membrane filtration, a quantitative test for the enumeration of microorganisms via solid phase cytometry (i.e., viability staining followed by laser excitation) and a PCR-based microbial identification technique.

Separately and in 2013, the PDA published its long-awaited revision of Technical Report Number 33 (TR33)[4]. The new document, *Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods*, provides significantly enhanced guidance on a number of topics, including, but not limited to, risk assessments, user requirements, supplier considerations, implementation and technology transfer strategies, global regulatory expectations, equipment and software qualification, method suitability testing, the demonstration of equivalence, use of statistics and an updated technology overview.

All three guidance documents have been successfully utilised by multinational firms to support their alternative and rapid method validation and implementation approaches. However, recent changes to USP <1223> warrant the need to take a closer look at this revised chapter and understand the similarities and differences that exist with PDA TR33 as well as the proposed modifications to Ph. Eur. 5.1.6.

Instrument qualification and validation

The new USP chapter provides guidance on how to qualify equipment and instrumentation associated with an alternative microbiological method and specifically references another chapter, USP <1058>, *Analytical Instrument Qualification*, for additional details[5]. PDA TR33 and Ph. Eur. 5.1.6 provide similar recommendations for instrument qualification.

After the instrumentation has been qualified, USP <1223> recommends using a standardised panel of microorganisms against specific validation criteria in order to validate the analytical technique. At this stage, actual product is not used and nor is there a comparison to a compendial method. Similar procedures are recommended by PDA TR33 and Ph. Eur. 5.1.6; however, there are some differences worth mentioning. For example, only USP <1223> identifies repeatability as a separate validation criterion, which is actually a subset of *precision* in all three guidance documents. Additionally, the term *ruggedness* is not found in Ph. Eur. 5.1.6 but the same concept (as presented in USP <1223> and TR33) is addressed in chapter 5.1.6 as *intermediate precision*. *Limit of detection* is also not identified as a validation criterion for quantitative methods in Ph. Eur. 5.1.6.

Table 1: Validation criteria for quantitative (enumerative) methods

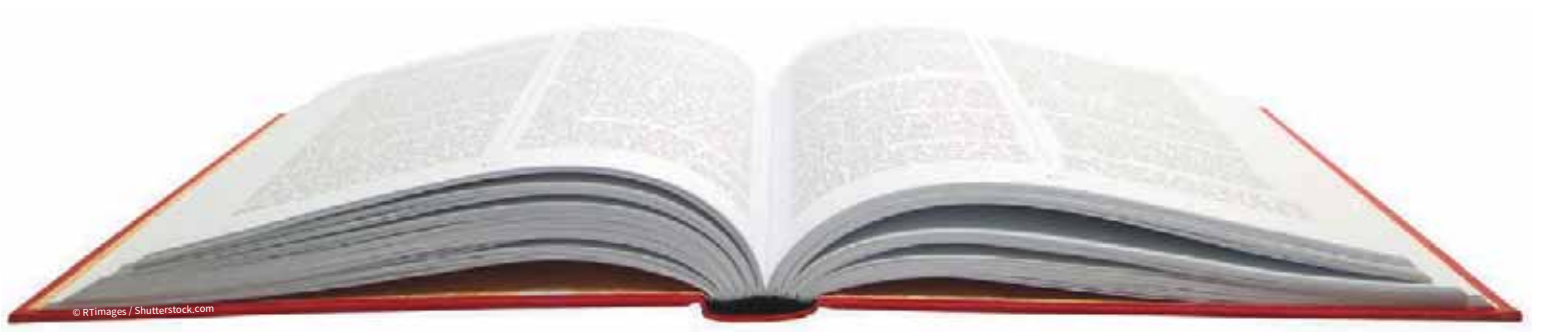
Validation Criteria (as defined in USP <1223>)	USP <1223> (2015)	PDA TR33 (2013)	Proposed Ph. Eur. 5.1.6
Accuracy	✔	✔	✔
Precision	✔	✔	✔
Specificity	✔	✔	✔
Limit of detection	✔	✔	
Limit of quantification	✔	✔	✔
Linearity	✔	✔	✔
Operation (dynamic) range	✔	✔	✔
Ruggedness	✔	✔	Addressed as 'intermediate precision'
Robustness	✔	✔	✔
Repeatability	✔	Addressed under Precision	Addressed under Precision
Equivalency	✔ (demonstrated in the *absence* of product)	✔ (demonstrated *with* product)	✔ (demonstrated *with* product)

Table 1 and Table 2 (page 41) compare each of the validation criteria to be assessed for a quantitative and qualitative method, respectively.

Equivalency changes

A significant difference exists between USP <1223> and the other two documents in terms of *equivalence*. USP <1223> defines equivalency as "when the test results from two procedures are sufficiently close for the intended use of the procedures. Demonstration of equivalence requires a pre-specified measure of how similar the test results need to be." It can also be understood that the demonstration of equivalence in USP <1223> is conducted in the absence of actual product or test samples. Essentially, a panel of relevant microorganisms is used to compare the alternative method with the compendial method. Conversely, product is separately utilised during *method suitability* studies, which is defined in USP <1223> as a "demonstration of lack of enhancement or inhibition by the product on the signal generated by the method." A more detailed discussion of USP's method suitability strategy is presented in a subsequent section within this article.

PDA TR33 and Ph. Eur. 5.1.6 also utilise actual product during method suitability studies; however, the product is also required when



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demonstrating equivalency. TR33 and chapter 5.1.6 define equivalency as follows:

- TR33: "Equivalence or comparative testing involves the use of actual product and other sample matrices that will be routinely tested using the alternative or rapid method once it is validated and implemented."
- Chapter 5.1.6: "A direct approach to demonstrating the equivalence of two qualitative methods would be to run them side-by-side and determine the degree to which the method under evaluation leads to the same pass/fail result as the pharmacopoeial method. This parallel testing shall be performed based on a pre-specified period of time or number of samples."

Considering a practical strategy for demonstrating equivalence, TR33 suggests employing similar procedures and data analyses to those previously utilised for assessing validation criteria with standardised cultures, such as accuracy, precision, limit of quantification, limit of detection, linearity or range. For a qualitative method, chapter 5.1.6 advocates demonstrating the same pass/fail result as a qualitative compendial method. For a quantitative method, chapter 5.1.6 states that if the result of the alternative method can be expressed as a number of colony-forming units (CFU) per weight or per volume, statistical analysis of the results shall demonstrate the results of the alternative method are at least equivalent to those of the compendial method. Otherwise, if the result of the alternative method cannot be expressed as a number of CFU, then statistical analysis shall demonstrate the results of the alternative method are at least equivalent to those of the compendial method.

USP <1223> describes four options that may be used to demonstrate an alternative method is equivalent to a compendial method. These options are based on a 2009 stimuli article published in USP's *Pharmacopeial Forum*[6]. Three options (recognised as *performance equivalence*, *results equivalence* and *decision equivalence*) allow for the direct comparison with a compendial method. Multiple characteristics are compared when using the performance equivalence option and this is the strategy synonymous with the recommendations in PDA TR33 and the proposed Ph. Eur. 5.1.6. Alternatively, a single characteristic is compared when using the results or decision equivalence options. Interestingly, a fourth option (identified as the *acceptable procedure*) does not require a comparison between an alternative and a compendial method; only a minimum performance or acceptance condition is required. USP provides examples of how to conduct studies based on the results equivalence option (using a quantitative method) and the decision equivalence option (using a qualitative method); however, there is no specific guidance on when each of the four options would be appropriate for use.

> "USP <1223> has undergone a significant revision with the intent of offering stakeholders greater flexibility in validating alternative and rapid microbiological methods"

Method suitability

USP <1223> teaches that for each new product to be evaluated with a validated alternative method, suitability testing should be performed using the same sample preparation, quantity and number of units appropriate for the product and the required level of assay sensitivity. Furthermore, method suitability testing should demonstrate the alternative signal is not quenched or increased in the presence of the product being evaluated. Essentially, the user is demonstrating the product is compatible in the validated alternative method. Accuracy and precision are required to be evaluated for quantitative methods and the recovery of microorganisms according to USP <62>, <71> and <1227> is demonstrated for qualitative methods. And since method suitability is generally the only time actual product is used within USP's validation approach, this phase would be similar to what is termed *equivalence* testing in TR33 and chapter 5.1.6.

USP also states that once an alternative method has been shown to be equivalent to a compendial test for a single product, there is no need to repeat the equivalency parameters for every new product (i.e., only method suitability is to be verified for each new product). For the purpose of clarity, this author assumes the 'equivalency parameters' USP is referring to are the accuracy, precision and detection studies as previously mentioned, considering actual product or test samples are not necessarily identified as being used during *equivalence* testing. However, if the USP intended for the product to be tested according to one of the four equivalence options, in addition to what is described under method suitability, this should have been unmistakably stipulated in the revised chapter.

In PDA TR33 and the proposed Ph. Eur. 5.1.6, method suitability confirms the compatibility of a product or test sample in the alternative procedure:

- TR33: "To demonstrate that the new method is compatible with specific product or sample matrices that will be routinely assayed, each material should be evaluated for the potential to produce interfering or abnormal results, such as false positives (e.g., a positive result when no viable microorganisms are present in the test sample) or false negatives (e.g., a negative result when microorganisms are present in the test sample). This may also include evaluating whether cellular debris, dead microorganisms or mammalian cell cultures have any impact on the ability of the new method and accompanying system to operate as it is intended to."

Table 2: Validation criteria for qualitative (presence/absence) methods

Validation Criteria (as defined in USP <1223>)	USP <1223> (2015)	PDA TR33 (2013)	Proposed Ph. Eur. 5.1.6
Accuracy			
Precision			
Specificity	✔	✔	✔
Limit of detection	✔	✔	✔
Limit of quantification			
Linearity			
Operation (dynamic) range			
Ruggedness	✔	✔	Addressed as 'intermediate precision'
Robustness	✔	✔	✔
Repeatability	✔		
Equivalency	✔ (demonstrated in the *absence* of product)	✔ (demonstrated *with* product)	✔ (demonstrated *with* product)

- Chapter 5.1.6: "The alternative method must be applied according to the specified procedure and with the samples to be analysed under the responsibility of the user. The method must be shown to give comparable results as characterised in the model system used by the supplier. Compatibility of the response with the product prepared as needed by the user, evaluated using pharmacopoeial test strains."

Therefore, the guidance provided in TR33 and chapter 5.1.6 focuses method suitability on the potential for a product or test sample to generate an incorrect response (i.e., false positives, false negatives or interference). Although no specific criteria are identified by TR33 when conducting method suitability studies, similar strategies as described above for the validation criteria may be employed (e.g., evaluating accuracy and precision for quantitative methods; limit of detection or inclusivity/exclusivity for qualitative methods). For suitability testing, the proposed revision to Ph. Eur. 5.1.6 recommends assessing the detection limit for qualitative methods and accuracy, limit of quantification and linearity for quantitative methods.

It is important to remind the reader that the PDA and Ph. Eur. strategies outlined in the prior paragraph apply to method suitability testing and are not a demonstration of equivalence, even though actual product or test sample is used in both analyses. To reiterate, method

Table 3: Comparison of Validation Phases

Validation Phases (as defined in USP <1223>)	**USP <1223>**	**PDA TR33**	**Proposed Ph. Eur. 5.1.6**
Instrument qualification	■ Refer to USP <1058>	■ Similar strategy as USP	■ Similar strategy as USP
Validation of the analytical method	■ Refer to Table 1, 2 ■ Adds 'repeatability' ■ Use panel of microorganisms ■ No comparison with compendial method ■ No product	■ Refer to Table 1, 2 ■ Use panel of microorganisms ■ Includes comparison with compendial method ■ No product	■ Refer to Table 1, 2 ■ 'Primary validation' performed by supplier ■ 'Verification of primary validation' performed by user ■ No 'Ruggedness' but same as 'intermediate precision' ■ No 'Limit of Detection' for quantitative methods ■ Use panel of microorganisms ■ Includes comparison with compendial method ■ No product
Demonstration of equivalence	■ 'Acceptable procedure' (not compared with compendial method; meets minimum performance) ■ 'Performance equivalence' (multiple characteristics) ■ 'Results equivalence' (single characteristic; quantitative) ■ 'Decision equivalence' (single characteristic; qualitative) ■ Compared with compendial method ■ No product	■ Use similar validation criteria as in validation of the analytical method, as appropriate for the method (qualitative or quantitative) ■ With product	■ Qualitative method (use same pass/fail result as compendial method) ■ Quantitative method (statistical analysis shall be at least equivalent to compendial method) ■ With product
Method suitability	■ With product ■ Assess compatibility with method ■ Quantitative (test accuracy, precision) ■ Qualitative (test recovery according to USP <62>, <71>, <1227>	■ With product ■ Assess compatibility with method (false positive, false negative) ■ Can use criteria as appropriate for the method (qualitative or quantitative)	■ With product ■ Assess compatibility with method (false positive, false negative) ■ Qualitative method (test limit of detection) ■ Quantitative method (test accuracy, limit of quantification, linearity)

suitability evaluates incompatibilities with a test material when examined in an alternative method; equivalence demonstrates a statistically similar, non-inferior or better response between an alternative and a current/compendial method in the presence of a test material. Therefore, TR33 and chapter 5.1.6 teach that method suitability and equivalence are evaluated on their own.

Based on this comparative review of the new USP <1223>, PDA TR33 and the proposed Ph. Eur. 5.1.6, the reader may be somewhat confused in attempting to understand the similarities and differences between the three documents. To simplify what each is guidance is communicating, **Table 3** (page 42) provides a streamlined summary of what has been examined in this article.

Summary

USP <1223> has undergone a significant revision with the intent of offering stakeholders greater flexibility in validating alternative and rapid microbiological methods. Some of the changes are comparable to the strategies presented in PDA TR33 and the proposed Ph. Eur. chapter 5.1.6 while others are appreciably different. Therefore, end-users should thoroughly review each of the validation guidance documents to determine which ones offer the most appropriate options for a successful validation program. The reader is also encouraged to visit http://rapidmicromethods.com for additional guidance on qualification strategies and regulatory expectations during the validation and implementation of these novel technologies.

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Dr. Michael J. Miller is an internationally recognised microbiologist and subject matter expert in pharmaceutical microbiology and the design, validation and implementation of rapid microbiological methods. He is currently the President of Microbiology Consultants, LLC (http://microbiologyconsultants.com) and owner of http://rapidmicromethods.com, a website dedicated to the advancement of rapid methods.

For more than 25 years, he has held numerous R&D, manufacturing, quality, business development and executive leadership roles at multinational firms such as Johnson & Johnson, Eli Lilly and Company and Bausch & Lomb. In his current role, Dr. Miller consults with multinational companies in providing technical, quality, regulatory and training solutions in support of rapid methods, sterile and non-sterile pharmaceutical manufacturing, contamination control, isolator technology, environmental monitoring, sterilisation and antimicrobial effectiveness.

Dr. Miller has authored more than 100 technical publications and presentations. He currently serves on the editorial and scientific review board for *European Pharmaceutical Review*. Dr. Miller holds a PhD in Microbiology and Biochemistry from Georgia State University (GSU), a BA in Anthropology and Sociology from Hobart College, and is currently an adjunct professor at GSU.

