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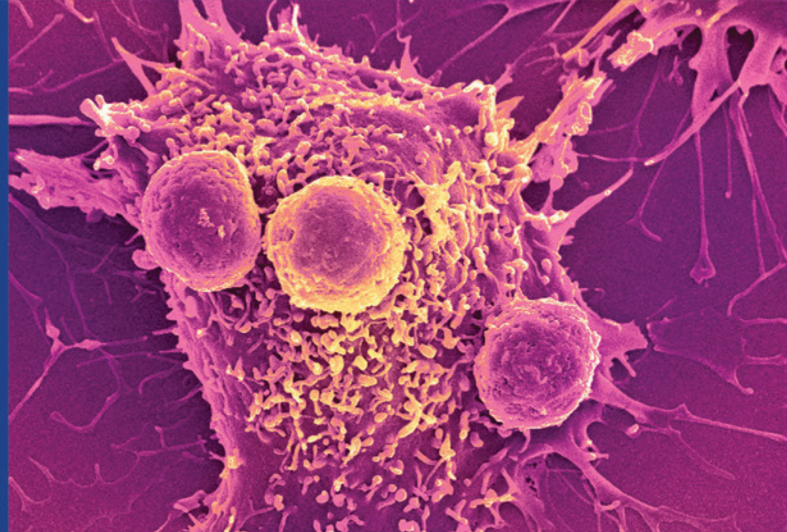

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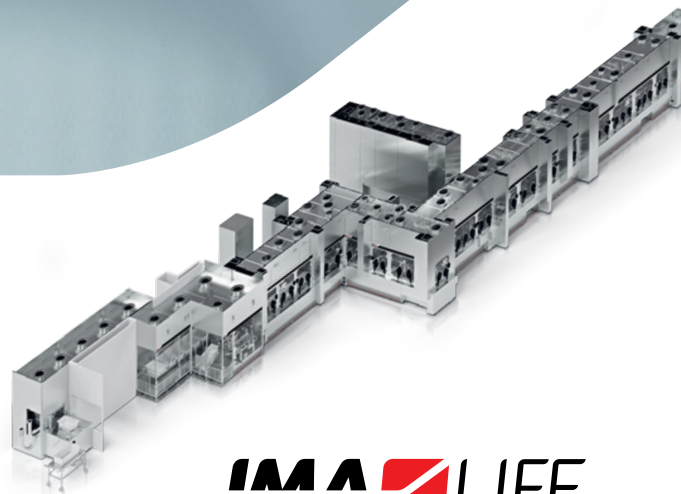

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Sales

ACCOUNT EXECUTIVE| Ashley Poste
aposte@aprpub.com • 650-243-5639

Production

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jcampbell@comparenetworks.com

LAYOUT DESIGNER, TRADE SHOW COORDINATOR
Allison Chelminiak
achelminiak@comparenetworks.com

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acoleman@comparenetworks.com

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CompareNetworks, Inc.
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Message from the Editor »

Inevitable AI

Have you ever had the feeling you were heading too fast towards something that was inevitable?

Maybe in a dream? Maybe on a roller-coaster?

Maybe unsure of where this journey might take you? Maybe feeling a little out of control?

I'm beginning to feel that way about Artificial Intelligence (AI).

I have used ChatGPT on numerous occasions – and when it's good – it's great. When it's not – well – how can you ever trust it again?

Anyway, as 2023 ends, and 2024 is right around the corner it seems AI is poised to make an even bigger impact on our lives – probably mostly good (fingers crossed) yet not without drawbacks – as with any new technology.

Recently, I've come across a few stories that I think illustrate both sides of the AI coin – and one that shows it's here and only getting bigger.

First – the outlook.

In a recent Yahoo Finance article, TECHnalysis president Bob O'Donnell was quoted saying:

"2024 is going to be the year when it really explodes, because every day people are going to use [AI]," TECHnalysis president Bob O'Donnell told Yahoo Finance.

Think PCs and smartphones running generative AI programs, and generative AI-powered video and audio platforms.

That's the basic outlook – and really – it makes sense. 2023 has turned into the launch pad for AI. 2024 will see the take-off.

But what about the bad stuff? Stories abound about how AI will take over society, jobs, etc. I found an interesting article which summarizes the results of a study conducted by the American Society of Health-System Pharmacists (ASHP) which found that:

"ChatGPT's answers to nearly three-quarters of drug-related questions reviewed by pharmacists were incomplete or wrong — in some cases providing inaccurate responses that could endanger patients, according to a study presented at the American Society of Health-System Pharmacists Midyear Clinical Meeting Dec. 3-7 in Anaheim, California. When asked to cite references, the artificial intelligence program also generated fake citations to support some responses."

Seems like pretty scary stuff.

But certainly, beyond all this doom and gloom – there must be some promising uses of AI – especially in the pharmaceutical industry, where (hopefully), its used by knowledgeable and detail-oriented professionals.

We all know that the pharmaceutical industry is very conservative, and slow to adopt new technologies. Preferring to adopt a wait and see attitude.

Yet, recently, Merck announced the launch of their AIDDISON™ drug discovery software. In the press release, Merck says it's the first software-as-a-service platform that bridges the gap between virtual molecule design and real-world manufacturability through Synthia™ retrosynthesis software application programing interface (API) integration. The press release adds:

"It combines generative AI, machine learning and computer-aided drug-design to speed up drug development. Trained on more than two decades of experimentally validated datasets from pharmaceutical R&D, AIDDISON™ software identifies compounds from over 60 billion possibilities that have key properties of a successful drug, such as non-toxicity, solubility, and stability in the body. The platform then proposes ways to best synthesize these drugs."

"With millions of people waiting for the approval of new medicines, bringing a drug to market, still takes on average, more than 10 years and costs over 1.9 billion Euros1" said Karen Madden, Chief Technology Officer, Life Science business sector of Merck. "Our platform enables any laboratory to count on generative AI to identify the most suitable drug-like candidates in a vast chemical space. This helps ensure the optimal chemical synthesis route for development of a target molecule in the most sustainable way possible."

So, are there any conclusions we can make from all this? From my point of view AI is here to stay. It will get refined, maligned, and used for good and bad. Where it leaves us as humans remains to be seen.

Let me know what you think.

Mike Auerbach
Editor-In-Chief
mauerbach@comparenetworks.com

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Filtration Systems
In Design and Practice

Robert Dream
HDR Co., LLC

The principal advantage of the three different filtration/separation techniques are that the operation is achieved without change or interphase transfer, thus any desired product is continually maintained in an aqueous environment. Size differences provide one basis on which the separations occur. The three available separations are Microfiltration (MF), Ultrafiltration (UF), and Nanofiltration (NF), respectively from large pore size retainment to smaller pore size retention. These techniques separate molecules of different sizes based on their size by retaining the larger size and permeating the smaller size. Product (mostly biologic) purification is broken down into four steps.

- First step is removal of insoluble
- Second step is isolation
- Third step is primary purification
- Fourth step is polishing or final purification

Microfiltration, Ultrafiltration and Nanofiltration

These three filtration types are similar in principal and operation but depending on the function they are used for there are subtle differences between them. Also, the pore size of the membranes is different. The microfiltration and nanofiltration operations are employed to concentrate products and/or switch carrier solvent. In this case the retentate is the product. Usually, the ultrafiltration process is employed to purify products from suspended contaminants and permeate is the product. Microfiltration usually fits within the first category, ultrafiltration fits within the second and third categories, and nanofiltration fits within the fourth category.

Microfiltration retains particles 0.1-10 microns in diameter, for example, whole cells or cell debris. Common materials used for the manufacture of micro-filters include paper, polymers, and ceramics. In addition, most micro-porous membranes are symmetric or isotropic, that is, the membrane pores are the same size throughout the depth of the filter.

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Ultrafiltration, which exclude particles and macromolecules of 1,000–500,000 Dalton, are usually asymmetric or anisotropic. Anisotropic membranes consist of an extremely thin “skin” of homogeneous polymer supported upon a much thicker, spongy substructure. The pores of the skin layer are markedly smaller than the pores through the rest of the membrane. Consequently, the thin surface layer constitutes the major transport barrier and governs the filtration characteristics of the entire membrane.

Nanofiltration, is for concentration of compounds with molecular weights of 250-2000 Daltons, and removing monovalent salts, methanol and/or Ethanol from aqueous solutions of these compounds.

Analyzing Transmembrane Flux

During membrane separation processes, a set of feed pressure forces the solvent, and certain solutes, to flow through the membrane, while undesired solutes are retained, Figure 1.

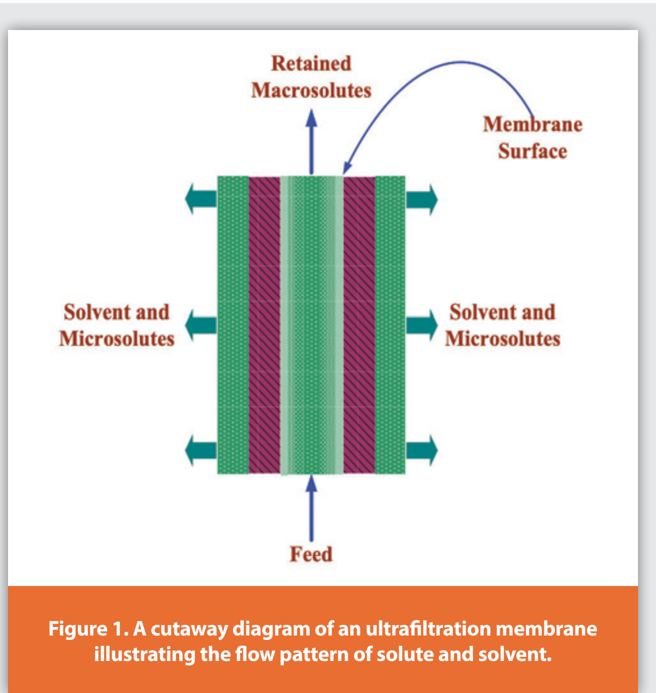

Figure 1. A cutaway diagram of an ultrafiltration membrane illustrating the flow pattern of solute and solvent.

To be effective the feed pressure must be greater than the osmotic or bulk pressure of the solution. For dilute solutions and with pressure exerted on one side of the homogenous, isotropic membrane, the steady-state, transmembrane solvent flux can be approximated by the following relations;

$$J_w \cong L_p(\Delta P - \sigma \Delta \pi) \quad (1)$$

The flux of the solute can be expressed by the following relation;

$$J_s \cong P_s \Delta C + J_w(1-\sigma)\bar{C} \quad (2)$$

Where;

J_w = Solvent flux [cm³ (cm².s)]

J_s = Solute flux [gm (cm².s)⁻¹]

L_p = Membrane permeability for the solvent [cm³ (cm².s.atm)⁻¹]

P_s = Membrane permeability for the solute (cm/s)

ΔP = Hydrostatic pressure difference across the membrane (atm)

Δπ = Osmotic pressure

σ = Reflection coefficient

ΔC = Solute-concentration difference across the membrane (gm/cm³)

[sometimes referred to as the solute-concentration difference between the upstream and downstream solutions]

$\bar{C}$= Average concentration of solute in the upstream solution

(1-σ) = the quantity represents the fraction of the solvent flux carried by pores large enough to pass the solute

If the reflection coefficient (σ) is small (σ≅0), the membrane will be highly permeable to both solute and solvent; if σ is large (σ≅1), the membrane will reject all solute.

The first term on the right-hand side of equation (2), corresponds to the diffusive flux of solute through the membrane. The second term represents the “convective flux” or “coupled transport” of solute driven by the net flux of solvent (it is sometimes interpreted as a consequence of frictional drag between moving solvent molecules and solute molecules within the membrane). In addition, a high diffusive flux of solvent will result in momentum exchange between solvent and solute molecules, due to collisions, which could serve to increase the net flux of solute. Such momentum interchange would not be expected to cause a significant reduction in the diffusive flux of the more abundant solvent molecules, however, no term accounting for coupled transport is included in equation (1).

From mass conservation, the solute flux, J_s, can also be written in the form,

$$J_s = C_{perm} J_v \quad (3)$$

Where Cperm is the concentration of permeate or ultrafiltrate (the concentration in the downstream solution), and J_v, is the total flux of permeate (usually assumed to be equal Jw for dilute solutions). Alternatively, J_s can be expressed in terms of C_∞, the concentration of solute in the upstream solution:

$$J_s = (1-R) J_v C_\infty \quad (4)$$

Where R is the rejection coefficient, which is equal to the fraction of solute present in the upstream solution that is rejected by the membrane. In terms of concentrations, the rejection coefficient is defined as;

$$R = (C_\infty - C_{perm}) / C_{wall} \quad (5)$$

Finally, we should note that for a purely diffusive-type membrane (for which J_w is negligible), the solute mass flux is;

$$J_s = [\frac{K_s D_e}{\delta}](C_\infty - C_{perm}) \quad (6)$$

Where K_s is the distribution coefficient of solute between the membrane and solution (assumed to be constant), D_e is the effective diffusivity

of solute through the membrane, and δ is the membrane thickness. Equation (6) models the membrane as a continuous "solvent" in which the solubility of solute is described by the distribution coefficient K_s. An alternative approach is to treat the membrane as a sieve with distinct pores and a specific porosity.

Concentration Polarization

During concentration of the feed, pressure exerted on the upstream solution in contact with the membrane causes solute to flow toward the membrane surface. Initially, the convective flux of solute to the membrane exceeds the rate at which solute passes through the membrane, this result in accumulation of solute at the membrane, with the maximum solute level at the membrane surface. Such phenomenon is known as concentration polarization, Figure 2.

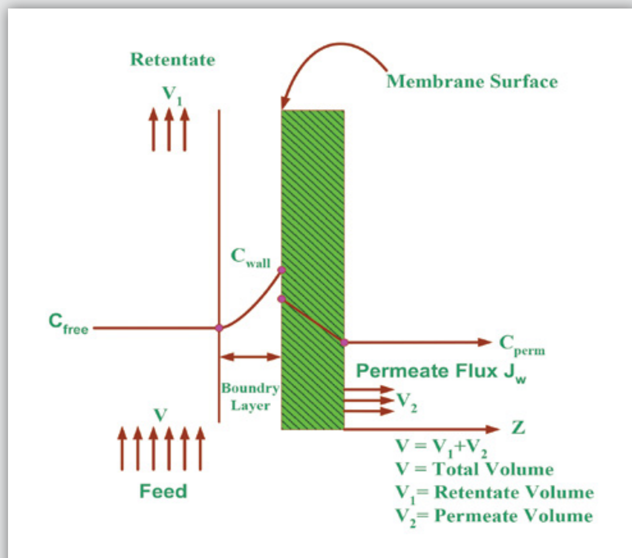

Figure 2. This figure illustrates the concentration gradient that results from concentration polarization, a phenomenon which occurs when solute is rejected by the membrane and accumulates on the feed side of the membrane's surface.

If the membrane is not completely impermeable to the solute, such polarization can cause solute leakage through the membrane, or anomalously low reflection efficiency. In addition, the high concentrations of solute can increase the osmotic pressure difference (i.e., the pressure difference across the membrane wall), and thus reduce the effective driving force for solvent transport through the membrane. In many cases particularly with macromolecular solutes, concentration polarization is the limiting factor governing flux rates.

Concentration polarization is often unavoidable but should be minimized. In practice, a nitrogen pulse or other method is supplied on the permeate side of the membrane, creating a shock wave that causes accumulated solute to dislodge from the feed side of the membrane and become part of the retentate stream, Figure 2.

During this activity, the permeate valve will be closed. Cross-flow filtration or vigorous mechanical stirring of the upstream liquid is also employed to alleviate the effects of concentration polarization.

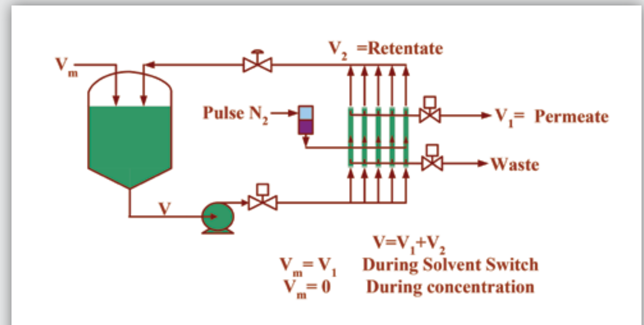

Figure 3. In this batch filtration unit, the feed solution is pumped through the hollow-fiber membrane modules. The permeate is collected (V_1) and the retentate is recycled (V_2). Makeup volume is added (V_m) to compensate for the removal of permeate. Periodic pulses of nitrogen gas are applied to the permeate side of the membrane to prevent concentrate polarization, or buildup of solute, from occurring on the feed side.

Gel Polarization

If the concentration of solute at the membrane surface is high enough, the polarization layer exhibits gel-like properties. At this point, gel-polarization is said to occur. The layer of concentrate assumes the form of a slime or cake at the membrane's surface, and the adherent layer presents a hydraulic barrier in series with the membrane. In fact, the gel (when present) usually contributes the dominant resistance to mass flow.

The concentration of solute at which gel filtration takes place will vary with the size, shape, and degree of the solvation of the solute particles.

For proteins and nucleic acids, concentrations as high as 10-30 percent by weight are often required before gelation is observed. In contrast, with rigid-chain, solvated macromolecules like polysaccharides, concentration below 1% by weight may be sufficient.

Effects of Polarization

To analyze the effects of polarization on solvent flux, we recall that the volumetric flux of solvent, J_w, can be written as;

$$J_w = L_p(\Delta P - \sigma\Delta\pi) \quad (1)$$

And;

$$\Delta\pi = RTC_{wall} \quad (7)$$

Where the osmotic pressure, Δπ, has been expressed in terms of C_{wall}, the concentration of solute at the surface of the membrane. Integration of a steady-state mass balance on solute in the polarization layer leads to;

$$J_w = \frac{D_s}{\delta} \ln \frac{C_{wall} - C_{perm}}{C_\infty - C_{perm}} \quad (8)$$

Where D_s is the diffusivity of solute (assumed here to be independent of solute concentration).

Furthermore, if σ=1, that is, if C_{perm}=0, then;

$$J_w = \frac{D_s}{\delta} \ln \frac{C_{wall}}{C_\infty} \quad (9)$$

This equation provides a relationship between the boundary layer thickness, δ, and the concentration of solute at the membrane surface, C_{wall}. Note that if gel polarization occurs, $C_{wall} = C_g$, the gel concentration of the macrosolute. The gel concentration of course places an upper limit on the solute concentration at the membrane surface.

At this point we should consider another subtle yet important distinction between concentration polarization and gel polarization. In the former, C_{wall} is less than C_g, and $C_{wall}/C\infty$ will adjust itself to an imposed J_w. Nonetheless, the polarized region presets a resistance to mass transfer and can be modeled as a membrane in series with original membrane. The resulting flux is given by

$$J_w = \frac{\Delta P}{(1/L_p) + R_p} \quad (10)$$

Where ($1/L_p$) is again the membrane resistance R_p is the resistance of the polarized boundary layer. Increasing the transmembrane pressure drop will increase c_{0s} and consequently, R_p. Thus; concentration polarization causes the flux, J_w, to increase less than proportionately with ΔP. On the other hand, gel polarization results in a constant value of $C_{wall} = C_g$. Equation (10) then predicts that the flux will be independent of the applied pressure. Such behavior has indeed been observed for many systems, particularly at high pressures.

