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Reasons behind critical key drug shortages in the EU and potential resolutions

Questioning whether viability methods effectively detect all viable organisms

Are capsosomes the revolutionary enzyme carriers for drug delivery?

Integrity in Pharma

Does your data stand up to scrutiny?
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Integrity is key

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DATA IS ONE of the most important assets for companies and organisations worldwide; and data integrity in pharma and biopharma is especially critical, as it underpins the development of potentially life-saving treatment. It is clear, however, that the industry must take further steps to address deficiencies in this area, evidenced by recent analysis of FDA FY2018 drug GMP Warning Letters, which identified that data integrity deficiencies are cited in 57 percent of all warning letters – a number which, in my opinion, is surprisingly high. Of note, this number is down from 79 percent in FY2016, but it is still a concern for an industry that is looking to use digital approaches to aid in risk reduction and allow for greater innovation in pharmaceutical lifecycle processes. For more information on solutions and advice relating to these issues, turn to our Informatics and Data Integrity Guide To series on page 67.

Another hot topic (still) is Brexit, but something that isn’t often discussed is future-proofing digital technologies. "Developing technology that can enable new and improved ways of working, drive increased efficiencies and visibility across organisations and supply chains, reduce costs and ensure legal compliance will minimise the impacts of Brexit," according to Neal Singh, Chief Operations Officer at Icertis in his article on ‘Brexit-tech’ on page 26.

Our regular columnist, Dave Elder, also discusses problems in the EU by exploring the reasons behind critical key drug shortages and what processes are being implemented to resolve the shortfall. He also notes several initiatives that have had a negative impact on drug shortages and their repercussions.

This issue sees the introduction of our ‘revamped’ editorial format, where each issue includes In-depth Focuses across four key topic areas – Formulation, Development & Delivery; Bioprocessing & Bioproduction; Manufacturing, Packaging & Logistics; and QA/QC & Analytical Techniques. Articles include discussions on higher order protein structure, RMM, FMD compliance and host cell protein contamination.

I hope you enjoy the issue and please get in touch if you have any research or work you would like to share.
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Focuses on supporting fisheries worldwide and ensuring the genetic diversity of the horseshoe crab.

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For more information about other industry events, go to: europeanpharmaceuticalreview.com/events
Inadequate supply: drug shortages in the EU

Dave P Elder
JPAG Member and David P Elder Consultancy

For nearly two decades, the US has been beset by critical shortages of key drugs and there are now similar issues within the EU. Here, Dave Elder explores the reasons behind the shortages and what processes are being implemented to resolve the shortfall.

RUG SHORTAGES are defined as, a situation in which the total supply of all clinically interchangeable versions of a regulated drug is inadequate to meet the current or projected demand at the use level. There are many different, inter-related reasons for drug shortages, but quality issues affecting the complex supply chains of many of these medicines are the most common. The American Society of Health-System Pharmacists' (ASHP) national drug shortages statistics for 2018 have identified the key reasons for drug shortages as being manufacturing (30 percent), supply and demand (8 percent), natural disaster (3 percent), raw materials (1 percent), discontinuation (10 percent) and unknown reasons (51 percent). In addition, there is often an over-reliance on single suppliers.

A single supplier will often produce 90 percent of the total supply of a medicinal product. In addition, it is very common for these suppliers to have a single source for active pharmaceutical ingredients (APIs) and key excipients in their supply chains. Moreover, 80 percent of APIs are manufactured in India and China and 60 percent of finished products are made outside the US. This can exacerbate drug shortages when such companies fail to meet US/EMA cGMP requirements or exit the market entirely. For example, the recent issues with sartans caused by N-nitroso contamination can be attributed to the fact that the sartan supply chain was heavily dependent on small numbers of impacted API suppliers. Similarly, manufacturing issues with Hospira in 2017 led to shortages of many critical drugs, particularly injectable narcotics and local anaesthetics, eg, lidocaine. There are currently significant issues with sterile injectable drugs across all drug classes and with antimicrobials, chemotherapy, cardiovascular (particularly the sartans'), CNS and nutritional fluids. There is also clear evidence that the situation is worsening; occurrences are increasing, duration is longer and public health impact is high. Some drug shortages have lasted for more than eight years, eg, liotrix tablets, with no imminent signs of resolution.

Ironically, some initiatives have had a negative impact on drug shortages. The US Food and Drug Administration (FDA) introduced the Unapproved Drugs Initiative (UDI) in 2006, which allowed companies to obtain regulatory approval and limited exclusivity for common, previously non-approved genericised drugs. Whilst the objective of the UDI was to improve overall safety and quality, it unintentionally increased drug prices resulting in drug shortages. Because many of the issues affecting drug shortages span multiple US agencies, an inter-agency task force was initiated in July 2018. An example of why this was deemed necessary is that the US Federal Trade Commission (FTC) isn’t currently required to assess public health considerations when assessing company mergers and acquisitions (M&A).

During drug shortages, market forces often exacerbate the issue by driving up prices of the existing suppliers. Similarly, hoarding of existing supplies can often be a result, again negatively impacting supply and demand logistics. Furthermore, the US pricing model for key drugs may be sub-optimal. For example, the current pricing model aims for maximal overall safety and quality, it unintentionally increased drug prices resulting in drug shortages.

Against this background, the FDA’s demands and public health impact is high. Some drug shortages have lasted for more than eight years, eg, liotrix tablets, with no imminent signs of resolution. Ironically, some initiatives have had a negative impact on drug shortages. The US Food and Drug Administration (FDA) introduced the Unapproved Drugs Initiative (UDI) in 2006, which allowed companies to obtain regulatory approval and limited exclusivity for common, previously non-approved genericised drugs. Whilst the objective of the UDI was to improve overall safety and quality, it unintentionally increased drug prices resulting in drug shortages. Because many of the issues affecting drug shortages span multiple US agencies, an inter-agency task force was initiated in July 2018. An example of why this was deemed necessary is that the US Federal Trade Commission (FTC) isn’t currently required to assess public health considerations when assessing company mergers and acquisitions (M&A).

During drug shortages, market forces often exacerbate the issue by driving up prices of the existing suppliers. Similarly, hoarding of existing supplies can often be a result, again negatively impacting supply and demand logistics. Furthermore, the US pricing model for key drugs may be sub-optimal. For example, the current pricing model aims for maximal overall quality with lowest achievable cost; consequently, there is no spare capacity, there is a systemic underinvestment in manufacturing facilities and there are long and over-extended supply chains. Against this background, the FDA’s demands (via audit programmes) for improvements in quality will often result in suppliers exiting the market as it can be too costly to implement remedial actions.

The FDA held a public meeting in November 2018 for the purpose of “Identifying the Root Causes of Drug Shortages and Finding Enduring Solutions”. This will hopefully facilitate the implementation of robust resolutions to the problem in the US.
Once-per-month HIV drugs could soon be available

HIV

ACCORDING TO a recent article, pharmaceutical companies are developing injectable HIV drugs that target different components of the HIV virus and can be administered once every few weeks.

This is because the current therapy for HIV – antiretroviral drugs in pill form, taken once a day – can be an issue as patients don’t always remember to take them.

When given as an intramuscular injection, the therapy was as effective as pills and persisted in the body for at least one month. In addition, more than 97 percent of study participants said in a survey that they preferred the long-acting injectable therapies for HIV are in clinical development. Recently, ViV Healthcare released data from two Phase III clinical trials of a combination treatment of two drugs that inhibit different parts of the virus.

If it proves effective in larger-scale trials, the vaccine, developed by researchers at Jefferson, could train the patient’s immune system to attack the colon cancer that had already spread before the surgery.

If it proves effective in larger-scale trials, the vaccine, developed by researchers at Jefferson, could train the patient’s immune system to attack the colon cancer that had already spread before the surgery.

Novel colorectal cancer vaccine shows positive Phase I results

VACCINES

A NEW VACCINE for colorectal cancer has proven to be safe in a small sample of human subjects, paving the way for the next phase of testing. Patients treated during Phase I of the clinical trial for the vaccine showed no signs of serious adverse events. The samples of their blood also contained markers of immune activation, which is an early indication that the vaccine could activate immune cells to fight colorectal tumours and metastases.

If it proves effective in larger-scale trials, the vaccine, developed by researchers at Jefferson, could train the patient’s immune system to attack the colon cancer that had already spread before the surgery.

Tumour vaccines have historically been developed against a sort of molecular signpost for cancer. Because they come from normal cells, cancer cells share nearly all of the same molecules, making it difficult for the immune system to differentiate normal from cancerous. Tumour antigens are molecules that the immune system can recognise as different from normal. In colorectal cancer, one such molecule (GUCY2C) was identified by Dr Scott Waldman, the Samuel MV Hamilton Professor and director of the Gastrointestinal Cancer Program of the Sidney Kimmel Cancer Center – Jefferson Health.

Phase I of the clinical trial enrolled 10 patients with stage I or II colon cancer. Patients were given one dose and came back for blood draws 30, 90 and 180 days after immunisation. Patients experienced some discomfort at the injection site, but reported no serious side effects of the vaccine.

The blood samples showed activation of ‘killer T cells’, the immune cell type the researchers had expected. These cells are responsible for finding and killing colon cancer cells that are responsible for causing the cancer to come back.

Ten big pharma companies collaborate on data sharing AI

ARTIFICIAL INTELLIGENCE

TEN TOP pharmaceutical companies have agreed to collaborate to train their drug-discovery, machine-learning algorithms on their shared data. The Machine Learning Ledger Orchestration for Drug Discovery (Melloddy) project represents the first time the companies have shared data with one another.

Owkin, a Google Ventures-backed start-up based in New York and Paris, has developed the artificial intelligence (AI), which is a secure, blockchain-based system. The algorithm is programmed to analyse data that the companies input without revealing trade secrets to rivals.

The AI will be used to improve the drug discovery process by predicting how molecules will react in certain conditions.

“We want absolute traceability of all operations made on the platform. It is very important that each pharma partner knows they are being treated on equal grounds, to make sure what is happening to their data is transparent so they can check it afterwards,” said Mathieu Galtier, co-ordinator for Owkin.
GSK and Novartis ‘misled’ consumers, says Federal Court of Australia

REGULATION

THE FEDERAL Court of Australia has found that the subsidiaries of pharmaceutical companies GlaxoSmithKline (GSK) and Swiss drug manufacturer Novartis breached Australian Consumer Law by promoting false claims or misleading assertions about their pain-relief products.

The Australian Competition and Consumer Commission (ACCC) held an investigation from January 2012 to March 2017 into the products. It found that Voltaren Osteo Gel claimed to be a more effective treatment against osteoarthritis-induced pain and inflammation than Voltaren Emugel, despite the two products having the same active ingredients.

The findings also showed that Voltaren Osteo Gel was sold at a higher price than Voltaren Emugel.

GSK has since corrected the packaging of the Osteo Gel to include the wording: “Same effective formula as Voltaren Emugel.”

The ACCC has stated that a separate hearing at a later date will take place to determine penalties.

Patent litigation for adalimumab resolved in the US

DRUG LICENSING

ABBVIE HAS announced that it has resolved its US HUMIRA (adalimumab) litigation with Boehringer Ingelheim. As reported by The Centre for Biosimilars, AbbVie first sued Boehringer Ingelheim for patent infringement in 2017, saying that Boehringer Ingelheim would infringe on more than 70 patents covering adalimumab in developing its biosimilar, which was approved just months later by the FDA under the brand name Cyltezo.

Under the terms of the resolution, AbbVie will grant Germany-based Boehringer Ingelheim a non-exclusive license to its HUMIRA-related intellectual property in the United States. Boehringer Ingelheim will pay royalties to AbbVie for licensing its HUMIRA patents and acknowledges the validity and enforceability of the licensed patents.

AbbVie will make no payments of any form to Boehringer Ingelheim.

“This is an important settlement as it resolves all HUMIRA-related patent litigation in the US and provides access for another biosimilar manufacturer seeking to enter the US,” said Laura Schumacher, Vice Chairman, External Affairs and Chief Legal Officer at AbbVie.

The US license for Boehringer Ingelheim will begin on 1 July 2023.
Tadalafil UKSC decision: evolution of the multi-factorial approach

Dr Matthew Royle
Partner, Taylor Wessing

The Supreme Court unanimously dismissed an appeal from the Court of Appeal in Actavis Group PTC EHF & Ors v ICOS Corporation & Anor, upholding its decision that a patent relating to the use of tadalafil in a 1 to 5mg dosage form is invalid for lack of inventive step. This is a case where the judge found invention at first instance and was overturned by the Court of Appeal. Matthew Royle, lead lawyer on this case, talks through the details.

Introduction
Actavis v ICOS [2019] UKSC 15 is one of very few patent cases to be considered at the highest level in the UK and represents the first instance in more than a decade that the Supreme Court has considered the issue of obviousness. In common with other patent cases before the Supreme Court, Actavis v ICOS raised issues of fundamental importance to patent holders and potential generic entrants alike because, as well as the four companies that were parties to the litigation, there were interventions from four industry...
bodies. The conclusion of the Supreme Court is, if anything, an example of evolution rather than revolution, but given the level of engagement it is interesting to consider the issues and how the court addressed them.

Background
The case related to the drug tadalafil, which is marketed by Lilly ICOS as Cialis® and is used to treat erectile dysfunction. The relevant patent protected the use of tadalafil to treat erectile dysfunction at doses up to 5mg per day. Cialis® is marketed at four doses, 2.5mg, 5mg, 10mg and 20mg; with only the lower two doses being protected by the patent. These doses are used for chronic daily administration, which affords the patients the benefit of spontaneity, although as the Judge noted, the claims are not limited to such use. Three companies – Actavis, Teva and Mylan – sought to revoke the patent in order to launch a generic tadalafil product at the lower doses. They argued that the patent was obvious in light of the claim that the dose response was something that the skilled person would have to do and would inevitably lead to the use of a 5mg dose and so there was no invention. ICOS argued that there were several value judgments that the skilled person would need to make to arrive at the invention and that it would be a surprise to the skilled person that a 5mg dose was efficacious (ie, there was no expectation of success).

At first instance, the Judge held that it was ‘very obvious’ to take tadalafil forward into clinical trials and that once the skilled person had identified that doses of 10mg, 25mg, 50mg and 100mg had the same efficacy (ie, were on a therapeutic plateau), it was very likely but not inevitable that the skilled person would try lower doses including 5mg. However, he held that the claim was inventive because it would be a surprise to the skilled person that a 5mg dose would be effective and because arriving at such a dose would require value judgments by the skilled person.

The Court of Appeal overturned the Judge’s decision. The purpose of the dose ranging studies is to ascertain the dose-response relationship and this purpose would not have been achieved when the therapeutic plateau including 10mg was identified. In the Court of Appeal’s view, this was not a case in which the skilled team was faced with parallel avenues of research. As Lord Justice Kitchin said:

“...the claimed invention lies at the end of the familiar path through routine pre-clinical and clinical trials process. The skilled but non-inventive team would embark on that process with a reasonable expectation of success and in the course of it they would carry out Phase IIb dose ranging studies with the aim of finding out...the dose-response relationship. It is very likely that in doing so they would test a dose of 5mg tadalafil per day...”

What were the issues?
There were two issues that the Supreme Court had to consider. The first was the correct approach to obviousness in the context of a patent to a dosage regimen and the second, which is not considered in this article, was whether or not the Court of Appeal was entitled to overturn the Judge in the context of this case. This article looks only at the first of these issues.