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Challenges with NIR reflectance measurements of solid pharmaceuticals

Dimuthu Jayawickrama, Gary McGeorge, Tim Stevens and Douglas Both
Bristol-Myers Squibb

Near-infrared (NIR) is a well-established spectroscopic tool for analysing pharmaceutical solids, with attractive features being its non-destructive nature and ability to evaluate solids without physical sampling. Although it lacks well resolved peaks, advances in chemometrics have helped to establish NIR as an excellent quantitative and qualitative spectroscopic method. As a result, it has been adopted as a vital analytical means for developing and commercialising pharmaceutical products. In addition, many NIR applications have been developed as real-time analytics (RTA) and to support process analytical technology (PAT).[1-3] NIR reflectance measurement, a measurement mode that relies on NIR signal recordings reflecting back to the NIR instrument after interacting with a sample (diffuse reflectance), is widely used in solid analysis. However, there are challenges associated with these measurements. This article discusses three such challenges: determination of NIR interrogation volume/amount and the nature of what is being analysed, and the impact of window fouling during real-time NIR measurements.

NIR interrogation volume

In pharmaceutical drug product analysis, the analytical sample is generally correlated to the final unit dosage size. For example, in tablet potency measurements, a tablet is completely dissolved and a portion of the homogenous solution is analysed. This guarantees the analysis of a sample (now in liquid phase) that is representative of the tablet. As a result, high performance liquid chromatography (HPLC) analysis of solids is trivial and straightforward. However, evaluating solids using NIR reflectance mode raises an important question: how much and what do we analyse?

The behaviour of NIR light penetration into solids during reflectance measurements has been well documented[4] and a theoretical discussion on diffuse reflectance has been presented by Dahm and Dahm.[5] **Figure 1a** illustrates a decreasing NIR polystyrene signal with increasing thickness of a micro crystalline cellulose (MCC) powder layer placed on top of a polystyrene disk (experimental details are similar to those given in [4]). NIR intensity of the polystyrene peak decreases exponentially with the MCC powder thickness. As shown in this example, a 450µm layer of MCC powder is responsible for diminishing 95% of the polystyrene NIR signal. Such a thin analytical layer is not suitable for representing a whole sample (i.e., powder blends, granules or tablets). So what can be done to overcome this intrinsic behaviour of diffuse reflectance light?

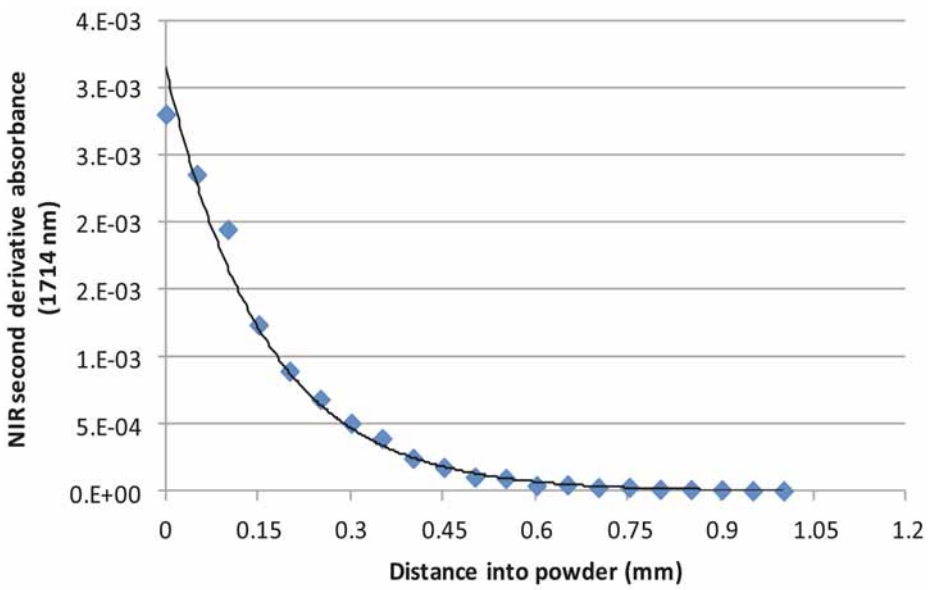

Figure 1a: Polystyrene NIR intensity (second derivative absorbance) as a function of MCC powder thickness

One straightforward approach is to utilise an NIR transmission measurement mode where only the NIR light passing through a sample is detected. The light transmitting through the sample will interact with it to a greater extent than that of a comparable diffuse reflectance measurement. This approach is typically used when analysing tablets, however, it is not practical with online/inline NIR measurements. NIR instrument hardware modification can assist in increasing the NIR sampling volume. For example, the NIR light penetration into the sample can be increased using NIR spectrometers with high intensity light sources.[6] Higher intensity light penetrates deeper into solid and increases overall NIR sampling volume.

> "The relationship between sampling amount and illumination radius can be used as a guidance to select the appropriate NIR instrumentation for a particular analysis"

It is also possible to manipulate NIR light illumination spot size to change the NIR sampling volume. The NIR sampling amount increases exponentially with the NIR illumination spot radius (**Figure 1b**). The relationship between sampling amount and illumination radius can be used as a guidance to select the appropriate NIR instrumentation for a particular analysis.[7] It has been recommended that analysis of a blend sample size of one-to-three of the dosage size is deemed as suitable to represent blend homogeneity.[8] As shown (**Figure 1b**), an NIR instrument with an illumination spot radius of 20mm provides an NIR sampling amount of 240mg, which is within two-to-three times the 100mg dosage size. Therefore, this NIR instrumentation is suitable to determine blend uniformity of blends that produce 100mg tablets. The light penetration depth and therefore the NIR sampling volume also depends on wavelength[4], so NIR sampling volume should be discussed with respect to the wavelength of interest. This raises a challenge when working in the multivariate environment where more than a single wavelength is involved in the volume of interrogation.

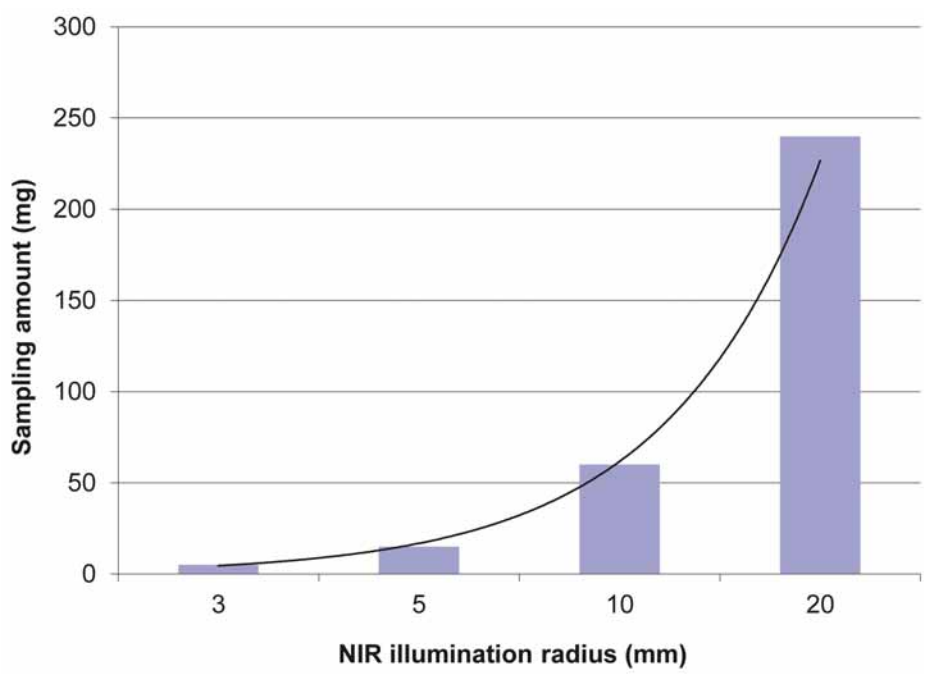

Figure 1b: NIR sampling amount vs. NIR illumination radius

Representation of the sample by NIR

The NIR analytical volume and the picture provided by NIR go hand in hand. **Figure 2** (page 48) illustrates the analysis of a hypothetical sample by three different NIR illumination spot sizes with a single NIR instrument. This sample consists of equal sized red and blue particles arranged in a single line. The smallest illumination spot size is comparable with the size of a single particle. A reflected NIR spectrum

recorded under the illustrated condition is dominated by the characteristic NIR spectrum of the red particle. However, in analytically representing the sample as a pure red particle, this spectrum does not represent the actual sample.

An NIR spectrum recorded with a wider illumination spot size that is twice the size of a particle may represent the sample as a mixture of red and blue particles. However, the widest illumination spot covers the entire sample and an NIR spectrum recorded with this shows the sample to be dominated by blue particles. This example demonstrates that the right adjustment of an appropriate NIR instrument is necessary to obtain correct information about the analyte. For example, knowledge about the particle size of the analytes and the pixel size information of the NIR imaging system is necessary to understand and interpret NIR imaging data.

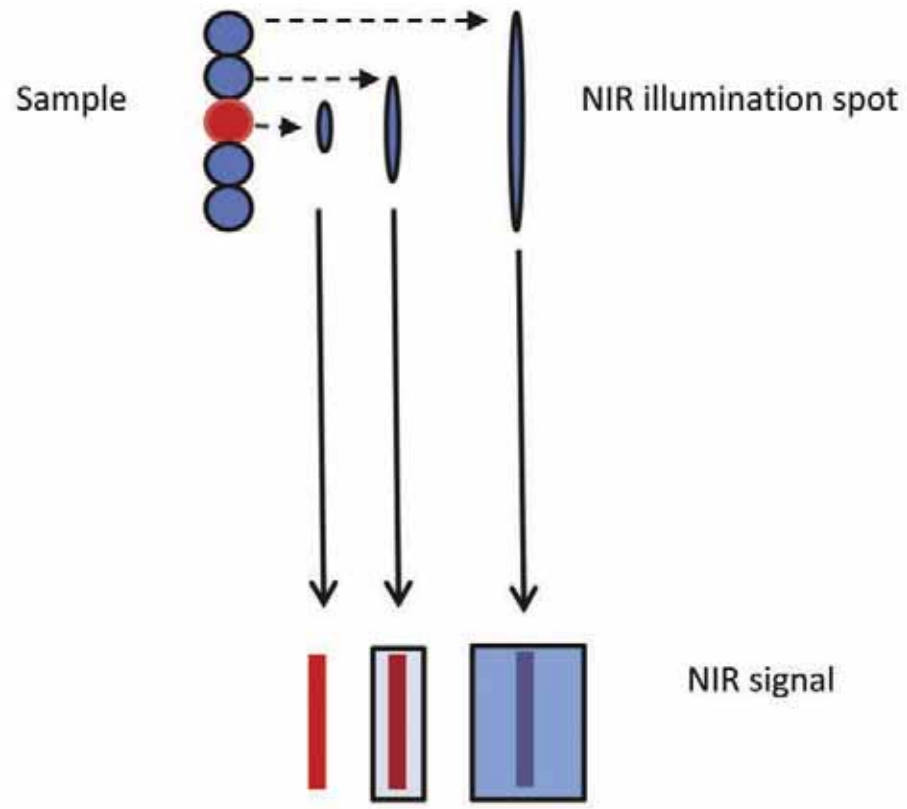

Figure 2: Illustration of NIR signal produced by different NIR illumination spots

Effect of window fouling on NIR

In real-time NIR process monitoring, the NIR probes are housed within the processing equipment and in contact with the sample directly (contact probes) or indirectly (non-contact probes). One challenge in real-time monitoring with contact probes is NIR detection window fouling (coating). This occurs because of the adherence of the powder material on the detection window. As a result, NIR detects the same materials over and over again. This leads to NIR recording inaccurate quantitative

and qualitative information about the bulk material.[9]

Non-contact NIR probes are widely used in blending, fluid blend processes, tablet feeder monitoring, etc. Most of the non-contact NIR technology is based on acquiring NIR data through a detection window of the processing equipment and keeping the NIR instrument outside of the processing equipment. NIR data are only acquired when the sample is in front of the detection window. At each NIR measurement, the sample in the front of the detection window should be refreshed with the progress of the manufacturing process to record a meaningful NIR value that is representative of the bulk of the sample. Unfortunately, the detection window can also be fouled during powder processing. Figure 3a depicts online NIR blend monitoring as a function of blender revolutions. The NIR profile shows typical macro and micro mixing followed by an atypical increase in potency beyond 100% potency (target potency) after 125 revolutions. Afterwards, the potency remains around 130-135% for the rest of the blending time. This behaviour can be due to actual high potency caused by weighing errors (i.e., low excipients or high active pharmaceutical ingredient [API]), calibration error/robustness issues and/or window coating by blending materials rich with API.

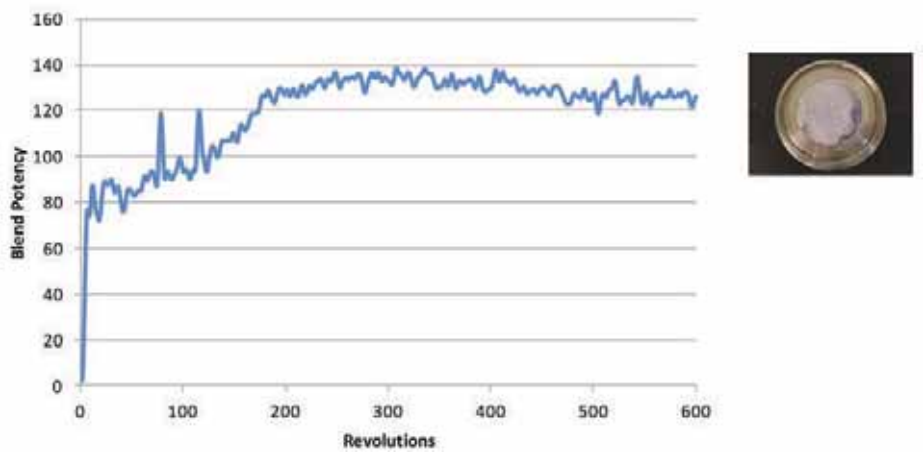

Figure 3a: An NIR blending profile showing high potency due to window coating of API

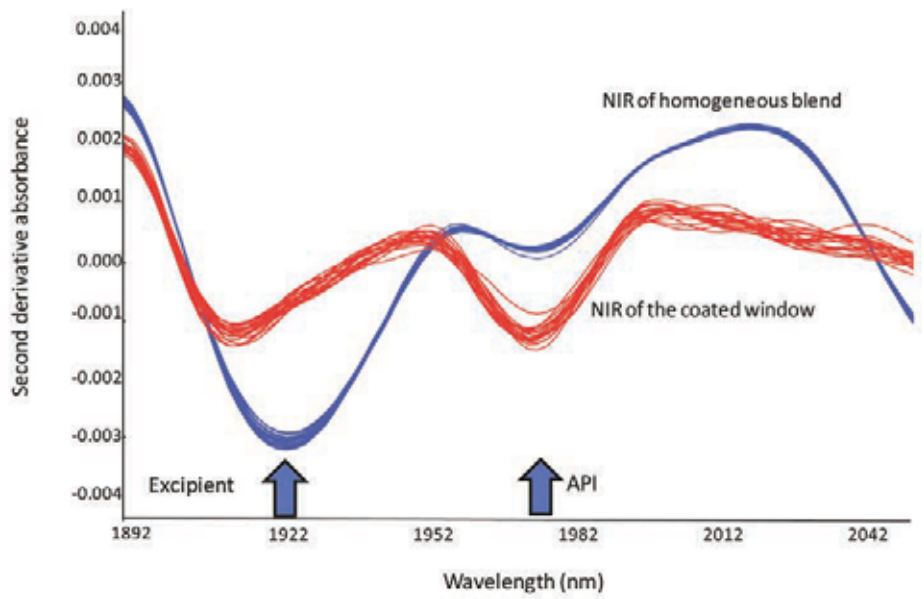

Figure 3b: NIR spectral comparison of a homogeneous blend and a powder coated window

A photograph (inset in Figure 3a) of the NIR detection window at the completion of the blending process shows a significant build-up of powder on the window. The NIR spectral comparison of a typical homogeneous blend and this coated window is shown in Figure 3b. The NIR spectrum of the coated window shows the presence of an elevated amount of API compared to a homogeneous blend at the 100% target potency. These observations confirm that fouling of the detection window with powder containing predominately API caused the unchanging high potency value during online NIR measurement. Thus, although window fouling is undesirable for NIR monitoring, it can provide valuable insight into powder properties and behaviour under processing conditions.