Returning to equation (10) the leading terms on the right-hand side, D_s/δ, can be viewed as a mass transfer coefficient, k_s, which allows us to write;

$$J_w = k_s \ln \frac{C_{wall}}{C_\infty} \quad (11)$$

Mass transfer coefficients for fluid flow in narrow channels conventionally are described by expressions of the form;

$$\frac{k_s d_h}{D_s} = A(\mathrm{Re})^a (Sc)^{\frac{1}{3}} \quad (12)$$

Where;

$$\mathrm{Re} = \frac{d_h u \rho}{\mu} \quad (13)$$

$$Sc = \frac{\mu}{\rho D_s} \quad (14)$$

d_h = Equivalent diameter of the channel, or

d_h = 4(Cross-sectional area / wetted perimeter)

u = Average velocity of the fluid along the channel

ρ = Density of the fluid

μ = Viscosity of the fluid

Re = Reynolds Number

Sc = Schmidt number

And, A and a are constants.

Time Required for Ultrafiltration

Suppose we wish to concentrate a protein solution by ultrafiltration in a batch system. The permeability of the membrane is L_p liter $m^{-2}hr^{-1}atm^{-1}$, and the total membrane area is A m^2. The initial volume of solution is V_0 liter and the amount of protein present is n_1 mmoles. The pressure drop is ΔP atm and the temperature of operation is T°K assumes that the membrane completely rejects the solute and that concentration polarization is negligible.

a. Derive an expression for the rate of change of the batch retentate volume with time (dV/dt).

b. Derive an expression for time required for ultrafiltration of the solution (reduce the volume from V0 liter to V liter).

Solution:

a. In our analysis of ultrafiltration, we would most like to have the time required to filter a given volume of feed. To find this time we first must find the solvent velocity through the membrane. This velocity is given by equation (1)

$$J_w = L_p(\Delta P - \sigma \Delta \pi) \quad (1)$$

If the solute is completely rejected by the membrane, the reflection coefficient σ=1; if the solution is dilute, the osmotic pressure $\Delta\pi = RTC_{wall}$, where C_{wall} is the solute concentration at surface of the membrane.

But what is C_{wall}? If the mixing in the holding tank were complete, then the concentration at the membrane surface C_{wall} should equal the concentration in the tank C∞. However, mixing is incomplete and these concentrations are not equal. Instead, the concentration at the membrane's surface may be much more than that in the bulk because the solvent flux through the wall strands solute near the wall. This high solute concentration, called concentration polarization, is illustrated schematically in Figure 3. It augments the osmotic pressure and, hence, reduces the flux through the membrane. To estimate this flux reduction, we recognize that solute carried toward the membrane by solvent convection must equal that diffusing away from the wall by diffusion:

$$CJ_w = -D\frac{dC}{dz} \quad (15)$$

This differential equation is subject to the boundary conditions that at the wall, the concentration is C_{wall}-C_{perm}:

$$Z=0, \quad C = C_{wall} - C_{perm} \quad (16)$$

and at,

$$Z=\delta, \quad C = C_\infty - C_{perm} \qquad (17)$$

Where, δ is the thickness of a thin layer near the surface.

Solving first order differential equation (15) and applying boundary conditions (16) and (17),

$$\int_0^\delta \frac{J_w}{D_e} dz = -\int_{C_\infty - C_{perm}}^{C_{wall}-C_{perm}} \frac{dC}{C}$$

Or

$$J_w = \frac{D_e}{\delta} \ln \frac{C_{wall} - C_{perm}}{C_\infty - C_{perm}} \qquad (8)$$

Furthermore, if σ=1, that is, if C_{perm}=0, then

$$J_w = \frac{D_e}{\delta} \ln \frac{C_{wall}}{C_\infty} \qquad (18)$$

In other words, a plot of flux versus the logarithm of reservoir concentration C∞ should be a straight line.

The variation of flux with reservoir concentration suggested by equation (18) can be checked experimentally. The small scatter in these data is typically of the success of the analysis. Note that the slope of this plot equal to (-De/δ), is a type of mass transfer coefficient.

Now we return to our original goal of estimating the time required to filter a given volume. In general, this analysis is complicated, involving transcendental equations. The most useful simple case occurs when there is little concentration polarization, that is, when

$$\frac{D_e}{\delta J_w} >> 1 \qquad (19)$$

In this case, $C_{wall} = C_\infty$.

We now make a mass balance on the solvent and combine the result with equation (1):

$$\frac{dV}{dt} = -AJ_w \qquad (20)$$

$$\frac{dV}{dt} = -AL_p \Delta P (1 - \frac{RTC_\infty}{\Delta P}) \qquad (21)$$

Because the membrane rejects all the solute, the total solute ($n_1 = C_\infty V$) is constant and equation (21) becomes:

$$\frac{dV}{dt} = -[AL_p \Delta P](1 - \frac{[RTn_1/\Delta P]}{V}) \qquad (22)$$

b. This equation is easily integrated using the initial condition

$$t = 0, V = V_0 \qquad (23)$$

$$t = [\frac{1}{AL_p\Delta P}]\{(V_0 - V) + (\frac{RTn_1}{\Delta P})\ln(\frac{V_0 - RTn_1/\Delta P}{V - RTn_1 \Delta P})\} \qquad (24)$$

Examples

Example 1: We wish to concentrate and achieve a solvent switch of a solution by cross flow microfiltration in a batch system. The flux for this ceramic membrane is 10 gal/hr-ft². The initial volume of the solution is 1800 gal and the final volume is 360 gal, the amounts of protein present is 18.0 Kg, and the molecular weight is 1213.43 gr./mole. The pressure drop is 30 psig and the temperature of operation is 277°K. If we want the operation to be completed in 2 hours calculate the required area to achieve this task.

Solution:

R=0.082-liter atm/gr.mole°K

T=277°K

n_1=(18x10³gr/1213.43 gr./mole)

Δp=30 psig

V_0 = 1800 gal

V = 360 gal

t = 2 hrs, assumed

j_v = 10 gal/hr-ft²

The time required for Micro-filtration is;

$$t = \left[\frac{1}{AL_p \Delta p}\right]\left\{(V_0 - V) + \left(\frac{RTn_1}{\Delta p}\right)\ln\left(\frac{V_0 - \frac{RTn_1}{\Delta p}}{V - \frac{RTn_1}{\Delta p}}\right)\right\} \qquad (1)$$

Then the term,

$$\frac{RTn_1}{\Delta P} = \frac{[0.082 liter.atm/gr.mole^\circ K](277^\circ K)\left(\frac{18x10^3 gr.}{1213.43 gr./mole}\right)}{2atm} = 168.33 liter$$

and from equation (1), the term,

$$\Pi = \left\{(V_0 - V) + \left(\frac{RTn_1}{\Delta p}\right)\ln\left(\frac{V_0 - \frac{RTn_1}{\Delta p}}{V - \frac{RTn_1}{\Delta p}}\right)\right\}$$

can be calculated;

$$\Pi = \left\{(1800 galx3.78 lit./gal. - 360 gal.x3.78 lit./gal.) + 168.33 lit.\ln\left(\frac{1800 gal.x3.78 lit./gal. - 168.33 lit.}{360 gal.x3.78 lit./gal. - 168.33 lit.}\right)\right\}$$

$$\Pi = 1363.56 gal.$$

Therefore equation (1) reduces to;

$$t = \left[\frac{1}{AL_p \Delta p}\right](1363.56 gal)$$

Also;

$$\frac{dV}{dt} = -Aj_v = -AL_p \Delta p\left(1 - \frac{RTc_1}{\Delta p}\right) \qquad (2)$$

But;

$$n_1 = c_1 V$$

or

$$c_1 = \frac{n_1}{V} = \frac{\frac{18x10^3 gr.}{1213.43 gr./mole}}{1800 gal.x3.78 lit./gal.} = 0.002 mole/lit.$$

Substituting;

$$\frac{RTc_1}{\Delta P} = \frac{[0.082 lit.atm/gr.mole^{\circ}K](277^{\circ}K)(0.002 mole/lit.)}{2atm} = 0.02 gr.^{-1}$$

Rearranging equation (1);

$$A = \frac{\Pi}{L_p \Delta pt} \quad (3)$$

And the term $\frac{RTc_1}{\Delta P}$ is a small value, therefore from equation (2) we have.

$$j_v = L_p \Delta p$$

then equation (3) reduces to:

$$A = \frac{\Pi}{j_v t}$$

or

$$A = \frac{1363.5 gal.}{(2hr)X(10 gal/hr - ft^2)}$$

And therefore;

A= 68.18 ft^2, is the area required to cross flow 1800 gal. to 360 gal in two hours.

Example 2: If we assume that the membrane completely rejects the solute and that concentration polarization is negligible, then repeat example 1.

Solution:

V_0 = 1800gal

V =360 gal

t = 2 hrs

j_v =10 gal/hr-ft^2

R=0.082 lit.atm/gr.mole.°K

T=277°K

n_1=(18x10^3gr./1213.43gr./mole)

Δp=30 psig

MW=1213.43

The time required for Micro-filtration is;

$$t = \left[\frac{1}{AL_p \Delta p}\right]\left\{(V_0 - V) + \left(\frac{RTn_1}{\Delta p}\right)\ln\left(\frac{V_0 - \frac{RTn_1}{\Delta p}}{V - \frac{RTn_1}{\Delta p}}\right)\right\} \quad (1)$$

If we assume the term,

$$\frac{RTn_1}{\Delta P}$$

Is small, then equation 1, reduces to:

$$t = \left[\frac{1}{AL_p \Delta p}\right](1800 gal - 360 gal)$$

Assume the term $\frac{RTc_1}{\Delta P}$ is small, then:

$$j_v \cong L_p \Delta p$$

Because,

$$\frac{dV}{dt} = -Aj_v = -AL_p \Delta p\left(1 - \frac{RTc_1}{\Delta p}\right)$$

And $n_1 = c_1 V$.

Then equation (1) reduces to;

$$A = \frac{\Pi}{L_p \Delta pt}$$

Or

$$A = \frac{\Pi}{j_v t}$$

$$A = \frac{(1800 gal. - 360 gal.)}{(2hr)X(10 gal/hr - ft^2)}$$

And therefore;

A= 72 ft^2.

Example 3: We wish to concentrate and achieve a solvent switch of a solution by cross-flow Nano-filtration in a batch system. The flux for this membrane is 5 gal/hr-ft^2. The initial volume of the solution is 667 gal and the final volume is 119 gal, the amounts of protein present is 18.0 Kg, and the molecular weight is 1213.43 gr./mole. The pressure drop is 400 psi and the temperature of operation is 277°K. If we want the operation to be completed in 2 hours calculate the required area to achieve this task.

Solution:

R=0.082 lit.atm/gr.mole°K

T=277°K

n_1=(18x10^3gr/1213.43 gr./mole)

Δp=400 psig

V_0 = 667 gal

V = 119 gal

t = 2 hrs, assumed

j_v = 5 gal/hr-ft^2

The time required for Nano-filtration is;

$$t = \left[\frac{1}{AL_p \Delta p}\right]\left\{(V_0 - V) + \left(\frac{RTn_1}{\Delta p}\right)\ln\left(\frac{V_0 - \frac{RTn_1}{\Delta p}}{V - \frac{RTn_1}{\Delta p}}\right)\right\} \quad (1)$$

Then the term,

$$\frac{RTn_1}{\Delta P} = \frac{[0.082 liter.atm/gr.mole^{\circ}K](277^{\circ}K)\left(\frac{18x10^3 gr.}{1213.43 gr./mole}\right)}{27atm} = 12.48 liter$$

and from equation (1), the term,

$$\Pi = \left\{ (V_0 - V) + \left(\frac{RTn_1}{\Delta p} \right) \ln \left(\frac{V_0 - \frac{RTn_1}{\Delta p}}{V - \frac{RTn_1}{\Delta p}} \right) \right\}$$

can be calculated;

$$\Pi = \left\{ (667 gal x 3.78 lit./gal. - 119 gal.x3.78 lit./gal.) + 12.48 lit. \ln \left(\frac{667 gal.x3.78 lit./gal. - 12.48 lit.}{119 gal.x3.78 lit./gal. - 12.48 lit.} \right) \right\}$$

$$\Pi = 542.23 gal.$$

Therefore equation (1) reduces to;

$$t = \left[\frac{1}{AL_p \Delta p} \right] (542.23 gal)$$

Also;

$$\frac{dV}{dt} = -Aj_v = -AL_p \Delta p \left(1 - \frac{RTc_1}{\Delta p} \right) \quad (2)$$

But;

$$n_1 = c_1 V$$

Or

$$c_1 = \frac{n_1}{V} = \frac{\frac{18x10^3 gr.}{1213.43 gr./mole}}{667 gal.x3.78 lit./gal.} = 0.01 mole/lit.$$

Substituting;

$$\frac{RTc_1}{\Delta P} = \frac{[0.082 lit.atm/gr.mole ^\circ K](277^\circ K)(0.01 mole/lit.)}{27 atm} = 0.005 gr.^{-1}$$

Rearranging equation (1);

$$A = \frac{\Pi}{L_p \Delta pt} \quad (3)$$

And the term $\frac{RTc_1}{\Delta P}$ is a small value, therefore from equation (2) we have.

$$j_v = L_p \Delta p$$

Then equation (3) reduces to:

$$A = \frac{\Pi}{j_v t}$$

or

$$A = \frac{542.23 gal.}{(2hr) X (5 gal/hr - ft^2)}$$

and therefore;

A= 54.23 ft^2, is the area required to cross flow 667 gal. to 119 gal in two hours.

Example 4: If we assume that the membrane completely rejects the solute and that concentration polarization is negligible, then repeat example 3.

Solution:

V_0 = 667 gal

V =119 gal

t = 2 hrs

j_v =5 gal/hr-ft^2

R=0.082 lit.atm/gr.mole. °K

T=277°K

n_1=(18x10^{3}gr./1213.43gr./mole)

Δp=400 psig

MW=1213.43

The time required for Nano-filtration is;

$$t = \left[\frac{1}{AL_p \Delta p} \right] \left\{ (V_0 - V) + \left(\frac{RTn_1}{\Delta p} \right) \ln \left(\frac{V_0 - \frac{RTn_1}{\Delta p}}{V - \frac{RTn_1}{\Delta p}} \right) \right\} \quad (1)$$

If we assume the term,

$$\frac{RTn_1}{\Delta P}$$

Is small, then equation 1, reduces to

$$t = \left[\frac{1}{AL_p \Delta p} \right] (542.23 gal)$$

Assume the term $\frac{RTc_1}{\Delta P}$ is small, then:

$$j_v \cong L_p \Delta p$$

Because,

$$\frac{dV}{dt} = -Aj_v = -AL_p \Delta p \left(1 - \frac{RTc_1}{\Delta p} \right) \quad (2)$$

And $n_1 = c_1 V$.

Then equation (1) reduces to;

$$A = \frac{\Pi}{L_p \Delta pt} \quad (3)$$

Or

$$A = \frac{\Pi}{j_v t}$$

$$A = \frac{542.23 gal.}{(2hr) X (5 gal/hr - ft^2)}$$

And therefore;

A= 54.8 ft^2.

System Data Digitization of the Input/ Output Hierarchy

Digitalization of any manufacturing industry is a key step in any progress of the production process. The process of digitalization includes both increased use of robotics, automatization solutions and computerization, thereby allowing to reduce costs, to improve efficiency and productivity, and to be flexible to changes. The biopharmaceutical Industry has however been resistant to digitalization, mainly due to fair experience and complexity of the entailed development and manufacturing processes. Nevertheless, there is a clear need to digitalize as the demand in both traditional and new drugs are constantly growing.

An exponential progress and growth in innovation and development of new technologies today have reached new heights in creating and implanting digital platforms, e.g.; PAT (process analytical technology), QbD (quality by design), digital sensor, IIoT (industrial internet of things), and AI (artificial intelligence) are few to note that creating a paradigm shift and acceptance by industry and regulatory.

Today we can avoid all the manual equation solving, data manipulations and the tedious effort to qualify the obtained results by using statistical models. We can use input/output models by using mechanistic models to gain the same results. By developing unit models starting from process development through clinical and product launch (throughout the lifecycle), figure 4. Mechanistic modeling has evolved from the following steps; studying *one-factor-at-a-time* (OFAT), *design of experiments* (DoE), *high-throughput process development* (HTPD), *raw material insights and quality by design (QbD), platform knowledge,* and *mechanistic modeling*.

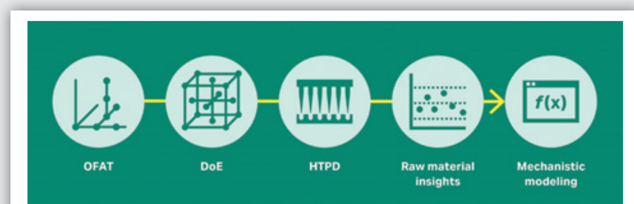

Figure 4. The evolution of process development, from one-factor-at-a-time (OFAT), through design of experiments (DoE), to high-throughput process development (HTPD), raw material insights and quality by design (QbD), and now mechanistic modeling [2].

Since mechanistic models are based on natural laws, they are valid far beyond the calibration space. In practice, this means that the process setup and parameters can easily be changed. Such as switching and changing from batch to continuous processing, changing type, size, dimensions, and much more. As the parameters (CMAs, CPPs, CQAs) are based on natural principles, mechanistic models allow you to generate mechanistic process understanding and thus fulfill QbD obligations (ICH Q8), which is not the case with statistical models.

- This opens a wide range of applications using the same mechanistic model without any further experimentation, including early-stage process development, process characterization and validation, and process monitoring and control.
- Even completely different scenarios can be simulated with no additional experimental effort, such as overloaded conditions, flow-through operations, or continuous filtration.
- The model will evolve with the proceeding development lifecycle and account for holistic knowledge management, enabling a fast and lower cost replacement of lab experiments with computer simulation.

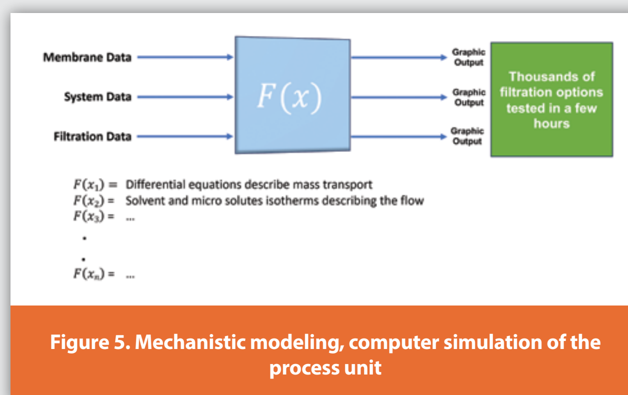

Figure 5. Mechanistic modeling, computer simulation of the process unit

A mechanistic model is a type of model that assumes a complex system can be understood by examining the workings of its individual parts and the manner in which they are coupled. Mechanistic models are typically based on mathematical descriptions of mechanical, chemical, biological, or other phenomenon or processes. A good example of a mechanistic model is the propagation of sound in random media, which can be described by stochastic differential equations.