What did the Supreme Court decide?
The Supreme Court reviewed the tests that are often applied by the courts in England and Wales and the EPO – Windsurfing/Pozzoli and the problem-and-solution approach. It highlighted that these tests should not be applied mechanistically and that they were glosses on the statutory language of the EPC and the Patents Act, which set out the ultimate question to be applied:

“An invention shall be considered as involving an inventive step if, having regard to the state of the art, it is not obvious to the person skilled in the art.”

Lord Hodge, with whom the other Lady and Lords agreed, referred to the multi-factorial approach
that is typically applied but found that this list was not exhaustive. He then set out relevant considerations to the present case:

1. Whether at the priority date something was "obvious to try", in other words, whether it was obvious to undertake a specific piece of research, which had a reasonable or fair prospect of success.
2. The routine nature of the research and any established practice of following such research through to a particular point. This is said to be only one of several factors and has no primacy or paramount status. On the facts of this case, it would appear to have significantly influenced the court's decision.
3. The burden and cost of the research programme. Depending on how it is interpreted, this is a potential change in the law because historically commercial factors have not typically been relevant to an assessment of the inventive step. Here, the cost of the clinical trials is high and this points towards the need for patent protection to encourage pharmaceutical research but this was not a determinative factor.
4. The necessity for and nature of the value judgments, which the skilled team would have in the course of a testing programme.
5. The existence of alternative or multiple paths of research will often be an indicator that the invention was not obvious, but more than one avenue of research may be obvious.
6. The motive of the skilled person is a relevant consideration. The notional skilled person is not assumed to undertake technical trials for the sake of doing so but rather because he or she has some end in mind.
7. The fact that the results of research that the inventor carried out are unexpected or surprising, may point to an inventive step.
8. One must not use hindsight, which includes knowledge of the invention, in addressing the statutory question of obviousness. This applies to a step-by-step analysis, which may be arrived at by hindsight. Perhaps with the facts of this case in mind, Lord Hodge says that it is legitimate to take a step-by-step analysis where the pattern of the research programme can clearly be foreseen.
9. It is necessary to consider whether a feature of a claimed invention is an added benefit in a context in which the claimed innovation is obvious for another purpose.
10. The nature of the invention. In this case, it is a dosage form patent and in that context, Lord Hodge quoted Jacob LJ in Actavis v Merck that "nearly always such dosage regimes will be obvious".

The Supreme Court does not provide any guidance to how much weight each of these considerations should be given and it will no doubt vary depending on the facts of the case, leaving the discretion to the trial judge. Here, it seems that the routine nature of the studies, the absence of other avenues
of research and perhaps the nature of the invention had considerable weight. Other factors listed by Lord Hodge, including the cost and burden of the research (however that is interpreted) and the surprising nature of the results, may have pointed towards an inventive step but were not sufficient to affect the outcome.

Interveners
As mentioned above, the Supreme Court received interventions from four industry bodies, all of which commented on the correct approach to obviousness of dosage regimen patents. The BIA and the IP Federation were concerned that the Court of Appeal’s approach may be taken too far to mean that the use of routine investigations could not lead to an invention. The Supreme Court agreed with the concerns of the BIA and IP Federation but disagreed that any judges of the Court of Appeal had suggested that this was or should be the case.

Does Actavis v ICOS change anything?
This decision does not change a great deal. Structured approaches to obviousness such as Windsurfing/Pozzoli and the problem-solution approach are put into context as glosses on the statutory language, which should not be applied mechanistically. The decision clarifies that dosage regimen patents may be inventive and that not all inventions arrived at through routine experimentation will lack inventive steps. The main evolution is through its expansion of the multi-factorial approach to inventive step. Rather than quoting Lord Justice Kitchin, it will now be the factors identified by Lord Hodge that will be used and considered when assessing an invention. These factors are not exhaustive, and their relative importance will depend on the facts of the particular case, meaning that a large amount of discretion will remain with the first instance judge. Perhaps these factors will become a new framework that the court should not apply too mechanistically.

European medical device regulation
“Managing a nimble and conform transition”


The regulations clarify the obligations of the economic operators (manufacturers, authorised representatives, importers and distributors) that place their products on the European market. Moreover, the inclusion under the scope of the MDR of aesthetic devices, having the same risk profile as medical devices, extends the obligation to many companies hitherto unfamiliar with the medical device requirements.

The regulations, with the scope to provide a high level of health and safety protection for EU citizens, retain all the requirements of the current directives, and add some new obligations. Annex I of MDR specifies the general safety and performance requirements, while Annexes II and III specify the requirements of the technical and post-market surveillance documentation. As a result, the technical file of legacy devices will be updated providing evidence of the fulfilment to the new regulations requirements.

Significantly reinforced are the clinical evaluation requirements, such as the post-market clinical follow up, which is intended as a continuous process that updates the clinical evaluation, as well as vigilance and post-market surveillance requirements. The MDR sets out new rules for determining risk classes and, therefore, Class I devices may be upper-classified and may require the intervention of a notified body.

Being ready for the transition becomes crucial. Medical devices companies and economic operators need to prepare a transition plan to identify discrepancies and allocate resources to manage the identified gaps. Gap analysis is already a must to face NB inspections.

Plan with the designated notified body early enough; times and modalities for the release of the new EC certificate is key to ensure uninterrupted circulation of the products in the European market.

PQE Group supports all the “economic operators” in the transition period, carrying out gap analysis, preparing a transition plan and reviewing the technical file for new and existent medical devices and in vitro diagnostics of all classes, focusing on the high-risk devices, and building compliance at the beginning of the design and development phase.

pqegroup.com
Cell and gene therapies: the future is now and it’s (about to) happen in plastics

The Future is Now is the theme of the 2019 BPSA Single-Use Summit to be held in Washington, DC on 22-24 July, 2019. Below are the highlights for the upcoming event.

THE HEADLINES are common now and are steeped in both promise and perplexity. They read something like this: “Gene therapies come at exorbitant costs: who’s going to pay?” Good question. Driving costs out of cell and gene therapy (CGT) production processes are hugely important to their eventual commercial success. That success will be determined by perceived affordability (remember: these are cures, not chronic treatments) as we transfer CGT from the experimental research phase onto the menu of patient options in the medical suite.

BPSA addresses these cost concerns in its recently published white paper, The Role of Single-Use Polymeric Solutions in Enabling Cell and Gene Therapy Production: “successful commercialisation of cell therapies relies on the development of a scalable manufacturing process that can produce products of appropriate quality on a routine basis in a cost-effective manner”.

Most importantly, as the BPSA paper outlines, there is a daunting “but” here – ie, “on the process front, while the challenges associated with establishing robust manufacturing processes can be therapy-specific, issues arise from the fact that CGT therapies, when manufactured, are of significantly higher complexity than standard biologics, such as monoclonal antibodies or recombinant proteins”.

As daunting a challenge as this last statement represents, what we do know, as evidenced now in the nascent emerging plastic components and systems will be CGT. In other words, the future of CGT is now and it’s going to involve polymeric processing platforms – but probably unlike any “standard biologics” systems we are now familiar with.

While CGT will take centre stage in our programme, it is worth noting that in the context of CGT production, there is no “free lunch.” All the legacy challenges inherent in large-molecule biologic production come to bear in CGT: extractables, particulates, system integrity, change control, standards development, innovation around new CGT systems, resin quality and regulation. All these challenges figure into “costs” of production – and most importantly, to the safety to the patient. These topics are all on the docket at the BPSA 2019 Summit.

Our agenda for the programme can be found on our website. The BPSA 2019 Summit highlights the critical need for innovation in the polymer-based bioprocessing industry, to accelerate and advance life-saving cell-growth and cell-harvested immunotherapies, as well as serve the existing monoclonal antibody market. So yes, the future is now and it’s going to happen, one way or another, because of plastics. Find and download your complimentary copy of BPSA’s CGT White Paper, The Role of Single-Use Polymeric Solutions in Enabling Cell and Gene Therapy Production on BPSA’s website.

We hope you can join us for further discussion on this critical topic at the BPSA Single-Use Summit, 22-24 July, 2019 in Washington, DC.
BIOPROCESSING & BIOPRODUCTION

The efficacy, safety and pharmacokinetic properties of a protein therapeutic substantially depend on the molecule having the right structure. This article reviews current methods used for obtaining higher order structure information of biotherapeutics.

Purification of a drug from host cell protein contaminants can be challenging, with low-level contamination often remaining after purification. Vanda Dolabela de Magalhães shares discussions on the subject from six Brazilian companies working in the biotech field.
Analytical methods used in obtaining higher order structure information from protein therapeutics

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The efficacy, safety and pharmacokinetic properties of a protein therapeutic substantially depend on the molecule having the right structure. This article reviews current methods used for obtaining higher order structure information of biotherapeutics.

WITH REGARD to protein structure, the correct amino acid sequence and glycosylation (primary structure) are critically important, as are the secondary, tertiary and quaternary structures, which define how the protein presents in three dimensions. The latter are collectively referred to as having higher order structure (HOS) and contribute to the quality attributes of a biotherapeutic. Protein HOS can be affected and perturbed by changes in manufacturing, formulation and storage and must be measured and monitored, as per regulatory requirements.

Protein secondary structure is maintained primarily by hydrogen bonds between amino acids and results in coiling of the polypeptide backbone (α-helix) and stacking of amino acid side chains (β-sheet). The tertiary structure involves protein folding through the formation of disulfide bonds and salt bridges between amino acids, resulting in the lowest energy state (high stability) of the molecule. These globular proteins are water soluble, as folding exposes hydrophilic amino acids to the outer surface of the protein and shields hydrophobic amino acids in the interior. The quaternary structure comprises the arrangement of two or more folded protein subunits into a multi-subunit complex. Except for insulin and therapeutic antibodies, which consist of different subunits or chains linked by disulfide bonds, this is not a usual arrangement in biotherapeutics. Efforts are even made during manufacturing and through the selection of appropriate formulation and storage conditions to keep the formation of the aggregates to a minimum, as they are often associated with adverse immune responses.2

High-resolution methods
A variety of biophysical methods have been developed and refined to measure and characterise protein HOS. X-ray crystallography has arguably been the “gold standard” for obtaining high-resolution three-dimensional structures of proteins;3,4 however the formation of large protein crystals suitable for detailed analysis is complex and time consuming. Also, X-ray crystallography is not routine in biopharmaceutical characterisation. Cryo-electron microscopy requires no crystallisation which are relatively easy to obtain. This newer technique is gaining traction and is likely to become widely used soon. High field nuclear magnetic resonance (NMR) of proteins is another high-resolution method for obtaining HOS information,5 but its routine adoption for the characterisation of biopharmaceuticals has been impeded. The difficulty in interpreting the resulting data for larger proteins (e.g. antibodies), the relatively large sample requirements and because, similar to X-ray crystallography and cryo-electron microscopy, the equipment is costly and its use, analysis and interpretation of the data require specialised operators, are the main reasons for its slow acceptance.

Spectroscopic methods
Spectroscopic methods are commonly used in the HOS determination of biotherapeutics, as sample requirements are generally modest, data acquisition and analysis are relatively uncomplicated and the corresponding software streamlined. The cost of equipment is similar to other laboratory instruments. In general, these methods provide HOS information by inference compared to imaging of structural elements obtained by X-ray or electron diffraction methods outlined above. Circular dichroism (CD) spectroscopy has been used extensively to measure secondary and tertiary structure features of proteins. CD data are obtained...
from the differential absorption of circularly polarised ultraviolet (UV) light. Far UV (180nm-290nm) CD spectra relate to secondary structure elements (helices and sheets) and near UV (>290nm) CD spectra, which side chains of aromatic amino acids absorb, relate to the tertiary structure of a protein.5 Using appropriate models for protein structures that are similar to the molecule being analysed, estimates of helix, sheet and unordered sequence can be obtained. These data are useful in comparing different modelled structures of a biotechnological protein and assessing stability in different formulations and storage conditions, as loss of HOS features suggests protein unfolding, denaturation and aggregation.9

Fourier-transform infrared (FTIR) spectroscopy is also used to measure elements of a protein that comprise its secondary structure.6 Different bonds absorb at different wavelengths and those associated with stretching of the amide carbonyl (C=O) bond ca. 1,650cm⁻¹ (Amide 1 band), bending of the amide N-H bond ca. 1,540cm⁻¹ (Amide 2 band) and stretching of the amide C-N bond ca. 1,240cm⁻¹ (Amide 3 band) can be correlated to hydrogen bonding, which defines sheet and helix formation. As with other spectroscopic data, estimates of helix, sheet and unordered sequence can be generated from FTIR data using appropriate models. Recent developments using infrared laser spectroscopy, coupled with microfluidic sample introduction and continuous subtraction of the formulation buffer background, allow for increased sensitivity, extended range of protein concentration and automated analysis of samples. All of these are significant improvements over traditional FTIR spectroscopy.10

Raman spectroscopy is based on inelastic light scattering by molecular bonds (the Raman effect). It uses the same amide bands as IR spectroscopy to generate data that can be related to protein secondary structure features.11 Additional information can be obtained from stretching of disulfide bonds as well as from aromatic amino acids. The vibrational frequencies recorded in Raman spectra of proteins are sensitive to the local environment of the molecule and frequency shifts can be related to the unfolding of the structure due to strain. The method can be used to compare different batches of material or monitor changes in HOS due to formulation or storage conditions.

Intrinsic protein fluorescence is due to aromatic amino acids, chiefly tryptophan. The aromatic side chain absorbs UV light (280-290nm). An electron is promoted to an excited state and upon its return to the ground state light is emitted at a longer wavelength than the absorbed light (ca. 340nm). As with other spectroscopic methods, intrinsic protein fluorescence can be used to compare different batches or to monitor denaturation, since the hydrophobic tryptophan would typically be shielded from the aqueous environment. Therefore, changes in fluorescence intensity and emission wavelength between different protein samples would indicate differences in their tertiary structure.12

**Light scattering**
Molecules in solution will scatter high-intensity monochromatic light, usually from a laser, and the scattering angle and intensity correlate with the

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1. Resolution refers to the detail that can be obtained for various elements of the protein higher order structure and not to the resolution of the analytical technique itself (e.g., chromatographic resolution).
2. S: secondary structure; T: tertiary structure; Q: quaternary structure.
3. The difficulty is assessed from combining the relative difficulties of sample preparation, data acquisition and data interpretation.
4. Low: less than $20,000; Medium: between $20,000 and $100,000; High: greater than $100,000.
5. Low: less than 5μg protein; Medium: between 5 and 100μg protein; High: greater than 100μg protein.

**BIOGRAPHY**

SHANNON RENN-BINGHAM is Manager of Analytical Development at Celldex Therapeutics. She has been with the company for nine years and is responsible for method development and qualification of release and stability assays used in Quality Control and for generating characterisation data of drug substance for inclusion in Investigational New Drug (IND) applications. Shannon holds a BS in Biology from Brandeis University and an MS in Molecular, Cellular and Developmental Biology from Yale University.
The vibrational frequencies recorded in Raman spectra of proteins are sensitive to the local environment of the molecule and frequency shifts can be related to the unfolding of the structure due to strain

Mass spectrometry
Mass spectrometry (MS) and tandem mass spectrometry (MS/MS) of peptides, generated by enzymatic digestions of proteins, has been used almost since the emergence of the biotechnology industry to confirm the primary sequence of protein biotherapeutics and to characterise post-translational modifications as well as changes to the structure brought about by handling and storage. Additionally, characterisation of aspects of tertiary structure, specifically the connectivity of disulfide bonds, has also been developed using similar methodology, digestion of a protein without prior reduction of disulfides and under conditions that do not promote disulfide scrambling.