> “Although window fouling is undesirable for NIR monitoring, it can provide valuable insight into powder properties and behaviour under processing conditions”

But how can one avoid window coating? There may be a number of reasons behind its occurrence, including the properties of processing materials under the processing condition used and NIR detection window surface characteristics. Often, the powder properties of materials and processing conditions are fixed and therefore cannot be tweaked to avoid window fouling. One option is to manipulate the powder contact surface of the window. The material fouling the window can be removed after each measurement through engineering solutions such as using a wiper or air purging system. The window surface can also be modified by coating the powder contact surface with a material to avoid/minimise fouling.

Figure 4a (page 50) shows an increase in potency with successive measurements during blending, which implicates window fouling. However, once the window is coated with a small amount of magnesium stearate (MgSt) powder, the blend coating on the window decreases (Figure 4b; page 50). The degree of window fouling depends on the amount of MgSt applied as shown in the Figure. The window fouling gradually decreases when increasing amounts of MgSt are applied and reaches a plateau as indicated by unchanging potency values. A permanent chemically-bonded coating window surface may also be suitable to avoid window fouling, however, the coated material can be stripped off with usage and time and may have a

negative impact on the measurement and the material being analysed. The example given here shows the MgSt's ability to reduce the propensity of materials sticking.

Conclusion

The intrinsic nature of the NIR diffuse reflectance measurement and the NIR instrumentation dictates the qualitative and quantitative information extracted from solid samples. The NIR sampling amount can be estimated/calculated with the knowledge of the NIR light penetration depth and NIR light absorbing properties of the sample. Knowledge about NIR hardware (i.e., illumination spot size) is also required when determining the NIR penetration depth. The material coating of the NIR detection window during online/inline measurements can be avoided or minimised by modifying the powder contact surface of the detection window.

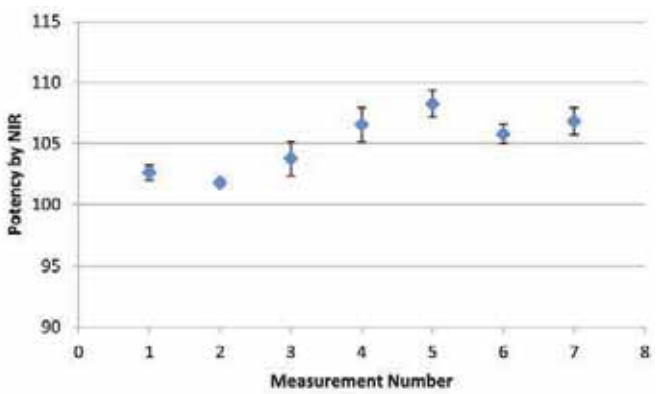

Figure 4a: Effect on potency increase (beyond target value of 100) with each measurement

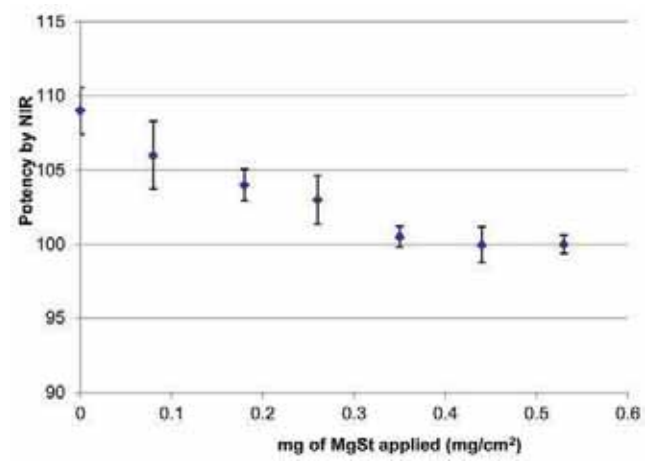

Figure 4b: Effect of MgSt on the NIR detection on potency

Dimuthu Jayawickrama is a Senior Research Investigator in the Department of Analytical and Bioanalytical at Bristol-Myers Squibb (BMS) in the US. He is a core member of an interdisciplinary team which is responsible for developing measurement tools based on real-time analytics/statistics for process monitoring, understanding and controlling of drug product processes. Dimuthu earned his BS in Chemistry from the University of Peradeniya, Sri Lanka and PhD in Chemistry from the University of Kansas, US. Before joining BMS, he worked as a postdoctoral researcher at the University of Illinois, Urbana-Champaign, US, and as a Senior Lecturer in the Department of Chemistry at the University of Peradeniya in Sri Lanka.

Gary McGeorge received his PhD in solid-state NMR spectroscopy (ssNMR) at Durham University in England. He then held a Post Doctoral position at the University of Utah using ssNMR for the analysis of pharmaceutical and natural products. Gary is a Sr. Principal Scientist within the R&D organisation at Bristol-Myers Squibb. He has been responsible for the analysis of polymorphs within solid pharmaceuticals using various spectroscopic tools. Most recently he became one of the technical leads for development and implementation of process analytical technology for drugs products. He is on the editorial advisory board for the *Journal of Pharmaceutical Sciences* and *Spectroscopy* magazine.

Tim Stevens received his PhD in Electrical Engineering from the Pennsylvania State University, studying electro-optic instrumentation and active laser remote sensing where he developed instrumentation techniques operating from the UV to NIR portions of the electromagnetic spectrum. His research focused on application of these instruments and techniques to atmospheric remote sensing. Before joining Bristol-Myers Squibb (BMS) Tim worked at SciTec of TRW developing electro-optic measurement and sensor systems for the military, including UV and NIR missile tracking systems, missile countermeasures, laser mine detection systems and optical receivers for foreign laser range finders and bomb designators. Tim developed complete system models using first principles, system noise and measurement variables to aid in instrument design, calibration and to predict performance. Tim has been with BMS for over 10 years and is currently a Sr. Principal Scientist working in Analytical and Bio-analytical Development (ABD) where he leads a group developing real-time analytical technologies for drug products. Tim's focus is on in-process measurements and controls to better understand drug product unit operations and support new product development control strategies including real-time release strategies.

Douglas Both is a Research Fellow at Bristol-Myers Squibb with more than 30 years of pharmaceutical R&D development experience. Doug leads the Biopharmaceutical Process Analytical Science Development (BPAS) Group in New Brunswick, New Jersey. His group's role has been to support drug product scale-up with respect to non-invasive analytics to enhance drug product and process understanding and process control. Doug holds a BS and MA in Chemistry from Montclair State University. He is the author and co-author of more than 14 publications and 20 presentations at national and international conferences. He is on the Editorial Advisory Board of *Journal of Pharmaceutical Innovation*. Doug can be reached at douglas.both@bms.com

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Application of NIR spectroscopy in linking velocity profiles of a binary granular system

Oumaima Chaib, Nicolas Abatzoglou and Ryan Gosselin
Université de Sherbrooke

Previous publications have shown that residence time distribution (RTD) of gravity-driven free flowing powders through hoppers can successfully be measured using a near-infrared spectroscopy (NIRS)-based methodology. The experimental setup consists of a lab-scale hopper equipped with an automated high-speed gate-valve (guillotine). RTD can then be used to evaluate radial velocity profiles. An extended semi-empirical mathematical model based on a laminar flow pattern is presented herein. It considers the effects of powder rheology via the internal (particle-particle) and external (particle-wall) angles of friction measured in shear cells. These tests were performed with a cylindrical hopper. The results show that when both angles of friction are known and used as model constants, a two-parameter model, which expresses the relative importance of these two angles of friction and the deviation from classical fluids laminar flow, can be used to predict with satisfactory precision such granular flow velocity profiles.

Introduction

Granular materials are known for their complexity since they combine characteristics approaching those of solids (hardness, elasticity, friction), liquids (free flowing) and gas (compressibility)[1]. In general, their behaviour is very difficult to predict because of various phenomena occurring during flow, such as agglomeration and segregation. However, knowledge of their flow properties is of the utmost importance when developing manufacturing processes and handling procedures. It is generally known that the hopper material and geometrical characteristics, operating conditions and powder characteristics all affect the granular flow[2].

Since the 1950s, several works have proposed mathematical models

seeking to quantify the flow of granular materials through hoppers and silos, focused on the flow velocity profile[3]. Nedderman and Tuzun[4] proposed a mathematical model based on the fact that the radial velocity is proportional to the downward velocity gradient (i.e., the shear rate) with an inertial term. Meanwhile, Moreera *et al.*[5] predicted the velocity distribution of cohesionless materials based on the method of characteristics, and Abdulmobeen *et al.*[6] and Ketterhagen *et al.*[2] predicted the flow of cohesive powders as a function of the cohesion parameters and hopper angle. These works were in good agreement with experiments.

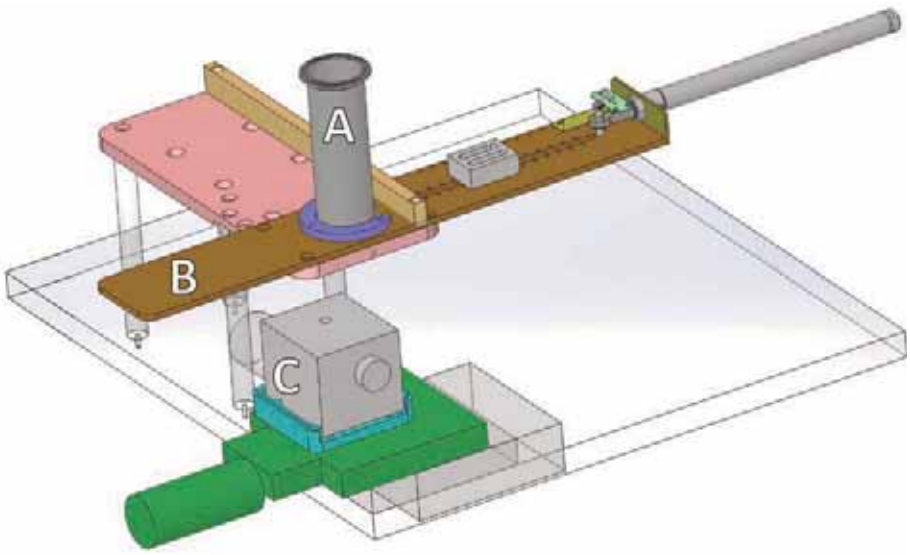

Figure 1: Equipment set-up illustrating cylindrical hopper, NIR probe and guillotine

Recently, Abatzoglou and Simard[7,8] proposed an experimental method inspired by pulse injections used in reactor engineering to predict the velocity profiles of granular mixtures; this approach relies on the experimental estimation of the mean residence time distribution and its variance. The method consists of introducing a discrete powder pulse inside the bed of another powder, then calculating the pulsed powder concentration at the hopper outlet over time. To measure this concentration, our research group[9,10] developed a new methodology based on NIR to simultaneously measure both radial and axial profiles. An NIR probe was placed at the outlet of the hopper while the flow was sliced using a high-speed gate-valve (guillotine). This approach seeks to monitor radial concentrations of the compounds over time of flow and makes it possible to estimate the RTD of the hopper for every particular binary granular flow.

> “Hopper material and geometrical characteristics, operating conditions and powder characteristics all affect the granular flow”

Inspired by the pulse injection method explained in the previous paragraph and the flow velocity profile of classical fluids, the aim of the present work is to experimentally measure the velocity profile of a binary powder mixture using NIR spectroscopy and to prove the feasibility of using a two-parameter phenomenological mathematical model based on the

velocity profile equation of classical laminar fluids to predict efficiently the experimental observations. Said phenomenological model was first proposed by Abatzoglou[10] and relies on the assumptions that powder-wall angle of friction ($\varphi_{p\text{-}w}$) and the powder internal angle of friction ($\theta_{p\text{-}p}$) could be used in taking into account these interactions, the same way viscosity influences classical fluid flow patterns.

Materials and Methods

Materials

Lactose and microcrystalline cellulose (MCC), two common pharmaceutical excipients widely present in oral solid dosage forms, were used in this study:

- MCC: AVICEL PH 101, FCM Biopolymer, Newark, Delaware, USA, Lot P109820690; and
- Lactose 316 N.F. Fast Flo Foremost Farms, Rothschild, Wisconsin, USA, Batch 2000271553.

These excipients were sifted through 10cm diameter brass pan sieves (ASTM E-11 standard test sieve, Gilson Company, Lewis Center, Ohio, US) in order to obtain different particle size ranges MCC (0-75µm) and lactose (180-250µm); this giving an average particle size ratio Lactose:MCC of 3:1.

Equipment

The design of the set-up was based on the equipment described in a previous work[10] with a conical hopper operated inside a humidity-controlled enclosure. The in-house apparatus consisted of a lab-scale bin (SS 316, internal walls polished to Ra=0.28-0.81µm) equipped with an automatically controlled gate-valve system (guillotine), allowing for simultaneous radial and axial sampling of the flowing powder in the form of thin cylindrical slices (**Figure 1**; page 52). An Axsun OH-CH NIRS with lab scale diffuse reflectance accessory kit was used to provide quantitative powder analysis at the outlet of hopper. A shear-cell tester (FT4 rheometer), operated inside a controlled-humidity enclosure, was used to measure the angles of friction.