A digital processor to perform numerical calculations on sampled values of the signal, figure 5.

The processor may be a general-purpose computer such as PC, or a specialized DSP (Digital Signal Processor) chip. The analog input signal must first be sampled and digitized using an ADC (analog to digital converter).

Digitalization of any manufacturing industry is a key step in any progress of the production process. The process of digitalization includes both increased use of robotics, automation solutions and computerization, thereby allowing to reduce costs, save time, to improve efficiency and productivity, and to be flexible to changes. To implement digitization it is required to plan, digitization planning/process of a manufacturing operation in the biopharmaceutical industry (Figure 6).

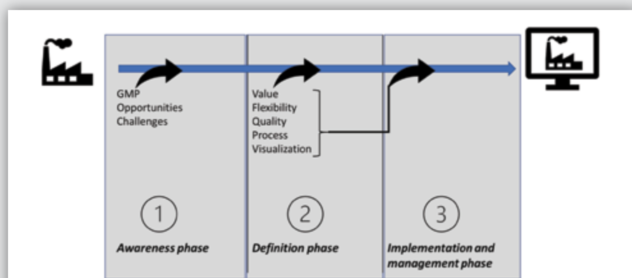

Figure 6. Digitization planning/process of a manufacturing operation in the biopharmaceutical Industry.

Figure 7. Opportunities and challenges related to digitalization process in an organization.

Figure 8. Five principles of Good Manufacturing Practice, schematic overview.

GMP Planning and Implementation

Digitization and data harness are new sources of data, fed into systems powered by machine learning (ML) and Artificial Intelligence (AI), are at the heart of this transformation. The information flowing through the physical world and the global economy is staggering in scope. It comes from thousands of sources: sensors, satellite imagery, web traffic, digital apps, videos, and credit card transactions, just to name a few. These types of data can transform decision making. In the past, a packaged food company, for example, might have relied on surveys and focus groups to develop new products. Now it can turn to sources like social media, transaction data, search data, and foot traffic; all of which might reveal that Americans have developed a taste for Korean barbecue, and that's where the company should concentrate.

The US Food and Drug Administration (FDA) now has many guidelines for GMP in the biopharmaceutical business, which cover process validation and data integrity. The FDA defines current GMP as systems that provide proper design, monitoring, and control over manufacturing processes and facilities in the pharmaceutical industry. These systems are intended to assist organizations in ensuring the identification, strength, purity, and quality of drug substance and products.

In other regulatory bodies, e.g., GMP inspections are carried out by National Regulatory Agencies within the European Union, the European Medicines Agency (EMA) oversees inspections to ensure that these standards are followed and are significant players in standardizing GMP activities across European Union (EU). GMP must be followed by any manufacturer of pharmaceuticals for the EU market, regardless of where they are based in the world. The Health Products and Food Branch Inspectorate oversee GMPs in Canada, while the Medicines and Healthcare Products Regulatory Agency (MHRA) in the United Kingdom conducts GMP inspections. Routine GMP inspections are conducted by each inspectorate to guarantee that drug items are manufactured safely and correctly. In addition, many national bodies across the world conduct routine GMP inspections to verify that drug products are manufactured safely and correctly. Many countries also conduct pre-approval inspections (PAI) for GMP compliance before the marketing authorization of a new medicine. The digitization and digital data collection will streamline the inspection and review process and improve record keeping that is required by the regulatory. It will assist in CAPA and OOS investigation as well.

There are challenges and opportunities in the digitization of drug product operation and manufacture, Figure 7 depict a summary outline of these opportunities and challenges.

Regulations allow medicine, medical device, food, and blood products, processors, and packagers to take proactive actions to guarantee that their goods are safe and effective. GMP regulations, codes and standards demand quality-oriented approach, risk-based validated drug product manufacturing. Allowing businesses to reduce or eliminate contamination, mix-ups, and errors, figure 8 depict simplified look at the requirement.

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What Microbial Tests Should be Considered Stability Test Parameters?

Tony Cundell, Ph.D.
Microbiological Consulting, LLC
Rye, New York

Introduction

In the absence of definitive guidance on microbial testing in R&D and Marketed Product Stability Programs for Drug Substances and Drug Products, the pharmaceutical industry makes inconsistent and sometimes misguided choices with respect to microbiology. These choices are further confounded by the fact that stability programs are almost always managed by analytical chemists or pharmacists without training and limited experience in the field of microbiology.

This article will review the current regulatory guidance and provide recommendations on the role of microbial testing as stability test parameters, and when other physicochemical tests will be preferred to microbiological tests. To be comprehensive, a product life cycle approach will be taken for drug substances and both sterile and non-sterile drug products.

Guidance Documents

Stability testing to establish the appropriate storage conditions and expiration dating are good manufacturing practice requirements as described in 21 CFR 211.137 *Expiration Dating* and 211.116 *Stability Testing*. The regulatory expectations are that each strength of a drug product and each primary package and closure system in which it is distributed throughout its shelf life must be supported by stability testing.

The content of the FDA good manufacturing practices is reproduced as follows:

21 CFR 211.137 *Expiration dating*

a. To assure that a drug product meets applicable standards of identity, strength, quality, and purity at the time of use, it shall bear an expiration date determined by appropriate stability testing described in 211.166.

b. Expiration dates shall be related to any storage conditions stated on the labeling, as determined by stability studies described in 211.166.

c. If the drug product is to be reconstituted at the time of dispensing, its labeling shall bear expiration information for both the reconstituted and un-reconstituted drug products.

d. Expiration dates shall appear on labeling in accordance with the requirements of 201.17 of this chapter.

21 CFR 211.166 *Stability testing*

a. There shall be a written testing program designed to assess the stability characteristics of drug products. The results of such stability testing shall be used in determining appropriate storage conditions and expiration dates. The written program shall be followed and shall include:

    1. Sample size and test intervals based on statistical criteria for each attribute examined to assure valid estimates of stability.

    2. Storage conditions for samples retained for testing.

    3. Reliable, meaningful, and specific test methods.

    4. Testing of the drug product in the same container-closure system as that in which the drug product is marketed.

    5. Testing of drug products for re- constitution at the time of dispensing (as directed in the labeling) as well as after they are reconstituted.

FDA Guidance for Industry provide information on how the U.S. regulation may be met including FDA *Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics,* 1987; FDA Guidance for Industry *Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications,* January 2020, FDA Guidance for Industry *Container-Closure Systems for Packaging Human Drugs and Biologics,* July 1999, and FDA Guidance For Industry *Container and Closure System Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products,* February 2008.

The keystone guidance document is the International Conference on Harmonization (ICH), Harmonized Tripartite Guideline Q1A (R2) *Stability Testing of New Drug Substances and Products* while supporting documents include ICH Q5C *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products and ICH Q6A Specifications: Test Procedures and Criteria of New Drug Substances, and Product.*

Other useful ICH, WHO, and regional guidance documents include ICH Q1B *Photo-stability Testing of New Active Substances and Medicinal Products;* ICH Q1C *Stability Testing: Requirements for New Dosage Forms;* ICH Q1D *Bracketing and Matrixing Designs for Stability Testing of Drug Substances and Drug Products;* ICH Q1E *Evaluation of Stability Data;* World Health Organization (WHO) Technical Report Series, No. 863, 1996 Annex 5 *Guidelines for stability testing of pharmaceutical products containing well established drug substances in conventional dosage forms* and Association of South East Asian Nations (ASEAN) *Guideline on Stability Study of Drug Products.*

Given the importance of water activity in the microbial stability of non-sterile drug products, and container-closure integrity for sterile products, the author would direct the reader to USP <922> *Water Activity, <1112> Application of Water Activity Determination to Nonsterile Pharmaceutical Products, and USP <1207> Package Integrity Evaluation—Sterile Products.*

USP <1112> Table 1 lists water activity (Aw) values required to support the growth of microorganism species. If water activity falls below that value, microbial growth is suppressed.[2-4] USP <1207> and its companion chapters provides comprehensive guidance on the best application of container-closure integrity tests (CCIT) based on head space composition. CCIT is preferred to sterility and bacterial endotoxins tests as the microbiological stability does not change in a sterile product unless the container integrity is loss.

Next the author will review stability issues associated with drug substances, product development, and marketed products.

Drug Substances

Drug substances used to make drug products are diverse and range from synthesized chemical entities to mammalian cell-derived products to processed animal or plant-derived materials. The bioburden of these drug substances will reflect their origin and manufacturing process. For example, a synthesized chemical entity typically is recovered from organic solvents, crystallized, dried, and milled resulting in a low bioburden material, whereas animal or plant-derived materials may be potentially contaminated by fecal pathogens depending on the degree of processing. A chemical drug substance will typically have a low moisture content and if stored in lined fiber or plastic drums that protect the drug substance from elevated temperatures and humidity will have excellent microbiological stability as result of its low water activity. Drug substances used for the manufacture of sterile drug products will require a low bacterial endotoxin content and if aseptically assembled must be sterile. Drug substances for the manufacture of non-sterile drug products must meet the absence of specified and objectionable microorganism requirement for the proposed dosage form, which is not usually included in compendial monographs. Although they do not have expiration dating, drug substances have re-certification date at which they are re-tested against their specifications. Open dish, accelerated and long-term stability studies are conducted with drug substances to support product development.

Product Development

During product development the formulation, product attributes, manufacturing processes, and packaging of the drug product are established. Accelerated and long-term stability studies are conducted to support successful product development. In early development analytical methods may be qualified as fit for use, but may not be fully validated, and methods will be broader in scope than the eventual product specifications to fully characterize a product and investigate it shelf-life stability. For multiple-use aqueous drug products the antimicrobial preservative effectiveness of the product must be addressed during formulation development. After the first lot of production-scale product successfully passes preservative effectiveness testing at expiry revert to chemical assay as the test parameter, in place of the microbial test.

Microbiological stability will be promoted by a reduced water activity of the drug product and the protection from humidity of the container-closure system. For example, compressed tablets and liquid-filled and powder-filled capsules with a low water activity would not require microbial testing on stability.

Marketed Products

Drug products will have expiration dating that reflect their labelled storage conditions and primary packaging that are supported by long-term stability studies. Typically, one batch in each packaging style is entered into the stability program each year. The stability testing parameters often are a smaller subset than the release tests, which may not all be stability indicating. For example, content uniformity and absence of specified microorganisms which are release criteria would not be included in a stability study. Low water activity dosage forms like compressed tablets and powder-filled capsules protected from moisture by their packaging will have microbial stability hence would not be subject to microbial testing. Likewise, sterility and

bacterial endotoxin content in a sterile drug product protected by the packaging would not reflect the stability of the product and container-closure integrity may be substituted for these two tests.[1,5]

Critical Quality Attributes of Drug Products

The author recommends that the critical quality attributes with respect to microbiology be established for each dosage form and the appropriate stability test parameters selected from them. It should be re-emphasized that some release tests may not be considered stability indicating and may not be a stability test parameter. For example, with biotechnology products like monoclonal antibodies, identity, peptide mapping and sequencing, content uniformity, residual DNA, mycoplasma content, bacterial endotoxin, and sterility would not be stability tests.

The first division for discussion would be between non-sterile and sterile drug products.

Non-Sterile Drug Products

- Microbial enumeration and absence of specified microorganisms (Initial time point only)
- Antimicrobial Effectiveness (Aqueous, multiple-dose products only)
- Water Activity
- Container Closure Integrity

Sterile Drug Products

- Sterility
- Bacterial Endotoxins
- Antimicrobial Effectiveness (Aqueous, multiple-dose products only)
- Water Activity
- Headspace Composition
- Container Closure Integrity
- Reconstitution and Storage Studies

Tables 4 through 8 provide broad guidance for stability study protocols for a drug substance and different pharmaceutical dosage forms.

ICH Stability Storage Conditions and Time Intervals

In designing your stability protocols, it is important to select the stability temperature and humidity storage conditions and time intervals for testing. For the latter, for accelerated studies samples are pulled for testing at 1, 3, and 6 months whereas for long term studies samples are pulled at 3, 6, 9, 12, 18, 24 and 36 months. Release testing may be used as the zero-time point provide the samples are placed in the stability chamber promptly, so that the 36-month time point coincides with the projected expiration date.

USP <1112> Table 1 lists the water activity values required to support the growth of microorganism species. If water activity falls below that value, microbial growth is suppressed. Microorganisms likely to be found in pharmaceutical drug products will not grow at Aw less than 0.75, furthermore the compendial methods found in USP <61> and <62> will not detect halophilic bacteria, osmophilic yeast, and xerophilic molds due to the high-water activity of the microbiological culture media.

Table 1. The appropriate climate zone based on the marketing plans for the drug product.

Zone	Type of Climate	Regions	Example Countries
Zone I	Temperate zone	North and Central European Nations	Germany, Russia, Sweden, United Kingdom, The Netherlands, Rumania
Zone II	Mediterranean/ subtropical zone	Southern European Countries, parts of Africa, South America and Asia	Spain, France, Italy, Greece, Turkey, Israel, Argentina, Chile, South Africa, Australia, Japan, United States, China
Zone III	Hot dry zone	North Africa, Middle East	Iraq, Jordan, Botswana, Chad
Zone IV	Hot humid/ tropical zone	Central and Southern America, Parts of Africa and Asia	Brazil, Saudi Arabia, India, Nigeria, Singapore, Taiwan, Thailand, Puerto Rico, Uganda
Zone IVb	ASEAN testing conditions hot/ higher humidity	Southeast Asia	Indonesia, Malaysia, the Philippines, Singapore, and Thailand

Role of Water Activity in Moisture Transmission of Blister Packaging

Tablets and capsules may be stored in blisters for the convenience of the patient especially when they are out of the house but the level of protection from humidity will vary by material of construction.

Table 2. Temperature and humidity requirements of different climatic zones

Climatic Zone	Temperature	Humidity	Ability to Support Microbial Growth
Zone I	21 ± 2ºC	45 ± 5% RH	No Growth – ERH too low
Zone II	25 ± 2ºC	60 ± 5% RH	No Growth – ERH too low
Zone III	30 ± 2ºC	35 ± 5% RH	No Growth – ERH too low
Zone IV	30 ± 2ºC	65 ± 5% RH	No Growth – ERH too low
Zone IVb	30 ± 2ºC	75 ± 5% RH	Fungi Growth Possible – ERH Marginal
Refrigerated	5 ± 3ºC	No Humidity	No Growth – Temperature too Low
Frozen	-15 ± 5ºC	No Humidity	No Growth – Temperature too Low

ERH = Equilibrium Relative Humidity

Note: Sterile drug products stored in stoppered and sealed glass vials do not require humidity control in the stability chamber as they are fully protected by their primary packaging.

Table 3. Water Activity for Growth of Representative Microorganisms of Interest to the Pharmaceutical Industry (from USP <1112>)

Bacteria	Water Activity (a_w)	Molds and Yeast	Water Activity (a_w)
Pseudomonas aeruginosa	0.97	*Rhyzopus nigricans*	0.93
Bacillus cereus	0.95	*Mucor plumbeus*	0.92
Clostridium botulinum, Type A	0.95	*Rhodotorula mucilaginosa*	0.92
Escherichia coli	0.95	*Saccharomyces cerevisiae*	0.90
Clostridium perfringens	0.95	*Paecilomyces variotti*	0.84
Lactobacillus viridescens	0.95	*Penicillium chrysogenum*	0.83
Salmonella spp.	0.95	*Aspergillus fumigatus*	0.82
Enterobacter aerogenes	0.94	*Penicillium glabrum*	0.81
Bacillus subtilis	0.90	*Aspergillus flavus*	0.78
Micrococcus lysodekticus	0.93	*Aspergillus niger*	0.77
Staphylococcus aureus	0.86	*Zygosachharomyces rouxii (osmophilic yeast)*	0.62
Halobacterium halobium	0.75	*Xeromyces bisporus (xerophilic fungi)*	0.61

Moisture ingress of formed and sealed blisters (PVC, PVDC, Aclar Ultrx 2000 and cold form foil) when stored at 23°C/75% and 40°C/75% RH may be measured by weight gain or more functionally as an increase in water activity of the dosage form. USP <671> *Containers – Performance Testing* uses water-filled blister packs instead of desiccant-filled ones, which has been the standard test method since the 1970s, but the author believes that water activity determination of the stored drug products is a better choice in a stability program.

Conclusions

As marketed product stability protocols are binding regulatory commitments, companies should get them right and if necessary, discuss them with the national boards of health in advance of their finalization. With many dosage forms, microbial enumeration, bacterial endotoxin assay, and sterility testing are not stability-indicating tests and should be replaced by the more useful water activity determination (no-sterile products) and container-closure integrity testing (sterile products).

Table 4. Model Drug Substance Stability Protocol

Stability Storage Conditions	Time Points							
	Initial	3 Month	6 Month	9 Month	12 Month	18 Month	24 Month	36 Month
Reserve	R	R	R	R	R			
Long Term	X, Z, Y	X	X	X	X, Z, Y	X	X, Z, Y	X, Z, Y
Accelerated		X	X					

Key:
- R - For Phase I (First in Man) studies where the long-term storage condition is 25°C/ 60%RH, a quantity of reserve samples must be placed on stability storage at 5°C to support investigations, if needed.
- X - Appearance, Assay, Degradation Products, Moisture, Chiral Purity (if applicable), Other Physical Tests (if applicable)
- Y – Endotoxin (for parental grade drug substance only). At initial and annual time points only.
- Z - Microbial enumeration and/or water activity (if applicable)

Table 5. Model Topical Drug Product Stability Protocol

Stability Storage Conditions	Time Points								
	Initial	1 Month	3 Month	6 Month	9 Month	12 Month	18 Month	24 Month	36 Month
Long Term	X, Y, Z, L		X, Y, L	S, X, Y, L	X, Y	S, X, Y, Z, L	X, Y	S, X, Y, Z, L	S, X, Y, Z, L
Intermediate			O	O	O	O			
Accelerated b		X, Yc	X, Y, L	S, X, Y, L					

b Lower temperatures (e.g., 35°C) should be considered for semi-solids prone to changes in physical attributes.
c If required for trending purposes. Alternate intervals (e.g., 2, 4, and 6 months) at accelerated conditions are acceptable.

Key
- O - Optional, e.g., perform testing if Accelerated condition has a significant change as defined in ICH Q1A (R2).
- S- Antimicrobial Effectiveness Test (multiple use preserved products only)
- X - Appearance, Package Observations, Assay, Degradation Products, Preservative and Antioxidant Content (if applicable)
- Y - Weight Loss, Viscosity, pH
- Z - Microbial Enumeration, Homogeneity, Water Activity, Polymorphism (if applicable)
- L – Leachables (if applicable)

Table 6. Model Oral Liquid Stability Protocol

Stability Storage Conditions	Time Points								
	Initial	1 Month	3 Month	6 Month	9 Month	12 Month	18 Month	24 Month	36 Month
Long Term, Upright	X, Y, Z, L		X, Y	S, X, Y, L	X, Y				
Long Term,	S, X, Y, Z, L	X, Y							
Inverted	S, X, Y, Z, L	S, X, Y, Z, L							
Intermediate, Upright			X, Y, L	L, S		S, X, Y, Z, L		S, X, Y, Z, L	S, X, Y, Z, L
Intermediate, Inverted			O	O	O	O			
Accelerated,			O	O	O	O			
Upright		X, Y	X, Y, L	S, X, Y, L					
Accelerated,			X, Y, L	S, X, Y, L					
Inverted									
c ICH requires only 6, 9, and 12 months for the intermediate condition.									