More recently, hydrogen-deuterium exchange (HDX) in protein samples, originally used with NMR, has been coupled with MS analysis to identify those regions of a protein that are exposed to the aqueous environment and those that are shielded from it. Incubation of a protein in deuterated water buffers leads to exchange of labile hydrogens (those on amides, amines and hydroxyls) that are exposed to the solvent. Rapid proteolysis under conditions that slow down amide hydrogen back exchange, followed by high performance liquid chromatography (HPLC) and MS, yields data that can be used to identify those regions of the protein molecule where deuterium was incorporated with resolution of a few amino acids. This can be related to the secondary and tertiary structures of the protein but also to quaternary structure in cases of non-covalent complexes between proteins or between a protein and a small molecule. The regions of contact will yield little or no hydrogen-deuterium exchange as they are not exposed to the aqueous environment.

Native MS is another relatively recent application in which the intact biomolecular structure of folded proteins and non-covalent protein assemblies can be studied. This is accomplished using buffers that do not significantly disrupt the native structure of biomolecules and do not adversely affect the ionisation process in the MS ion source. The distribution profiles of the multiply-charged electrospray ion envelopes of the same protein in native (folded) and partially or fully unfolded states are different and can be related to the basic amino acid side chains exposed to the solvent, so are available for protonation.

Chromatography and electrophoresis
High performance liquid chromatography (HPLC) of proteins can be carried out under conditions (buffers, temperature) that cause minimal or no disruption to the higher order structure of biological molecules. Species can be separated based on charge (ion exchange chromatography – IEC) or size (size exclusion chromatography – SEC) and the relative abundance of these species, as measured by UV detection, is thought to reflect their relative abundance in solution under native conditions. Similarly, polyacrylamide gel electrophoresis (PAGE), capillary gel electrophoresis (cGE) and capillary isoelectric focusing (cIEF) under native conditions allow for the separation, based either on charge or size, of native protein complexes. Such methods do not provide any information on the actual structure of these molecules but are useful in ascertaining the presence and measuring the comparative abundance of charge variants or aggregates, from which perturbations to the HOS can be inferred. Often, chromatography, especially SEC and to a lesser extent CE, is carried out in-line with light scattering detection or native MS, thus augmenting and confirming information obtained from the chromatographic separation.

Often SEC and cGE, which are easy and quick to perform, are validated by more involved ‘orthogonal’ methods for obtaining information regarding the presence and distribution of protein aggregates, such as analytical ultracentrifugation (AUC) and field-flow fractionation (FFF). The various analytical methods outlined above are summarised in Table 1.

Conclusion
A significant collection of analytical methods and associated commercial instruments are available for the analytical scientists tasked with generating higher order structure information of a protein biotherapeutic. Such information is essential in understanding the structure of such a molecule; identifying suitable formulation, handling and storage conditions that do not perturb the structure; and establishing that the manufacturing process yields the same product within specified parameters, all of which contribute to the quality of a protein biotherapeutic.
Eshmuno® CP-FT Resin

EXPLORE EFFICIENCY

Eshmuno® CP-FT resin is the first-of-its-kind to efficiently remove mAb aggregates in flow-through frontal chromatography mode with loading capacities 10x higher than traditional bind/elute chromatography. Eshmuno® CP-FT cation exchanger features more efficient impurity removal, significantly reducing resin and buffer needs. As part of the BioContinuum™ Polishing Platform, Eshmuno® CP-FT resin offers greater manufacturing flexibility, process intensification, and higher performance.

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HOST CELL proteins (HCPs) are impurities found in biotechnological products that can cause immunogenicity and reduce the potency or stability of a drug.\textsuperscript{1,2} Reducing HCPs is therefore a matter of safety. Historically, the bioindustry has used Enzyme Linked Immunosorbent Assay (ELISA) to quantify these contaminants in a drug substance and it is considered the gold standard approach due to its sensitivity and selectivity. During drug development, regulatory authorities accept the use of commercial generic ELISA kits to quantify HCPs, but as the drug progresses to Phase III, they demand a proprietary ELISA is developed and used thereafter. This demand originated when an HCP that caused anti-human growth hormone antibody wasn’t detected by the commercial kit.\textsuperscript{3} Here we present the discussion and conclusions made by Brazilian biopharmaceutical companies who met recently to discuss this issue. The companies involved in this discussion were Biolab, Bionavis, Blau, Cristália, Eurofarma and Libbs, with support of the group FarmaBrasil.

Mammalian and bacteria cells are among the most commonly used platforms for biopharmaceutical production. The heterogeneity of an HCP profile, which includes proteins with differences in molecular weight, hydrophobicity...
and pl, poses a challenge to detection and quantification. Genome sequencing studies identified 24,000 predicted genes in Chinese Hamster Ovary (CHO) cells and approximately 4,300 genes in Escherichia coli (E. coli) chromosomal DNA.4,5

Although different processes can result in varying clearance of HCPs, such differences are thought to be of minor consequence.6 As technology improves, authorised manufacturing changes are often implemented.7

Other techniques have emerged for analysing HCPs, such as 2D-SDS-PAGE, Western Blot (WB), CE and LC-MS. It is noteworthy that immuno-specific methods, like ELISA or WB, have drawbacks related to the recognition capacity of the polyclonal serum. On the other hand, non-specific methods, like 2D-SDS-PAGE and mass spectrometry (MS), can detect any protein. The bioindustry has relied on this orthogonal approach to qualify the manufacturing process, and track and identify HCPs in the drug substance.6,7

Chapter 1132 of USP focuses on these orthogonal methods to demonstrate that a proprietary ELISA assay is fit for use after modifications in the manufacturing process. The suggested approach is approximately the same one used for ELISA development. If such a strategy can show the fitness of a proprietary kit after manufacturing changes, it is conceivable that the same approach could also demonstrate the fitness of a generic ELISA kit for a specific process. This supposition relies on three arguments: a) the protein profile of a null cell does not necessarily mimic the protein profile of a producing cell; b) development of an ELISA assay is not trivial and will not cover all HCPs; c) the protein profile of different strains of the same species are relatively conserved.

A null cell is derived from the same cell line used for production but does not contain the sequence expressing the recombinant protein. The use of a null cell is important to develop an ELISA kit for HCP detection because any contamination by the recombinant protein might generate antibodies against the product. Recombinant proteins produced by bacterial cells precipitate as inclusion bodies in the cytoplasm, dragging HCPs randomly in the process. Since the null cell does not express the recombinant protein, there is no inclusion body formation and no stress related to overproduction of a heterologous protein.

In the case of mammalian cells, a parental cell can be used as a null cell, or they can be transfected with the empty vector (without the recombinant protein coding sequence). Usually, vector insertion can be mapped but not always controlled. Moreover, after transfection, cells are adapted to grow in special conditions and uncontrolled mutations can occur.

The protein profile of bacterial or mammalian null cells will not be the same as the production clones. Moreover, scientists have shown that drug substances eluted from protein A chromatography showed a higher HCP concentration than null cells eluted at the same step. This was explained by an association of HCP to the antibody that would not be detected in a process using null cells despite the method sensitivity.6

Challenges concerning the development of an immunoassay must also be considered. A pool of characterised HCPs are used to immunise more than one certified germ-free animal to guarantee a broader immunological response, considering the expected biological variability.13 Nevertheless, some HCPs might show higher antibody affinity while others might be less immunogenic, resulting in over- and under-representation of some HCPs in the antiserum. The absence of reproducibility is a drawback in 2D-SDS-PAGE that is used to characterise the obtained antiserum.14 The WB technique establishes the coverage of the antiserum but presents limitations of transfer efficiency to the membrane and of matching spots between gel and blot due to spur stains.13 Moreover, the denaturing conditions of 2D-SDS-PAGE will destroy native epitopes that are shaped by the three-dimensional structure of the protein, drawing amino acids apart in the protein sequence. Such epitopes will not be recognised in the WB.13

Consequently, a cascade immunisation protocol has been described to improve coverage.15 The ideal situation would identify by MS the non-recognised proteins, determine the most immunogenic epitopes of these proteins and use peptides containing this sequence in a new immunisation cycle. Even then, however, over- or under-representation is often the rule in immunoassays and no polyclonal antiserum recognises all HCPs.

Knowledge of the homogenous genetic nature of different strains of the same species is reported in various genomics studies. Wild-type strains of E. coli of different origins revealed a small number of sequence types.16 The complete genome sequence of an E. coli strain identified 234 lateral transfer events in the last 100 million years from when this species diverged from Salmonella.17 More recently,
comparative analysis of genomes, transcriptomes and proteomes identified phenotypic differences between E. coli strains, including two B-derived strains and two K12-derived strains. Protein profiles were very similar and most differences were in the spots intensity.19 Proteomics studies also showed minor differences in the HCP profile of E. coli hosts grown in different fermentation processes.19 Whole genome comparison of LS5218 E. coli strain to another 22 strains of the same species showed similarity of most genes. Accessory genes were found in two strains and unique genes in a single strain.21

Among a thousand proteins resolved by 2D-SDS-PAGE from three different CHO cell lines, only 11 qualitative changes and 26 quantitative changes were detected. Most qualitative differences represented post-translation modifications, reflecting cell adjustments during growth.2 Another study compared two CHO cell lines after one suffered selective pressure known to result in chromosome rearrangements and only five differences in gene expression were detected.21

Genentech scientists saw more similarity than differences in HCP profile by LC-MS/MS of three CHO cell lines grown in different upstream processes.22 Knowledge regarding the homogenous genetic nature of closely-related strains supports the probability of the commercial kit fitness for most biotechnological processes. One ELISA commercial kit for E. coli, for instance, is derived from six different E. coli strains, while a third-generation CHO commercial kit was developed using two strains of CHO cells that react to approximately a thousand HCPs.

Conclusions
ELISA, the gold standard method for HCP quantification in biotechnological drug substances, has some limitations relating to the method itself and its development. Any ELISA kit, whether specific or generic, cannot detect all possible HCPs, even using a careful cascade strategy to develop the immune antiserum.

The null cell suggested by the pharmacoeypa to produce HCPs for immunisation doesn’t necessarily produce a protein profile identical to the production cell. Genetic homogeneity among different strains of the same species has been demonstrated by genomic studies. Differences in processes have shown small impacts on the protein profile.

The findings described above and the restraints of developing an ELISA kit corroborate the idea that a proprietary ELISA kit might not detect such minute differences. There is no assurance that a proprietary kit will perform better than a commercial one. The better strategy to mitigate potential adverse effects of HCPs will be continuing the development of a robust process that consistently removes HCPs to the lowest possible level. An orthogonal approach must prove the robustness of the manufacturing process in removing HCP. At specific steps, HCP must be evaluated by 2D-SDS-PAGE, WB, MS and ELISA, as exemplified above. A proprietary ELISA kit is only required in the event that the orthogonal approach doesn’t show good performance in tracking HCP along the manufacturing process and quantifying it in the drug substance. These issues must be discussed case by case with the regulatory agencies in order to assure the required patients’ safety along with a successful biodrug development.

REFERENCES

Eshmuno® CP-FT resin – developed for the flow-through removal of aggregates using frontal chromatography

Eshmuno® CP-FT cation exchange resin is specifically designed to provide efficient removal of monoclonal antibody (mAb) aggregates in the flow-through frontal chromatography mode of operation enabling loading capacities 10-times higher than traditional bind/elute CEX chromatography. It facilitates greater manufacturing flexibility and process intensification while reducing the overall cost for the downstream purification of mAbs.

MAbs are a successful class of therapeutic proteins increasingly used in the biopharmaceutical industry, but the manufacture of safe and effective mAb drug products as well as increasing productivities of upstream processes provides many challenges to downstream purification.

One of these challenges is the tendency of mAb molecules to aggregate. Aggregates must be cleared during downstream chromatography processes as they pose a significant risk to patients by increasing the potential of an immunogenic response and reducing drug efficacy. However, aggregates are one of the most difficult impurities to remove from mAb feeds due to their similarity to the monomeric target product.

Eshmuno® CP-FT resin is the first-to-market cation exchange (CEX) resin specifically developed for the flow-through removal of aggregates using frontal chromatography.

Benefits on resin volume and buffer volume with Eshmuno® CP-FT resin

In the flow-through mode, Eshmuno® CP-FT resin demonstrated that it can reduce the volume of resin and buffer required to remove aggregates from a mAb feed by tenfold compared with traditional bind/elute CEX resin.

For instance, while a mAb feed might be loaded up to 80g/L with a traditional bind/elute chromatography process, we have demonstrated efficient removal of aggregates at a loading of 1,000g/L by flow-through frontal chromatography with Eshmuno® CP-FT resin. Switching from a bind/elute chromatography step to a high loading flow-through frontal chromatography step with Eshmuno® CP-FT resin offers the potential for a tenfold reduction in resin and buffer consumption, allowing greater manufacturing flexibility and reducing the footprint of the downstream purification process by requiring less resin and smaller buffer tanks.

Frontal chromatography mechanism

Eshmuno® CP-FT resin operates under strong binding conditions (pH 4.0-5.5, 3-7mS/cm) that favour strong electrostatic interactions between the positively charged mAb monomer/aggregates and the negatively charged CEX tentacle surface chemistry. Under these conditions, both the mAb monomer product and the mAb aggregates will initially bind to the Eshmuno® CP-FT resin. The resin’s novel CEX tentacle surface chemistry facilitates displacement of the bound monomer by the larger aggregates enabling efficient removal using a frontal chromatography mechanism. The mAb monomer elutes from the column in the first front and the column can continue to be loaded with the mAb feed until its full capacity for the mAb aggregates is exhausted.

The low salt process conditions eliminate the need for dilution before subsequent ion exchange steps and the significant reduction in processing volumes improves virus filtration and ultrafiltration processing economics. Furthermore Eshmuno® CP-FT resin’s rigid base bead allows higher flow rates and easier column packing. This enables biopharmaceutical manufacturers to establish more flexible and connected downstream purification processes while reducing their costs and manufacturing footprint.

Eshmuno® CP-FT resin is part of the family of Eshmuno® CEX polishing resins that have surface chemistries optimised for specific applications. These include our Eshmuno® CPX resin designed for the efficient separation of aggregates using bind/elute chromatography and our Eshmuno® CPS resin which was designed specifically for the capture of recombinant proteins directly from clarified cell culture.

For more information, please visit: merckmillipore.com/eshmunocpft
WILE THE decision by the UK to leave the EU will certainly have major consequences for the transport, logistics and supply chain sector, pharma companies will be in a significantly different position to those businesses who are merely moving unregulated goods across borders. Deal or no deal, there will be serious repercussions; with biopharma R&D set for a particularly trying time. So how can businesses prepare for whatever comes their way?

The biggest challenge relates to supply chains and clinical trials, with EU regulation requiring all drugs to be tested within the EU and for all relating paperwork to be held there. Considering this, one practical step organisations can take to reduce implications for research funding and clinical trials is to increase their focus on future-proofing technology investments. Developing technology that can enable new and improved ways of working, drive increased efficiencies and visibility across organisations and supply chains, reduce costs and ensure legal compliance, will minimise the impacts of Brexit.

Cost reduction and operational efficiency is particularly important as navigating different sets of guidelines will inflate costs and study times. For example, between 2010 and 2017, the average cost of developing a pharmaceutical compound, from discovery through launch, rose by 82 percent from $1.19 billion to $2.17 billion. While these costs can be decreased by speeding up time to market, this requires jumping multiple legal hurdles before a company can generate revenue; finding ways to speed up clinical discovery can be extremely challenging.

As 31 October draws closer, organisations across all sectors are putting contingency plans in place for whatever the impact of Brexit may be. However, for pharmaceutical organisations already operating in a heavily regulated, highly competitive landscape, the future looks a lot more uncertain than for most. Here, Neal Singh discusses digital initiatives that could help companies to get ahead of the curve.