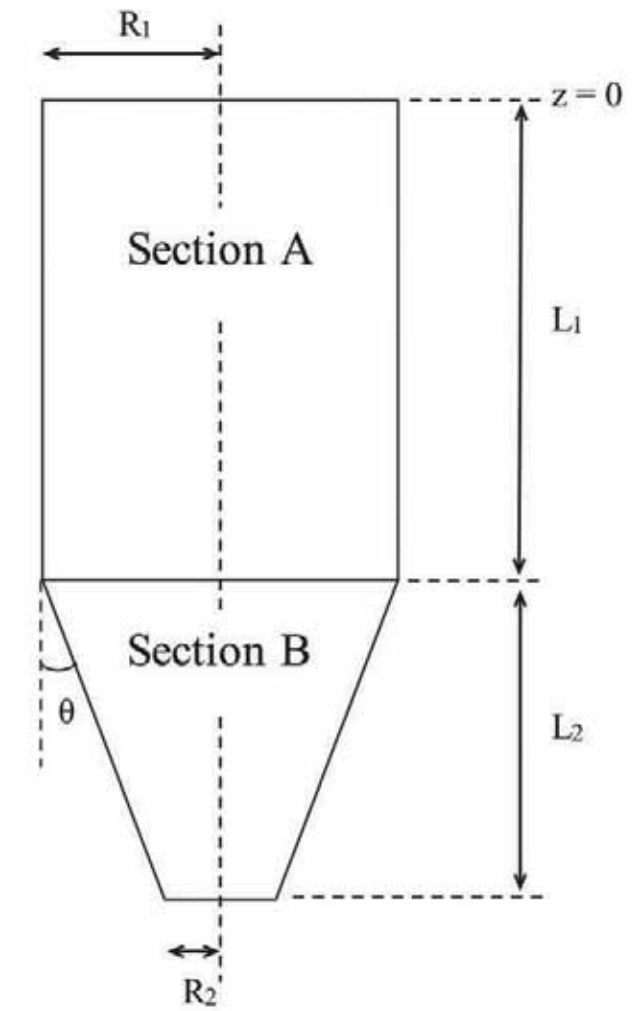

Figure 2: Cylindrico-conical sections in powder flow hopper

Methods

The experimental protocol used was as follows: a 10g layer of MCC was placed on top of a 100g bed of lactose (100mm in height). To avoid the unconfined movement of the top of the MCC layer, 20g of lactose was then poured on the top of MCC. The powder was set down carefully to maintain a flat pulse interface. The average residence time was calculated using the following equations:

$$t_m = \int_0^\infty t\,E(t)dt \qquad \text{Equation 1}$$

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt} \qquad \text{Equation 2}$$

Theoretical calculation of the velocity profile

The model development is based on the binary powder flowing inside a cylindrical bin (Section A) of radius R_1 and length L_1 (**Figure 2**). The present work is based on the hypothesis that the axial velocity is constant over the vertical length (L1) of a cylindrical

bin. The equation used for calculating the velocity profile of fluids is based on the fact that the fluid is viscous, homogeneous and isotropic and does not take into account the particular characteristics of the powders such as cohesion and adhesion. In a previous publication[10], the authors proposed the semi-empirical equation introducing the internal angle of friction between particles $\theta_{p\text{-}p}$ and the friction of the powder with vessel wall $\phi_{p\text{-}w}$ (Equation 3):

$$U_A = U_{A,max} * \left[1 - \frac{\sin\theta_{p-p}^{(1-r/R)}}{\sin\varphi_{p-w}^{(r/R)}} * \left(\frac{r}{R}\right)^{\zeta}\right] \quad \text{Equation 3}$$

This expression follows from the assumption that the wall-powder interactions decrease while the powder-powder ones increase as r decreases from R (cylinder radius) to 0 (center line).

Small values of $\theta_{p\text{-}p}$ and $\varphi_{p\text{-}w}$ representing low cohesive and adhesive forces cause the $\frac{\sin\theta_{p-p}^{(1-r/R)}}{\sin\varphi_{p-w}^{(r/R)}}$ ratio to tend to 1. When combined with a ζ value of 2, the model essentially reverts to an ideal laminar flow. However, this model has proven to be insufficient since it implies that the impact of powder/powder friction and powder/wall is equal and well known. To make up for this, an additional multiplying parameter (ω) was introduced to express the relative importance of these phenomena (Equation 4) on the velocity profile. The smaller the ω values, the closer to plug flow behaviour. Equation 4 has been reformulated as:

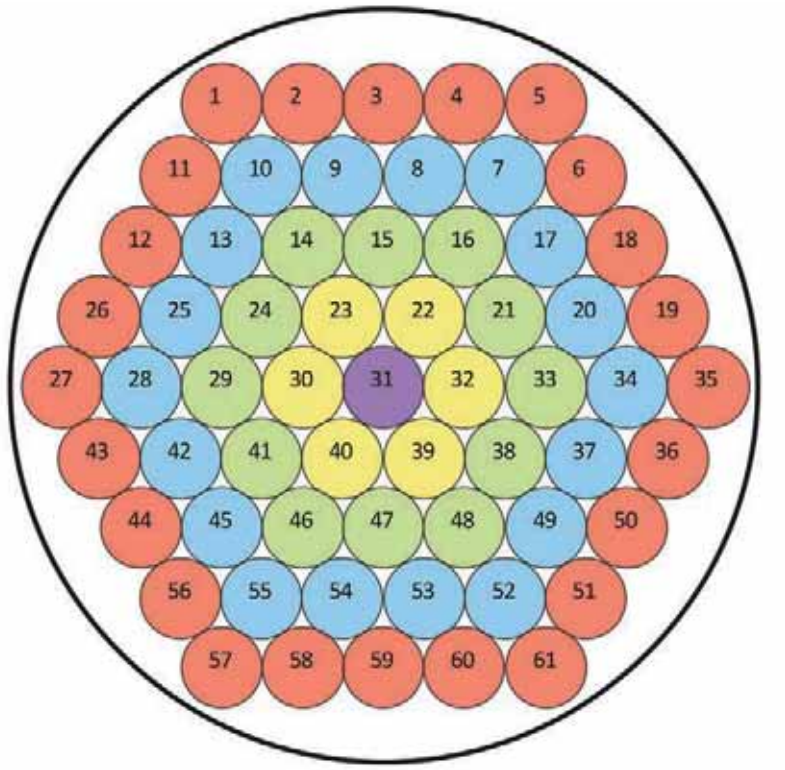

Figure 3: Schematic view of a collector and its effective sampling area
r = 18mm, r = 9.5mm, r = 4.5mm, r = 4.5mm, r = 0mm

$$U_A = U_{A,max} * \left[1 - (sin\theta_{p-p})^{(1-r/R)} * \omega * (sin\varphi_{p-w})^{(r/R)} * \left(\frac{r}{R}\right)^{\zeta}\right] \quad \text{Equation 4}$$

Results and discussion

Effective surface area sampled by NIR methodology

Preliminary experiments have shown that the effective area sampled by the NIR probe of 9mm in diameter was 15.90mm^2 based on an illuminated spot of 4.5mm of diameter. Thus, an overlapping of 61 points (4.5mm diameter) is recommended to increase the resolution of the NIR probe (**Figure 3**; page 54).

Experimental measure of velocity profile

A calibration curve was needed to link NIR measurements with components' concentrations in the sampled flow. For this, three replicates of 20g standard binary mixtures of MCC (0-75µm) and lactose (180-250µm) were prepared with the following concentrations: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 w/w % MCC, with an accuracy of ±0.02mg. The particle size ratio (lactose particle size/MCC particle size) was kept constant (3:1) during all tests (calibration curves and experiments) to minimize extraneous spectral variability. Relative humidity was also maintained under strictly controlled conditions (20% RH). Samples were deposited in a collector and fixed at the outlet of the hopper to obtain the same experimental environment. 61 spectra of each standard were collected, and their averages were plotted as a function of mixture concentrations (**Figure 4**). These curves were used to evaluate the concentration of MCC during its gravitational flow through the bed of lactose using PLS regression. The residence time (Equations 1 and 2), and the velocity profile (Equation 5) were then calculated for the 61 positions. Five radial zones were then chosen and the average velocity of each zone was calculated.

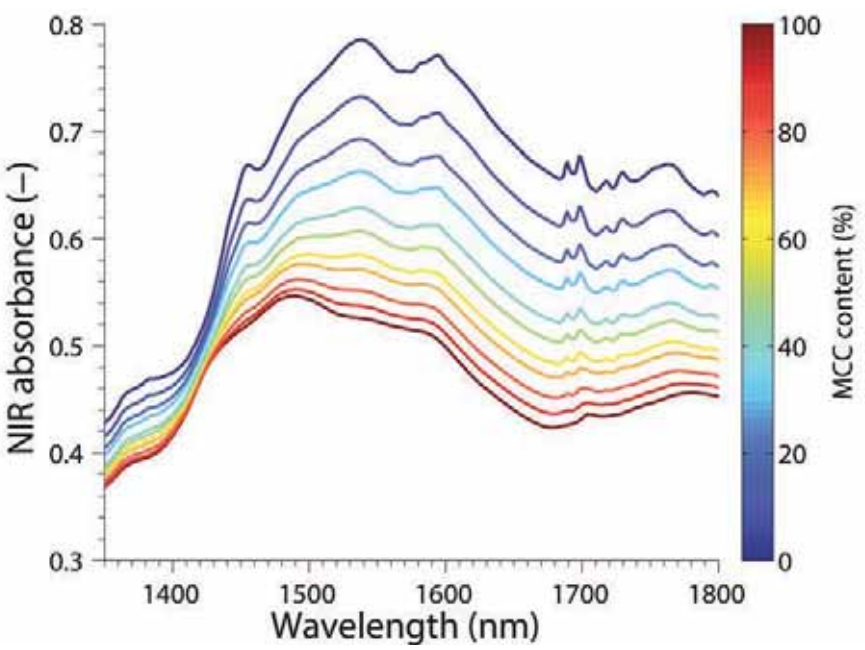

Figure 4: Average NIRS of pure MCC, L316 and standard mixtures of both components (calibration curves)

$$U = \frac{L_1}{t_m}$$ Equation 5

Figure 5 (page 56) illustrates that the velocity profile of the flowing granular bed is parabolic. Contrary to classical fluids, flowing powders do not have zero velocity at the hopper walls and they move as function of powder and hopper properties. In the following calculation, the angles of cohesion and adhesion of the pulsed component are taken into account and used as constants in the tested model.

Theoretical measurement of velocity profiles

The velocity profile was predicted using the proposed Equation (5). Friction angles of MCC (pulsed component) were measured using FT4 shear cell at a controlled humidity of 20% HR. The internal friction angle was

measured from a shear test, and the wall friction angle was measured from a friction test using friction head (316 stainless steel, roughness Ra = 0.28µm). Results show that $\varphi_{p\text{-}w}$ = 11.1° and $\theta_{p\text{-}p}$ = 35.2°. Based on these measurements, model parameters for Equation 4 were obtained via non-linear regression: ζ = 1.95±0.41 and ω = 0.14±0.02 (95% confidence intervals). The model is shown in **Figure 5**.

These results show that the velocity profile predicted using the proposed equation is in agreement with the experimental values. This parabolic profile is due to the fact that the relative importance (ratio) of 'powder-powder' and 'wall-powder' interactions decrease as r increases from 0 (center line) to R (cylinder radius), and vice-versa.

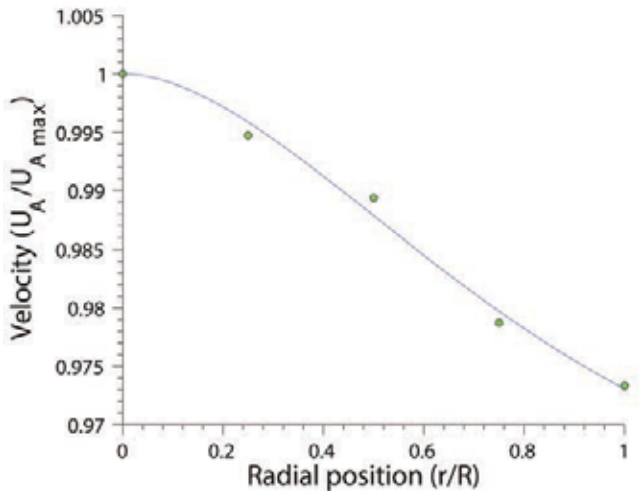

Figure 5: Normalised velocity profile in function of the radial zones (r/R)

Conclusion

NIRS-based methodology and the 'pulsed material test' have been used to evaluate the velocity profile of a binary granular system (microcrystalline cellulose and lactose) under gravitational flow inside a cylindrical hopper. The velocity profile has been shown to follow a near-parabolic profile; similar to that of laminar flows of classical fluids. In the present work, a new phenomenological equation taking into account the powder's macroscopic parameters of cohesion and adhesion (angles of frictions) has been tested as a means to predict the velocity profiles of a granular system under a gravity flow. This model was validated only in the case of cylindrical geometry hopper.

Next steps include an endeavor to check the applicability of the proposed model in the case of conical geometry hopper. The principal difference between the two geometries is that the axial velocity in conical hoppers varies as a function of both diameter, depth and angle of inclination of the hopper. So, axial velocity flows inside conical hoppers can be expressed by Equation 6. Introducing this equation in Equation 4, radial velocity profiles inside the conical hopper maybe predicted using the Equation 7.

$$U_B = U_A * \left\{\frac{R_1^2}{[R_1-(Z-L_1)\ tan\theta\ ]^2}\right\} \qquad \text{Equation 6}$$

$$U_B(Z,r) = \left[1-(sin\theta_{p-p})^{(1-r/R)} * \omega * sin\varphi_{p-w}{}^{(r/R)} * \left(\frac{r}{R}\right)^{\zeta}\right] * \left\{\frac{R_1^2}{[R_1-Ztan\theta]^2}\right\}$$

Equation 7

Oumaima Chaib is a PhD student in Chemical and Biotechnological Engineering at Université de Sherbrooke in Quebec, Canada. In 2009, she obtained a Masters degree in pharmaceutical innovation at Université Claude Bernard Lyon 1, in France. Since 2013, she has been working under the supervision of Professor Nicolas Abatzoglou and Professor Ryan Gosselin on the granular flow project, using process analytical technologies. This project, funded by Pfizer Industrial Research Chair, aims to correlate the gravitational flow velocity to the friction properties of the granular system.

Dr. Nicolas Abatzoglou is full Professor and ex-Head of the Department of Chemical & Biotechnological Engineering of the Université de Sherbrooke. He is Adjunct Professor at the University of Saskatchewan and Laval University. He is a Fellow of the Canadian Academy of Engineering. He is a specialist in Process Engineering involving particulate systems, and is the Director of the PIFIR/UdeS Research Centre GREEN-TPV (Groupe de Recherche en Énergie/Environnement-Technologies et Procédés Verts). Since May 2008, he has held the Pfizer Industrial Research Chair in process analytical technologies in Pharmaceutical Engineering. He is the Leader of Pyrolysis Project in Canada's NCE Network BioFuelNet on Biorefining. He is co-founder of the company Enerkem Technologies Inc., precursor of Enerkem Inc., a spin-off commercialising technologies in the field of energy from renewable resources. He has written 100+ publications, reviews, conferences, keynote, plenary and invited lectures, patents and three book chapters.

Dr. Ryan Gosselin is an Assistant Professor at the Department of Chemical & Biotechnological Engineering of Université de Sherbrooke, Canada. He is a specialist in Process Engineering and in-line quality optimisation through the use of multivariate data analysis and chemometrics. As a member of the Pfizer Industrial Research Chair on process analytical technologies in Pharmaceutical Engineering, his present work focuses mainly on issues relating to the production, monitoring and handling of non-reactive particulate systems.

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Henry Langston
Product Manager,
Ocean Optics

Nada O'Brien
PhD, Director, Product Management and Marketing, MicroNIR™ Spectrometers, VIAVI Solutions (formerly JDSU)

Dr Stuart Tindal
Product Manager of PAT,
Sartorius Stedim Biotech

Moderator
Dimuthu Jayawickrama, Senior Research Investigator, Bristol-Myers Squibb

What challenges do we face when using NIR spectroscopy as an online/inline or atline/offline analytical tool for biopharmaceutical analysis and how can we resolve these issues?

Henry: The biggest challenge is managing the consistency of the sampling geometry and therefore the results across different factories, laboratories and equipment. NIR chemometrics calibrations are complex and can be sensitive to many environmental factors. Keeping the sample presentation, equipment and optical geometries consistent is a key part of minimising errors. When developing models from well characterised reference models, validating them in the lab and then deploying them online/inline, it is very challenging to keep things consistent. Modular spectroscopy solutions offer a flexible toolkit of solutions that make it easy to deploy the same equipment in the lab or on the line.