Key
O - Optional, e.g., perform testing if Accelerated condition has a significant change as defined in ICH Q1
S- Antimicrobial Effectiveness Test, USP and Ph. Eur. (multiple use preserved products only)
X - Appearance, Package Observations, Resuspendability, Assay, Degradation Products, Dissolution, Preservative and Antioxidant Content (if applicable); Y - Weight Loss, Viscosity, pH, Particle Size/ Distribution
Z - Microbial Limits (long term only), Polymorphism (if applicable)
L – Leachables

Table 7. Model Oral Solid Drug Product Stability Protocol

Stability Storage Conditions[1]	Time Points								
	Initial	1 Month	3 Month	6 Month	9 Month	12 Month	18 Month	24 Month	36 Month
Long Term	X, Y, Z		X, Y	X, Y	X, Y	X, Y, Z	X, Y	X, Y, Z	X, Y, Z
Intermediate			O	O	O	O			
Accelerated			X, Y	X, Y					

Key
O - Optional, e.g., perform testing if Accelerated condition has a significant change as defined in ICH Q1A (R2).
X - Appearance (including brittleness for capsules), Package Observations, Assay, Degradation Products, Dissolution (immediate release), Release Rate (extended release), Moisture
Y - Hardness (uncoated tablets), Friability (tablets, if applicable)
Z - Microbial enumeration and/or water activity (if applicable)

Table 8. Model Sterile Drug Product Stability Protocol

Stability Storage Conditions[1]	Time Points								
	Initial	1 Month	3 Month	6 Month	9 Month	12 Month	18 Month	24 Month	36 Month
Long Term	X, Y, Z	W	X		X	W, X, Y, Z	X	W, X, Y, Z	W, X, Y,Z
Intermediate			O	O	O	O			
Accelerated				W					

Key
O - Optional, e.g., perform testing if Accelerated condition has a significant change as defined in ICH Q1A(R2).
W – Antimicrobial Effectiveness Test. USP and Ph. Eur. (multiple use preserved products only)
X - Appearance
Y – Sterility. Note: Container-Closure Integrity Testing may be used in lieu of sterility testing
Z – Bacterial Endotoxins Test (Optional)

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Unleashing the Potential of AI: Revolutionizing Rare Disease Research and Drug Development Through Diversity and Innovation

Deepti Dubey, PhD and Harsha Rajasimha, PhD

IndoUSrare

Rare diseases present substantial challenges in terms of diagnosis and care navigation. For 25% of patients, obtaining an accurate diagnosis can take an average of five to seven years from the onset of the disease, necessitating the involvement of a proficient and comprehensive clinical team. What further complicates the journey with rare diseases is that the diagnosis marks not the culmination but rather the commencement of the odyssey. From a prognostic and therapeutic standpoint, there exist considerable gaps yet to be bridged. The challenges at the prognostic level stem from the dearth of reliable parameters and/or biomarkers, as the molecular pathophysiological mechanisms remain largely elusive. Additionally, the limited number of patients data points with a given rare disease hinders the derivation of statistically significant parameters. Conventionally, bringing a drug to market takes 10 to 15 years, with an average research and development cost of $2.6B.[1] These factors pose a bottleneck in the drug discovery process for rare disorders; research expenses are high while revenues remain low due to the small patient population. This consequently leads to the protraction of new drug and treatment development, compounded by insufficient data and funding.

Artificial Intelligence (AI)-driven tools have the potential to streamline the research and development process for orphan therapies for rare diseases, reducing both the time and costs by accelerating the timelines, minimizing manual repetitive tasks, and make sense of complex variety of data. In recent years, numerous AI systems utilizing Machine Learning (ML) and Deep Learning (DL) algorithms have been developed to address the diagnostic, prognostic, and therapeutic gaps essential for achieving patient-centric care for individuals with rare diseases.[2]

AI-Driven Diagnostic Tools

The accurate diagnosis of rare diseases holds crucial importance in patient triage, risk stratification, and targeted therapies. Due to their infrequency, symptoms of rare diseases often present as unfamiliar and atypical to clinicians. This increases the likelihood of patients not receiving an appropriate diagnosis and subsequently missing out on successful therapy. The variability inherent in rare diseases further compounds the challenge of timely identification, owing to the limited accessibility of clinical diagnostic procedures.

A standard diagnostic approach for rare diseases entails a comprehensive assessment of medical history, physical examination, and genetic testing, which may unveil specific mutations associated with the condition. Additionally, imaging studies like X-rays, MRI, or CT scans may be employed. In this context, AI emerges as a potential game-changer, albeit a complex one. Through the development of ML algorithms capable of scrutinizing vast datasets, AI can discern patterns and markers characteristic of specific rare diseases. ML and DL models have demonstrated efficacy in aiding diagnostic decisions based on phenotypic characterization.[2] Knowledge graphs, leveraging historical data, medical knowledge, and genetic tests, have been widely utilized for disease classification.[3] DL-based approaches have been instrumental in gauging disease severity using pathological features such as gait analysis in conditions like Huntington Disease (HD).[4] Multiple studies have successfully employed ML and DL techniques to differentiate disorders with overlapping clinical manifestations, like Parkinson's disease and multiple system atrophy, utilizing MRI, CT scans, or X-rays.[2] ML algorithms, leveraging specific biomarkers or multiomics data, are now at the forefront of early detection efforts for many rare diseases. In a recent study, researchers applied a trained

neural network called ConvNetACh, which analyzed heart rate variation data from Rett syndrome patients, effectively distinguishing them from subjects with typical development. This has potential applications as biomarkers for early detection of neurodevelopmental spectrum disorders.[5] Additionally, DeepMind's AlphaMissense has made strides in predicting the molecular effects of genetic variants on protein function, contributing to the identification of pathogenic missense mutations and previously unknown disease-causing genes. This development is poised to increase the diagnostic yield for rare genetic diseases.[6]

AI-Driven Prognosis

Most rare conditions are chronic and lifelong, making predictive prognosis crucial for patients. AI can significantly contribute to the prognosis of rare disorders by bridging gaps in data and experience. Through the analysis of extensive datasets, including electronic health records, genomic data, and imaging studies, ML algorithms can discern patterns and forecast outcomes for individuals with rare diseases, offering valuable insights to shape prognoses and guide decisions.

Numerous studies employing ML and DL techniques have identified genetic and protein biomarkers for adrenocortical carcinoma, enabling the prediction of prognosis for this rare and aggressive cancer.[7] AI aids in comprehending disease progression and predicting survival times using medical data. For instance, researchers utilized immune cell frequency profiles, along with clinical and serological data from patients with juvenile-onset systemic lupus erythematosus (jSLE), to pinpoint predictive disease outcome signatures through AI tools.[8]

AI-Driven Treatment

Nearly 95% of rare diseases lack FDA-approved drug treatments, and the rising number of rare diagnoses places significant pressure on scientists and clinicians to characterize these conditions and align patients with suitable treatments.[9] With the continuous influx of biomedical data, AI presents an opportunity to convert this knowledge into a usable format for identifying therapeutic strategies. Utilizing ML-based software like Assay Central, researchers are screening compounds in silico before conducting in vitro testing. This approach has proven successful in identifying novel compounds with potential for disease modulation in the treatment of sialidosis.[10]

Recently, artificial intelligence (AI) has revolutionized drug toxicity prediction by offering more precise and efficient methods for identifying potentially harmful effects of new compounds before subjecting them to human clinical trials. This not only saves time but also conserves financial resources.[11]

Lack of Diversity

It has been 22 years since the landmark completion of the draft human genome sequence, resulting in an unprecedented volume of genomic data. This data is scrutinized through genome-wide association study (GWAS)/phenome-wide association study (PheWAS) methods to unveil connections between genotype and phenotype. These discoveries have significantly contributed to pharmacogenomics and enhanced clinical decision support in numerous healthcare systems. However, managing the influx of genomic data from sequencing and clinical information from electronic health records (EHRs) presents formidable challenges for data scientists.

With the emergence of artificial intelligence (AI) technologies like machine learning and deep learning, an increasing number of GWAS/PheWAS studies have successfully harnessed this technology to surmount the challenges.[12] Yet, it is important to note that most genomics studies, including genome-wide association studies (GWAS), have been conducted in individuals of European descent (86.3%), followed by East Asian (5.9%), African (1.1%), South Asian (0.8%), and Hispanic/Latino (0.08%) populations. Data from the International HundredK+ Cohorts Consortium (IHCC), a recently established consortium of international cohort studies, highlights this ancestral disparity, with approximately 22.5 million participants from North America and Europe compared to a mere 0.3 million from South and Southeast Asia (Indian subcontinent).[13] Significantly, the Indian subcontinent alone constitutes a quarter of the global population, underscoring the potential impact of increased representation in clinical research and genetic databases.

It is imperative to recognize that if training data lacks representation of population diversity, AI may inadvertently perpetuate bias, potentially leading to misdiagnoses in historically underrepresented patient groups. This is exemplified by a case where Face2Gene, an automatic deep-learning algorithm, predicted facial phenotypes of Noonan Syndrome (NS) and Neurofibromatosis type 1 (NF1) syndromes in a Latino-American population with accuracies of 66.7% and 10%, compared to 100% accuracy in the European population.[14] Studies like these underscore the critical importance of incorporating diverse populations in genetic studies and clinical trials to enhance diagnostic methods and therapeutic interventions for rare disorders.

The potential of AI in accelerating rare disease research and drug development is immense. However, this potential is currently compromised by the lack of diversity in the available patient data, participation, and limited focus on specific populations in clinical genomic studies. Recognizing and addressing this challenge is crucial to unleashing the full power of AI in advancing healthcare. This can be accomplished through cross-border collaboration, active engagement with the rare disease patient community, and sharing of resources and knowledge. Organizations like IndoUSrare are instrumental in initiating these cross-border collaborations between the USA and densely populated countries such as India and providing a platform for discussions crucial for rare diseases and orphan drug development. Key highlights and insights from the inaugural Indo US Bridging RARE Summit 2023 can be found at https://www.prweb.com/releases/indo-us-bridging-rare-summit-heralds-a-new-era-of-cooperation-for-rare-diseases-orphan-drugs-development-301978670.html

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Author Biographies

Deepti Dubey, PhD, *is an accomplished researcher with expertise in Molecular Genetics, Neurological Disorders, and Rare Diseases from esteemed institutions. In collaboration with non-profit patient organizations, she has driven research initiatives for accelerating breakthroughs for rare diseases. With a Clinical Trials Specialization, Deepti is a scientific writer at IndoUSrare.*

Harsha K Rajasimha, PhD, *is a serial entrepreneur, investor, and philanthropist. He serves as the Founder and Executive Chairman of the Indo US Organization for Rare Diseases (https://IndoUSrare.org) with the mission to make rare disease research and clinical trials universally accessible. Harsha is the Founder and CEO of Jeeva Informatics Solutions Inc. (https://jeevatrials.com) an AI-driven unified platform for efficient and universally accessible clinical trial management. Harsha serves as the chairperson of the annual Indo US bridging RARE Summit (https://summit.indousrare.org).*

CHALLENGES AND SOLUTIONS FOR VIRAL PRODUCT DEVELOPMENT AND MANUFACTURING

By Todd Ranheim – Advanced Analytics Core, and Joseph Shultz -Technical Head – Biologics & Vaccines, Resilience

The proliferation of recombinant protein therapies has driven the need for new capacity in the biotherapeutic manufacturing space. Monoclonal antibodies (mAbs), high-value products that require large volumes of material, are one such modality that has motivated a surge in capacity. Many large contract development and manufacturing organizations (CDMOs) are standing up new equipment and facilities to meet the demand for these products.

Additionally, the potential for viruses and viral vectors to address rare and intractable diseases makes them an essential modality for the future of the vaccine, cell, and gene therapy fields. However, developers of live virus, attenuated live virus, oncolytic virus, or viral vectors have narrow partnering options for manufacturing. This is largely due to the complexity and diversity of these viral products and the bespoke nature of their production. For some of these viral products, the platform technologies and suspension culture approaches that have enabled scale for mAb manufacturing are impractical or unavailable. A service provider with the ability and capacity to execute such complex manufacturing requires expertise in suspension and adherent cell culture capabilities, cell line productivity, and a deep understanding of the intricacies involved in manufacturing these products.

The Challenges of Virus and Viral Vector Manufacturing

One of the key differences between viral products and conventional biologics is the need to infect cells and have multiple rounds of replication to produce the active component. Viral tropism – viruses will only infect certain types of cells – often inhibits the establishment of a platform, such as the suspension CHO cells that are commonly used for monoclonal antibody production. Instead, these viral products are typically developed at bench scale in adherent cell lines specific to certain virus types, and the heterogeneity of virus types has impeded the investment and efforts to create universal suspension cell platforms that enable viral infectivity.

For most developing virus programs, the leap to suspension would require a step backward, as most research programs have already selected an adequate or optimal cell line, that often started in adherent cell culture, during pre-clinical development. Developing a comparable next-generation process using a suspension cell line capable of performing as well or better than the initially selected line is burdensome, lengthy, and can stall clinical progress. Until suspension cell lines with the required characteristics are widely available to preclinical developers, adherent capacity that can scale to meet a program's needs is crucial.

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Although new manufacturing technology has primarily been focused on suspension processes, there is still a significant need to advance adherent manufacturing technology to support commercial demand. Additionally, any attempt to change the cell line of a product during late-stage development can necessitate clinical rework, as the health authorities will consider it a distinct new process. As a result, some organizations have committed to scale utilizing CF40 cell factory systems and, occasionally, next-generation adherent bioreactors to achieve higher yields and productivity.

The unique and diverse features of each virus type often introduce added complexity for developers and manufacturers. For example, factors such as the size of a virus can preclude sterile filtration as viruses larger than 0.2 microns would be filtered out along with the intended removal of contaminants. These nuances, coupled with other features such as the shape of certain viruses, can preclude the possibility of a one-size-fits-all downstream processing paradigm. Other factors, such as whether a virus is lytic or intracellularly produced, necessitate a bespoke approach to process development. Expertise in areas such as aseptic process simulation (APS), which allow operators to evaluate the capabilities of an aseptic process using microbial growth-promoting media, are tools that can address the need to process large viruses without sterile filtration. These tools are examples of what organizations should look for in a manufacturing partner.

Moving the Needle to Further Biomanufacturing Innovation

Resilience, a unique technology-based biopharmaceutical manufacturing company that works across multiple therapeutic modalities, including cell and gene therapies, viral vectors, vaccines, and recombinant proteins, was formed to address the challenges that have stymied technological advancement within biopharmaceutical manufacturing. With a network of 11 sites across North America, its biomanufacturing services span platform technology and development, process and analytical development, and cGMP manufacturing.

To meet demand and to enable the scale of high-volume vaccine production, Resilience has invested significant resources in developing expertise in various bioreactor systems. Its Center for Biologics, Vaccines, and Gene Therapies in Alachua, FL, houses a wealth of technical capabilities related to viral products, including several state-of-the-art, fixed bed iCELLis and Univercells adherent cell bioreactors and multiple single-use suspension bioreactors ranging from 10L-2000L. Additionally, the site boasts expertise in cell and viral banking, small batch fill/finish, bioanalytical development and testing, and experience working across mammalian, microbial, and insect cell lines.

To tackle the issue of yield, Resilience's technical experts have worked to optimize its own Vero cell line and proprietary media for viral products, resulting in greater yields when compared to other commercially available cell lines. The combination of the Resilience Vero cell expression system and expertise in large-scale adherent cell bioreactor systems allows the development of highly scalable viral expression platforms that are robust across multiple viral products (figure 1).

The company has developed significant expertise around live virus, attenuated live virus, oncolytic virus, and viral vectors because of the promise these modalities hold in

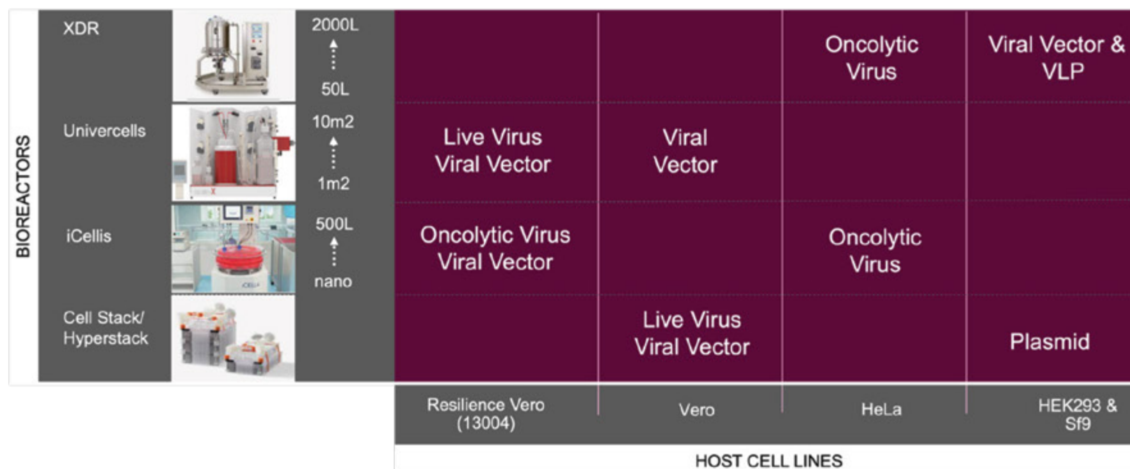

Figure 1. Resilience's manufacturing experience

gene delivery for intractable diseases. Oncolytic virus products, as one of the more promising treatments for various cancers, require capacity and support in the pursuit of their development.

Though researchers are continuing to pursue the optimization of these viruses and their manufacture through viral engineering strategies, their continued progress hinges on meeting goals linked to safety, efficacy, and commercial scale-up. These goals include high virus yields, highly reproducible key quality attributes, genetic stability, formulation, and product stability.

Quality	Attribute	Current Techniques
Identity	Confirmation of presence and identity of viral vector	ddPCR, SDS-PAGE, CE-SDS, Western blot
Potency	Physical viral titer	ddPCR, Optical density (A260/A280), Virocyte, HPLC/UPLC
	Functional viral titer	Plaque assays, FFU, TCID50, Bio-impedance (Maestro Z), Laser force cytology (Radiance)
Purity	Process-related impurities	Chromatography, MS, TEM
	Host cell-related impurities	ddPCR, ELISA, HPLC
	Capsid content	Physical/functional titer ratio
Safety	Endotoxin	LAL method, Rabbit pyrogen assay
	Mycoplasma	PCR, Cell culture-based assays
	Replication competent virus	PCR, Cell culture-based assays
	Adventitious agents	PCR, Cell culture-based assays
Stability	pH	Potentiometry
	Osmolality	Osmometry
	Aggregation	DLS, SEC-MALS, FFF-MALS

Figure 2. Resilience's analytical development capabilities.