Neal Singh
Chief Operations Officer at Icertis

Brexit-tech: overcoming post-brexit challenges by driving operational and cost efficiencies
One example of a digital initiative that is already being undertaken at many of the world’s leading pharmaceutical companies like AbbVie and Sanofi, is re-imaging contract management as an enterprise-wide system. These companies are finding that at the heart of challenges, like complex drug trial processes and supply chains, are broken contract management processes that slow business velocity, reducing agility and leaving them open to increased risk.

Removing the need for manual management of contracts can save a significant amount of time and money, while helping to avoid contractual risks like missed entitlements, unwanted expiries/renewals and non-compliant clauses that can damage important business relationships and slow down progress of getting new drugs to market.

Looking specifically at the drug trial process, this typically involves relationships with many external parties including sponsors, Contract Research Organisations (CROs) and sites. Each of these relationships is business critical, so simplifying and automating processes such as contract negotiations can deliver enormous value.

By using technology to better manage contracts around clinical trials and R&D work, pharmaceutical firms can provide greater access to contracts and easier tracking of budget consumption, as well as address four key areas:

Supply chain compliance: large pharmaceutical companies must monitor work completed across multiple jurisdictions to ensure products are delivered to market safely and on time. A single trial can involve multiple CROs and it is often the sponsor’s responsibility to ensure all sites are compliant. Manual contracting processes means visibility into these complicated supply chains is impossible. Especially difficult to track are back-to-back contracting situations when a vendor agrees with another to fulfil an obligation for the end buyer. If companies do not have full visibility into multi-tier supply chains, they expose themselves to regulatory liabilities, without any control or knowledge of the compliance regulations suppliers are subscribed to.

Cost: traditionally, the pharmaceutical industry has been revenue focused. However, in the past decade companies have placed greater emphasis on controlling costs as reimbursement pressures and drug development costs escalate. Manual contracting processes impede a company’s ability to optimise spending. It is not uncommon for two different divisions of the same pharma company to unknowingly contract with the same supplier but on different terms. As the departments have no visibility of each other’s contracts, inefficiencies persist.

Internal visibility: enterprise contract management creates a central repository of agreements, providing better visibility for all departments across an organisation. By establishing a single source of truth, executives, legal teams, business users, compliance officers, etc, all know where they can find the fully executed version of a contract and all amendments. Companies are not slowed down by having to search for or wait to receive the documents they need to move forward.

Supply chain management: with full visibility into their contracts, pharmaceutical companies gain deep visibility into their supplier relationships, including regulatory compliance, renewal and expiry data as well as back-to-back contracting risks. This can help them to understand their exposure not only to Brexit-related contracting but also future governmental and regulatory changes that may impact the business.

Current contract management systems integrate the latest technologies to help unlock R&D bottlenecks and ensure pharmaceutical companies can become more responsive and ultimately better prepared for the challenges posed by Brexit.

The application of artificial intelligence (AI) to contract management turns once unstructured documents into live, strategic assets. AI-infused contracts understand the meaning of complex contract clauses and can identify what contract language could pose risks or opportunities to an organisation. AI can also unearth data to enhance contract data input and visualisations. With AI, companies can look at every contract – historical, in-flight and new – and ask where there is opportunity to save and make money. Where can we reduce business risk? What terms might accelerate contract acceptance? How can we learn from previous mistakes?

And this is just the beginning of how contracts are getting ‘smarter’. Blockchain presents the next step in the evolution of the contract management space. It has the capacity to effortlessly automate, manage and control processes. It can record transactions based on key pieces of metadata such as rules, entitlements, clauses and obligations, in turn triggering actions based on pre-programmed activity and improving visibility.

It is clear that the conversations and negotiations that have happened since the Brexit referendum have been disorientating, with many potential situations and impacts being painted by different sources. The fact is that post Brexit, anything can happen and the pharmaceutical industry has valid concerns about what lies ahead. Much of the potential impact will be inevitable, but there is also a lot that can be done organisationally for companies to get ahead of the curve in terms of operational improvements and cost cutting initiatives enabled by digitalising traditional and inefficient processes like contracting.

Blockchain presents the next step in the evolution of the contract management space

BIOGRAPHY

NEAL SINGH has invested more than 25 years in enterprise software and leadership roles. Prior to joining Icertis, he served as the President and CEO for Caradigm where he helped create their SaaS product offerings in Enterprise Population Health Management, Analytics and Identity Access Management. Prior to Caradigm, Neal was at Microsoft for over a decade and, as a member of the Business Leadership Team, helped build the Microsoft Dynamics Enterprise ERP product portfolio.
FORMULATION, DEVELOPMENT & DELIVERY

The ADDoPT collaborative consortium has been working to explore how emerging process understanding and predictive models can be applied and embedded within industrial workflows to enhance decision making and accelerate development. This article delves into their discussions.

Nature can offer inspiration for the synthesis of more effective drug delivery platforms. In this article, Marc Baiget Francesch discusses the potential of capsosomes as a novel, biocompatible, drug delivery system.
Digital design for pharmaceutical product and process development

As well as cost benefits, outsourced manufacture brings associated supply chain risks and mitigation costs.

**BIOGRAPHY**

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In an increasingly digitalised world, it is evident that pharmaceutical manufacture is still built on heuristics. Process and product development is mostly achieved by “making and testing”, development cycles are long and processes efficiency is sub-optimal. As the sector transitions from the era of “blockbuster” drugs and market expectations change with the drive towards personalised medicines, sector leaders clearly recognise the need to transform the capability, flexibility and speed of their development workflows and supply chains.

ADDoPT (Advanced Digital Design of Pharmaceutical Therapeutics) is a major UK-based supply chain project established in response to the challenges faced by the industry. The four-year, £20.4m government-industry-academia collaboration has enabled partners from across the pharma value chain to define a system for top-down, knowledge-driven digital design and digital operation for drug products and their manufacturing processes. The aim is to integrate a wide range of predictive models and insight from industrial case studies at four major pharmaceutical companies, allowing more targeted future experimentation, a better understanding of risk and subsequent improved design and scale-up for robust products and processes. In the words of the former chair of the Medicines Manufacturing Industry Partnership, ADDoPT’s objective is to “create virtual medicine manufacturing systems to make sure they are effective and efficient before creating them in the real world”.

This desire for change is set in the context of a regulated industry, which has used outsourcing/offshoring extensively to reduce costs. Whilst these may be regarded as braking forces, this is not necessarily so. Regulators like the FDA and EMA support Quality by Design (QbD), an approach that aims to ensure the quality of medicines by employing statistical, analytical and risk-management methodology in their design, development and manufacture. QbD calls for all sources of variability to be managed by appropriate measures, ensuring that the finished medicine is consistently delivered “right first time”. Unfortunately, the assurance provided by QbD generally demands substantial amounts of supporting data, which is a significant challenge under the current heuristic, experimentally-driven paradigm. As well as cost benefits, outsourced manufacture brings associated supply chain risks and mitigation costs. Manufacturers want to hand over robust, predictable, reproducible process systems to CMOs with peace of mind. In both cases, digital design tools offer a potential change in approach through reduced data requirements as well as the ability to consider many more factors and identify true critical process parameters and material attributes.

Due to the cost and risk pressures, and the need for quality assured products, the pharma sector is actively exploring solutions such as flexible manufacturing and advanced process modelling and control. However, these approaches are not routinely embedded at this point. Manufacturers...
are nevertheless gradually, but increasingly, coming to regard their development and process data as key assets in the journey towards digital operations. The challenge now is how to systematically harness the potential of advanced materials and process modelling and data analytics to transform decision making in product and process design and control.

Case studies in pharmaceutical digital design
The ADDoPT definition of digital design combines research insight and qualitative and quantitative mechanistic modelling to provide links between raw materials, manufacturing processes and the needs of the patient. It spans all unit operations, processes and procedures during the manufacture of medicines, as well as their impacts, both upstream on the efficiency of product and process design and downstream on product performance. The project partners have worked together to co-ordinate the development of a suite of case studies providing coverage of relevant application areas across this space, in order to evaluate and illustrate the use of new modelling tools and approaches to address a series of relevant, real-world challenges. The following examples provide a flavour of the work undertaken.

Example 1: using computational modelling and big data to overcome tablet sticking
Adherence of an active pharmaceutical ingredient (API) to tablet punches during compression, often referred to as tablet sticking, has been identified as a common and long-standing processing challenge for the pharmaceutical industry that impacts manufacturing efficiency. Resolution of a tablet-sticking issue is usually complex and rooted in a multi-factorial cause, which therefore needs a holistic approach to finding a solution as part of continuous process improvement. In this case study an approach combining computational modelling at a molecular and materials level with ‘big data’ analysis of the manufacturing process and materials is being applied (see Figure 1). The study covers most of the breadth of the ADDoPT remit from API properties through to drug product manufacturing performance.

‘Big data’ gathering and analysis
Process data
In order to evaluate a commercial drug product process where sticking issues are observed, a manufacturing site has collected a wide range of process parameters, including punch cleaning times.

Materials characterisation
All in going material (API and excipients) have been tested using 16 characterisation techniques. This will cover three formulation strengths and multiple API suppliers, producing data that is a mixture of numerical results, patterns, traces and images. In-process samples from four sampling points for 49 batches of product are being tested using a suite of material characterisation techniques. In addition, characterisation will be completed on the final tablet.

Data analysis
A range of analytical and statistical tools are being employed, firstly to determine the similarity/consistency of material properties of ingoing API and excipients, in-process samples and tablets across batches. The results will then be used to identify the characteristics that link through the process to the processing behaviour of the batch. For any hypothesis that relates to the API properties, the consistency of the prediction with the material modelling described below will be considered. The intention of the analysis is to deconvolute the relative importance of the material attributes and process parameters to sticking.

FIGURE 1
ABOVE: Linking material and processing data analysis to molecular modelling

FIGURE 2
ABOVE: Actual and predicted powder performance (shear cell angles) for a range of pharmaceutical materials

BIOGRAPHY
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BIOGRAPHY
DR HERVÉ BARIAT obtained a PhD in physical chemistry, before working in translational science departments within the pharmaceutical industry for 14 years, concentrating on the development and use of pre-clinical imaging models.
Material modelling
This consists of studies starting at the molecular level, examining predicted surface properties, how the surfaces may interact with excipients and the effect of crystal morphology on the relative proportions of key features. Initial work has indicated that the surfaces have different behaviours providing some key hypotheses that can be tested using the data above. In addition, modelling of the impact of solvents on the predicted morphology of the API would provide a good starting point for API crystal engineering studies, if required.

Achieving these combined computational modelling activities has involved a multi-disciplinary approach including a team from the University of Leeds and colleagues from both R&D and commercial manufacturing groups at Pfizer. This type of multi-disciplinary, orthogonal approach to a computational tool is a powerful way to solve multi-factorial issues such as tablet sticking.

Example 2: from powder properties to powder flow
Continuous manufacture of oral solid dosage forms is now a reality but has not been fully optimised, and some of those “continuous” processes may require enabling by batch pre-blending. For continuous direct compression, a primary requirement is that powders flow reliably and predictably within the loss in weight feeders and mixers that lead to the mixers and press. If an API has poor flow it may have to be premixed with another component in a batch stage prior to the continuous process, a sub-optimal arrangement. A better understanding is needed of what characteristics lead to reliable flow; however, direct measurement of these characteristics often requires testing on ‘real life’ equipment, which requires kilograms of material to make a reliable assessment. At development costs and scales this can be a prohibitive requirement.

In addition, the measurement of flow and its correlation to performance in feeders and mixers has not been fully aligned in the industry. The fundamental powder properties linked to this performance have been examined, but generally on model materials with properties significantly different from active APIs. In many cases the latter are micronised to a size of around one micron, whereas model materials may be in the hundreds of microns range, and in silico modelled systems tend to be based on single sizes or shapes.

Within ADDoPT the consortium has been able to attack this challenge. The unique synergy between large pharmaceutical companies, academic groups and data centres has allowed the programme to:

- Define the characteristics of a powder that could be successfully fed in a continuous feeder, and how these can be assessed without using direct measurements in the feeder
- Agree harmonised methodologies on how to measure the fundamental properties (size, shape, surface area and surface energy) of powders, which either met, or did not meet, these criteria. This was done on state-of-the-art apparatus available at all the industrial partners
- Devote resources to making both the flow measurements and the fundamental measurements using the harmonised methods, with a significant number of materials with both acceptable and unacceptable properties
- Analyse the large body of data generated, using the data systems and capabilities of the STFC Hartree Centre, to build innovative models of powder properties likely to lead to success in a feeder.

With these models available it was possible to predict, for the first time across a range of pharmaceutically relevant materials, which systems would be likely to have the necessary flow characteristics to deliver the required performance in a loss in weight feeder (Figure 2). Most importantly, this could be applied not only to actual materials where measurements were available (even on a small amount of material) but also to profile materials, the properties of which (eg, size, shape) could realistically be made, allowing the formulator to direct manufacturing colleagues to make, procure and specify suitable materials capable of performing in continuous direct compression.

Example 3: utilisation of a process model for twin screw granulation
Traditional pharmaceutical manufacturing has frequently made use of batch wet granulation processes to manufacture oral solid dosage forms. More recently, twin screw granulation (TSG) has gained importance as a continuous, scalable form of wet granulation with many associated benefits. In TSG, powdered formulation components are transported through a barrel containing two co-rotating screws, contacted with liquid and discharged into a fluid bed dryer. Typically, the layout of screw conveying, mixing and kneading
elements is highly configurable and this along with other variables (powder feed rate, screw speed and liquid feed rate) leads to a large number of possible combinations. Finding an optimal design and operational space for a new formulation can potentially consume significant time and material if done in a purely experimental manner.

Many parallel and competing rate processes, such as granule formation, agglomeration, breakage and consolidation, occur during wet granulation. A population balance modelling approach using compartmentalisation along the length of the TSG barrel has been developed to describe these processes. Through ADDoPT, a TSG model was implemented in gPROMS Formulated Products, based on previously published work. A case study utilising a dataset generated from a proprietary pharmaceutical formulation was developed, which sought to answer the following questions:

- Is it possible to calibrate and validate a model of the TSG process that is suitably predictive for industrial use?
- Would having a process model for the TSG change the current experimentally-driven process development workflow?

Data was available from 24 experiments where liquid-to-solid ratio, screw speed and powder feed rate had been systematically varied, along with five experiments that included changes in screw configuration and liquid-to-solid ratio.

Model validation

Experimental data was required to estimate rate parameters for the mechanisms occurring during TSG. Parameters were estimated for drop nucleation, breakage and layering, by fitting with dried granule size distributions. Other model inputs included the feed powder particle size distributions and bulk densities, the flowrates of powder and liquid, and the screw configuration and elements. The residence time distribution in each screw element was estimated from published data.

Following parameter estimation, the model was found to be able to capture the influence of liquid-to-solid ratio on the granule size distribution. In the experiments, an increase in liquid-to-solid ratio led to an increase in granule size, which was also captured by the model.

In the dataset containing different screw configurations, the configurations were made up of a single block of the kneading elements (3K), a single block of six kneading elements (6K) and two blocks of six kneading elements (66K). All remaining elements were conveying. The 3K and 66K experiments were used for parameter estimation and the 6K experiments for model validation. Figure 4 shows an excellent level of prediction for the 6K experiments.