Stuart: There are two key linked issues: 1) miniaturisation to benchtop/low volume scale to support process development; 2) management of large enough datasets to generate robust process models. Recording the NIR spectrum of a single process run only gives a snap shot to one possible process trajectory. Commercial biomanufacturers follow repeatedly that trajectory and control critical processing parameters (CPP). To generate an entire CPP map from multiple runs performed with experimental design and analysed with multivariate data analysis would show the complete process picture. However, using NIR spectroscopy in a process development environment has not yet been realised effectively.

Nada: As with small-molecule manufacturing, the biopharmaceutical industry is transforming towards an all-encompassing operational excellence to bring new products to market faster, reduce cost, and

produce high quality and safe medicines. This is achieved through investments in next-generation manufacturing that deploy continuous processes, single use systems and the implementation of process analytical technology. Small footprint and cost-effective NIR spectrometers that are relatively easy to use, transport, and mount are important. The biggest technical challenge is the presence of water in low concentrations. On-line and in-line probes require small path lengths. However, we are collaborating with pharmaceutical companies in devising solutions using our versatile miniature NIR technology.

What are the panel's thoughts on the recently issued European Medicines Agency's guideline for NIR (guideline on the use of NIR spectroscopy by the pharmaceutical industry and the data requirements for new submissions and variations)?

Henry: Well-defined processes are an important part of deploying NIR successfully, in terms of making sure the chemometric models are comprehensive, accurate and well validated against 'gold-standard' methods. The fear is that focusing on principal components or very specific methods of chemometric modelling can limit innovation with new methods. As long as regulators keep an open mind towards iterating and introducing new tools, then I think NIR, with its advantage of not needing sample preparation or expensive consumables, has a bright future in the pharmaceutical industry.

> "I think this is an exciting time for NIR technology as we see the development of smaller, lower cost solutions for NIR sensors"
>
> *Henry Langston*

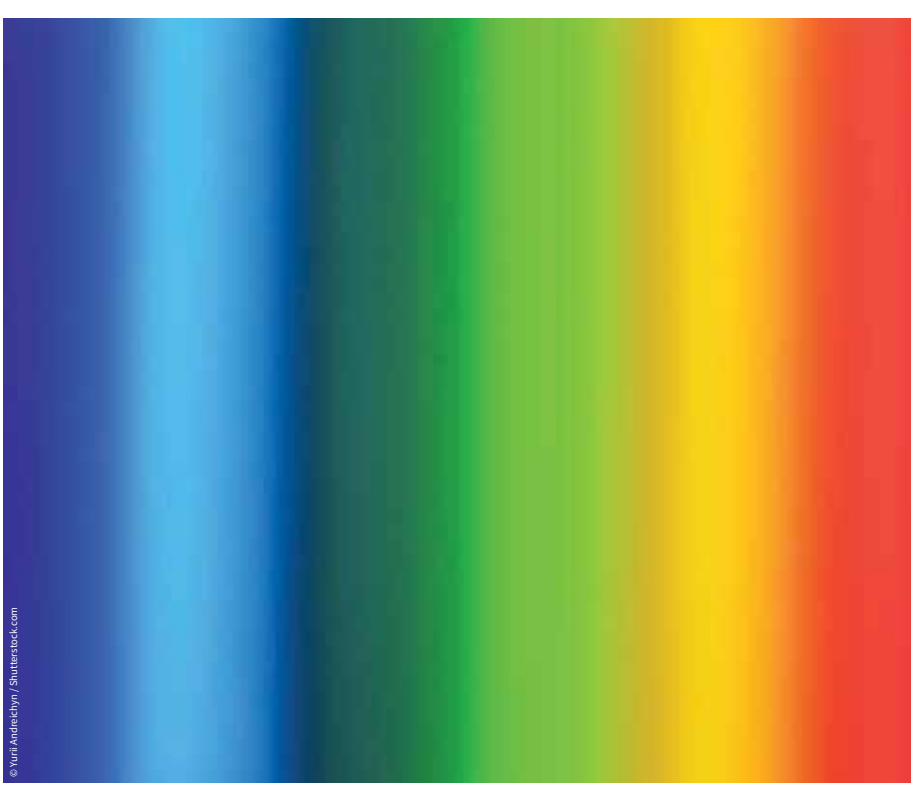
Stuart: As with the FDA draft guidelines that were issued in March 2015 there is more work to be done to understand the specific regulatory requirements of how NIR can support process and product improvement and quality. However, clearly both European and American agencies are moving in the direction of making this technology more than just 'nice to have' and rather making it obligatory for product and process control.

Nada: One of the changes in the guideline is the requirement to register the make and model of NIR instrument and the software version. This makes it harder to transfer the model to other sites post-registration if they do not have exactly the same kit. For us, from an instrument manufacturer point of view where we are making the small and cost-effective MicroNIR analysers, the new guideline addendum might encourage users to adopt those new fit-for-purpose analysers for their affordability aspect and thus ease of standardising on the same instrument across multiple sites.

Having multiple NIR sensors at different locations of a bin can provide valuable information about blending. What is the future of small NIR blend analysers?

Henry: I think this is an exciting time for NIR technology as we see the development of smaller, lower cost solutions for NIR sensors. This is making it easier and more cost effective to deploy NIR spectroscopy in blending applications. The more sensors, the better the data, and the more useful it becomes as a process control tool, the more others will be encouraged to use it too, creating a virtuous circle. I think the ongoing miniaturisation and lowering of costs will enable much more widespread application of multi NIR sensor platforms in the future.

Stuart: We do not see the advantage of multiple NIR sensors in one vessel. The end user focuses on the product inhomogeneity, which is accessible with a single device that gains the information by the variation over time. In contrast, multiple devices gather the information on variation over distance, revealing heterogeneities in the vessel. However, this is only noteworthy for the manufacturer of blenders. One factor that needs to be assessed is the spot size as it has to be larger than the particle size to confirm homogeneity. With light guided fibres this is not always guaranteed.



Nada: The ability to detect dead spots, product demixing phenomena and other process defects can be enhanced using multiple sensors on blenders. In general, as the number of measurement points increases, whether on a given unit operation or on a continuous process, the size, weight, cost and ease of implementation of the NIR spectrometer become more critical.

With our MicroNIR™ PAT, we have designed a small battery-powered NIR spectrometer that weighs less than 1.4kg and has the capability to interface with commercially-available MVA software tools and PAT data management systems. MicroNIR PAT represents a way for pharma companies

to take a Quality by Design approach without the large capital expenditure.

What technological innovations are available and/or possible to analyse a whole tablet by NIR?

Henry: Imaging technologies, multispectral and hyperspectral, are fast developing and NIR is no exception. I think the challenge here is developing meaningful methods of analysis that can make use of the overwhelming amount of information that imaging of whole tablets provides. You can have great hardware but the real value is in harnessing the right computational power to turn it into an answer. It adds an extra dimension of complexity to the problem, one that requires a new generation of expertise to solve.

Stuart: Spatially resolved NIR achieved with hyperspectral imaging technique is a promising candidate for the analysis of complete tablets. Beside the overall chemical composition, hyperspectral imaging in the NIR region reveals inhomogeneity within a single tablet, which is crucial in case of dosages of fractions of a single tablet.

Nada: NIR transmission has been the preferred method for intact tablet analysis because it takes a cross-section of the interior of the tablet. The success of this measurement depends on the tablet thickness and the measurement speed. An alternate approach is to move the analysis a bit more upstream in the process. Miniature NIR probes can be positioned in the feed frame of a tablet press to continuously monitor the API concentration of powder before tablets are produced. The result is an earlier detection of process deviation well before 1000s of tablets are produced.

> “SORS enables identifying materials a few millimeters deep through paper bags and containers without the need to have a prior knowledge of the packaging material”
>
> *Nada O'Brien*

Spatially offset (resolved) Raman spectroscopy is a promising analytical tool to analyse materials beneath obscuring surfaces. Is there any potential for similar development in NIR?

Henry: NIR spectroscopy is inherently well suited to through barrier techniques because the longer wavelengths penetrate deeper – it is for this reason it works so well in through-skin biomedical diagnostics applications. Through barrier Raman techniques are well suited to identification or rapid verification applications, especially where the barrier material is unknown or varying, for example, when verifying that the pill in that blisterpack is what it says on the label. NIR will be better suited to quantification applications but is perhaps going to need some pretty sophisticated models for dealing with various types and sizes of barriers.

Stuart: Spatially offset Raman Spectroscopy detects Raman signatures up to 2-4 millimetres deep. NIR radiation has higher wavelengths and thus achieves higher penetration depths. The common referred example for Raman measures the container and substance independently with a laser spot, detecting mainly the substrate a few millimetres apart. In NIR measurement this is done simultaneously. To measure the powder in a plastic container using NIR you first record the spectrum of the empty container and use that data to correct and baseline the powder/container spectrum. However, as the physics of the two measurement principle differ significantly, one will never be able to cover all features of the other.

Nada: SORS enables identifying materials a few millimeters deep through paper bags and containers without the need to have a prior knowledge of the packaging material. Conventional NIR spectroscopy has the inherent advantage of penetrating a few millimeters into surfaces depending on absorption characteristics of the materials and the NIR wavelength range used. However, the outside packaging material has to be taken into account by subtracting the material's spectrum from the spectrum of the sample underneath. This can be done readily in routine type analyses of known products for conformity testing, for example, as is usually the case in the pharma industry.

CPhI worldwide
where pharma intelligence gathers

CPhI Worldwide returns to Madrid in 2015, where the world's most prominent pharma executives will gather to learn, exhibit and network with the leading decision makers and innovators across this industry. Following the success of the 25th Anniversary in 2014, this year promises to be another record-breaking show with an expanded conference platform, increased content sharing and greater networking opportunities across the event's huge range of diverse platforms.

Between 13th and 15th October, the entire pharma community will return to IFEMA (Feria de Madrid, Spain) for the three days of networking, informative conferencing and meetings that will help shape the industry over the next year. In total, over 36,000 attendees are expected to attend and engage with some 2,500 exhibitors from over 150 countries.

But CPhI Worldwide is much more than a meeting platform; it provides an unrivalled melting pot of ideas and innovations, all designed to maximise business benefit, networking and sharing. More than 20 dedicated zones covering active pharmaceutical ingredients, excipients, finished dosage, contract services, packaging, biopharm, machinery and many more will be present at CPhI Worldwide, delivering world-class, industry-wide content.

Running alongside the pharmaceutical ingredients halls are three other sister brands, which help visitors to quickly identify the right halls for their needs. ICSE is an outsourcing focussed area designed to connect the pharmaceutical community with contract providers from clinical trials, contract research organisations, logistics providers, data management firms and contract manufacturing organisations. InnoPack brings together buyers and specifiers from the packaging and pharmaceutical industries. Finally, P-MEC Europe features exhibitors of traditional large-scale capital equipment to companies focussed on instrumental analysis, measuring and testing technologies, materials testing, quality control and laboratory services.

Pre-Connect

CPhI Worldwide 2015 officially begins with the CPhI Pre-Connect Congress (12th October), where senior executives and influential speakers discuss the latest innovations, trends and market developments from across the industry in a series of market-led educational modules. It is organised along two main tracks, with sessions in 'track one' including 'Formulation & Drug Delivery', 'Biologics, Biosimilars & Biobetters' and 'API Sourcing & Manufacture'. 'Track two' features modules across 'Generics', 'Pharmaceutical Packaging', and 'Mergers and Acquisitions'. Additionally, the main exhibition also includes key content features including the Innovation Gallery, Exhibitor Showcases and Innovation Tours – details of which can be found on the next page.

Pharma Forum

Another major event feature, new for 2015, is the CPhI Pharma Forum – a dedicated content village – that will provide a central hub to examine thought leadership from media partners and the CPhI Pharma Insights Reports. The Pharma Forum will also be the location for the exhibitor and visitor party, and will include exhibitor Innovation Galleries, the CPhI Pharma Awards and the Pharma Insight Innovation Briefings – offering impartial, in-depth sessions on regional updates and specialist topics covering regulation, QC, traceability, sustainability and health to name but a few.

Again returning in 2015 are the revamped CPhI Pharma Awards (with five new categories) and free sessions in Speaker's Corner – which provide exhibitors a forum to deliver first-hand presentations to senior pharma attendees from across the globe. To help attendees tailor their time at the show, CPhI Worldwide Global Meetings is a custom matchmaking programme that enables attendees to find and connect with specific companies tailored to their business needs.

Summary highlights for CPhI Worldwide 2015

CPhI Pharma Awards

Now in their 12th consecutive year, the CPhI Pharma Awards will honour companies and individuals driving the pharma industry forward through new innovations, approaches and strategies. The addition of five new categories in 2015 allows for a broader range of recognition of all the great advances coming out of the Pharma industry. The CPhI Pharma Awards jury panel will review all the entries before announcing all the winners during the show.

Global Angels

CPhI has partnered with Global Angels, a charity that connects with innovative projects to deliver tangible, life-saving results in developing countries. CPhI has already worked with a charity in Kenya and strongly encourages the pharmaceutical community to get involved with this amazing cause. No matter how big or small the donation, every penny received goes directly to Global Angels and will make a substantial difference, so please do have a look at the range of charitable packages we are offering.

Exhibitor Showcases

The Exhibitor Showcases are an invaluable opportunity for exhibitors to educate the industry across their expertise and services. Showcases last approximately 25 minutes and provide an opportunity for companies to present forward-thinking perspectives on their key products, innovations, services and more to visitors and the press. It also provides an open platform for speakers to directly interact with attendees and increase their networking contacts.

Innovation Gallery

After its successful launch in 2014, the Innovation Gallery has returned

to CPhI Worldwide for a second year, and allows for companies to showcase their innovations on an international platform. This forum presents the latest innovations and newest products on the market to top pharma executives in attendance.

Innovation Tours

Across each of the three days at CPhI, top industry experts will guide attendees through free-of-charge Innovation Tours. These hour-long bespoke tours give a brief overview of industry trends and recent innovations within the pharmaceutical industry. Starting from the Innovation Gallery, the tours travel throughout CPhI, ICSE and InnoPack providing perspective across the entire pharmaceutical supply chain.

Mobile App

CPhI worldwide will once again feature its hugely successful mobile app, providing exhibitors and attendees with a timetable of the day's activities, a list of exhibitors attending and their location and much more. In addition, this advanced service will allow for your company to directly promote its main messages, products and services.

Pharma Forum

The Pharma Forum is a dedicated content village, which will be the central hub for thought leadership from industry players, media partners and the CPhI Pharma Insights Briefings. Across the expansive space, event attendees will have free access to in-depth, industry-focus materials and insights. This new addition to CPhI Worldwide 2015 will also include the Exhibitors' Innovation Galleries, Global Angels and CPhI TV.

Pharma Insight Briefings

Taking place over the course of the three show days in our new Pharma Forum, the Pharma Insight Briefings are a series of 45-minute seminars on specialist topics and regional updates, allowing you to build your schedule around high value content that is relevant to you. All seminars are free to attend.

Tweet chat

Returning for its second year, the second day of CPhI Worldwide will host an industry wide tweet chat broadcasting a conversation between the CPhI Expert Panellists and the attendees. With the release of the annual report, attendees will be able to analyse the contributions of the panellists and contribute to a live discussion displayed throughout the screens at the show.