Advances in Analytics Ensure Development Velocity

Advanced analytical development capabilities are core to Resilience's commitment to the enablement of these therapies. While traditional assays can be used to assess viral production, Resilience has developed methods based on new technologies, that achieve a more precise and accurate estimate of the potency of samples, giving developers better precision around the productivity of a given batch and better stability estimates. Other techniques such as impedance-based measurements and laser force cytology are currently being investigated as next-generation potency assays by Resilience. In addition to specificity, many of these assays boast faster run times compared to their traditional counterparts.

By bringing the majority of testing in-house, Resilience has cultivated a comprehensive manufacturing and testing paradigm that can help partners scale their viral products faster and more efficiently. Its capabilities, technologies, and experience span adherent and suspension cell lines, viral vaccine manufacture, next-generation bioreactor platforms, APS, fill/finish, gene therapies, oncolytic products, and more. This framework is likewise supported by a diverse team of experts from across the biopharmaceutical manufacturing, research and development, quality and regulatory policy, AI, and venture finance spaces. Resilience's culmination of expertise and investment has resulted in a CDMO partner committed to providing a sustainable network of high-tech, end-to-end manufacturing solutions that free its partners to further advance the science behind biopharmaceutical manufacturing.

ABOUT RESILIENCE

Resilience is a technology-focused biomanufacturing company dedicated to broadening access to complex medicines. Founded in 2020, the company is building a sustainable network of high-tech, end-to-end manufacturing solutions to ensure the treatments of today and tomorrow can be made quickly, safely, and at scale. Resilience seeks to free its partners to focus on the discoveries that improve patients' lives by continuously advancing the science of biopharmaceutical manufacturing and development.

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The E 710 Smart was developed for high production volumes with low margins. This economical tablet press for mono- and bilayer production is based on Kilian's proven four-column design. The single-piece, deep-drawn process area ensures hermetical separation between the compaction and technical areas. A closed V-ring seal underneath the die table additionally prevents tablet dust from entering the "grey" area. With between 51 and 85 press stations and a pitch circle diameter of 712 mm, the Kilian E 710 Smart achieves a maximum output of up to 1,020,000 tablets per hour. Brake magnets permit absolutely homogeneous filling of the dies without any wear on the punch shafts. Moreover, special Kilian bellows protect the tablets from contamination in the form of black spots. The fill shoe is quick and simple to clean because the gear is external. Owing to the small number of interchangeable parts and the good accessibility to individual components, retooling and cleaning times are significantly reduced. The tablet scraper and chute are mounted together on a swivel arm for easy removal. Product loss is minimized thanks to the floating product scraper with magnets.

Romaco Group
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Child-Resistant Smart Blister Wallet

Schreiner MediPharm has partnered with Keystone Folding Box Co. to introduce a smart blister pack with especially child-resistant, senior-friendly features. Ideal for clinical trials, the new solution utilizes integrated electronics to convert blister packaging into a real time e-diary for capturing dosing history data. Schreiner MediPharm has developed a smart extension for the proven Key-Pak® wallet for oral solid dose medication, from Keystone Folding Box Company. The novel, customizable blister wallet combines a child-resistant paperboard package with state-of-the-art technology for digital adherence monitoring, enhancing clinical trial reporting accuracy. The distinguishing characteristic of the "smart" Key-Pak® wallet is the integration of conductive trace-patterns linked to each of the wallet card's cavities. As a result, data is generated at the instant the patient removes a dose from the wallet.

Schreiner MediPharm
www.schreiner-medipharm.com

Keystone Folding Box Co.
www.keyboxco.com

Fully Automatic Syringe Filling System

Designed with monoblock modularity for as-needed manufacturing, the company's TipFil™ Syringe Filling & Assembly Machine completely automates all processes – from syringe loading, filling and capping through inspection, labeling and printing. Incorporating a racetrack indexing system for rapid, reliable throughput of up to 50 pieces per minute, the servo-driven intermittent motion machine features interchangeable pucks to accommodate various syringe sizes from 1-60 mls. Loading is performed via elevator hopper to a vibratory bowl, which transfers syringes to a tracked placement mechanism. Plungers are inserted with an automatic tamping station equipped with position sensors. Dosing is performed via ceramic pump, with a no container/no fill sensor preventing product wastage. Metering occurs via plunger positioning sensors, with a plunger pullback mechanism reducing drips and assuring exacting fill levels. From there, three stainless steel shutoff nozzles execute through-the-tip filling, and press-on or turn cap placement is performed using a pick-and-place system incorporating a linear track and stainless steel vibratory bowl feeder.

TurboFil Packaging Machines LLC
www.TurboFil.com

Annex 1 Compliance Checklist

BioTrak™ Real-Time Viable Particle Counter

- Samples continuously
- Expedites detection
- Reduces risk

Are you meeting new EU GMP Annex 1 requirements?
Annex 1 now requires continuous airborne total and viable particle monitoring in Grade A. The TSI® BioTrak™ Real-Time Viable Particle Counter, a biofluorescent particle counter (BFPC), provides a complete solution.

To learn more, visit
tsi.com/biotrak

Continuous & Intervention-Free Microbial Monitoring

Innovation for a Changing Industry

Aseptic manufacturing is changing. Regulations like the EU GMP Annex 1 encourage modernization. Technologies such as gloveless isolators and single-use systems that automate processes and limit human interventions are becoming the norm. Regulators and manufacturers alike recognize that highly-automating manufacturing leads to improved quality, safety, and efficiency.

This new paradigm demands in-depth process understanding, fewer interruptions, and no operator intervention. After decades of reliable service, traditional microbial monitoring techniques (i.e. growth-based detection methods) are not capable of meeting these demands.

Annex 1 ready, the BioTrak® Real-Time Viable Particle Counter (a biofluorescent particle counter) provides real-time viable and total particle monitoring of critical environments, including the aseptic core, without the need for operator intervention. The BioTrak® Real-Time Viable Particle Counter complements manufacturing innovations to maximize process understanding and efficiency without introducing any risk to product

A Complete Solution for Environmental Air Monitoring

BioTrak® Real-Time Viable Particle Counter provides complete environmental monitoring of air in cleanroom spaces. Monitoring for both total and viable particles can be performed with this one instrument using a single isokinetic probe located within the test area.

1. **Total Particle Counter -** A 1 CFM (28.3 LPM) total particle counter provides the same trusted measurements users expect from all TSI particle counting instruments. Fully compliant to ISO 21501-4, it is suitable for use in all GMP applications.
2. **Biofluorescent Particle Counter (BFPC) -** Viable particles, also referred to as autofluorescence units (AFU), are detected using laser induced fluorescence (LIF). LIF works by detecting the fluoresce of metabolites in viable microorganisms that are excited as they pass through a laser beam. This requires no growth or reagents and makes viable results available in real-time.
3. **Particle Filter -** A highly efficient gelatin filter can be installed to capture the particles that pass through the BFPC. The filter can be transferred to growth media for an opportunity to identify culturable contaminants that were present in the sample

Key Applications

Continuous Process Monitoring

BioTrak® Real-Time Viable Particle Counter is the ideal instrument for continuous microbial monitoring in the aseptic manufacturing core. Real-time, interruption-free monitoring unlocks process efficiencies and improves quality. Seamless integration with TSI Facility Monitoring System enables complete environmental monitoring automation.

Non-Compliance Based Applications

These applications, such as root- cause investigation, room release, and gowning training/verification, offer immediate benefit for any manufacturing facility. BioTrak® Real Time Viable Particle Counter has features and accessories designed for easy operation and data analysis

Validation

TSI® has a dedicated team of professionals with knowledge and experience to help you confidently implement the BioTrak Particle Counter in a compliant manner.

TSI® has submitted a Type V Drug Master File with the FDA. It includes rigorous performance qualification studies. A summary is available upon request or via the website.

Unmatched Benefits

Regulations such as Annex 1 recognize that manufacturers need to move past traditional growth-based monitoring methods to better assure product quality. These methods are also incompatible with efforts to modernize and to make Pharma 4.0 a reality. BioTrak® Real-

Time Viable Particle Counter is ready to meet these demands in ways that are not possible with historically used microbiological methods.

Improve Process Understanding - Continuous monitoring reveals where and when microbial excursions occur. Time-resolved data demonstrates continuous control during normal operation. In the event an excursion does occur, immediate actions can be taken to resolve the issues with immediate feedback on action effectiveness. This allows for greatly improved, real-time, process control for improved quality.

Reduce Risk by Eliminating Operator Interventions - People are a primary source of viable contamination. Fully automating the air sampling within the aseptic core removes the need for operator interventions for microbial monitoring.

Reduce Loss of Product from Line Stoppages - Not only do line stoppages require interventions that increase risk, they often result in loss of product when the line is restarted. By eliminating the need to change plates, BioTrak® Real-Time Viable Particle Counter allows continuous operation during fill/finish operations.

Achieve High Level of Data Integrity - Data integrity is critical. BioTrak Real-Time Viable Particle Counter seamlessly interfaces with TSI Facility Monitoring Software (FMS). FMS is a fully compliant continuous monitoring software package that trends data, triggers alarms, and easily makes data available where and when it is needed.

Why Dual-Channel Laser Induced Fluorescence (LIF) Technology

Microorganisms contain fluorescent molecules that produce unique optical signatures. By measuring the size and fluorescent properties of individual particles, TSI's BioTrak® Real-Time Viable Particle Counter effectively distinguishes viable particles from non-viable particles.

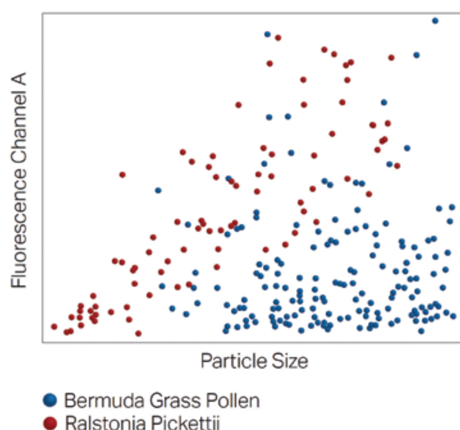

***Figure 1. Single Channel -** A single channel of fluorescence makes it very difficult to discriminate viable from non-viable particles.*

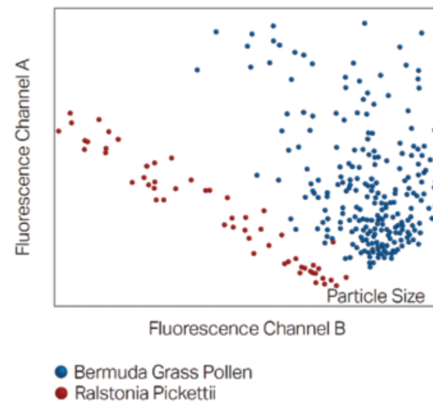

***Figure 2. Dual Channel -** TSI's two channels of fluorescence detection provides the better measurement by clearly discriminating the viable particles.*

At the heart of the BioTrak® Real-Time Viable Particle Counter is LIF technology. Simply stated, when microbial particles are exposed to ultra-violet laser light, they absorb and re-emit light at higher wavelengths; a process called fluorescence. Fluorescent cell metabolites associated with viability, such as nucleotides, flavins, lipids, and amino acids, are the primary markers targeted by the LIF technique.

Unlike products with just one channel of fluorescence detection, TSI's BioTrak® Real-Time Viable Particle Counter has two channels for better discrimination. In the example shown, it is impossible to differentiate the pollen particles from the microorganisms using a single florescence channel. But, when a second channel of detection is added, the differentiation becomes clear. By collecting and processing more optical data than other instruments, TSI® has produced the most discriminating measurement on the market today.

Specificity

A viable particle count in the absence of a microorganism is considered a false-positive. In critical environments, such as the aseptic core, false-positives can adversely impact processes. With dual-channel LIF and sophisticated discrimination algorithms, the BioTrak® Real-Time Viable Particle Counter reliably delivers essentially zero viable particle counts in rigorously hygienic spaces such as Grade A / ISO 5. The graph shows actual data from real-world manufacturing spaces. The BioTrak® Real-Time Viable Particle Counter shows little or no signal in extremely clean environments for as long as aseptic conditions are maintained.

Learn more at tsi.com/meet-the-biotrak

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Rapid Sterility Testing for Cell and Gene Therapy Treatments

Lamin Jallow

Microbiology Technology Specialist
MilliporeSigma; Burlington ; MA; USA
An affiliate of Merck, KGaA Darmstadt, Germany

Dr. Anne-Grit Klees

Lead Expert, Product & Portfolio Manager
BioMonitoring Environmental Monitoring
Merck KGaA, Darmstadt, Germany

Every day counts for manufacturers of short shelf life biologics/treatments who need to release their products and have them administered to patients as fast as possible. This is particularly the case for biologics as well as cell and gene therapies that are based on hybridoma or cultures of the patient's cells, for example T cells or stem cells. Classical sterility testing takes 14 days to complete following 21 CFR 610.12 and USP <71> due to lengthy incubation because microorganisms vary considerably with respect to their proliferation rate on or in the culture media used for sterility testing, so unfortunately it is the slowest growers that ultimately determine the time-to-result. In addition to the microorganisms that grow slow by nature, there may be others that are in a dormant stage or have been injured or stressed, for example by microbicidal compounds or depletion of certain nutrients during cell cultivation. These organisms need to recover and can take longer to grow than under optimal conditions. Manufacturers of advanced therapy medicinal products are therefore increasingly turning to alternative rapid microbiological methods to perform their sterility tests and reduce the time-to-result.

Rapid testing methods suitable for biologics

USP <1071>, titled "Rapid Microbial Tests for Release of Sterile Short-Life Products: A Risk-Based Approach", gives an overview of recommended rapid technologies. These are based on either adenosine triphosphate (ATP) bioluminescence, flow cytometry, isothermal microcalorimetry, nucleic acid amplification, respiration or solid phase cytometry. Scientists at the FDA's Division of Biological Standards and Quality Control examined three rapid systems for their suitability for sterility testing of biological products: the Milliflex® Rapid system for membrane filtration and microcolony detection as CFUs via ATP bioluminescence and two systems that detect the CO_2 released in the course of microbial growth after direct inoculation (contact us for study details).

The Milliflex® Rapid system achieved results the fastest, detecting all the 11 bacterial, yeast and mold strains in the panel within five days, crucially including slow growers such as *Cutibacterium acnes*, a bacterium that took the other two rapid methods almost as long as the compendial methods to detect. The Milliflex® Rapid method was found to be significantly more sensitive at detection than both other rapid methods as well as the compendial membrane filtration and direct inoculation methods. The Milliflex® Rapid filtration procedure rinses away inhibiting substances such as thimerosal so the method was unaffected by the addition of this preservative to the matrix, whereas the two other rapid methods did not consistently recover all strains under

this condition. The Milliflex® Rapid method also proved compatible with inactivated influenza vaccine and aluminum-containing adjuvants, with no interfering background bioluminescence.

Removing the ATP emanating from mammalian cells

ATP-based sterility testing of mammalian cell cultures has to take into account that ATP is contained in all living cells, not just microorganisms. When the density of cells in a sample is high (e.g. 108 cells/mL), the Milliflex® Rapid method may require an extra step to remove background luminescence. In a study of ours aimed at establishing a rapid sterility testing procedure for cell culture samples that does not harm microorganisms and that complies with USP and EP guidelines, we treated two different immune cell preparations with a proprietary mammalian cell lysis buffer (MCLB) and the enzyme apyrase. This selectively lyses mammalian cells and subsequent filtration removes the ATP they have produced, paving the way for selective detection of the remaining microbial ATP. The Milliflex® Rapid method captures the widest possible range of microbes using Schaedler Blood Agar/RSTM (Rapid Sterility Test Media) for aerobic and anaerobic incubation at 30 to 35 °C as well as aerobic incubation at 25 to 30 °C. The fast and slow growing bacteria, yeast and mold strains in our study all showed recovery rates above the 70% threshold criterion after one to three days of incubation (contact us for study details).

Although these results are an indication that the Milliflex® Rapid method will work with mammalian cell cultures in general, each manufacturer or contract lab must qualify it in their own laboratory using their own products, cell cultures and in-house microbial strains. Getting the method implemented as an alternative to compendial sterility testing usually reduces the time-to-result from 14 to 5 days or, depending on the individual risk assessment, to as little as four days. We offer expert services and consultation to support a successful validation.

Find out more about the 21 CFR Part 11 compliant, second-generation Milliflex® Rapid System 2.0 for rapid sterility and bioburden testing on a small footprint platform & get in touch with our sterility testing experts.

www.SigmaAldrich.com/milliflex-rapid

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Milliflex® Rapid System is back to speed up your microbial QC

What if you could dramatically speed up your sterility and bioburden testing with a proven rapid method?

That's exactly what the Milliflex® Rapid System 2.0 can do: it takes only a small fraction of the time that traditional methods do.

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47374 03/2023

Millipore®
Preparation, Separation, Filtration & Monitoring Products

Harnessing Microphysiological Systems to Bring Humanized Processes to ADME and Bioavailability Studies

Dr. Yassen Abbas

Lead Scientist
CN Bio Innovations Ltd

Article Synopsis

Absorption, distribution, metabolism, and excretion (ADME) studies are a critical component of preclinical drug discovery, providing important insights into the pharmacological properties of a drug, including indicators to a drug candidate's efficacy, and safety. ADME studies help to inform candidate selection for clinical progression as well as dosing regimens, to ensure clinical trials provide meaningful and actionable data and most importantly, at minimal risk to human health. Despite this, ADME studies are often held back due to a lack of human-relevant data obtained during preclinical studies.

In this article, the author discusses the common drawbacks to ADME studies using conventional methods, and how these issues can affect candidate selection and dosage. The article will also discuss emerging complementary technologies, such as organ-on-a-chip, and how these technologies allow for human-relevant in vitro *preclinical ADME studies that better inform* in vivo *experimentation. Finally, the article will discuss bioavailability, and how using organ-on-a-chip technology can support the determination of human bioavailability to determine drug dosing regiments, reduce side-effects and potentially recover flawed therapeutic candidates.*

Absorption, distribution, metabolism, and excretion, or ADME, studies are a critical component of preclinical drug discovery; designed to provide deeper insights into how a therapeutic behaves within a living organism. The findings are harnessed to inform the direction of clinical studies, providing early data on a drug's efficacy and the dosing regimen required for treatment. Conventionally, ADME studies are performed either using chemistry-based methods, such as determining the solubility of a compound, or using biological models that range in complexity from *in vitro* 2D cell culture to *in vivo* animal studies. However, recreating human representative biological models in the lab is challenging, making it difficult to accurately measure these important parameters and significantly impacting drug development processes. New approach methodologies (NAMs), such as organ-on-a-chip (OOC) technology, are helping to overcome these barriers, enabling researchers to reevaluate dosing regimens, reduce side effects and potentially even recover therapeutic candidates that failed due to poor ADME properties.