Utilisation of a process model

Rate parameters were estimated using the full 24 experiment dataset and then with a subset of five experiments. The simulation results showed comparable levels of prediction for variation of granule size with liquid-to-solid ratio. This shows how use of the process model could be used to reduce the experimental burden of process development. A smaller number of scavenging experiments could be used to build the model, which can then be used to help identify a suitable operating space. Exploring different screw configurations can be particularly time consuming and costly in material wastage due to both the large number of possible combinations and the associated equipment dismantling and reassembly. The results from the model show it may be possible to use experiments from just a couple of screw configurations to estimate the relevant model parameters that can then be used to explore other potential screw configurations in silico.

Overall, the case study demonstrates how development of a complex process can be made more efficient by an integration of digital design with development workflows. In this case, optimisation of the process around granule size was demonstrated. Further development of downstream unit operation and product models to enable simulation of the tablet properties will further aid the application of digital design to the pharmaceutical development process.

Conclusions

These case studies exemplify both the challenges and opportunities that exist in bringing together mechanistic materials and process modelling techniques with broader statistical analysis as part of the digital transformation in pharmaceuticals development and manufacturing. In addition, they illustrate the importance of an ecosystem that brings together colleagues from academia, industrial end-users and solution providers. This approach is being extended across further industrial case studies due for completion in 2019 (the final year of the ADDoPT project).

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IN THE CASE of any disease, having access to the right drug can make all the difference and having the adequate drug is one of the most important steps in disease treatment. However, merely having the drug is worthless if it never reaches the site of action. For this reason, equipping pharmaceuticals with a vehicle to make this possible is important; which is why there is a whole field of research around drug delivery.

Designing an appropriate drug carrier is not an easy task. The body environment is, in general, very hostile to any substance that is not natural to the organism – the so-called xenobiotics – or that comes from an unusual route of administration. The digestive tract, for example, is an especially tough environment, with different pH areas and lots of proteases lurking in it. These levels of protection are generally a positive feature and one of the reasons we make it through to old age. However, with our body being such an efficient sentinel, it is hard for many drugs to reach its site of action unscathed.

That's not to say that overcoming those barriers is entirely difficult; it depends on the case. For drugs that are meant to act quickly and protect the active ingredient, so it reaches its site of action. Once there, it gets degraded and, after approximately six hours, the substance is presumably disintegrated in full: its job is done, and the drug is gone. However, in other cases it is more complicated than that. Lysosomal storage disease, which is characterised by the body’s inability to produce certain enzymes, is one such example. For sufferers, the lack of a particular enzyme is critical and leads to severe problems, as their body is unable to perform certain essential physiological activities. In many cases, life expectancy is extremely low. For those affected, provision of the missing enzymes solves the problem for a short period, but a single administration isn't sufficient. As the saying goes, "give a man a fish and you feed him for a day, teach a man to fish and you feed him for a lifetime". In this case, teaching the man to fish would be the equivalent of providing the patient's body with the possibility of generating its own enzymes. While this presents an interesting challenge for scientists in the field of gene therapy, there is still a long way to go until a proper solution is achieved.

This begs the question, how do people with enzyme deficiencies deal with their diseases? In the case of phenylketonuria – a disease characterised by the lack of the enzyme that breaks phenylalanine into tyrosine – people normally opt for a phenylalanine-free diet to avoid complications. However, a research study showed that half of...
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that is not natural to the organism.

Light at the end of the tunnel

In order to tackle this problem, scientists were inspired by nature. They asked themselves where enzymes are naturally stored in the body and noted that cells possess small sacs called lysosomes to retain their structural integrity, as enzymes don’t cope alone in the cytoplasm. From this observation, scientists decided to try designing artificial lysosomes to carry the missing enzymes into the organism.

Liposomes have proven to be good structures to encapsulate enzymes while retaining their activity, but to provide a sufficient number of enzymes to replace the activity of the missing ones would require several liposomes. This prompted the idea for capsosomes.

Capsosomes are polymeric carrier capsules that contain liposomes as sub-cargo structures. Although there are several types of multi-compartmentalised vesicles, the combination of hydrophilic and hydrophobic systems affords capsosomes the characteristics of both types. While liposomes protect the enzymatic structure from misfolding or denaturation, small substrates can enter and leave the structure through the lipid bilayer allowing the enzyme to perform its activity over its target substrate. Meanwhile, the polymeric structure, while also semipermeable, confers structural protection to the liposomes, which are more susceptible to degradation in this environment.

Capsosomes are synthesised using the layer-by-layer technique. This involves the deposition of alternate layers of differently charged polyelectrolytes in order to build each layer around a silica core, to then attach the liposomes. The silica core can be removed after the structure is built. Using this technique, capsosomes can range in size from nanometres to a few micrometres. For instance, capsosomes with a diameter of 3μm can contain more than 150,000 liposomes between their polymeric layers. Due to their structure, enzymatic substrates can permeate through liposomes at body temperature. In this way, capsosomes not only act as enzymatic vehicles, but as micro-reactors inside the body.

While its clinical efficacy is yet to be proved, in vitro experiments are showing promising results. In 2015, a group of researchers from the University of Aarhus in Denmark and the National University of Singapore successfully assembled enzyme-loaded capsosomes in order to degrade alanine into trans-cinnamic acid. The group, led by Leticia Hosta-Rigau and Brigitte Städler, demonstrated that capsosomes can co-encapsulate different enzymes and allow consecutive reactions to occur inside. Also in 2015, a team from the University of Melbourne in Australia, led by Frank Caruso and James W. Maina, demonstrated the ability of capsosomes to perform protein loading and release in physiological conditions for more than two months, portraying capsosomes as potential candidates for sustained release.

If future clinical trials show positive results, capsosomes could revolutionise the medical field. The synergy between synthetic chemistry and molecular biology could provide insights into enzyme-replacement therapies, showcasing the importance of drug formulation in such treatments.

One of the main reasons for success is that capsosomes were inspired by natural structures. The creation of multi-compartmentalised structures is an example of cell mimicry – a field dedicated to the design of artificial cell structures and, ultimately, artificial cells. This is a promising scenario that demonstrates how replacement of defective or missing cell structures could be solved with the design of artificial but nature-inspired cellular structures. Coming back to the earlier analogy, for a man who can’t learn to fish, capsosomes at least ensure that fish reaches his belly.

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Customised approach

Step 1
Product target profile
- Project synopsis
  Reference product, targeted market, regulatory route
- Targeted patients
  Characteristics and needs
- Device selection supports, characterisation

Step 2
Setting DPI capsule specification
- Active principle ingredient (API) profiling
  Formulation approach discussion
- Support to QbD approach
  On critical capsule parameters

R&D added-value service
- Rapid supply of R&D capsule quantities
  For preliminary test
- On-site technical support
  For first filling trials

Step 3
Support throughout validation and dossier filling
- Capsule branding
  Colour matching
  Custom colour and print development
- Rapid supply of customised DPI capsule
  For validation/clinical batches
- On-site technical support
  Scale-up, filling process optimisation
- QA and regulatory support
  Required statements for dossier filling, support on questions from agencies

FURTHER INFORMATION:
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Don’t let difficult substances control your cleaning validation

In last month’s webinar, Daniel Kellner-Steinmetz provided examples of best practices for performing TOC recovery studies for cleaning validation applications, including comparison of pre-acidified and non-acidified vials. Here are examples of questions that arose from his presentation.

How do you set up limits for cleaning compounds, especially if they are toxic? How do you calculate the maximum allowable carryover?

The determination of acceptance criteria limits for TOC in cleaning validation can be easily calculated and defended when starting from justifiable clinical reference values. Limits need to be calculated based upon toxicological data of your compounds. This data is usually defined by a person with the relevant education (pharma or medical degree). For detergents, you should get the data from your supplier.

What difference in values should I expect between each day if I test the sample hold time for eight days?

This will depend on your sample matrix. Please see our report, DUCT Vial Performance and Stability. This report provides information about the effects of sample storage on TOC and conductivity levels, but you should verify the stability with each of your sample matrices.

Do you have any experience with protein-based samples/contaminants in cleaning validation?

Yes, Sievers TOC Analyzers have demonstrated quantitative recoveries with low standard deviations for diverse compounds, including proteins and large molecules. Please see detailed study information in the following report, Recovery of Industry Specific Insoluble/Difficult-to-oxidize Compounds using TOC Analysis. There are also examples where specific vial types, such as pre-acidified vials, may help with recovery of certain compounds such as monoclonal antibodies.

How do I go about testing my product families with different vials to maximise recovery and linearity? Is there an optimal vial type?

Different vial types may improve performance such as TOC percent recovery on specific product families. SUEZ has written a Vial Selection Guide that helps test three types of vials and identify the optimal vial choice for specific applications. After completing the test plan and data worksheet, the optimal vial choice can be selected based on TOC recovery and linearity.
Zafar Iqbal sheds light on how Raman spectroscopy is used to detect and understand the amorphous phase in a range of solids, providing examples of his own laboratory applications.

Many bacterial species have been found to exist in a 'viable but non-culturable' state. Jeanne Moldenhauer discusses this phenomenon and makes suggestions as to why we don’t often see an increase in viable cells when using viability-based methods.
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Abstract
The following is a short overview of the use of Raman spectroscopy, particularly in the low wavenumber lattice and intermolecular mode region, to detect and understand the amorphous phase in solids ranging from that in plasma-grown nanoscale silicon to organic molecule pharmaceutical drugs. Examples from work in the author’s laboratory on silicon and pharmaceutical drugs, acetaminophen, fenofibrate and griseofulvin are discussed.

Raman spectroscopy is used to detect and study the fundamental atomic vibrational modes of extended inorganic semiconductor structures, such as that of silicon. It is also used to similarly examine the fundamental Raman-active vibrational modes of molecular organic solids used as the bioactive ingredient in pharmaceuticals. In the face-centred cubic crystal of silicon there is only one type of fundamental atomic motion comprised of Si-Si vibrations that gives rise to a narrow Raman line at 520cm⁻¹. In pure organic solids where the molecules are held together mostly by van der Waals forces – and in some instances by hydrogen bonding – there are two types of Raman active modes: one referred to as
lattice or inter-molecular modes and the other as intra-molecular or internal modes associated with the molecular modes of the molecules forming the solid. The lattice modes lie in the low wavenumber region, typically below 200 cm⁻¹, whereas the intra-molecular modes range in wavenumbers from 300 to 3,300 cm⁻¹. In molecular solids, the lattice modes are either translational motions of the centre of mass of the molecules or hindered molecular rotations. Typical pharmaceutical and biomedical applications of Raman spectroscopy involve investigations of intra-molecular modes showing Raman shifts that are usually greater than 200 cm⁻¹. In the past, lower frequency modes were not investigated due to the limitations of optical filters used to remove the intense, elastic Rayleigh scattering from the samples. However, with recent advances in optical technologies, low frequency Raman spectroscopy (LFRS) to well below 200 cm⁻¹ can now be rapidly performed with the same sensitivity and resolution as higher frequency spectroscopy. LFRS enables the detection of crystalline features via the appearance of lattice modes in organic solids in the early or later stages of crystallisation from the amorphous phase and the appearance of short-range order in the amorphous phase of extended solids, such as silicon and diamond.

The concept of phonons as collective modes developed in 1912 by Debye who realised, in contrast to Einstein, that low energy vibrational excitations in solids are not just oscillations of single atoms, but are collective modes that propagate throughout a lattice and accept energy in discrete amounts within the formalism of quantum mechanics. Phonons therefore behave as quasi-particles with definite energies and directions of vibrational motions analogous with photons and likewise obey Bose-Einstein statistics. The theoretical underpinning of lattice dynamics associated with phonons, as formulated by Born and von-Karman, adopts the symmetry of the crystals in a harmonic potential under adiabatic conditions. Within the framework of thermodynamics, an adiabatic process involves one that takes place without transfer of heat or matter between a thermodynamic system and its surroundings. As a consequence of crystal symmetry, the properties of the entire crystal can be obtained from that of a small region of the crystal.

In crystalline, extended solids, phonons of only one type comprising interatomic vibrations exist as noted above. In bulk crystals, such as that of cubic silicon, only one sharp and symmetric fundamental line at 520 cm⁻¹ is Raman active. As shown in Figure 1, this line decreases in frequency due to phonon confinement with reduced crystal size and broadens asymmetrically due to increase in the surface-to-bulk ratio resulting in the initial stages of amorphous phase formation on the nanocrystal surfaces. Below the crystal size limit of about 35 Å (achieved at a plasma deposition temperature of 65°C, shown in Figure 1) the
crystalline state becomes thermodynamically unstable and transforms to a purely amorphous phase with a broad Raman spectrum peaking near 480 cm\(^{-1}\). The amorphous phase in silicon and similar structures can be considered to comprise of highly disordered atoms that can be modelled as a random ‘walk’ with regions of short-range crystalline order. The amorphous phase is ‘glassy’ with no indication of even short-range order. The Raman spectrum is therefore featureless as for the pharmaceutical fenofibrate shown in Figure 3. When intermolecular hydrogen bonding is present, as in the pharmaceutical acetaminophen, the Raman spectrum depicted in Figure 2 of the amorphous phase below 200 cm\(^{-1}\) shows a broad feature overlapping the lines associated with its lattice modes. This indicates the likely presence of short-range ordered regions in the amorphous phase. The higher frequency Raman spectra for both amorphous fenofibrate and acetaminophen retain relatively sharp features that are somewhat...
broadened and shifted from that observed in the crystalline phase. This indicates that intra-molecular order is largely retained in the amorphous phase of organic solids.

The broadening and wavenumber shifts reflect changes in potential energy due to the fundamentally metastable amorphous phase. Another feature of the organic amorphous phase is displayed in Figure 4 (top), which shows the normal Raman spectrum in the higher frequency region of the molecular modes of the pharmaceutical griseofulvin. The surface-enhanced Raman spectrum (SERS) taken at a low concentration from a nanostructured substrate provides the spectrum of a finite number of molecules. Note that the SERS molecular spectrum is essentially the same as the solid-state spectrum of the amorphous phase. This suggests that the amorphous phase in an organic solid, such as griseofulvin, is a random or glassy distribution of molecules.

Find, classify and identify microparticles with Raman imaging

High-resolution measurements of particles are of great interest in many fields of application. Combining confocal Raman microscopy with particle analysis tools makes it possible to find, classify and identify particles almost entirely automatically.

Pollen, dust, flour, metal flakes and pigments in paints, titanium dioxide in sunscreen and toothpaste, fat crystals in food emulsions – these and many more substances in our daily lives contain or consist of microparticles. Recently, the public and scientific community have directed their attention towards microplastic particles in the environment. Confocal Raman microscopy is ideally suited for finding, classifying and identifying microparticles because not only does it yield images with a resolution down to 300nm, but with Raman vibrational spectroscopy the chemical components of a sample can be identified. It is a nondestructive method that requires little, if any, sample preparation.

WITec, a pioneer of Raman imaging and correlative microscopy, has introduced ParticleScout, a revolutionary particle analysis tool for the alpha300 Raman microscope series. ParticleScout delivers a greatly accelerated workflow to the researcher investigating particulate samples while making full use of confocal Raman imaging’s abilities in fast, label-free and nondestructive chemical characterisation.

It begins by surveying samples with bright and dark field illumination to view the contained particles. Image stitching combines many measured areas for a detailed overview of large areas and focus stacking allows larger particles to be sharply rendered for accurate outline recognition. A Raman spectrum is then automatically acquired from each particle. The Raman spectra are evaluated and the particles they correspond to can be identified manually or by using the seamlessly integrated WITec TrueMatch Raman database software. This integration of a particle analysis tool with a Raman database is unique in the industry and offers a streamlined experimental environment to boost productivity.