VIP and Exhibitor Party

On the first day, the new addition of the Pharma Forum will house an exclusive VIP & Exhibitor Party. At this event, the industry's leading figures, thought leaders, innovators and exhibitors will meet together to network and discuss the latest developments across the industry. Additionally, the party will host the CPhI Pharma Awards Ceremony, where winners will be announced.

Women's Networking Breakfast

Uniting female executives from the global pharmaceutical network, this event will celebrate female leadership and encourage empowerment within the industry. Hear from senior level female executives on how to advance through the pharma industry and widen networks.

CPhI Worldwide holds a unique position in hosting the largest number of traditional pharmaceutical buyers, alongside an array of top pharmaceutical companies. Register now for CPhI Worldwide 2015 at: www.cphi.com/europe/visit or book exhibition space at: www.cphi.com/europe/exhibit

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Predictive monitoring and control approaches in biopharmaceutical manufacturing

Cenk Undey, Tony Wang, Bryan Looze, Yingying Zheng and Myra Coufal
Amgen

Predictive monitoring is a key feature of biopharmaceutical manufacturing; making predictions about the key process end points such as process performance indicators or quality attributes using a process model offers the unique advantages of process improvement and optimisation, and helps give insights into variability. However, whilst model-predictive monitoring is advantageous, it is also desirable to apply model predictions for closed loop control of biologics manufacturing using various process analytical technology (PAT) tools. We summarise some of our experiences with predictive monitoring, closed loop control using *in situ* Raman spectroscopy and state-space methods for model predictive control of cell culture bioreactors.

Introduction

As in other process industries, biopharmaceutical manufacturing processes generate a lot of data during the clinical and commercial production runs collected over many stages on many different variables. It is imperative to monitor these variables across the stages and batches in real-time to detect any developing trends that may affect batch performance or lead to loss of a batch. It also is of great interest to understand trends in process variables that may impact on product quality attributes (PQA) and key performance indicators (KPI). Regulatory Agency guidelines[1,2] also promote the lifecycle concept linking product and process development with the commercial manufacturing process. We have previously discussed and presented very powerful PATs such as application of real-time multivariate statistical process monitoring in the open-loop mode of operation

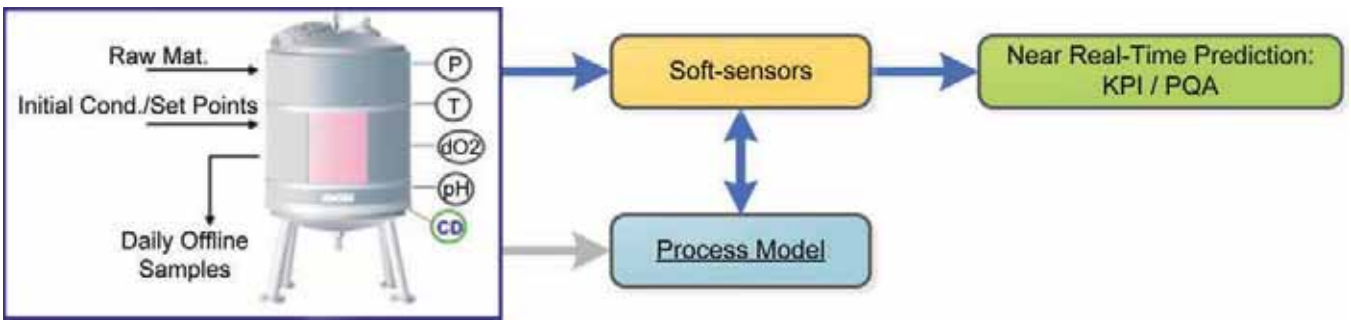


Figure 1: Predictive monitoring via soft sensors

where many variables across many stages in a biopharmaceutical manufacturing process are monitored and weak signals are detected[3,4].

While detecting weak signals is important to enable engagement in process monitoring and troubleshooting, it is also highly desirable to control the process performance and product quality via closed-loop approaches using model-based predictive techniques. As described in the PAT guidance[5], some of these control applications may involve process chemistry tools and analysers, whilst others may depend on soft-sensors and other predictive technologies. This aligns with a quality-by-design approach and ensures the target product profile is met by establishing an effective control strategy[6-8]. Ample literature exists in closed loop and model predictive control in various industries including fermentation technologies[9-12]. However, actual industrial applications of model-based or model-predictive control are not yet very common in biopharmaceuticals manufacturing.

We shall summarise some of our experiences with open- and closed-loop models in the following areas:

- Multivariate predictive monitoring;
- Raman probe-based monitoring and model predictive control; and
- State-space model-based monitoring.

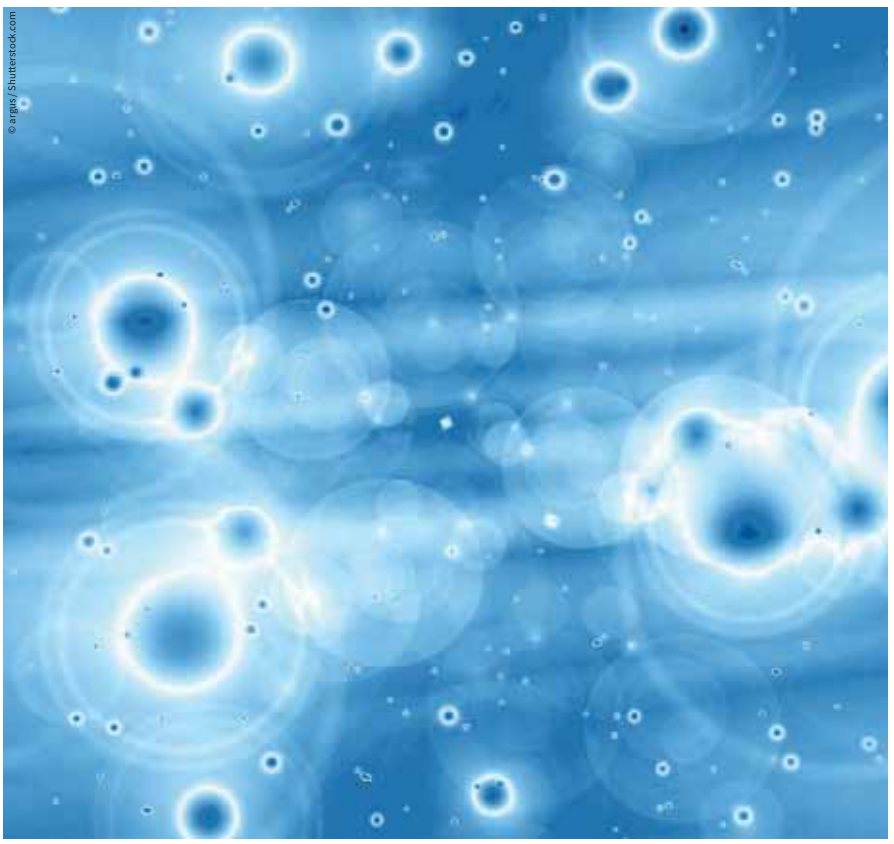
Multivariate predictive monitoring

This method involves using readily available and frequently sampled or discrete data on measured variables in the bioprocess equipment. These may include in-line probes, on-line instruments and offline sample assay results. For instance, in a bioreactor, typical frequently measured variables include various gas sparge rates (O_2, CO_2, Air), dissolved O_2, pH, pressure, working and feed volumes and temperature. Typical discrete variables measured infrequently are metabolites, viable cell density, cell viability and dissolved CO_2. Soft-sensors are often used to estimate variables that are unmeasurable, replace more time-consuming or costly techniques and improve frequency of data availability. Lastly, pre-processing and calculation of additional derived variables, such as specific growth rate

or metabolite production, can improve a model's predictive capability.

Predictive models may be developed only if each significant operational variable spans a sufficient range (and/or if there is a first principles model describing the relationship) that will allow for correlation to process performance and product quality[8,10,12-13]. These predictive models, acting as soft-sensors as shown in **Figure 1** (page 64), allow for predictions about KPIs and PQAs in near real-time.

One example of this methodology uses a predictive multivariate model to estimate KPIs and PQAs in near real-time, where they were traditionally measured through offline process sampling and analytics, for use in a commercial manufacturing facility to improve batch productivity, operational capacity and flexibility by optimising the harvest timing of the bioreactor. If the length of the bioreactor culture duration can be increased within the operating range, unit productivity will be increased. However, this must be balanced with key variables in the process and performance that must be met to ensure product quality.



In this example, the KPIs modelled are glucose, cell viability and a PQA. Glucose is critical to the growth and production within cells. Due to inherent variability within the cells and raw materials, consumption of glucose can vary from batch to batch. Therefore, it is imperative that glucose in the medium will not be depleted. Prediction of the time of glucose depletion confirms the longest the culture may be operated. Cell viability reflects the state and health of the cell culture. The prediction of this KPI ensures that the culture will not fail the KPI prior to harvesting. Lastly, one of the key PQAs (that is impacted by the cell culture conditions) measured in the downstream purification process was identified due to the significant correlation with the cell culture process. Predicting that the PQA is within its limits is the last step to determine the optimal culture duration.

The use of multiple environmental, KPI and PQA prediction models allows operations to make more informed business decisions earlier in the production of a batch. **Figure 2** illustrates how the predictions allow for the determination of whether an early harvest of the cell culture might be needed to ensure KPIs and PQAs meet limits or to extend the cell culture duration for productivity improvement.

A current use case (Batch A and B) demonstrates the ability to extend batches for increased production. First, the PLS scores, Hotelling's T^2 and Distance to Model X (DModX) values are verified to be within acceptable limits for assurance of prediction. **Figure 3** (page 66) shows that both batches meet data fit criteria. Next, viability, glucose and the PQA predictions determine batch extension capability. Only Batch B could be extended as Batch A had a viability prediction indicating that an extended culture would fall below its action limit.

Another example where predictive modelling provided commercial manufacturing with the tools to make more informed decisions comes from an investigation into a lower-than-expected KPI at the final stage of a multi-stage purification process. An evaluation into over 100 input variables from the entire process (**Figure 4a**; page 66) identified many

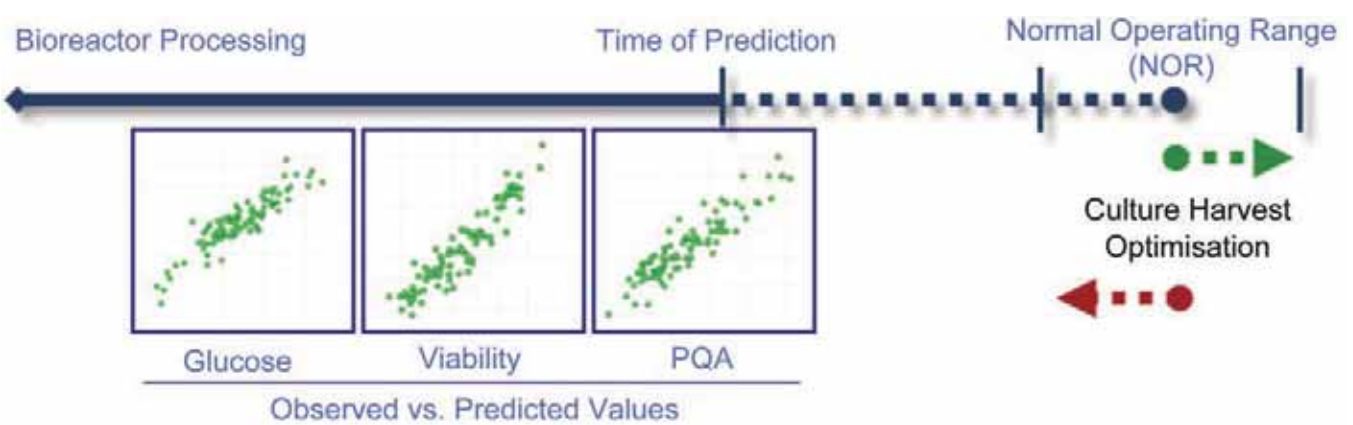


Figure 2: KPIs and PQAs are predicted to optimise bioreactor culture harvest

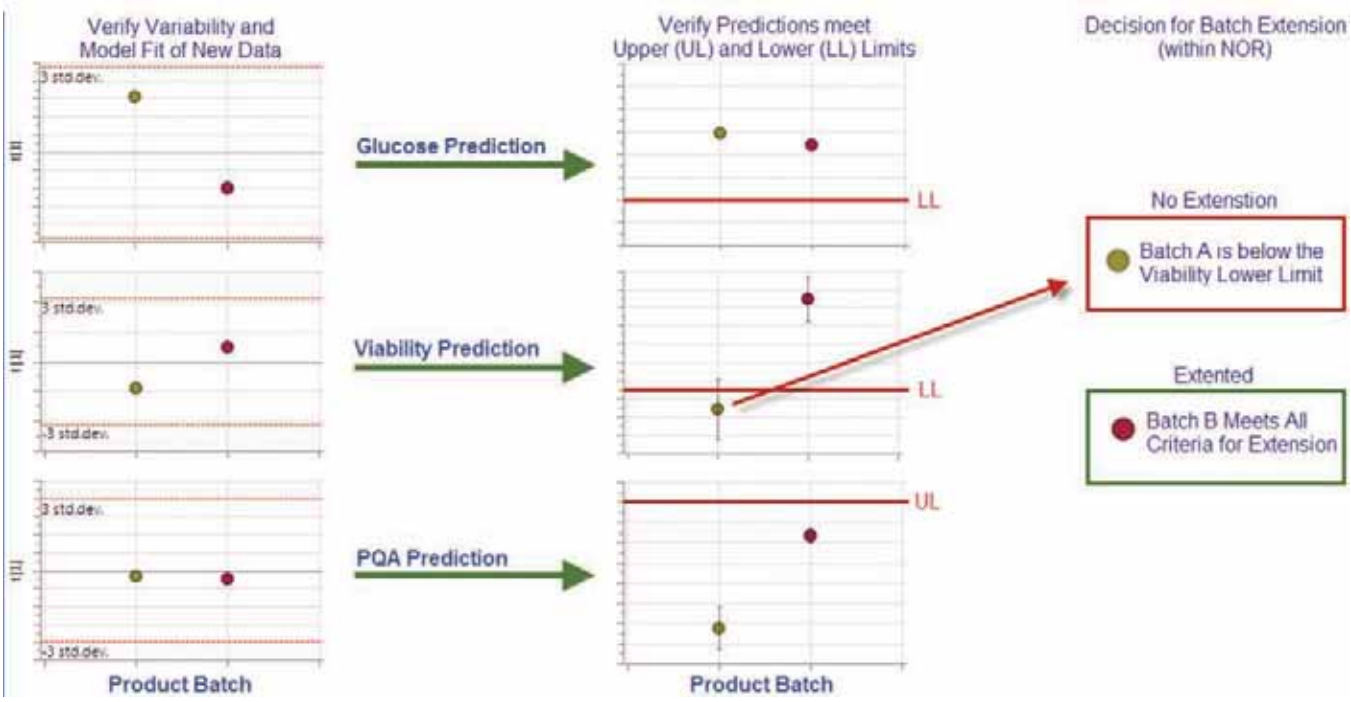


Figure 3: Data set and prediction verification

variables that contribute significantly to the KPI (green bars), and others that do not (red bars). Due to the large number of variables across all stages, understanding how the variables are related is difficult to discern. A more efficient way to tackle the problem is to understand how the KPI across the six different stages influence the final KPI (Figure 4b). This focuses the investigation away from stages that do not influence the final KPI (red bars), and allows a more concerted effort into understanding the stages that do influence the KPI (green bars). Stage-specific models can then be built to better understand which variables should be monitored and controlled to impact the KPI (Figure 4c).