Drug developers carry out ADME studies early in the development pathway to understand the pharmacokinetic behavior of a therapeutic candidate; they assess a series of critical parameters of its chemical properties and how it responds in the body. These include the rate the drug is absorbed through the gastrointestinal tract, rate of clearance by the liver and identification of metabolites, and whether it can achieve sufficient concentrations in the blood to be effective at the site of action.[1] These studies also factor into another essential measure of a drug's effectiveness – bioavailability. Defined as the proportion of a drug which enters circulation when introduced into the body and so can have an active effect, bioavailability enables researchers to predict dosages required for a patient to receive an efficacious amount of a therapeutic compound. Taken together, preclinical ADME and bioavailability studies are key to identifying lead compounds with optimal properties, optimizing efficacy, and minimizing safety issues. By providing an early insight into how a molecule may behave in human clinical trials, the findings help to ensure that only safe and effective therapeutics progress. Therefore, the accuracy and efficiency of the methods chosen by researchers hold huge significance for drug developers in determining the success of programs.

The Drawbacks of Conventional Methodology

Traditionally, biological-based ADME and bioavailability studies are conducted in two stages, beginning with *in vitro*, 2D cell culture. This method is cost-effective, utilizing very simple monocultures of cells to enable the researcher to assess initial parameters that are crucial to a drug's success, such as the extent of protein binding, the likelihood of inhibition of major drug metabolizing enzymes, and insights into its metabolic stability.

Following this, promising candidates can be further investigated using *in vivo* animal models. These complex studies enable researchers to assess a drug in the context of a living organism, providing deeper insights into its systemic effects, including across the intricate, multi-organ systems that are particularly hard to recapitulate in *in vitro* models, such as a complete immune system or circulatory system. When taken together, this two-stage system provides researchers with key data to make predictions about a drug's likelihood of clinical success, and consequently make the decision whether to progress a candidate to clinical trials. However, they are not without their drawbacks. Studies have shown that as many as 5-10% of clinical failures are due to issues with pharmacokinetics.[2] When considering estimates that place the total cost of progressing a drug candidate from discovery to market as high as $2.3B, the insights these studies can provide become significant, not only to maximize chance of candidate success, but also to reduce the financial burden of potential clinical failures on the industry.[3]

So, what limits the success of these methods in pharmacokinetic studies? In general, both *in vitro* 2D cell culture and *in vivo* animal models are limited by poor clinical translatability – inherently, the data they produce does not always accurately predict human responses. Whilst 2D cell culture can be a useful tool for initial analysis, the models lack physiological relevance and their simplicity does not facilitate researchers to account for a drug's effects across different tissues or cell types, nor replicate complex inter-organ and systemic effects, such as blood perfusion or immune responses.

Animal models offer this level of complexity; however, a host of interspecies differences limit their predictability in humans. Humans and animals differ in many ways, ranging from gene expression profiles to metabolic capacity, for example in the human cytochrome p450 enzymes which are key players in the detoxification of drugs, cellular metabolism, and homeostasis. Furthermore, considering *in vivo* trials can be conducted in a range of different species, the disparity increases. Because of this, it is very hard to extrapolate data reliably from animal into human, as evidenced by Musther et al. in 2014, where oral bioavailability was compared between various animal models and humans across a database of 184 drug compounds.[4] Commonly used animal models – mouse, rat, and dog – showed poor correlation with human bioavailability (R^2 = 0.25, 0.28 and 0.37 respectively). Non-human primate (NHP) models provide an improved bioavailability correlation with humans (R^2=0.69), although these models are not commonly used due to significant ethical considerations, stringent regulatory requirements, and high cost. Whilst these data do not eliminate the usefulness of animal models for pharmacokinetic analysis, it highlights that these studies are best used as qualitative indicators, rather than quantitative data.

In drug development, some of the most important innovations over the past few decades has been computational or *in silico* tools to screen for new compounds and to predict ADME behavior from a compound's chemical structure. Clinical behavior can be forecasted using physiological based pharmacokinetic (PBPK) modelling which combines a compound's chemical properties, the physiology-based properties of population being modelled, and data from ADME studies. These models are becoming increasingly important prior to trials in humans, however, their ability to accurately predict outcomes in the clinic will often be constrained by the quality of the data imputed.

Poor clinical translation can also result in significant knock-on effects in the latter stages of drug development. A lack of accurate pharmacokinetic data can lead to miscalculations of dosing regimens – an issue that, at best, can mean the drug shows a lack of efficacy, and, at worst, can cause serious adverse effects. Either way, this can result in the failure of a drug candidate. These drawbacks, considered alongside the increasing cost of *in vivo* animal studies (particularly NHP's) and the associated ethical considerations, demonstrates the distinct need for improved predictors of human outcomes in preclinical research.

Organ-on-a-Chip – The Solution to Human Relevance

As awareness regarding the limitations of conventional *in vitro* and *in vivo* models gains traction, many innovative new approach methodologies (NAMs) have been developed to address the gaps. One of the leading technologies in this field is organ-on-a-chip (OOC), which, over the last decade, has emerged to bridge the gap between traditional *in vitro* assays and *in vivo* tissue functionality in humans. OOC technology, or microphysiological systems (MPS), has several key advantages over 2D *in vitro* cell culture. Firstly, the systems are designed to feature constant fluid perfusion, which mimics blood flow by delivering nutrients and removing waste from the cells. Also, the technology can utilize multiple human cell types, to recapitulate the architecture and multiple processes of tissues or organs. Because of this, these models exhibit similar functionality to human organs, to better reflect drug responses in the body. Perhaps most critical to advancing the field of ADME and bioavailability studies in recent years has been the development of multi-organ MPS. Using fluidic interconnection, relevant organ models can be linked together, providing human relevant systems that more closely offer the complexity of animal studies.

As mentioned previously, initial *in vitro* assessment of metabolism and bioavailability is often carried out using 2D cell cultures. The standard practice for these experiments is to use immortalized intestinal cell lines and primary suspension hepatocytes, cells that are known to be poor predictors for this type of study due to low expression levels of metabolic enzymes, in addition to the short duration of culture for primary cells. Using OOC, complex multi-organ MPS have been created which more accurately replicate the interactions between the gut and liver (Figure 1). These models utilize liver and intestinal microtissues, formed via seeding primary human hepatocytes and gut cells, respectively, onto specially designed scaffolds that replicate the architecture of the human organ. Fluidic connection, via micropumps, is used to ensure the flow of liquid, drug and signalling molecules between the organs, to mimic blood circulation, including expression of a range of cytochrome p450s and transporters. By accurately recreating the process of drug absorption and first-pass metabolism, these advanced MPS can derive bioavailability, offering enhanced predictivity versus animal models.

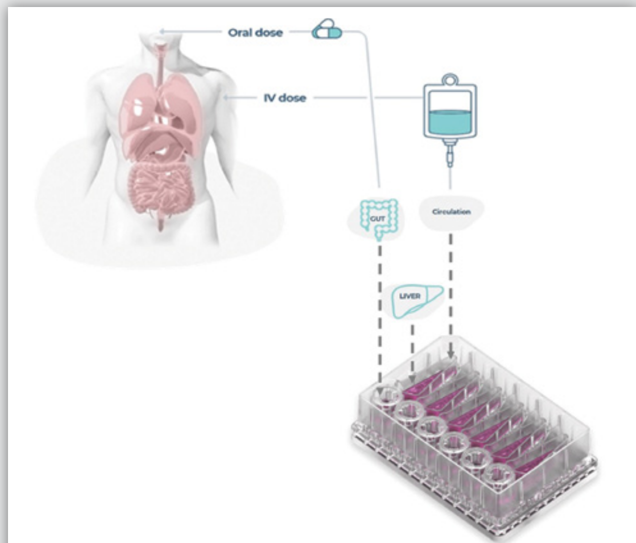


Figure 1. An example of a gut/liver multi-organ model, using micropumps to mimic blood perfusion and interlink the organ microtissues.

In a recent study highlighting the effectiveness and flexibility of these multi-organ MPS for ADME studies, a leading multi-organ MPS was operated as either gut only, liver only or the combination of the two organs: gut tissue interconnected with liver tissue. Diclofenac was used as an example whereby the contributions to clearance and production of metabolites by the gut and liver tissue could be assessed separately and when in combination. In a single experimental system, multiple key ADME parameters were shown to be estimated then extrapolated using *in silico* models for *in vivo* prediction.[5,6]

Oral bioavailability is a parameter that depends on both the extent of intestinal absorption and rate of hepatic clearance and is well suited for estimation by multi-organ MPS comprising of both gut and liver tissues. Here, the multi-organ MPS operated as either oral (both gut and liver tissues) or IV (liver only) (Figure 1) and the bioavailability estimated by determining the area under the curves of both dosing regimens (Figure 2).[6]

Despite being the primary absorption site for many drug products, the gut isn't the only organ that must be studied when seeking to measure absorption and bioavailability. Inhaled medications represent a growing portion of therapeutics due to an array of benefits offered by the lungs, including a large, thin surface area for rapid absorption, direct access to the bloodstream and a lack of required metabolic activity relative to the gut.

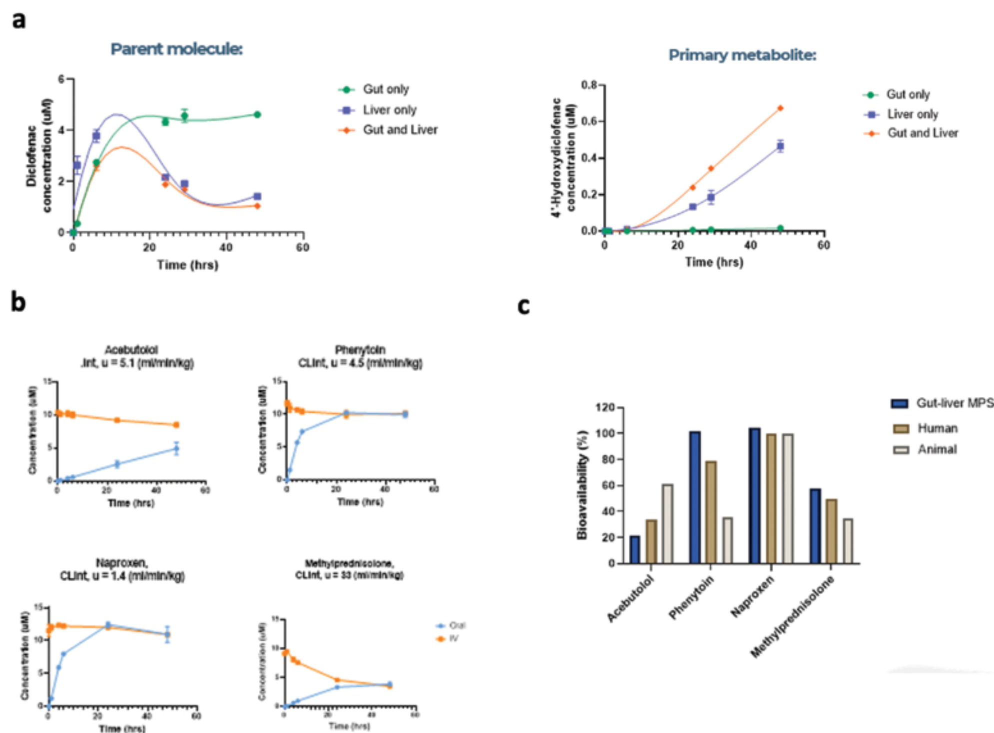


Figure 2. Gut-Liver MPS model for oral bioavailability investigations (a) The Gut/Liver multi-organ MPS can be operated as gut only, liver only or the combination of the two to study the organ contributions to absorption, clearance, and metabolite production. Oral bioavailability can be estimated (b) by first obtaining drug concentration versus time profiles from oral and IV dosing regimens. (c) Interlinked gut/liver OOC models provide more accurate representation of human bioavailability compared to previous data obtained using animal models.

However, the lung presents many challenges for ADME studies. Being a particularly large and complex organ, most *in vitro* models fail to account for the number of factors that can influence drug absorption here, for example, the size of a drug particle. Larger particles are likely to settle within the proximal lung – a region containing a thick mucus coating and beating cilia, presenting a significant barrier to entry unless a drug can rapidly dissolve and diffuse into the epithelium before it is cleared. By contrast, small particles will likely reach the alveolus, which contain extremely thin epithelium and provide an ideal point of entry, however these regions also contain alveolar macrophages that can engulf foreign bodies before they can enter the bloodstream.

These difficulties in modelling are not just limited to *in vitro* models. Studies conducted *in vivo* are often constrained by the physical act of inhalation. Poor technique, or poorly fitting inhalation apparatus, can lead to significant variation in the amount of drug product entering the body. Additionally, rate of breathing and volume per breath can vary significantly between individuals due to many factors. This is particularly pertinent during *in vivo* animal studies, where factors such as stress can have a serious impact on an animal's breathing rate.

Because of these difficulties, the lung has become an area of interest amongst the OOC community. Data presented by Richardson et al. in 2023 outlined the potential of the technology to develop more human relevant *in vitro* models for ADME and bioavailability studies of inhaled medications.[7] Given the physical variation between distinct regions of the lungs, the team developed separate models of both the alveolar and bronchial regions of the lungs using primary human cells that make up the epithelium of each region, alongside endothelium layers and a micropump to mimic blood perfusion (Figure 3). Each model was also developed to feature immune cells, enabling researchers to mimic drug loss via immune cell clearance.

The research concluded that both models showed a significant increase in permeability relative to static *in vitro* cell cultures (Figure 4 a).[7] These lung MPS models also showed conserved human phenotypes with both ATI and ATII cell types being expressed in the alveolar model, and mucus secretion in the bronchial model, along with the presence of goblet and club cells.

A recent study showcased the potential of lung MPS, using primary cells under perfusion, for ADME studies.[7] Three inhaled medications (Salbutanol, Olodaterol and Fluticasone) were used as an example to assess drug ADME prediction of the alveolar lung MPS model compared to standard *in vitro* preclinical models (ex vivo and static cell culture). The study highlighted the lung MPS model as more human and clinically relevant than current standard *in vitro* models (Figure 4b).

A Look to the Future – ADME and Bioavailability

Advanced NAMs hold the potential to transform drug discovery programs. By providing researchers with the opportunity to recreate human physiology in the lab, MPS can be used for a myriad of applications that further our understanding of disease mechanisms,

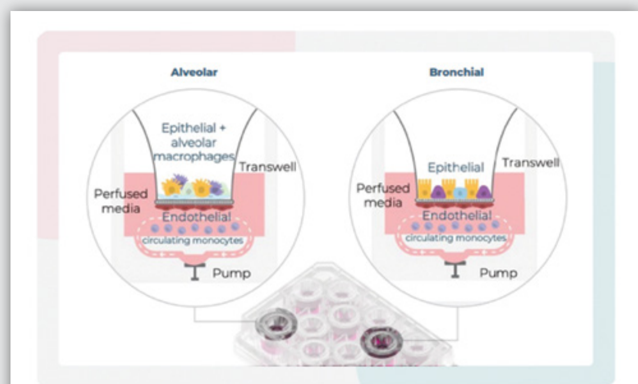


Figure 3. Lung MPS models designed to recapitulate distinct alveolar and bronchial regions of the lung. Models incorporate a micropump to mimic blood perfusion across the epithelium, and immune cells to model macrophage clearance.

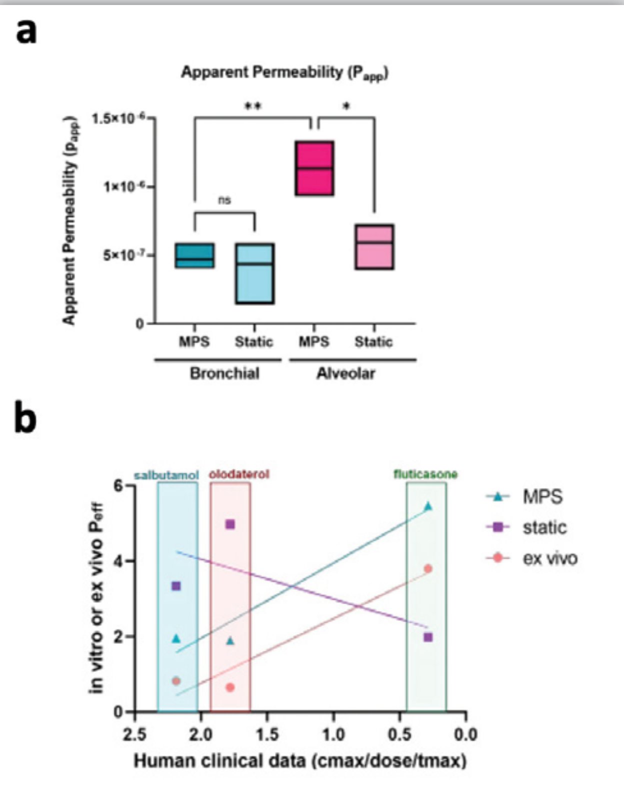
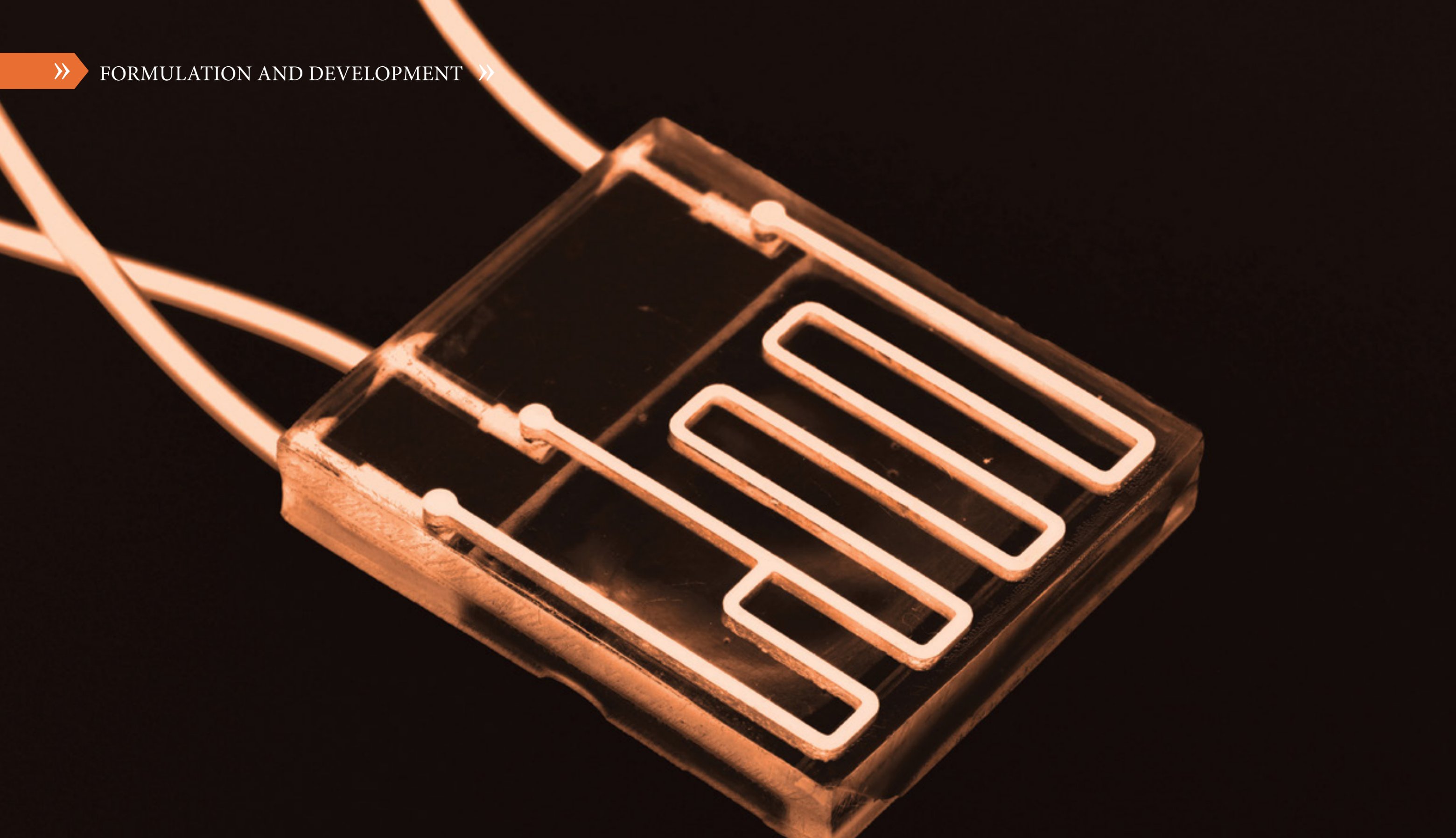


Figure 4. Lung MPS models are fit for ADME and efficacy assessment. (a) Lung MPS models show increased permeability over standard *in vitro* static models. (b) Lung MPS accurately predict the ADME properties of pulmonary inhaled medications.



uncover potential therapeutic targets, and assist with the safe and efficacious development of potential therapeutics.