Finally, ParticleScout generates a comprehensive report that features user-selectable combinations of filters and advanced algorithms to show the quantities of selected particles and their prevalence relative to other groups. These reports make ParticleScout the perfect tool for finding correlations between the physical and chemical attributes of particles.
Raman spectroscopy

Many researchers are keen to find analytical solutions that generate the chemical fingerprint of a sample at the molecular and cellular level. Overcoming these problems is made possible by label-free techniques such as Raman, SPRi or particle size analysis. This brief overview will identify the benefits of Raman spectroscopy.

Author: Dr Chiraz Frydman, Global Senior Product Manager at HORIBA France

CELL ANALYSIS is routinely used in pharmaceutical and biotechnology companies to understand the interaction phenomena, antibiotic impact, living behaviours, etc. It’s also used with high interest in cosmetology to investigate cell behaviour, leading to non-invasive study conditions.

On the other hand, in clinical research the information generated from microbial at a single-cell level can be crucial for disease understanding. The ability to identify cells within a sample remains a significant challenge, since many bacteria cannot be isolated and cultured quickly and easily. Equally, analysing samples in their native medium without purification or modification can become a difficulty.

Raman microscopy is a highly valuable technique for providing the chemical fingerprint of a sample in a label-free way. This approach helps to save time by shortening the sample preparation steps and offering information at the level of a single cell, without the need for culturing. This technique is based on light scattering, where a photon of light interacts with a sample to produce scattered radiation at different wavelengths. The frequency difference between the incident and scattered light characterises the molecular vibration, providing information on the chemical fingerprint of the sample (Graph 1).

Identification of contaminating microbes, discrimination between different bacterial species and follow-up of cell growth can be challenging, whereas Raman microspectroscopy can help provide relevant information. For some applications, whatever growth stage a specific cell is in, Raman can provide an easy method for species identification and clusters bacteria according to their species phenotypic differences, despite temporal variances in cellular physiology during growth steps (Graph 2).

Raman spectroscopy can be used to obtain information regarding the molecular composition of the skin down to several hundred micrometers below the surface. The stratum corneum is the skin’s outermost layer and the main protective barrier against water loss, microorganisms and toxic agents of the epidermis. Non-invasive confocal Raman spectroscopy can give insight into the structure and mechanisms governing the behaviour of this layer. Moreover, in-depth measurements allow the determination of relative concentration modifications of the major constituents (water, urea, proteins, etc.) in the thickness of the epidermis. Such information is of major interest for the development of in vivo diagnosis of skin diseases and the improvement of transdermal drug administration (Graph 3).

The large quantity and complexity of information generated in a Raman spectrum makes the data analysis complex and difficult. Thanks to the multivariate techniques (principal components analysis, discriminant functional analysis and hierarchical cluster analysis), the results can be easily understood.

Graph 1: Illustrating the Raman principle

Graph 2: Multivariate analysis of spectra from three bacterial species (Acinetobacter sp., ADP1 E. Coli DH10 and Pseudomonas fluorescens SBW25)

Graph 3: Skin spectra and fingerprint of “hydrated” and “non hydrated” region performed to study the water content variations versus depth (µm)

Dr Chiraz Frydman is currently Global Senior Product Manager for SPRi and life science instruments at HORIBA France. She has an engineering diploma in biology and a PhD in enzymatic engineering, biocconversion and microbiology. Chiraz set up her own company dedicated to developing an immunoaffinity kit, before joining Opticvalley, where she oversaw the Biophotonic Project. In 2007 she started at HORIBA and in 2015 she received the "Yoshihiro Ishikawa" HORIBA presidential Award, which is awarded to employees who have made outstanding achievements in technology management.
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Introduction
Moldenhauer\(^1\) discussed some changes in how clinical microbiologists are evaluating samples for viable cultures. Study of the gut microbiota has required the development of new culture and evaluation methods. Lagier, et al.\(^2\) indicated that as many as 80 percent of human gut bacteria are unknown and considered ‘unculturable’. This is like the term used by many pharmaceutical microbiologists – ‘viable but not culturable’ (VBNC). As a result of studies conducted on the human microbiome, new methods are being developed for culture and recovery of human viable microorganisms. The data being generated indicate that several previously used culture methods do not detect all of the organisms present. The previously non-culturable organisms require various treatments including longer incubation periods. The use of these types of methods has been called the “rebirth of culture in microbiology.”\(^1,2\)

The newer culture methods being developed incorporate the use of culturomics and metagenomics. Culturomics is an approach that utilises “multiple culture conditions combined with prolonged incubation”\(^2\) and application of these methods has resulted in the isolation of hundreds of new bacterial species from the gut in a short time.\(^2\) Once the recovered cultures are...

\(^1\) Jeanne Moldenhauer
Excellent Pharma Consulting, Inc.

Many bacterial species have been found to exist in a viable but non-culturable state. Jeanne Moldenhauer discusses this phenomenon and makes suggestions as to why we don’t often see an increase in viable cells when using viability-based methods.

BIOGRAPHY
JEANNE MOLDENHAUER is Vice-President of Excellent Pharma Consulting and has more than 30 years’ experience in the pharmaceutical industry. She chaired the Environmental Monitoring/Microbiology Interest Group of PDA for more than 15 years, served on the Scientific Advisory Board of PDA for 20 years, founded the Rapid Microbiology User’s Group™, and is a member of ASQ and RAPS. She is the author of many books and numerous publications.
properly isolated, sequencing is used to identify the organisms or identify that a new species exists and must be classified.

Metagenomics was developed before culturomics and refers to the study of genetic material. The genetic material of the microorganism comes directly from environmental samples of microorganisms and it is this material that is evaluated rather than pure colonies of organisms. After the culture has been isolated it is sequenced. The use of wild strains enables the detection of a largely unbiased sample of all genes from the sampled communities (ie, may not be pure cultures).³

As stated above, the use of culturomics has resulted in the isolation of hundreds of new bacterial species from the gut in a short time.² Following isolation, sequencing is used to identify the organisms or establish that a new species exists and must be classified. Most of the work that has been carried out with organisms from the gut microbiota has been performed in France. Studies conducted in the United States indicate development of new culture techniques for use with blood and urinary tract infection samples. These techniques have resulted in the discovery of 191 new bacterial species, 696 new bacterial subspecies and 105 known L-form bacteria as of the date of writing. As such, these newer techniques are not limited to only gut microbiota.¹,⁴

The obvious next question is, what do these new culture methods mean to the pharmaceutical microbiologist? Are we using appropriate methods for the culture and recovery of microorganisms?

Viable but non-culturtable

A common term used in pharmaceutical microbiology is VBNC. Fakruddin, et al.⁵ defined this term as a “unique survival strategy of many bacteria in the environment in response to adverse environmental conditions. VBNC bacteria cannot be cultured on routine microbiological media, but they remain viable and retain virulence. The VBNC bacteria can be resuscitated when provided with appropriate conditions.” This term became popular in the pharmaceutical industry as new rapid or alternative microbiological methods were introduced, since many of them used cellular viability as a detection method. As the new viability methods were introduced, vendors started using the VNbc term for any organism that could not be cultured using conventional methods.

Oliver,⁶ one of the world’s leading experts in VNbc organisms, indicated that: “Microbial ecologists have long recognised that large proportions of the microbial populations inhabiting natural habitats appear to be non-culturtable. Indeed, plate counts of bacteria in soil and aquatic environments typically indicate that far less than one percent of the total bacteria observed by direct microscopic examination can be grown on culture media.”

Oliver⁶ found that at least 30 species of organisms from 16 genera enter the VBNC state. Since this original article, many other papers have been published that identify yet more organisms.

However, not all scientists share the view that this VNbc state exists. Barer and Bogosian,⁷ for example, believe that the so-called VNbc state is part of the dying or injured process of cells. Regardless of whether you accept the term VNbc, it is noted that there are differences between the numbers of microorganisms counted by direct methods such as counting colonies on agar plates and other methods. This was specifically reported with urine samples.

In this article, the term VNbc is considered to be synonymous with the types of concerns expressed by clinical microbiologists, wherein they are now finding microorganisms that are grown and recovered using new culture methods – ie, these organisms were previously not detected or recovered, but are now recovered and growing (viable).

Rapid methods using viability as the detection method

In the 1980s and ’90s several instruments were introduced to pharmaceuticals for microbiology testing (which is not so say they were implemented). These early technologies were originally developed for the US Department of Defense to detect bioterrorism threats. ScanRDi was one of the first viability detection systems offered to the pharmaceutical industry. Early studies evaluated the use of this system for high-quality water monitoring. In these studies,
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Implementation of some rapid microbiological methods may necessitate changing monitoring limits

the data show counts approximately 0.5 log higher than the counts generated using traditional methods. While not much higher than traditional counts, many companies feared this would be a significant regulatory concern. As such, purchase and implementation of viability-based systems were not encouraged in many companies.

Later, the IMD-A was introduced for air monitoring, which also used a viability-based technology. Regulators in the US were interested in this technology as it provided real-time viable microorganism counts, which they considered to be consistent with their agenda for Process Analytical Technologies (PAT). Since that time other viability-based air monitoring systems have been introduced with subsequent evaluation studies being conducted at various companies. Those companies that tested these methods across various room classifications found that some classifications interfered with the detection methods, eg, pollen, and other biological materials might fluoresce in a similar way to a viable cell. Miller, et al. performed studies using the IMD-A in a manufacturing aseptic isolator. This group found the system to be appropriate for use in these conditions, without requiring a change in the monitoring limits.

In the last few years, viability-based technologies have been introduced for in-line water monitoring and a consortium of companies has been working together to evaluate these systems for use.

While many technologies have been introduced, implementation has traditionally been hindered by fears of higher counts and regulatory issues surrounding these “higher counts” while FDA regulators have asserted that limits are based upon technologies used. As such, they understand that implementation of some rapid microbiological methods may necessitate changing monitoring limits; it seems that pharmaceutical companies are not as open to change as the regulators, however, demonstrating reticence to implement some systems.

How viability is detected and reported

There are a variety of methodologies available to show viability and there is at least one commonality in all of the viability methods that also use computer imaging – that the software requires one or many parameters to assess whether the cell is viable. These parameters are called discrimination factors. Some systems use as few as a couple of discrimination parameters, while other systems utilise 20 or more.

Generally, increasing the number of discrimination parameters provides “better” data. Vendors generally design these parameters to ensure that the counts are being accurately reproduced.

Are all the viable cells being counted?

Looking at the clinical data, which seems to indicate that we are not detecting all the viable cells with the currently used culture methods; why don’t we see an increase in viable cells when using viability-based methods.

There are several potential reasons for these differences:

- Everything is working correctly and there are no undetected viable cells in the pharmaceutical environment
- Like the clinical culture methods, the viability detection method does not count all viable cells
- The increased counts have been documented in aquatic environments and clinical laboratories evaluating blood, urine and gut samples. The pharmaceutical industry counts are not typical of other increased samples: for example, in pharmaceuticals we monitor air, high-quality water, surfaces and personnel. Perhaps there is no difference in counts due to the cleanliness levels of the ISO 5 areas.
- Equipment vendors attempting to show equivalence have ‘over discriminated’ the counts and are inadvertently blocking out valid counts
- The clinical microbiologists are not really finding new viable cells, keeping in mind that they have successfully used these methods in many laboratories across countries (and oceans)
- Other, as yet unidentified, reasons.

This is not meant to indicate viability-based methods – they are useful tools in our environments.

Going forward

While not indicting the use of rapid methods, there is room for further evaluation and analysis. Continued research should be conducted to determine whether all viable cells present are being counted. We may need to consider whether some of the newer clinical methods would detect more counts in our pharmaceutical environments. Vendors must look critically at how they establish the discrimination parameters to ensure all viables are being counted, rather than achieving counts that are consistent with the traditional methodology. In the past few years, regulators have been looking at the use of rapid methods, as evidenced by the Warning Letters issued to those companies that use these methods. It can be important to understand how the system detects viability and how you could account for why some industries get much higher counts for viability while pharmaceutical companies do not.
Low Endotoxin Recovery (LER)

The Parenteral Drug Association (PDA) has recently published Technical Report No. 82 (TR82) on the topic of Low Endotoxin Recovery (LER), providing both consensus to the science and data behind the analytical issue, as well as to analytical and mitigation strategies. Dr Christian Faderl from bioMérieux explains the important implications of TR82 as well as presenting the ENDO-RS® sample preparation technology for demasking endotoxin in affected biologics, as described in Case Study 7 of TR82.

What is low endotoxin recovery?
First reported by Chen and Vinther in 2013, the phenomenon known as LER has been broadly studied and identified in biologics and certain therapeutic proteins. LER is a temperature- and time-dependent process and defined as loss of detectable endotoxin activity over time when using Factor C-based assays (LAL and rFC) when undiluted products are spiked with a known amount of endotoxin standards. Regulatory authorities also request hold-time studies to determine the validity of the endotoxin release test methods during the review of BLAs for biotech products, as well as new methods to overcome the issue.

What is PDA TR82?
In March 2019, the PDA published TR82 on LER. The report constitutes a much-needed source of information and guidance, including scientific findings on the mechanism behind the analytical issue. It also includes recommended procedures for analysis and mitigation of endotoxin masking commonly leading to LER in biologics. This comprehensive document is a result of three years intensive work completed by the PDA LER Task Force, which consists of members from the US FDA, academia, pharmaceutical industry and all endotoxin testing suppliers. TR82 is available in full for members and can be purchased directly from the PDA Technical Reports Portal.

What are TR82’s biggest takeaways?
TR82 is an important scientific and guidance document that will give pharmaceutical companies leverage for the successful mitigation of the LER phenomenon, with the ultimate outcome of increased process and product knowledge. Important takeaways of the report are: 1) a description of the underlying mechanisms and contributing factors of LER, 2) a summary of the potential clinical impact, 3) clear guidelines for conducting LER hold-time studies and 4) a choice of strategies for the mitigation of LER are made available for the industry.

What are the outcomes of Case Study 7: evaluation of an endotoxin demasking protocol, in TR82?
An important part of TR82 is the comprehensive appendix of case studies considering LER occurrences, encompassing both analyses of root-causes and endotoxin preparations, as well as methodologies for overcoming LER. In one of the studies, Case Study 7: evaluation of an endotoxin demasking protocol, the application of the ENDO-RS method, developed by Hyglos – a bioMérieux company, was assessed. This study used a sample known to be affected by LER to evaluate a sample preparation protocol using ENDO-RS reagents prior to detection of endotoxin using the LAL method. The results showed that the ENDO-RS protocol was able to reliably detect the endotoxin under all conditions tested, ensuring robust recovery and diminishing the risk of false-negative test results.

What is the ENDO-RS® method?
The ENDO-RS method is a patented set of reagents for sample preparation addressing the masking of endotoxin in biopharmaceutical formulations typically containing surfactants, chelating agents or specific active pharmaceutical ingredients (APIs). ENDO-RS enables full quantitative recovery of endotoxin prior to detection, independent of storage time and endotoxin concentration. bioMérieux provides specialised protocol development services applying ENDO-RS. Our work for leading pharmaceutical companies has resulted in validated methods for LER, fulfilling regulatory requirements.

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Continuous innovation is key to the advancement of medical device technology. To allow for innovation, whilst also assuring safety and effectiveness, global standards are required. This article provides information for companies manufacturing medical devices on the benefits of holding ISO 13485 certification and how to get certified.