Raman probe-based monitoring and control

Raman spectroscopy is a powerful PAT tool to enhance process monitoring capabilities in the biopharmaceutical industry. An online Raman spectroscopy probe can supplement existing process and operating data and enhance the predictive capability of online monitoring systems. To achieve online monitoring using Raman spectroscopy, calibration models need to be created that translate Raman spectra into useful process parameters (i.e., acting as a real-time soft-sensor). We achieved this by combining multivariate (MV) modelling tools with Raman spectroscopy[15-16]. An example of a calibration model – for glucose – is provided in

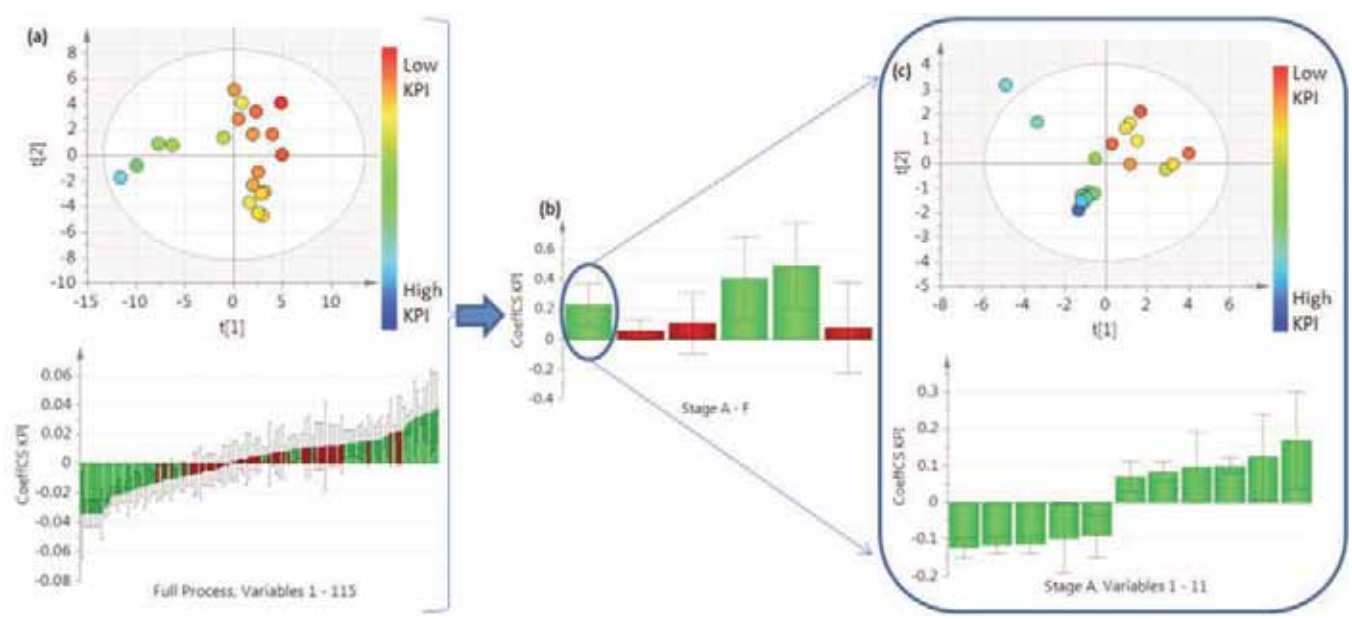


Figure 4: Focusing a KPI investigation from 115 variables down to 11 variables

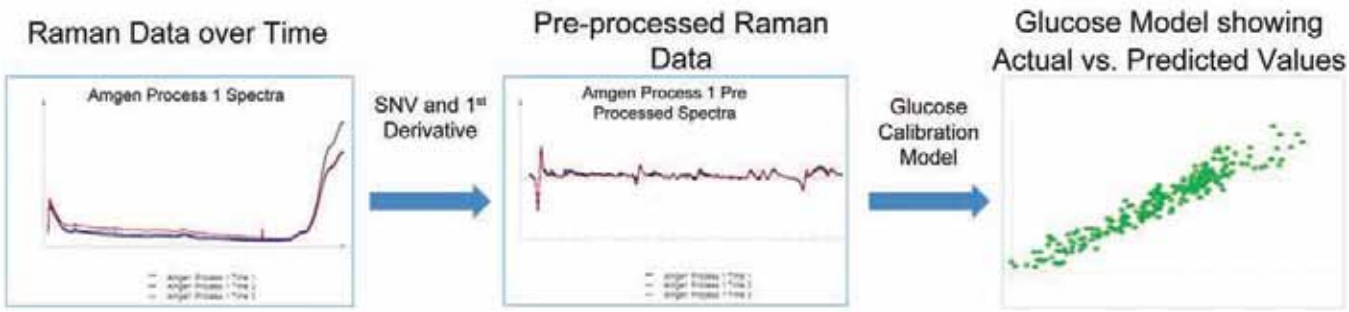


Figure 5: Glucose concentration Raman calibration model for a cell culture process

Figure 5. Similar calibration models are created for other critical cell culture parameters such as viable cell density, viability, lactate, glutamate, glutamine, ammonium and osmolality.

Once the calibration models are developed, they can be integrated into a Distributed Control System or Data Historian to perform online monitoring of the ongoing process (Figure 6).

Model predictive control (MPC) can be used to optimise batch trajectory based on historical and real-time results (Figure 7; page 68). The MPC model (batch trajectory optimisation) is a supervisory model which resides outside of the traditional open loop setup. The open loop may have additional MV models used to perform online monitoring which can be set up independently from the MPC model or fed into the MPC model as inputs. The MPC model takes information from the open loop as well as data from its internal training set to predict an optimised trajectory by suggesting new process set points.

> "Industrial applications of model-based or model-predictive control are not yet very common in biopharmaceuticals manufacturing"

Our MPC prediction model was used to optimise final day viable cell density (VCD) for a cell culture process run on a bench-scale seed bioreactor. In this example, temperature, pH and O_2 are operational parameters that the MPC model used to optimise the process trajectory. The Scores, Hotelling's T^2 and DModX results are calculated and available for the scientist. The MPC model uses sample measurements from the process that is running at the time to predict an optimised trajectory for the process. When we simulated the closed loop control conditions, an estimated 23% higher final VCD (Table 1) was predicted in comparison to open loop control case[14]. While other controlled and uncontrolled load disturbances (such as raw material variation) need to be taken into account, this initial study at bench-scale provided affirmation that the MPC approach was promising.

Table 1: MPC-simulated cell culture; final day viable cell density

Run	% Increase in VCD (open loop vs. closed loop)
1	25%
2	18%
4	6%
6	43%
7	24%
Average	**23%**

State-space model-based monitoring and control

For both predictive monitoring and model predictive control, a compact and accurate description of the dynamic behaviour of the system is desirable. Dynamic models describing the system of interest can be constructed using the first principles of physics, chemistry and biology. While they are very strong and predictive, the first principles models are difficult and expensive to derive, as they require a deep and full understanding and knowledge of the processes. Moreover, the first principles model is often complex and simulations take a considerable amount of time. Thus, the first principles model may not be suitable for fast on-line applications.

An alternative way of developing models is via system identification[17]. In system identification, the goal is to estimate the dynamic models directly from the observed input data and output data. System identification approach leverages the ample data that we acquire from modern control systems, PAT instruments and data acquisition tools. State-space models derived from system identification are flexible and yield compact and accurate

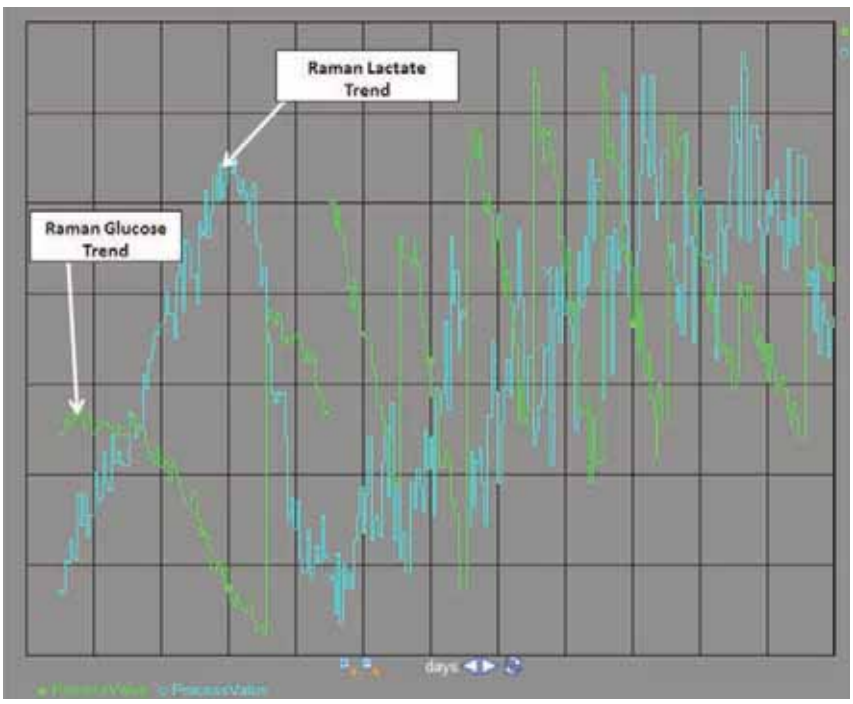


Figure 6: Glucose and lactate trends of a cell culture process from Raman probe spectra monitored in real-time

models that are suitable for fast on-line applications and for model predictive control.

The general form of a state-space model[17] is given as:

$$\begin{cases} x(t+1) = Ax(t) + Bu(t) + Ke(t) \\ y(t) = Cx(t) + Du(t) + e(t) \end{cases}$$

where u is the process input vector, y is the process output vector, x is the state vector and e is the noise vector. A, B, C, D and K are state-space matrices and t denotes the time step. Given the process input and output data, the state-spaces matrices can be estimated by system identification. Once the state-spaces matrices are estimated, the state-space model is ready for use. It is flexible in a way that the prediction of the output can be made at every time step.

We use the mammalian cell culture processes as an example here. The process input vector is:

$$u = \begin{bmatrix} gluc \\ PO_2 \\ PCO_2 \\ pH \end{bmatrix}$$

where *gluc* denotes the glucose concentration, PO_2 the partial oxygen pressure, and PCO_2 the partial carbon dioxide pressure.

The process output is defined as:

$$y = [VCD]$$

where *VCD* denotes the viable cell density.

Both the input and output data are collected from the bioreactor measurements. MATLAB system identification toolbox[18] was used to estimate the state-space matrices. After the state-space matrices are estimated from system identification, the estimated state-space model is applied to predict *VCD*. The predicted *VCD* and the experimental data are shown in Figure 8; page 69). The prediction shows agreement with the experimental data, with a root mean square error (RMSE) of 10.18.

MPC can be built on a state-space model. The objective function in our case is to maximize the *VCD* trajectory while penalised by the control action move. The *pH*, PO_2, PCO_2 and *glucose* are subject to the physical constraints. The objective function is expressed as follows:

$$Max: \quad \sum_{i=t}^{t+N_p} y_i - \sum_{i=t}^{t+N_c} \nabla u_i^T Q \nabla u_i$$

Subject to:

$$pH_{low} \le pH \le pH_{high}$$

$$PO_{2_{low}} \le PO_2 \le PO_{2_{high}}$$

$$PCO_{2_{low}} \le PCO_2 \le PCO_{2_{high}}$$

$$Gluc_{low} \le Gluc \le Gluc_{high}$$

where NP is the prediction horizon and NC is the control horizon.

We perform the following procedures:

1. In the time step *t*, compute an optimal input signal by maximising the objective function over a certain prediction horizon in the future using the state-space model;
2. Implement the first step of the optimal input signal;

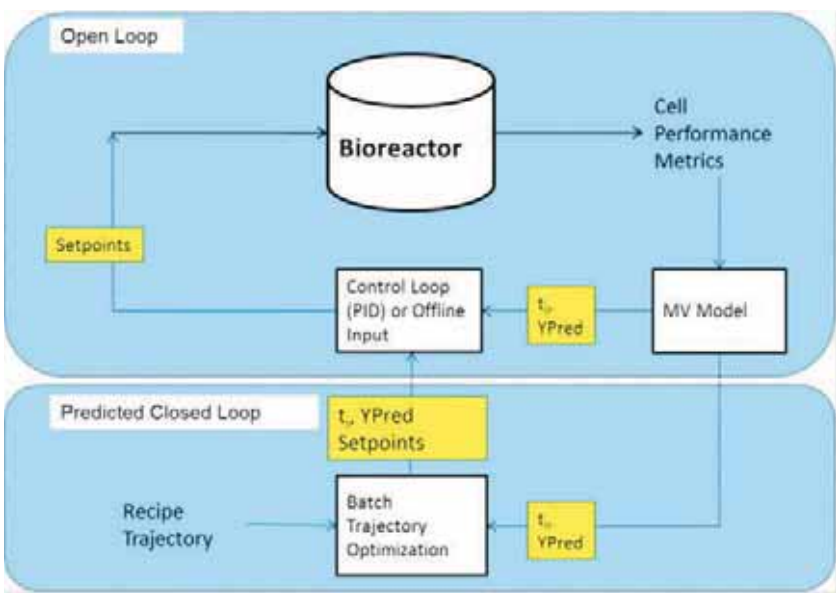


Figure 7: Model-predictive control framework for the bioreactor

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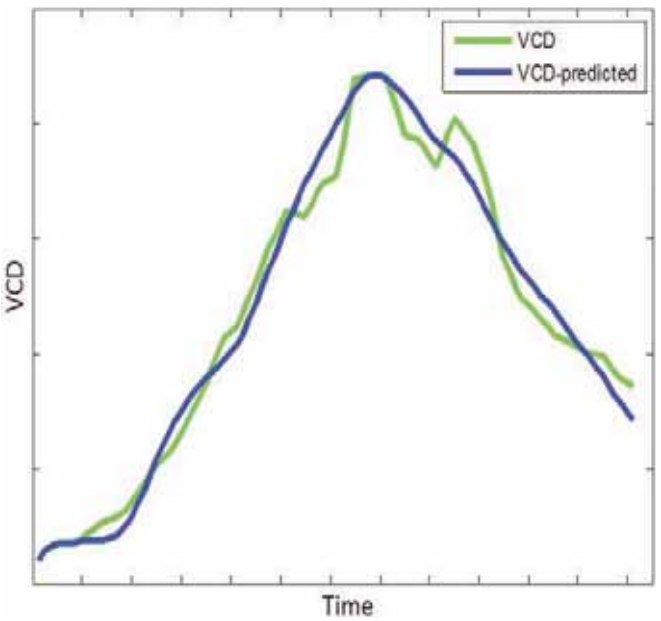

Figure 8: Predicted *VCD* from state-space model (blue line) and experimental data (green line)

3. Move to the next step *t+1*, compute an optimal input signal by maximizing the objective function over a certain prediction horizon in the future using the state-space model;
4. Implement the first step of the optimal input signal; and
5. Continue move on with the above procedures for the whole batch process duration.

The simulation results show that after MPC is applied, the final VCD could be increased by 20%, and daily-averaged VCD could be increased by 6%.