Determining the ADME and bioavailability properties of compounds is essential for lead optimization and candidate selection in early drug discovery, through to dose refinement and successful translation to clinic. The challenges of using traditional *in vitro* and *in vivo* methods demonstrate the huge potential of harnessing OOC technology throughout these investigations. Single- and multi-organ models closely predict human *in vivo* pharmacokinetics for more informed decision-making and provide deeper insights into the therapeutic window of a drug. Not only would this help to reduce drug attrition rates, an extremely costly issue within the pharmaceutical industry, but it could even enable drug developers to revisit candidates that were previously thought to be flawed.

A better understanding of how a drug interacts with the human body is integral to effective and streamlined drug development, the benefits to gaining these insights during the preclinical stage of research cannot be overstated. The potential of OOC technology on the world of preclinical drug development is yet to be fully realized, but as the field expands and adoption of NAMs continues to grow, we move closer to our goal of enabling safer and more efficacious drugs to get to market faster than ever before whilst reducing the number of animals required.

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Author biography

***Dr. Yassen Abbas** is a Lead Scientist at CN Bio Innovations. He completed an MEng in chemical engineering at The University of Edinburgh and joined the European Space Agency as a graduate engineer. He later received a PhD from the University of Cambridge and completed a postdoc fellowship, also at Cambridge, on the development of a tissue engineered model of the human endometrium. He has experience with real-time sensor technology, organoids and development of in vitro tissue models using human primary cells. Dr Abbas has published five peer-reviewed scientific articles, four as first author.*

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Enhancing Formulation Development: Leveraging Specialty Chemicals for Improved Drug Delivery

Dave Haase
ChemDirect

Pharmaceutical formulation refers to the process in which different chemical substances, including the active drug, are combined to produce a final medicinal product. This process is vital, as it can influence a drug's efficacy, stability, and patient acceptance. With scientific progress, this domain has grown in complexity and importance, constantly evolving to meet the therapeutic challenges of our age.

Today, the world of pharmaceutical formulation has become intricate and multidimensional. It encompasses a broad spectrum of activities ranging from selecting appropriate excipients to researching the increasingly complex chemical markets, and to designing the right delivery mechanism. The ultimate goal remains consistent: to create a product that delivers the medicinal agent effectively and safely to patients.

The Evolving Need for Improved Drug Delivery Systems

The landscape of medicine has shifted dramatically over the past decades. Diseases that were once deemed untreatable now have potential cures or management pathways. With this shift comes the necessity for drug delivery systems that can address these nuanced therapeutic requirements.

The challenge for formulators is to ensure that drugs are not only potent but also adaptable. As patients and practitioners alike seek more targeted and efficient treatments, the realm of pharmaceutical formulation becomes increasingly important in fulfilling these demands.

While traditional methods served their purpose during their time, the contemporary era demands a more sophisticated approach. The pharmaceutical industry has responded to this need, continuously innovating to create delivery mechanisms that balance efficacy with safety. The innovations aim to optimize therapeutic outcomes, allowing for targeted treatments and minimizing adverse effects.

The Rise of Specialty Chemicals

Historical context: traditional materials versus specialty chemicals

Basic materials in pharmaceutical formulations were the trusted staples, utilized for their familiarity and general applicability across various medicinal products. Their utility, while valuable, was often limited in scope and flexibility.

The 20th century, however, saw a paradigm shift in the materials used in drug development. The advent of specialty chemicals

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brought with it a promise of diversity, offering multifaceted functionalities tailored to specific therapeutic needs. These chemicals, often designed with precision, ushered in a new era of possibilities for the pharmaceutical sector.

With specialty chemicals, the industry was introduced to a wider array of formulation options. This shift was not merely about diversity but also about quality: the drugs could now be delivered more efficiently, safely, and in ways previously deemed unimaginable — marking a transformative phase in the industry's history.

Benefits and characteristics of specialty chemicals

The advantages of specialty chemicals are manifold. First and foremost, these chemicals often come with unique properties that enhance the solubility, stability, and bioavailability of drugs. This means drugs can be absorbed better and work more effectively in the body, an essential factor in therapeutic success.

Beyond efficacy, specialty chemicals often provide flexibility that traditional materials couldn't offer — meaning they can cater to a broad spectrum of patient needs, be it specific release timings, targeted delivery, or minimizing potential side effects. In essence, specialty chemicals have redefined what's possible in pharmaceutical formulations.

We also cannot overlook the economic and environmental benefits these chemicals bring. Specialty chemicals can, in some instances, lead to cost-effective formulations, reducing waste and streamlining the production process. Additionally, with sustainability becoming a global concern, the efficient use of these chemicals also points towards a more environmentally conscious pharmaceutical industry.

Here is a list of their characteristics, and how they tie in with various benefits:

- **Molecular precision —** enables enhanced solubility, ensuring drugs that previously suffered from poor water solubility can now be absorbed more effectively in the body.
- **Diverse functionalities —** offers versatility in drug design, leading to the development of a wide range of drug formulations, catering to diverse therapeutic needs.
- **Adaptability —** allows for tailored bioavailability. Drugs can be fine-tuned to release and absorb at optimal rates, making treatments more patient-specific.
- **High purity levels —** promote increased stability in pharmaceutical products. This purity ensures consistent drug performance over time and maintains patient dosage accuracy.
- **Environmentally considerate —** while this characteristic ensures a reduced ecological footprint of pharmaceutical manufacturing, it also indirectly supports a safety boost. By focusing on purity and reducing environmental contaminants, the likelihood of undesirable reactions in patients diminishes.

How Can Specialty Chemicals Optimize Drug Delivery Systems

The science behind enhanced efficacy

Deep within the realm of specialty chemicals, researchers find a trove of potential to amplify drug efficacy. Formulated at the very molecular level, these chemicals weave intricate dances with drug molecules, elevating their performance. Consequently, drugs benefit from improved routes of absorption, distribution, metabolism, and excretion, reaching their therapeutic potential with heightened precision.

Beyond the simple mechanics of better chemical bonds, there's a narrative of meticulous engineering. Specialty chemicals have been crafted to engage with drug compounds in distinct, targeted manners. Through such fine-tuned engagements, these chemicals champion the cause of drugs, allowing them to function with utmost efficiency and therapeutic effectiveness.

Versatility is one of the defining attributes of these chemicals. Adaptable and resilient, they mold themselves to suit a plethora of drug needs. Such flexibility propels the birth of cutting-edge drug delivery systems, each one uniquely crafted to promise unparalleled patient outcomes.

Specialty chemicals and improved safety profiles

Within the pharmaceutical industry, patient safety is the "true North" of all innovation efforts. Herein lies another domain where specialty chemicals make their mark: with their multifaceted properties, these chemicals fortify the safety ramparts of drugs, tempering side effects and championing tolerability.

Another feather in the cap of these chemicals is their knack for mitigating toxic aftermaths. Facilitating streamlined drug metabolism and excretion, they act as gatekeepers, ensuring drugs journey safely within our bodily confines. Such guardianship amplifies therapeutic results while guarding the well-being of patients.

Contemporary medication regimens, intricate in their composition, often teeter on the precipice of drug-drug interactions. Specialty chemicals, with their functionalities, hold the potential to temper such risks by finessing drug release and absorption dynamics — they offer a protective shield, diminishing the hazards linked to concomitant drug consumption.

The Case Studies

The proof, they say, lies in the pudding. Let's look into the genesis of nanoparticle systems and extended-release formulations. These systems have been designed to zero in on specific tissues, deftly sidestepping systemic side effects, while heightening therapeutic efficacy and improving the control over drug release.

- Specialty chemicals drive the innovation behind **nanoparticle systems,** a transformative approach in medical treatments. Flash nanoprecipitation, a technique that encapsulates drugs into nano-scale particles, is at the forefront of this revolution. With this method, drugs gain enhanced efficacy and a reduced risk of side effects.

  Specialty chemicals allow these nanoparticles to navigate the intricate environment of the human body, delivering the drug to its intended target without premature degradation or excretion. The precision of such nanoparticle systems highlights the potential of specialty chemicals in modern medicine, setting the stage for treatments that offer both heightened effectiveness and safety.

- The agricultural sector is witnessing significant advancements with the integration of specialty chemicals, especially in pesticide formulations. **Extended-release formulations** are one notable innovation.

  Traditional pesticides, while effective, often grapple with challenges related to stability, longevity, and environmental impact. Extended-release formulations, fortified by specialty chemicals, address these challenges by releasing the active ingredient in a controlled and sustained manner. This method not only prolongs the effectiveness of the pesticide but also reduces application frequency — leading to cost savings and a diminished environmental footprint.

  With a consistent therapeutic level over extended periods, these formulations offer reliable protection against pests, promoting healthier crops and higher yields. The role of specialty chemicals in enhancing these formulations underscores their indispensable role in shaping sustainable agriculture's future.

The Patient-Centric Approach

The demand for patient-focused therapies

Recent years have witnessed a significant shift in the healthcare landscape. Patients, once passive recipients of medical care, now stand at the epicenter of therapeutic decisions. A burgeoning demand for patient-focused therapies attests to this transformation. Individuals seek treatments tailored to their unique needs, pushing the pharmaceutical industry to evolve in response.

Deeper within this trend is a nexus between the patient's voice and the trajectory of drug development. Individuals no longer desire mere alleviation of symptoms — they yearn for treatments that resonate with their lifestyles, preferences, and genetic makeup. This nuanced understanding of patient needs demands an overhaul in how therapies are designed and delivered.

Contemporary society, bolstered by rapid advancements in technology and communication, has empowered patients like never before. Armed with information and a desire for autonomy, they champion the call for treatments that aren't just effective but are also personalized. It's a call that pharmaceutical industries, backed by the might of specialty chemicals, are heeding with renewed vigor.

How Specialty Chemicals Cater to Patient Needs and Preferences

But how do specialty chemicals fit into this patient-centric paradigm? The answer lies in their inherent versatility and adaptability. Specialty chemicals, with their diverse functionalities, offer formulators a broad palette to craft drugs that align with specific patient needs. Such tailor-made formulations promise enhanced therapeutic outcomes, seamlessly merging with individual lifestyles.

There's also a narrative of convenience. Specialty chemicals, owing to their unique properties, can be harnessed to design drugs with varied release profiles, dosage forms, and routes of administration. Whether it's a once-a-day pill, a topical gel, or a timed-release patch, these chemicals play a crucial role in marrying patient comfort with therapeutic efficacy.

The journey doesn't end at formulation alone. Specialty chemicals also lend themselves to the creation of diagnostic tools and personalized treatment plans. Through influencing drug pharmacokinetics and dynamics, these chemicals can be instrumental in crafting treatments that align with a patient's genetic makeup, setting the stage for truly individualized medicine.

Specialty chemicals in personalized medicine

Personalized medicine, the holy grail of modern healthcare, is on the cusp of a revolution. Specialty chemicals, with their multifarious attributes, are set to play a central role in this transformative journey. These chemicals promise treatments that resonate with individual genetic profiles, heralding an era of precision medicine.

Delicate as a ballet, the interplay between drugs and our genetic makeup can dictate therapeutic outcomes. Specialty chemicals hold the potential to maximize therapeutic benefits while minimizing adverse reactions. Through such nuanced interactions, they elevate the promise of treatments that are not only effective but also harmonized with our genetic symphony.

The road to personalized medicine, while promising, is riddled with challenges. Yet, with specialty chemicals as allies, researchers are better equipped to navigate this terrain. As tools in the quest for precision treatments, these chemicals bolster the industry's efforts, illuminating the path towards a future where medicine is as unique as the individual it serves.

Future Outlook and Implications

The potential for further innovations in drug delivery

Now firmly established on the horizon of pharmaceuticals, specialty chemicals — with their vast potential and ever-evolving capacities

— are promising to redefine the boundaries of drug delivery. This will happen primarily by enabling therapies that were once thought to be the stuff of science fiction.

Think of drugs that can target individual cells, or formulations that can adapt in real-time to a patient's metabolic rate. The role of specialty chemicals in enabling such marvels cannot be understated, as they serve as the linchpin around which these future therapies will revolve.

Yet, the journey ahead isn't merely about novelty. As researchers unravel the potential of these chemicals, they aren't just charting new territories; they're also refining existing methodologies, driving efficiency, and enhancing patient outcomes at every turn.

Challenges and considerations for pharmaceutical researchers

While the vista of opportunities is expansive, it's not devoid of challenges. Pharmaceutical researchers often grapple with complex questions: issues of scalability, safety, regulatory compliance, and cost-effectiveness frequently intermingle, demanding a delicate balancing act.

Beyond the laboratory, there's a world of real-world dynamics. How does one ensure that these innovations, rooted in specialty chemicals, are accessible to the broader populace? How does one navigate the intricate maze of global regulations, each with its unique set of stipulations?

Collaborative efforts will likely be the key. Bridging the gap between research, policy, and industry will be essential. Specialty chemicals, while potent, need an ecosystem of support to truly shine. Researchers, backed by a cohesive network of stakeholders, will play a critical role in ensuring that the promise of these chemicals translates into tangible healthcare outcomes.

Let's look at some of the challenges that will shape the future of the field:

- **Scalability challenges:** ensuring that innovations in the lab can be scaled up for mass production without compromising quality or efficacy; invest in advanced manufacturing processes and robust quality control measures to ensure seamless transitions from lab to market.
- **Safety concerns:** determining the long-term safety profiles of drugs formulated with new specialty chemicals; implement rigorous, long-term clinical trials, and real-world data monitoring to continuously evaluate safety post-market.
- **Regulatory compliance:** navigating the complex and diverse global regulatory landscape for pharmaceutical approvals; establish dedicated regulatory affairs teams with expertise in global pharmaceutical markets to ensure compliance and facilitate smoother approval processes.
- **Cost-effectiveness:** balancing the costs of using specialty chemicals with the goal of keeping treatments affordable; explore partnerships with stakeholders, benefit from economies of scale, and consider alternate financing models to reduce patient costs.
- **Accessibility:** ensuring that innovations reach a broad spectrum of the population, including those in low-resource settings; collaborate with public health organizations and governments to develop distribution strategies and subsidy programs.
- **Intellectual property (IP) issues:** protecting the proprietary nature of innovations while promoting scientific collaboration; develop clear IP strategies and consider licensing agreements that foster both protection and collaboration.
- **Patient acceptance:** ensuring that new formulations using specialty chemicals are accepted and adhered to by patients; engage in patient education, feedback mechanisms, and iterative design processes to ensure formulations align with patient preferences.
- **Environmental impact:** assessing the environmental footprint of manufacturing and disposing of drugs formulated with specialty chemicals; adopt sustainable manufacturing processes and promote recycling or green disposal methods for pharmaceutical products.

Conclusion

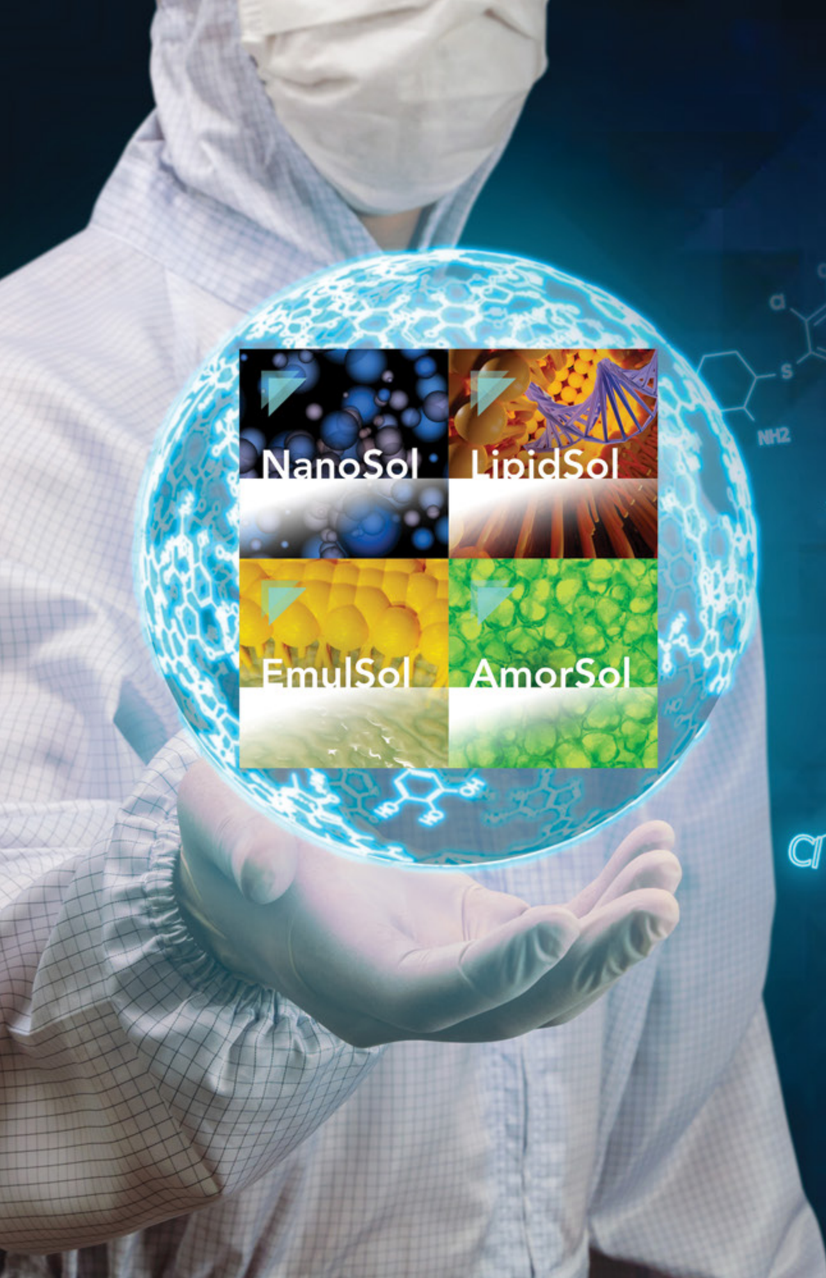
The significance of specialty chemicals in the realm of pharmaceuticals is abundantly clear: these aren't merely compounds, but the harbingers of a new era in drug delivery and formulation. Through their versatility, adaptability, and sheer innovative potential, they have reshaped the contours of what's possible in medicine.