Effective drug serialisation and identification is essential for improving the safety of medicines and combatting counterfeits. The Falsified Medicines Directive’s new requirements promise to crack down on the impacts of unsafe drugs. Neil Piper discusses the human cost of falsified medicines and how companies need to increase traceability of individual drug packets going forward.
ISO 13485:2016 is the latest standard from the International Organization for Standardization that sets out quality management system requirements, rules and guidelines for any company that designs, manufactures, installs, distributes or services medical devices. This includes companies that provide related services or components at any stage during a medical device product lifecycle, such as technical support, suppliers and external third parties. ISO 13485 allows a company to demonstrate that it consistently meets customer needs and medical device regulatory requirements and complies with local legislation. It is closely related to ISO 9001, which covers requirements for quality management systems, but emphasises areas such as risk management (demonstrated herewith), the work environment and medical device documentation and reporting.

What is a medical device?
A medical device under ISO 13485 covers any instrument, apparatus, equipment, implant, in vitro reagent or similar, which is used to diagnose,
Benefits of holding ISO 13485 certification
As the medical device industry is so highly regulated, the safety, effectiveness and performance of all products is crucial. Holding ISO 13485 certification clearly demonstrates to both customers and regulators a company's commitment to continual improvement, safety and quality. Companies that hold ISO 13485 certification:

- Show they comply with medical device regulations and legal requirements, eliminating uncertainty for all stakeholders
- Successfully manage risk
- Manufacture quality medical devices consistently
- Improve processes, efficiencies and overall performance with an effective quality management system
- Reduce costs through efficiencies in processes and supply chains
- Gain competitive advantage
- Have more opportunities to access global markets and build company growth.

How to get ISO 13485 certification
This is a brief overview of the main things your company must do in order to achieve ISO 13485 certification. There are of course nuances and further detail within each of these steps.

1. **Identify processes**: firstly, it's important to identify and collate all the processes your company carries out that are in any way connected with medical devices. Even if you don't manufacture something commonly considered as a ‘device’, such as a scalpel or piece of medical equipment, you may manufacture a chemical that helps the device to function. This is still classed as a medical device.

2. **Create a process flow**: you must then produce a process flow. Start at purchasing (and note that any raw materials you use must meet certain standards), all the way through to dispatch, installation and ongoing servicing, if that's something your company does. Look at all the processes in between your start and end points for the type of medical device your company works on. For example, this could be receipt of the purchased product, goods inwards verification, quality testing, storage, manufacturing and product release.

3. **Establish risks**: for each of your identified processes, you must compile a set of written procedures and establish where there is potential of risk to the quality of the product. This could be anything from risk of contamination or deterioration, control of records, employees not having undergone training and any suppliers that may need to go through an approval or checking process to ensure they are compliant. The next step is to identify and implement control measures that will reduce any possible risks.

4. **Monitor and measure**: you must monitor, measure and review all the processes and risks you've identified on an ongoing basis and record these reviews as they are carried out. Any equipment you use to monitor and measure your processes and products must also be controlled, calibrated and validated. Internal auditing is an important part of monitoring and measuring your processes, but can also include the number of complaints you may receive, any feedback from customers and any product non-conformances or deficiencies. This must all be documented and any improvement actions should be identified and then implemented.

5. **Manage change**: it's important to have a change control process in place for your quality management system. This should enable you to evaluate the impact of any changes to either the product or your processes and manage them appropriately. For example, if you are manufacturing an in vitro diagnostic reagent and you find the product is non-conforming, there must be procedures in place to correct this. You may have to stop dispatching the product, make corrections within the manufacturing process or even recall the product. You should also implement corrective and preventative action to address any future non-conformances.

6. **Document everything**: you must create a quality manual that references all the documents in your system and within each process. Each medical device must have its own file or record, which includes elements such as a product description, use, specifications, storage, handling, measuring, installation and servicing. These documents must be controlled, for example with version numbers and issue dates, and records must be kept for the lifetime of the device. You should be prepared to show any documentation to relevant regulatory authorities if applicable to the medical device you manufacture.

7. **Organise external audits**: it's a requirement to have an external quality management system audit on an annual basis. This audit will check...
your processes and internal audits. If you want to obtain full ISO 13485 certification this will need to be renewed every three years.

8. **Get management commitment:** To successfully achieve ISO 13485 certification, management support is required to ensure you have the necessary availability of resources and infrastructure. This could be anything from new equipment, workspace such as a clean or sterile environment, supporting services or budget. You should also undertake an annual management review. This should cover your quality management system (QMS) performance over the course of the previous year using results from your internal audits, investigating anything that may have gone wrong, the number of complaints, any non-conformances and delivery results. From this, improvement measures should be identified and implemented.

**ISO 13485 and risk management**

ISO 13485 is heavily biased to – and places great emphasis on – risk management, simply because medical devices are being used with people in some way. A risk-based approach is woven into the quality management system and is required through the lifecycle of the medical device. While this can be challenging for manufacturers of medical devices, it is a necessary focus that promotes safety, quality, compliance and product efficacy. For example, it’s a requirement that any employee working on an identified medical device process has a good working knowledge of ISO 13485 in order to minimise risk of human error and ensure they are able to make informed decisions.

**Advice for manufacturers planning certification**

Firstly, you should confirm that the device(s) your company manufactures can be defined as a medical device under ISO 13485 standards; or, if you provide a medical device service, that your service is related to a product defined as a medical device. Getting ISO 13485 certification is challenging and requires commitment so, secondly, it’s important that your leadership team confirms that holding the certification will add value to your company, meet its business objectives and support its strategy. While holding the full certification is not strictly necessary, as your company can still conform to and benefit from ISO 13485 standards without being externally certified, it does clearly demonstrate to all stakeholders that you comply with its requirements.

If you do want to become independently certified there are two phases; the first covers documentation, while the second implements your quality management system and audits it. You must carry out phase two within six months of completing phase one.

**ISO 13485 certification costs**

For companies who wish to be fully ISO 13485 certified, there are associated costs for external, independent certification. Although you may be under cost pressure, it’s important to work with an independent auditor who will add real value to your company during the certification process. In this highly regulated industry, choose a reputable auditing company with extensive expertise rather than one that enables you to gain certification at the lowest price.

**ISO 13485 and European Union medical device regulatory requirements**

Complying with ISO 13485 standards and gaining the certification is also a first step towards complying with the European regulations and requirements for Medical Devices and *in vitro* Diagnostic Medical Devices (EU Directives 93/42/EEC, 90/385/EEC and 98/79/EEC).
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Stability testing blister packs inspected with Sepha’s VisionScan test method

Author: Dr D. Dixon, University of Ulster, Northern Ireland

Introduction
Technology-based integrity testing offers pharmaceutical packaging operations increased efficiencies by utilising non-destructive techniques as part of the process. Calibrated and validated technology-based solutions offer a deterministic and repeatable test compared to traditional methods, which are often subjective, probabilistic and not as sensitive in their ability to detect a defect.

Many companies are still employing traditional methods for leak detection in blister packs and consequently miss out on opportunities to recover the cost associated with destructive testing and improve on the sensitivity of their testing whilst using a validated deterministic solution.

The challenge for companies considering alternative non-destructive leak test solutions is the lack of scientific data, which proves these techniques are truly non-destructive.

This whitepaper will test the hypothesis that the VisionScan is considered non-destructive. To test this hypothesis this paper will use the moisture status of tablets within inspected blister packs to prove there is no change in the status of the tablet during a typical period within a stability chamber, thus proving the non-destructive nature of the Vision with Vacuum test method.

Background
When considering current GMP (cGMP) guidelines within USP (United States Pharmacopeia) Chapter 1207 (Container Closure Integrity Testing) and ASTM F2338, there is growing awareness of the importance of packaging integrity testing within the pharmaceutical and medical device sectors. The increase in awareness is being driven not only by these guidelines but in part by FDA product recalls, and in 2018 lack of sterility assurance was the second biggest cause of FDA pharmaceutical recalls. Such recalls incur significant costs and damage brand reputation, and several major industrial players have left key markets as a result of product recalls. A robust leak detection system can assure packaging integrity, greatly reducing the likelihood of recalls. It is also a requirement under both FDA cGMP and EU regulations (eg: ISO 11607-1:2019 covering sterile medical devices) for manufacturers of medical devices and pharmaceuticals to demonstrate packaging integrity.

Packaging defects including pin holes, faulty seals, tears and pack misalignment can adversely affect product efficacy, shelf
life and may result in a loss of sterility. Leak testing packaging is therefore an essential part of the packaging process with companies having different options when considering how they do this, including blue dye, vacuum decay, gas analysis and vacuum with sensor techniques.

**Stability study of blister packs before and after VisionScan test method**

The stability study focuses on the Vision with Vacuum test method, using the Sepha VisionScan, and aims to confirm this method is truly non-destructive. In the study, packs of different material types were produced and inspected with the Vision with Vacuum method. The inspected samples were then compared to a control group of blister packs, which had not been inspected by the method to determine if the Vision with Vacuum test method had compromised the integrity of the blisters in any way.

**Moisture profiling™ and seal integrity**

To investigate the barrier performance of the blister packs, a moisture profiling™ technique from independent testing company Relequa (Waterford, Ireland) was used to measure the moisture levels of tablets, which would indicate a change in seal integrity. A breach of seal integrity will result in tablets with a higher moisture content caused by exposure of the blister pack to external high humidity.

In the Relequa technique the tablets are held in a chamber at a level of humidity higher than the humidity equilibrium point. The tablets then absorb moisture and the chamber gradually reaches the water vapour equilibrium point (WVEP).

A tablet with a low moisture content will absorb a large amount of moisture leading to a reduced WVEP.

Different types of blister material were used in the study to cover a range of moisture barrier protection types; these are given in the methodology.

**Methodology of testing**

The barrier performance of the packs before and after multiple tests was determined using moisture profiling™. Xylitol tablets were sealed inside blister packs with three different material types, including:

1. PVDC-coated PVC blisters sealed with aluminium 20µm hard lidding material
2. Aclar® (PVC/PE/PCTFE) pack sealed with aluminium 20µm hard lidding material
3. Aluminium cold-formed packs sealed with an aluminium foil (Alu/Alu) comprising a polyamide/aluminium/ PVC laminate.

The blister packs were split into two groups; one group of blister packs were inspected with the Vision with Vacuum technique and a control group with blisters, which were not inspected with Vision with Vacuum.

The inspected, together with corresponding control samples, were then aged for 12 weeks at two elevated humidity conditions (25°C/60 percent RH and 40°C/75 percent RH).

For each of the three pack types, Xylitol tablets were removed from the inspected and control blister packs, at different time points, and tested for moisture uptake.

Positive control packs with 15µm sized defects were also tested. The presence of any leaks in the packs would cause the Xylitol to absorb moisture during storage.

**Results**

Results up to 12 weeks at 25°C/60 percent RH and 40°C/75 percent RH show no difference between inspected or control blister packs of any of the three types. The tablets from all the blister pack types did show changes in moisture status over time, but the changes were consistent for both inspected and control blister packs and were within the expected moisture transmission rate of the materials.

Statistical analysis based on WVEP results of inspected and control blister packs as independent variables showed that there was no significant difference at the 99 percent level. For the positive control, it can be seen that the WVEP was typically unreadable, indicating that the tablets had absorbed very high levels of moisture.

For the Xylitol tablets sealed in the PVC/PVDC packs it can be seen that the WVEP rises during the 12 weeks of storage at 40°C/75 percent RH, gradually increasing from 60.1 percent to 65.8 and 65.3 percent for the inspected and control samples. It is widely known that even when coated with PVDC, PVC has inferior barrier properties to higher coats alternatives.

There was no significant increase in moisture uptake over the 12 weeks for the PVC/PVDC samples stored at the lower temperature and humidity condition (25°C/60 percent RH).

For the Aclar® and Alu/Alu packs there was no significant increase in moisture over the 12 weeks for either the inspected or control packs.

**Conclusion**

There was no significant difference in moisture uptake between control samples and those inspected with the VisionScan test method for any of the three pack types investigated; thus, demonstrating the VisionScan test has no impact on the integrity of the blister pack and is non-destructive. Vacuum-based integrity test methods provide manufacturers with a non-destructive cost-effective solution, assuring package integrity and sterility.

Dr Dorian Dixon is a senior lecturer in the School of Engineering at Ulster University. His research is focused on polymer materials, medical devices, packaging and nanomaterials and he has published over 45 journal papers. He also works extensively with industry and has completed funded projects with more than 40 companies.
Implementing global standards to ensure end-to-end FMD compliance

Neil Piper
GS1 UK

Effective drug serialisation and identification is essential for improving the safety of medicines and combatting counterfeits. The Falsified Medicines Directive’s new requirements promise to crack down on the impacts of unsafe drugs. Neil Piper discusses the human cost of falsified medicines and how companies need to increase traceability of individual drug packets going forward.

The global impact of falsified medicines

The number of falsified and counterfeit medications infiltrating the supply chain has become a growing problem for global health organisations and pharmaceutical manufacturers in recent years. As a result, there have been cost implications and risks to patient safety.

The World Health Organization (WHO) defines falsified medicines as “medical products that deliberately/fraudulently misrepresent their identity, composition or source.” According to WHO, approximately one in 10 pharmaceutical products in low- and middle-income countries are either substandard or falsified. As of November 2017, they reported that “twenty global medical product alerts and numerous regional warnings” had been issued, with “technical support provided in more than 100 cases.”

Neil Piper
GS1 UK

The introduction of a standardised, unique identification system for drugs or medical devices will enable the authentication and traceability of products.
Accounting for compounding factors such as the impact of loss of income to pharmaceutical companies and governments, as well as remedial expenses, it has been estimated that the cost to the European Commission equates to €950m per year. By 2020, it is predicted that the falsified market in Europe could be worth €3.3bn, highlighting the sheer scale of the problem. The financial consequences are real, but the most compelling and worrying implications arise when considering the severe level of risk to human health.

The human cost
Annually, hundreds of thousands of children in lower-income countries die as a direct result of having consumed drugs for treating malaria, pneumonia and other diseases that are either substandard or falsified. However, high-income countries aren’t immune from the challenges of counterfeit medicines. Perhaps the most prominent news story in recent months has been the identification of a counterfeit form of the drug Xanax, which is reported to have claimed more than 200 lives in the UK and 126 in Scotland alone.

How could standards help?
Maintaining and enhancing the integrity of the end-to-end pharmaceutical supply chain is vital to help prevent the infiltration of falsified medicines. The introduction of a standardised, unique identification system for drugs or medical devices will enable the authentication and traceability of products. This would help to combat the ability of counterfeiters to integrate their products into the healthcare supply chain.

Adopting a single, global standard in healthcare would provide key supply chain stakeholders with complete visibility and the ability to track products from manufacturer to patient. The benefits would include critical patient safety and cost savings.

A recent McKinsey study highlighted just how important standards are:

- Implementing global standards across the entire healthcare supply chain could save 22,000–43,000 lives and avert 0.7 to 1.4 million patient disabilities
- Rolling out standards-based systems globally could prevent tens of billions of dollars’ worth of counterfeit drugs from entering the legitimate supply chain
- Healthcare costs could be reduced by $40–$100bn globally by the implementation of global standards.

International organisations are working collaboratively to prevent counterfeiters from reaching the patients. For example, Interpol Foundation, WHO and the World Customs Organization are working alongside regulators such as the US Food and Drug Administration (FDA) and the EU Commission. Collectively, they are working to raise global awareness, introducing sanctions and introducing measures to secure the supply chain.

The Falsified Medicines Directive
To ensure the integrity of the pharmaceutical supply chain, the European Union has introduced the Falsified Medicines Directive (FMD), which came into force on 9 February 2019. The rules have become mandatory for the majority of countries in the EU. Beyond Europe, the battle against falsified medicines is a worldwide struggle. Legislation is being rolled out in the US and Canada, and many countries such as Argentina, India and China already have serialisation systems in place.