Conclusions

Applying model predictive monitoring and control has unique advantages in biopharmaceutical operations which can be generalised into a broader set of other biopharmaceutical manufacturing applications. The key aspect is having a robust model that would be representative of the process behaviour and could handle the controlled and uncontrolled disturbances. Finally, the models used for prediction could take various forms such as empirical, input-output, state-space, first principles mechanistic and a combination of these. A well-developed predictive control model offers advantages on controlling KPIs and PQAs, improving process capability and productivity.

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Cenk Undey, PhD, is an Executive Director of Process Development at Amgen. He leads the Digital Integration and Predictive Technologies group within CMC Lifecycle Management, advancing the process analytical technologies, implementation of real-time multivariate statistical process monitoring and control for biopharmaceutical manufacturing processes. His responsibilities include establishing digital integration of data, information and knowledge produced during process and product development. His group also focuses on first principles modelling, applying machine learning, pattern recognition and data mining technologies to identify, track and control variation in the value chain. He has conducted his postdoctoral studies at Illinois Institute of Technology, Department of Chemical and Biological Engineering in the area of Intelligent Systems. He has BS, MS, and PhD all in Chemical Engineering from Istanbul University in Turkey. He can be contacted at cundey@amgen.com

Tony Wang is a Senior Engineer in Process Development at Amgen in the Digital Integration and Predictive Technologies group within CMC Lifecycle Management. He is responsible for multivariate data analysis and advancing process analytical technologies, including Raman, NIR applications in raw materials and cell culture monitoring, as well as model predictive control. Previously he has worked in cell culture within Process Development and also as a process engineer within Facility Engineering at Amgen. Tony earned his BS degree in Chemical Engineering and MS degree in Biomedical Engineering both from University of Calgary in Canada. He is a licensed Professional Engineer within APEGA.

Bryan Looze is a Senior Engineer in Process Development at Amgen in the Digital Integration and Predictive Technologies group within CMC Lifecycle Management. His responsibilities in process analytical technologies include implementing real-time multivariate statistical process monitoring, multivariate data analysis and remote monitoring systems. He obtained his BS degree in Chemical Engineering from the University of Massachusetts.

Yingying Zheng, PhD, is a Senior Engineer in Process Development at Amgen in Digital Integration and Predictive Technologies group within CMC Lifecycle Management. Her responsibilities include implementing real-time multivariate statistical process monitoring, multivariate data analysis and developing and deploying model-based predictive control technologies. Prior to joining Amgen, she worked in The Dow Chemical Company and in Invensys Operations Management. Yingying holds a BS degree in Polymer Materials and Engineering from Tsinghua University in China, an MS degree in Electrical Engineering and a PhD degree in Chemical Engineering, both from University of Southern California.

Myra Coufal, PhD, is a Principal Scientist in Process Development at Amgen in Digital Integration and Predictive Technologies group within CMC Lifecycle Management. Her responsibilities include developing multivariate models to support commercial manufacturing investigations and process improvement projects, as well as internally promoting the use of multivariate and first principles models during process and product development. Previously, she led a Process Development cell culture team supporting mammalian commercial manufacturing. Myra earned a BA degree in Biochemistry from Harvard University, and a PhD degree in Biology from the Massachusetts Institute of Technology.

An evaluation of pharmaceutical co-payment reforms in Spain

Jaume Puig-Junoy Pompeu Fabra University
Beatriz G. Lopez-Valcarcel and Santiago Rodríguez-Feijoó University of Las Palmas de Gran Canaria

The sudden fall of public revenues after the long-lasting economic crisis that began in 2008 has led many public health systems in European countries to cut public health financing through high copayments or coinsurance rates on drug prescriptions dispensed in pharmacies. This is especially the case in Spain, where until July 2012 nearly three out of four prescriptions were dispensed free of charge, Spain being until then one of the European countries with a relatively high number of prescriptions per capita[1]. Spanish health authorities have long sought to control public-sector pharmaceutical expenditure, but the economic crisis exacerbated this, and severe pressures were exerted on the public sector. After more than three decades of medicines being offered free to the elderly, this led in mid 2012 to a new co-payment policy being adopted.

Bountiful backing until 2012

From 1978 to July 2012, the Spanish national health system (NHS) provided generous free healthcare coverage to all Spanish residents, with the exception of a non-refundable coinsurance rate for outpatient prescription pharmaceuticals. The general co-payment rate had been 40% of the retail price since the early 1980s. A lower coinsurance rate of 10% was applied to medicines mainly prescribed for chronic diseases, with a price cap of €2.64 per prescription. Thus, effective coinsurance rates for insured patients ranged from 40% to a rate slightly above zero for highly priced medicines under the lower coinsurance rate. In addition, drugs provided to hospitalised patients were provided free of charge.

Pensioners and their dependants were exempted from the coinsurance scheme, so the aforementioned coinsurance rates were applied only to economically active people and their dependants, independently of their socio-economic characteristics. Caps or ceilings on maximum out-of-pocket expenditure did not exist either. Thus active individuals who transited into retirement or received an incapacity pension, independently of their age, as well as all their dependants, were automatically exempted from the pharmaceutical coinsurance

scheme and got free access to outpatient prescription medicines[2]. It is worth noting that civil servants were the exception to the general rule, since they incurred a co-payment rate of 30% of the full retail price, which was applied to both active individuals and pensioners.

Nominal coinsurance rates (40% and 10%) had remained unchanged in the two decades prior to the 2012 reform, although the effective average coinsurance rate had halved since the eighties (from 15% in 1980 to 7% in 2009). The increasing ageing population might explain the reduction in effective cost sharing, as well as the increasing number of medicines with a 10% coinsurance rate and the fraud (pensioners could obtain prescriptions for other household members who were not exempt from copayments)[3].

Puig-Junoy, García and Casado[4] previously examined the impact of the coinsurance exemption for prescription medicines applied to elderly individuals in Spain after retirement using an administrative dataset that linked pharmaceutical consumption and hospital discharge records for the full population aged 58 to 65 years in January 2004. This population was covered by the public insurer in Catalonia. In the study, a 'difference-in-differences' strategy was used and the eligibility age for Social Security to control for the endogeneity of the retirement decision was exploited.

The published results showed that this uniform exemption increased the consumption of prescription medicines on average by 17.5%, total pharmaceutical expenditure by 25% and the costs borne by the insurer by 60.4%, without evidence of any offset effect in the form of lower short-term probability of hospitalisation.

Free medication for all Spanish pensioners has also been shown to be clearly inequitable. Since it was independent of financial circumstances, a pensioner who received a large pension or had assets worth millions would pay nothing, while an unemployed person or a family with young children and an income of barely €1,000 per month, would pay their share. Half of all the cost sharing contributed by patients is concentrated in a small group of sick people: it was provided by just 5% of users, for whom it can represent a heavy burden.

> "Nearly half (40%) of the population think that the new scheme is more fair"

Three-pronged reform approach

In June 2012 the co-payment for outpatient prescription drugs was reformed in depth, and three types of policies ('three-payment reforms') came into effect nearly concurrently between late June and early October 2012. These policies were: (i) the temporary introduction of a regional one-euro fee per prescription in Catalunya and Madrid until it was suspended by the Constitutional Court; (ii) reform of national co-payment provisions, in which cost-free arrangements for all pensioners' drugs were replaced with a 10% co-payment subject to a monthly cap, and non-pensioners' 40% co-insurance rate with a 50 or 60% co-payment, depending on income; and (iii) the de-listing of a broad spectrum of over 400 drugs, including most in certain categories (nearly all for minor ailments).

The main aim of the reform, in a country where drug consumption rates per capita are among the world's highest, has been to enhance public awareness that 'universal' does not mean cost-free. However, there are shortcomings to the reform. For example, the existence of differential treatment within each income and need for patients with serious diseases are issues, since the co-insurance rate is very high and there is no cap on total expenditure in place. Another shortcoming is non-pensioners' co-insurance, contrary to the intention, does not depend on income. The initial inability to apply pensioners' cap at the point of sale is not only embarrassingly expensive, but overrides the reduction of financial risk pursued.

Despite issues like these, the reforms did induce a spectacular decline in the number of prescription drugs dispensed by pharmacies for the first time in over 30 years. A study of prescriptions and nation-wide spending in Spain between January 2003 and August 2013[5] revealed that the number of post-reform prescriptions was 12.8% lower than the counterfactual number assuming absence of reforms.

Puig-Junoy *et al.*[6] ran 17 univariate ARIMA analyses, one for each autonomous region, covering the period from January 2003 to July 2013. Dynamic forecasts were calculated to estimate the counterfactual number of prescriptions that would have been issued in each region in the absence of reform measures. The response variable was the joint impact of the measures adopted in each region calculated as the difference, expressed in per-centage, between the cumulative number of prescriptions actually recorded after 3, 6, 12 and 14 months, and the (contrafactual) number predicted by the respective models.

The findings revealed that after the steep and steady 10 year climb in the number of prescriptions

dispensed in Spain before the reform, there has been: (i) a drastic decline of over 20% of prescriptions in the 14 months after the reform in Catalunya, Valencia and Galicia; (ii) drops of over 15% in nine other regions; and (iii) drops larger than 10% in 15 of Spain's 17 autonomous regions.

Puig-Junoy *et al.*[6] also detected substantial inter-regional variability in the impact of Royal Decree 16/2012 on the number of prescriptions, because its provisions were not uniformly applied (the Basque Country did not apply the change in co-payments in the period studied) and because some regions established one-euro per prescription co-payments of their own (subsequently overruled by the Constitutional Court). The study provided evidence of the high price-sensitivity of prescription drug demand and the huge potential impact of a small linear co-payment (€1 per prescription) on drug use. These results were consistent with the hypothesis that the first euro of co-insurance has a sizeable effect on drug consumption[7].

Nonetheless, by the end of the time series, the effect of the Royal Decree appeared to have been 'diluted', although this observation was not statistically conclusive at the time.

Additional analyses showed short-lived effect

Subsequently, throughout February 2014, the same authors[8] analysed prescription numbers over a longer time series, running ARIMA segmented regression analyses for each autonomous region and for Spain as a whole. A significant finding was that the effect of higher co-payments was short-lived: they induced a drastic but transient decline in NHS prescriptions without varying the underlying upward trend. While the number of prescriptions was observed to be lower than it would have been if co-insurance had not been reformed, the model predicted that the effect of the reform on prescriptions would disappear entirely in a few years' time in certain regions and in Spain as a whole. In other words, although the co-payments introduced in mid-2012 managed to reduce NHS prescriptions drastically in the short term, since they had no impact on the prior upward trend, the numbers would tend to creep back up to former levels.

A survey to the general population questions the social acceptance of the new copayment stablished in the RDL16/2012[9]. Opinions on the justice of the new regulation, on the protection to disadvantaged social groups and on the adequacy of the copayment burden to the economic level of the patient were gathered. It was found that nearly half (40%) of the population think that the new scheme is more fair and that it better protects the disadvantaged groups. However, most of the population consider than there should be more defined income brackets to differentiate copayment rates. 73% of those who had used the public NHS and 81% of non users answered in that direction.

The survey also questions whether the individual failed to follow a medical treatment prescribed by a public doctor in the last 12 months because he could not afford it economically. A small but noteworthy 5.3% of the surveyed answered affirmatively (6.3% of those that had used the public healthcare network). Since we do not have data for the year before the new regulation, we cannot stablish a cause-effect relationship. We profiled the groups experiencing economic barriers to medicines; they are predominantly active (employed and unemployed) with low income, as one would expect given the design of the copayment scheme.

Final comments

Given the high sensitivity to prescription prices, information is urgently needed on which groups of patients and drugs contributed most to the aforementioned drastic reduction. Such data are instrumental to assessing the potential decline in overuse attributable to zero cost and its impact on adherence to treatment, access to necessary and effective treatment, and ultimately health. Health authorities' scant understanding of and lack of interest in the impact of a measure with such far-reaching social effects (the typical "why waste time evaluating?" attitude) is surprising. Little or nothing is known about patients' and doctors' decision-making mechanisms when it comes to reducing the number of prescriptions dispensed or their effects on necessary/unnecessary consumption, adherence to treatment and the use of other healthcare services or health.

Jaume Puig-Junoy (PhD) is Associate Professor in the Department of Economics and Business of Universitat Pompeu Fabra, Barcelona. He is the Director of the Health Economics and Pharmacoeconomics International Master's Degree (UPF) and he has been Director of the Research Centre for Health and Economics (CRES-UPF) in 2010-11 and 2014-15.

Beatriz G. Lopez-Valcarcel has a PhD in Economics and is a Professor at the University of Las Palmas de GC, Spain. She is Vice-President of the Socidad Española de Salud Pública y Administración Sanitaria (SESPAS) and of the Section of Public Health Economics of the European Public Health Association (EUPHA) and an International Consultant in Mexico, Brasil, Argentina, Chile, Uruguay, Costa Rica, Panama and Mozambique. She conducts research on health economics and quantitative methods for economics and management. Pharmaceuticals is among her research topics. See http://www.ulpgc.es/index.php?pagina=bvalcarcel.dmc&ver=inicio

Professor Santiago Rodríguez-Feijoó studied Economics at the University of Santiago de Compostela and received his PhD in Economics from the University of Las Palmas de Gran Canaria in 1990. His research has focused on regional econometric modelling and studying prices. Since 2012, he has focused his studies on measuring the effects of the global economic crisis on the Spanish health system.

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Increasing throughput and sensitivity of LC-MS assays with a focus on MS optimisation and novel LC strategies

SCIEX sponsored the latest webinar, broadcast live on the *European Pharmaceutical Review* website on 28th July 2015. It aimed to give an insight into the world of LC-MS assays, and how they can be used to their best potential. As an alternative to ligand binding assays (LBAs), LC-MS/MS methods can be a great addition to support the expanding protein component of pharmaceutical companies' drug pipeline.

To tell us more about SCIEX's software and its application in LC-MS assays we invited two speakers from the company.

First up was Jason Causon, Senior Applications Specialist at SCIEX, who introduced the audience to the DiscoveryQuant™ 3.0 software. DiscoveryQuant™ is an automated MRM tuning and optimisation application and is an excellent tool to achieve maximum sensitivity. The software works by collecting product ion spectra and allows for the optimisation of compound dependent mass spectrometer parameters (DP, CE, CXP and EP) in under 1.5 minutes via infusion or flow injection analysis. In Jason's presentation he gave a live demonstration of the DiscoveryQuant™ 3.0 in action, and introduced some new features.

Jason Causon

Focusing on the pharmaceutical and bioanalytical markets within the UK & Europe, Jason is responsible for the development and implementation of analytical methods and workflows within this arena. He has experience with high-throughput method development and UHPLC-MS/MS applications, both within SCIEX and previously within the GLP environment.

Remco Van Soest

Our second presentation was from Remco Van Soest, Product Manager at SCIEX. Remco holds an MS degree in Analytical Chemistry from Vrije Universiteit in The Netherlands. He has more than 20 years of experience with the development of nano-and micro-HPLC instrumentation and applications in the life sciences. Drawing on this knowledge, Remco discussed another SCIEX system, the microLC 200™; a dedicated microLC system for increased sensitivity and throughput. After defining what microLC is, he went on to outline the microLC 200™'s applications in Bio-Analysis, gave examples of the data you can collect from this system, and explained the advantages, e.g., decreased solvent consumption.

Some of the questions these insightful presentations raised were:

What is the typical lifetime of a microLC column?

How difficult is it to run at low flow rates?

How can you adapt the column when working with complex samples?

This webinar was sponsored by SCIEX

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