The FMD integration is compulsory for any company selling or buying drugs – failure to comply means that pharmaceutical companies cannot sell their products. The directive requires the serialisation of prescribed drugs; every pack can be verified and decommissioned. If anything needs recalling, it can be identified down to the individual packet – a radical change for pharmacies and for manufacturers.

All medicinal products for human use need to have and adhere to two key safety feature requirements. The first supports the use of a single global standard, validating the comments in the McKinsey report. The second is the implementation.

BIOGRAPHY

NEIL PIPER is an Auto ID expert and business consultant, with over 25 years’ experience working predominantly in the Retail and Healthcare sectors. Skilled in GS1, ISBT standards, barcode technologies (printing and scanning), RFID, EPCIS, regulatory compliance (FMD, UDI both FDA and EU MDR/IVDR, UK MHRA GS1 compliance and more). He is currently redrafting ISB 1077 and ISB 0108 standards to comply with NHS Digital requirements. He has provided consultancy to numerous NHS trusts, pharmaceutical and medical device suppliers.
of an anti-tampering device on a product’s packaging. In accordance with the first principle, each product package is required to carry a unique identifier, a unique sequence carried by a two-dimensional barcode allowing the identification and authentication of the individual pack on which it is printed.  

What next for pharmaceutical manufacturers? Pharmaceutical manufacturers need to consider and invest in technology that can cope with significantly different requirements from global organisations. They need to be able to report into different regulatory databases, which may have varying requirements of data to be updated at various points in the supply chain.

With every medicine receiving a different serial number, systems are needed to manage and track information related to the product and its movement. Systems infrastructure will need to be able to condense data from vast networks and upload all information to a national or European database. Each manufacturer should also ensure they have aggregation solutions in place to allow for a full track-and-trace model, as this could potentially become a requirement in Europe.

Despite the considerations and investment that must be made by pharmaceutical manufacturers, serialisation and the use of standards provide opportunities for the future. These have already been validated and are in use in the retail sector, with very tangible benefits.

Serialisation in the UK Serialisation in the UK’s NHS system would have unprecedented benefits, particularly in acute trust settings to create a safer and more efficient service. Medications need to be tracked and traced throughout the patient pathway with ease, even to patient application. There are tangible benefits and cost savings in operational efficiencies such as the ability to automatically validate expiry dates, preventing the use or sale of expired medicines.

Identifying every product empowers healthcare professionals by allowing them to easily access accurate and transparent product information, facilitating precise ordering, improving product availability and lowering transaction costs. However, the real power comes in the ability to effortlessly identify and remove all recalled products across trusts, down to the patient level, once they have been discharged.

Conclusion As the development of falsified medicines becomes increasingly sophisticated, the healthcare system needs to constantly evolve to ensure it has robust processes in place to reinforce the supply chain.

Global efforts are being made to transform the pharmaceutical supply chain, increasing visibility and traceability throughout. Ultimately, the aim is to reduce the risk of harm to patients. To achieve this, standards need to prove their critical worth in ensuring a seamless, lean and secure end-to-end distribution practice.

REFERENCES

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The role of an integrated LIMS solution in supporting data integrity in current good manufacturing practice (cGMP) environments

During this webinar, we will consider and clarify the role of data integrity in cGMP for drugs as required in 21 CFR parts 210, 211 and 212. We will also discuss how a LIMS can aid your compliance efforts and enhance the value of your data.

THE US Food and Drug Administration (FDA) was perhaps understating a point when it said in its April 2016 Draft Guidance: Data Integrity and Compliance with cGMP that the increasing number of data integrity-related violations identified during its inspections was ‘troubling’. In fact, 21 out of 28 warning letters issued by the agency between January 2015 and May 2016 involved data integrity issues in drug manufacturing.1

Ultimately, whether you are a drug manufacturer, clinical research organisation (CRO) or pharmaceutical R&D company, FDA compliance as well as the accuracy and completeness of data is critical for safe product development, and any breach of data integrity could have serious implications for human health.

Attend this webinar and learn more about:
- Why is data integrity important?
- What is data integrity?
- Manual laboratory workflows
- How a Laboratory Information Management System (LIMS) can aid your compliance
- How a LIMS can support your organisation on saving time and money
- How STARLIMS can enhance the value of your data.

KEYNOTE SPEAKER:

TAMARA McKENNA
Sales Executive & Accounts Manager, Abbott Informatics

After graduating from the University of the Witwatersrand with a BSc (Hons) in Chemistry, Tamara spent some time working as a process metallurgist, in both hydrometallurgical processing and thermochemistry for a mining research and technology business, before moving into the LIMS industry where she has been for the past 18 years. Prior to taking on a business sales role Tamara was involved in implementation and project management of LIMS applications in a diverse range of industries and laboratories.

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1. Data Integrity and Compliance With Drug CGMP: Questions and Answers Guidance for Industry
2. Current Expectations and Guidance, including Data Integrity and Compliance With CGMP

WEBINAR PREVIEW
Welcome to European Pharmaceutical Review’s Guide to Informatics & Data Integrity. In this edition, two companies showcase their services and highlight how they stand out from the crowd.

The exponential speed of information technology evolution is rapidly being accepted and integrated into our everyday life, yet it remains a challenge in the laboratory. To introduce this ‘Guide to’ series, Isabel Muñoz Willery discusses emerging trends and software solutions.

PARTICLE MEASURING SYSTEMS is the only partner that can offer complete contamination monitoring solutions combined with a modern data management system. Plus, our system is preconfigured and validated to connect with our Facility Monitoring system for quick integration, saving time and money. With a continual focus on data integrity and helping our customers meet regulations while generating actionable insights, we provide a complete range of products and services.

ACD/LABS helps scientific R&D organisations assemble digitised analytical, structural and molecular information for effective decision making, problem solving and product lifecycle control by their scientists. Our enterprise technologies enable automation of molecular characterisation and product development, and facilitate chemically intelligent knowledge management, data analytics and collaboration – both within organisations and between partners. ACD/Spectrus – our analytical data management platform – is compatible with most major instrument vendor formats and industry standard, open source and emerging formats.
The digitalised laboratory

Introduction by: Isabel Muñoz Willery, Owner NL42 Consulting, Paperless Lab Academy

The laboratory informatics landscape has seen significant changes over the last 10 years. Here, we discuss emerging trends and software solutions.

WHILE INDUSTRIES are surfing the waves of the fourth industrial revolution, laboratories are raising their voices and asking for change. But they don’t just want automation and digital optimisation; they want to make a significant contribution to company business objectives. The scientific data generated in laboratories and the extracted outcomes are extremely valuable to companies, providing relevant insights to decision-making processes. Industry 4.0 can’t really be sustainable without adequate access to scientific data; meaning that the lab of the future needs to happen today.

When a laboratory reaches full automation, data, methods and processes are seamlessly shared between software applications and analytical instruments. This is the ultimate goal for a digitalised laboratory. The data then provides insights and business intelligence to decision makers at any step of the data-lifecycle. Such laboratories become a knowledge centre, with data at their core, so it’s fundamental to manage, control and protect data adequately along its entire lifecycle – from the source through to final archiving.

During the Paperless Lab Academy annual editions, we have defined four layers of the eData lifecycle: eConnect, eManage, eDecide and eArchive. Figure 1 provides an overview of the typical system mapping present in a laboratory and defines four layers of data gathering, manipulation, review, approval and archival.

Along with data management tools and methodologies, data integrity is one of the most important concepts covered at the academy. The complexity of the drug manufacturing process requires use of dedicated, specialised instrumentation along the product lifecycle. Trust in the data generated along the production line and the final product itself is fundamental. If there is an area of concern regarding data integrity in chromatography laboratories, for example, this will come down to the first moment the raw data was generated and managed. Alongside potential manipulation of the data, simple gaps between instruments and...
software might open the door to human translation errors and lack contemporaneity and protection.

Lately, FDA auditors have become skilled in tracking gaps and identifying misuse of informatic applications and misalignment with ALCOA+ principles. Data integrity is amongst the top issues cited on FDA warning letters. Indeed, the FDA has cited data integrity on 79 percent of warning letters over the last five years. The fact is that if you can’t trust the data, then the integrity of the company quality management is questionable.

Dedicated software applications are key assets when looking for adequate data management. Whether the laboratory is dedicated to research, development or quality control, whether it provides services to internal or to external customers, whether the origins of the samples to be analysed are biological or chemical, the available software solutions are numerous and differ from one to another.

The laboratory informatics landscape has seen significant changes over the last 10 years. Some of the key players in the market have positioned themselves as a one-stop-shop due to their accumulated experience; through running customer projects and acquisition of smaller companies proposing the latest innovative solutions. However, no longer is there just one company proposing the best software solution for the management of laboratory data – hundreds of companies now propose specialised solutions for a given process and offer alternative approaches to everyday laboratory routines.

The solutions offered in today’s market have evolved, with addition of new features, components and processes that were not available in the early days. More importantly, the technology used to support these systems has changed considerably in the last 20 years. Considering the dramatic evolution of IT systems, this is to be expected. In the ’80s the combination of hardware, basic software and application was offered by a single vendor. Nowadays, most of the providers have moved their products to the latest technology, including the possibility to run the application in the cloud. The applications are accessible from virtually everywhere, using smartphones or public network connections.

Discussions around introducing security measures often suggest blockchain as a possibility, potentially providing strong protection of information. We expect these technologies will be available for scientific data management very soon. Simulations designed on digital twins allows companies to evaluate the impact of digitalisation on their processes without affecting ongoing activities.

The original goal of software implementation in the laboratory was to substitute paper-based processes, increase reliability and decrease response time. The amount of information generated in the laboratory (eg, terabytes of data per day in certain research centres) and the immense power of computers and tools designed in the last few decades enables analysis of huge amounts of information in just a few seconds. Data analytics allows the evaluation of information present in different systems in a seamless way, cross referencing data and rapidly providing the required business or technical information.

The exponential speed of the information technology evolution is rapidly being accepted and integrated into our everyday life, yet it remains a challenge in the laboratory. Moving from on-premise solutions to cloud-based solutions remains a hot topic in laboratories that handle sensitive data. In the meantime, market leaders envisage the possibility of using blockchain technologies in the lab, while the Internet of Things is still booming. The laboratory is no longer a mysterious series of operations generating data that ends in a ‘go’ or ‘no-go’ decision. Instead, it is a production centre of scientific data where transparency and deep analysis brings fundamental added value to the business decisions of the company.
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Overview of services/solutions provided
ACD/Labs helps scientific R&D organisations assemble digitised analytical, structural and molecular information for effective decision-making, problem solving and product lifecycle control by their scientists. Our enterprise technologies enable automation of molecular characterisation and product development, and facilitate chemically intelligent knowledge management, data analytics and collaboration – both within organisations and between partners.

ACD/Spectrus – our analytical data management platform – is compatible with most major instrument vendor formats and industry standard, open source and emerging formats (eg. ICAMP, ASCII, AniML and ADF). ACD/Labs has offered machine learning for more than 20 years and is committed to ensuring that our technological developments are future-proof.

ACD/Labs solutions apply to a variety of industries with a concentration in large-to medium-sized pharma and biotech. We provide worldwide sales and support and bring 25 years of experience and success helping organisations innovate and create efficiencies in their workflows.

How do you integrate your solutions with legacy systems?
ACD/Labs software architecture supports convenient integration with existing informatics systems via various services, including RESTful web services.

What additional services/solutions do you provide?
ACD/Labs’ professional services team helps our customers automate their workflows and build solutions by configuring our commercially available off-the-shelf products. We are actively supporting customers with migrations to highly distributed and cloud computing platforms.

How do your solutions meet current data integrity requirements?
ACD/Labs software importantly stores not only data, but in the interests of data integrity, the lineage of knowledge from results and interpretations back to data. ACD/Labs software also has features that support 21 CFR Part 11 requirements. Some of these features include requiring user logins, providing permission control and providing audit trails or change histories.

What makes your company stand out in the field?
The ACD/Spectrus Platform uniquely digitises analytical data from various techniques (LC/UV/MS, GC/MS, NMR, chromatography, Raman, IR and more) and most major instrument vendors together in a single interface. ACD/Labs customers are able to assemble and connect chemical information with analytical data to support critical decision-making. Conveniently being able to store, report and share that data in context helps drive innovation through collaboration as well as enhancing regulatory compliance.

The ACD/Percepta Platform offers industry leading molecular property predictors that are used extensively in drug discovery for library screening, drug design and lead optimisation. Calculators include physicochemical properties – logP, logD, pKa, Lipinski’s Rule of five, etc.; ADME properties including oral bioavailability, CYP inhibition, P-gp specificity and blood brain barrier permeation; and toxicity endpoints including hERG inhibition, acute toxicity, mutagenicity and more.

Together, this software portfolio provides an extensive set of tools for innovative pharmaceutical R&D.

Other information you think would be helpful?
ACD/Labs software is uniquely able to extract and transform many different types of analytical data from a broad spectrum of instruments and apply a variety of advanced algorithms to perform essential processing and interpretation. At its core, the ACD/Spectrus software portfolio helps enable consolidation and simplification of the analytical data landscape.

ACD/Labs’ recent product introductions support vital activities in pharmaceutical development. In keeping with the trend towards web interfaces, these new applications offer a thin client browser-based interface for easy deployment, support and access. Luminata is a CMC decision support tool that helps teams make data-driven decisions about processes, impurities, stress testing and batch genealogy through effective data management. Katalyst 2D is purpose built to support the end-to-end workflow of high-throughput experiments and parallel synthesis, from design to decide. Katalyst is designed to integrate into your existing informatics infrastructure and automates planning, execution and analysis of array-based chemistry in a single interface.

COMPANY DETAILS
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Overview of services/solutions provided:
With a continual focus on data integrity and helping our customers meet regulations while generating actionable insights, Particle Measuring Systems provides a complete range of products and services:

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- Particle counters (remote and mobile)

**Services**
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  - Risk assessment
  - Sample point location
  - Quality assurance support
- GMP services
  - Measurement
  - Thermal validation
  - Smoke studies
- PMS product calibration and maintenance
- Validation and installation
- Project management

**Training and education**
- Particle College
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**Environmental monitoring systems**
- Monitor control system
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Our PharmaIntegrity Data Management provides our customers with a uniquely combined solution of products and services to ensure data integrity, helping not only with data management but also workflow management, all with portable integration and real-time connection.

How do your solutions meet current data integrity requirements?
Particle Measuring Systems offers the PharmaIntegrity Data Management system, which helps companies effectively manage their data with 21CFR Part 11 compliance in a paperless environment. We work with the customer from start to finish to ensure a smooth migration from legacy systems.

Not only do we provide the instruments and software (in a plug-n-play package) but our customers also have access to our advisory team (e.g., risk assessment, sample point locations, process improvement analysis), installation and validation services and global support.

What makes your company stand out in the field?
Particle Measuring Systems is the only partner that can offer complete contamination monitoring solutions combined with a modern data management system. Plus, our system is preconfigured and validated to connect with our Facility Monitoring system for quick integration, saving time and money.

What additional services/solutions do you provide?
Together with the PharmaIntegrity Data Management system, Particle Measuring Systems can support all phases of the project, including process mapping, process improvement and quality management evaluation to enhance compliance with the latest regulatory requirements and mitigate any risk of nonconformities.

Our installation, validation and support teams are located globally and offer after-sale support to ensure operators are well trained, updated and capable of performing daily activities without interruption.

COMPANY DETAILS

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