Today's pharmaceutical landscape, dotted with cutting-edge therapies and precision treatments, owes much to these chemicals. They've enabled drugs to be more effective, safer, and in tune with individual patient needs. The fusion of science and innovation, exemplified by specialty chemicals, is yet another step in the ongoing pursuit of better healthcare solutions.

The story of specialty chemicals is obviously far from complete. With each passing day, researchers unearth new potentials, explore novel applications, and dream of future possibilities. The journey ahead is rife with promise, and specialty chemicals, with their myriad capabilities, will undoubtedly play a starring role in the unfolding saga of modern medicine.

Author Biography

__Dave Haase__ has been a leader for nearly 20 years in consumer products and pharmaceuticals and now runs ChemDirect, a B2B marketplace for Chemicals. He has been awarded as a top performer in Marketing, Sales, and Finance. He has an MBA from Stanford and loves building early-stage businesses having taken multiple businesses from <$1m to over $10 million in revenue.



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Tackling the Challenging Molecules by Spray Drying

Shaukat Ali, PhD, FAAPS

Sr. Director,
Scientific Affairs & Technical Marketing
Ascendia® Pharma

Over 80% of new molecular entities (NMEs) are poorly soluble, often making it impossible to formulate them using the conventional technologies, such as micro-milling, salt formation, or complexation. That has triggered a range of enabling formulation options, including non-conventional technologies, such as amorphous solid dispersions (ASDs) or liquid dispersions and co-precipitation. Of several marketed ASD drugs, spray drying remains the most popular approach to convert crystalline drugs into amorphous powder, mainly due to a simpler downstream process to formulate ASDs into oral dosage forms.

Successful spray drying takes more than equipment. Ascendia® Pharmaceuticals blends modern equipment with its B.E.S.T. (Brilliant Technology, Excellent Service, Superior Quality, Trust) approach to provide advanced small-batch spray drying services. In fact, Ascendia® has a proven track record of spray drying molecules that have not traditionally been thought to be candidates for the process.

Spray Drying Process

Spray drying is a gentle one-step continuous manufacturing process that involves creating dry powder directly from a fully dispersed one-phase mixture of drug and polymer dissolved in a common solvent or slurry mixture of drug and polymer. The slurry is subjected to spray as fine droplets by atomization controlled with a stream of hot drying gas (nitrogen) typically carried out between 50°C - 100°C. The spray dried powder dries rapidly, as the solvent evaporates and product is collected in a cyclone, and the solvent is reconciled after condensing through a chiller.

Spray drying factors affect the product's quality in general. Figure 1 outlines some of the main factors.

- Feed Rate
- Flow Rates
- Humidity
- Inlet Air Temperature
- Nozzle Size
- Organic Solvents
- Outlet Air Temperature
- Polymer & Drug Concentrations
- Spray Rate
- Viscosity

Figure 1. Critical processing parameters for spray drying formulations.

Spray Drying Considerations

Spray drying requires polymers or blends of polymers to enhance miscibility of drugs fully dispersed in the matrix. The composition and nature of the polymers and polymeric solubilizers are critical for processing and manufacturing of drugs in ASD powder. Therefore, polymer selection remains one important criterion in creating and developing a robust ASD formulation.

A number of polymers are available commercially and also marketed in ASD drug products. The criteria for selecting polymers for spray drying include:

- Solubility of polymer and drugs in compatible organic solvents
- Thermal stability
- Ease of processability

All these criteria affect the in vitro and in vivo performances of drugs. The glass transition temperature (Tg) and chemistry and functional group of polymers and melting and Tg of drugs are all important factors in selection of appropriate polymeric excipients for spray drying.

Polymeric excipients used for ASDs are often amorphous, while the drugs are highly crystalline. The API's compatibility with polymers depends on the physico-chemical properties of APIs. In cases in which the drugs are like "brick dust" or highly "lipophilic," finding the appropriate polymers with understanding of higher drug loading and maintaining thermodynamic and kinetic stability - in powder and aqueous solution/biorelevant media, is challenging because all factors may impact the critical quality attributes of a robust formulation. The greater solubility of an amorphous drug, compared to its stable crystalline form, is primarily due to minimal energy barrier required to dissolve in water.

One of Ascenda's GMP spray dryers.

Screening of APIs with Polymers

Since APIs are available in small quantities, an efficient screening method is required to identify the appropriate polymers with higher drug loading or exposure. It is important because marketed ASD drugs are typically available in large pills (>1 g). Thus, when screening the APIs with a range of polymers structurally different from each other and having different physio-chemical properties, their high-solubilization characteristics must be considered.

It is achieved by dissolving the compounds and polymers in polar organic solvents and by casting clear films on drying in an oven at 50°C. This process is simple and rapid, which allows screening of multiple compounds with polymers at different drug:polymer ratios simultaneously within a short time.

Polymer selection and choice of compatible solvents are an important first step in spray drying. It may bring enormous challenges, as the processing conditions for spraying amorphous powders out of solvents/co-solvents can lead to immediate re-crystallization of drugs to their most stable state. Thus, spray rate, temperature, and atomization rate can all impact the outcome of amorphous dispersion powder.

Solvent Selection for Spray Drying

Spray drying requires a significantly large amount of solvents, which can be an impediment in developing an amorphous drug because of incomplete drying of ASD powder. At a smaller scale, it is highly feasible to save time and cost, but for scale-up, large amounts of solvent are required; therefore a more efficient drying process is necessary to control the residual solvents per ICH guidelines. In such cases, solvents with low boiling points and APIs with higher solubility are preferred to control particle size and produce higher ASD yield.

The drug's stability in the solvent feed requiring a longer spray drying process can lead to generating impurities by thermal degradation; therefore, care must be taken to minimize the undesired side reactions and related impurities.

The Ascendia® Difference

Ascendia has become the "go-to" CDMO for small batch spray drying projects, using advanced expertise and experience to implement processes that lead to effective scale up. Our proficiency helps us recognize formulations that will have issues – such as viscosity – later in the drug development process and optimize them for success.

Ascendia is adept at conducting spray drying in a number of different environments, including aseptic. Our expertise includes transitioning formulations from non-cGMP environments to cGMP environments at small scale. Such proficiency helps create a seamless transition of formulations from Ascendia to pharmaceutical companies for larger scale production.

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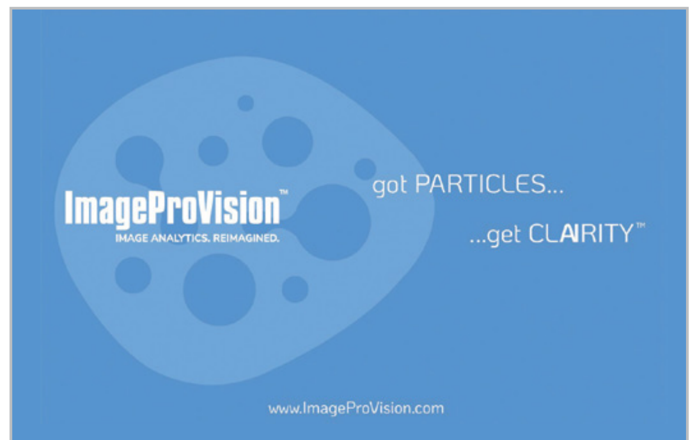

forms as well as the assessment of product quality, safety, and efficacy. Specific applications include characterization of drug product and drug substance, excipients for particle and/or globule size analysis as well as shape, texture and other morphological parameters providing component specific identification of particulates. This software platform can be used to perform analysis of samples for both particle counting and classification according to USP standards <776>, <695>, <788>, <789>, and <790>, <1776> with full 21 CFR – Part 11 compliance.

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Analytical Considerations for mRNA-based Therapies

Mark Rogers, PhD
Global Scientific Director
SGS Health & Nutrition

Messenger RNA (mRNA) technology was discovered in 1961, but it was not until the 1990's that the concept of mRNA-encoded drugs was demonstrated and another several decades before the first mRNA product was approved by the USFDA.

The development of mRNA-based therapies has followed a long and somewhat unique historical path. Today, one such therapy, a COVID-19 vaccine, is one of the most widely used and globally recognized medicines. In 2023, Katalin Karikó and Drew Weissman were awarded the Nobel Prize in Physiology or Medicine, an acknowledgment of their work in the development of mRNA-based vaccines and a testament to their many years of resilience against some harsh skepticism.

The positive influence on mRNA therapeutic development resulting from the Covid pandemic cannot be overstated. However, the need for strong and detailed analytical examination of such products has also recently been highlighted. Some reporting suggests unexpected, delocalized biodistribution of mRNA and protein products after inoculation with the mRNA vaccine and DNA plasmid contamination has been observed in certain mRNA vaccines.[1]

Currently, there are three major applications for mRNA-based therapies: prophylactic vaccines, therapeutic vaccines and therapeutic drugs which have been comprehensively reviewed in a recent publication by Wang.[2] The manufacture of such products ranges from the use of cellular systems to fully synthetic regimens.

Most conventional therapies work by binding and inhibiting hyperactive disease-causing proteins while mRNA therapies restore protein activities or, in the case of vaccines, may elicit new protein entities thereby reversing or obstructing the disease state. The specificity of this modality provides for low, off-target effects and minimal risk of causing genetic mutation since mRNA does not enter the cell nucleus.

To date, two main forms of mRNA vaccines have been developed: non-replicating and self-amplifying mRNA (SAM), the latter containing sequences that encode for replicases that direct intracellular mRNA amplification.

The development of mRNA as the basis of a therapeutic class has been largely challenged by the molecule's somewhat poor stability, immunogenicity, and issues involving it's *in vivo* delivery. However, while still being refined, some of these problems have been addressed by modifications to the mRNA sequence which may be segregated into four defined components: a 5′ end cap, upstream and downstream untranslated regions (UTR), a coding region, and a poly(A) tail.

Referenced in Figure 1 below, the production of mRNA drug substance is performed utilizing an *in vitro* chemoenzymatic process with many of the delivery issues overcome by incorporation of delivery elements[3] in the drug substance such as Lipid Nanoparticles (LNP), cationic polymers and cationic polysaccharides. Today, the most widely used are LNP's, which consist of an ionizable cationic lipid, lipid-linked polyethylene glycol, cholesterol, and a number of phospholipids, each with a specific mechanistic role.

The growth of mRNA therapeutics has inevitably led to a desire for a harmonized approach to provide quality attribute definition to RNA-based products and the analytical testing regimes necessary. Several organizations have already created such guidelines. In 2021, the WHO released an *"evaluation of the quality, safety and efficacy of messenger RNA vaccines"*, which provides a description of potential methods for characterization and control of various key quality attributes.[4] With continued focus on mRNA vaccines, in 2023, the EMA began an initiative on the development of a guideline for five specific quality aspects of this therapeutic class which is expected to be published in 2024/2025.[5]

Also in 2023, the USP released a second edition of their mRNA draft guidelines which offer a relatively comprehensive inventory of methodologies for both DS and DP including analytical approaches to LNP's.[6]

As with any therapeutic, the quality attributes of mRNA-based products can

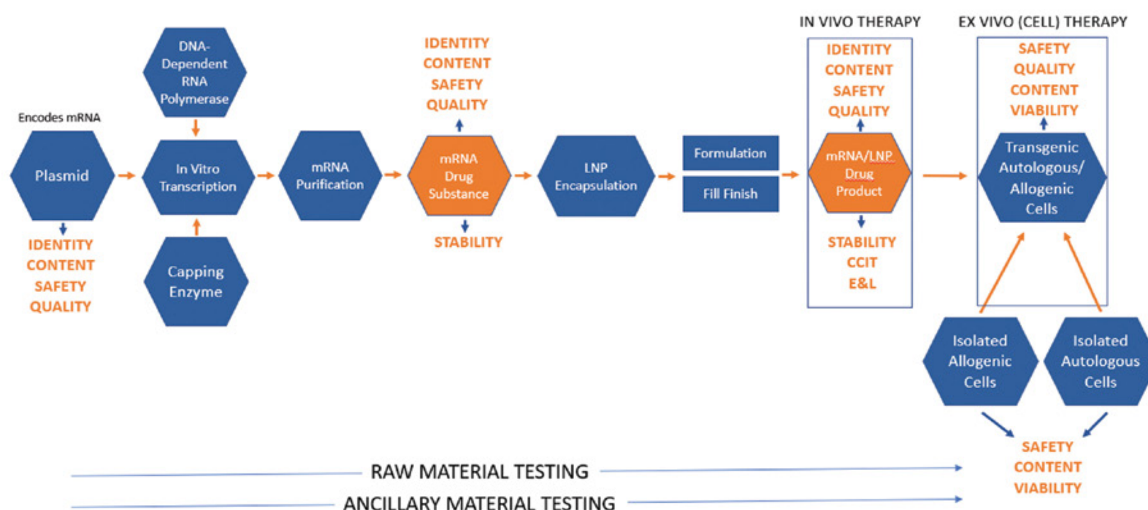

Figure 1.

be segregated into categories which address purity, potency, quality, identity and safety.

Purity: Analyses that define purity are expected to include sensitive and reliable assays for process- and product-related impurities with strict, specified upper limits. It is important that techniques are based on a wide range of physicochemical, biological and/or molecular properties. The results from forced degradation studies are often used as a guide to the choice of impurities that need to be monitored during production, release and/or in stability protocols.

Liquid chromatographic methods are often chosen for both DP and DS. For mRNA, several modes of RP-HPLC have been described for process related impurities and assessment of 3′ and 5′ modifications, while SEC can provide evaluation of aggregation. Kanavarioti,[7] published an excellent review of such methods as applied to both mRNA and tRNA.

Other potential impurities such as residual DNA may be monitored and assessed by quantitative PCR while impurities such as dsRNA, which has the ability to trigger an innate immune response, may be examined by ELISA methodologies.

Potency: Potency measurements are tests of functional integrity and may be determined by *in vitro* and *in vivo* methods that provide a quantifiable biological response. For mRNA-based vaccines and other therapeutics what is measured is the expression of a functionally active antigen (encoded by the mRNA sequence). For example, using flow cytometry or immunoblotting techniques, the potency of mRNA vaccines against COVID can be determined by measurement of the encoded Spike protein (S-protein) in transfected cells.

LNP or other liposomal formulations in the finished DP can be directly tested in cell-based potency assays, as the expressed protein antigen is released and accessible in antibody-based methodologies. The observation of antigen release by itself does not guarantee that virus-neutralizing antibodies will be induced upon administration of the mRNA-based vaccine but is a valid basis for the expectation that antibodies targeting the encoded antigen will be produced.

During development, non-clinical animal models are typically used to determine if there is a dose-dependent correlation between antigen expression and antibody production. The presence of such a correlation strengthens the rationale for cell transfection-based potency assays for the lot release of nucleic acid-based vaccines. Indeed, Patel[8] *et al.* have recently described an *in vitro* cell-based assay that is able to predict the potency of mRNA–LNP-based vaccines.

Quality: As with many of therapeutics, the quality of mRNA-based products is usually assessed using a variety of standard test methods, such as those described by the USP. Typically, for example, pH and appearance are recommended for the DS. The assessment of residual solvents is often part of the "quality" portfolio and can be satisfied using methodologies such as those described in USP 467 and guided by toxicity data as provided in ICH QC3(R8).

Testing may be expanded for the DP to include osmolality and extractable volume. Particle analysis is also critical, particularly for injectables and may be evaluated by a variety of techniques including Light Obscuration, Flow Imaging Microscopy, Resonant Mass Measurement and Nanoparticle Tracking Analysis.[9] Raman spectroscopy can be useful for particle identification. Final product testing may also include container closure integrity and extractable and leachable assessments.

Identity: The determination of molecular identity for mRNA therapeutics can be challenging due to the structural complexity and size of these products. In some cases, modifications to structure may require the development of new techniques or at least adaptation of existing platform methods. The size and sequence of the mRNA product are critical features. Confirmation of sequence may be accomplished using methods such as Sanger sequencing, RT-PCR and other, high throughput forms of sequencing. Several methods are available for size evaluation including capillary gel (CGE), capillary electrophoresis (CE) and mass spectrometry which can be useful for tracking modified nucleotide incorporation. Such methods may require oligonucleotide "mapping" to provide the accuracy necessary for meaningful mass assignment.[10]

Certain specific structural features are of particular importance due to their predicted heterogeneity and effect on performance. As examples, the 5' capping and polyA tail should be areas of analytical focus and can be monitored using well-described methods which combine sample manipulation followed by LC-MS(MS) analytics.

Similar analytical approaches to identity can be applied to the DP and the DS although the latter may require additional sample preparation. It is also important in the DP that attention is given to ID of the delivery system package, and thus, in the case of LNP's, assessment of lipid identity, content and polydispersity as well as RNA encapsulation should all be assessed.

Safety: In the context of this article, safety testing refers to product evaluation in terms of potential microbiological contamination. For mRNA-based products using LNP delivery systems, a "conventional" biopharmaceutical testing portfolio is generally applied involving bioburden and endotoxin testing for the DS and endotoxin and sterility for the DS. All methods are described in the USP.

However, many of these testing methodologies are dated and now often challenged by regulators and the industry itself, particularly in terms of speed and sensitivity. In response, investigators such as Terayama[11] have recently described an improved endotoxin method that reduces analysis times and improves accuracy. A rapid microfluidic assay has been described by Surrette[12] which reduces microbiological screening to a 3hr analysis time.

As the development of mRNA therapeutics continues, the need for robust and detailed analytical examination of the products' safety, quality, purity, potency, and identity remains critical. Strong scientific expertise and a deep understanding of the required testing techniques can help to accelerate the development process while ensuring the quality attributes of these important, life-saving treatments.

The future development and advancement of RNA-based therapies is inevitable. Machine learning is already being applied to develop predictive models and engineer *de novo* sequences that optimize protein expression.[13] Concurrently, innovative analytical technologies will be required to support our understanding and control of these products. Recently, Gunter[14] described a protocol, known as "Vax-seq" that is able to measure key mRNA quality attributes, including sequence identity, integrity, 3'-poly(A) tail length and DNA and RNA contamination in a single method.

Despite the worldwide use of vaccines based on mRNA-LNP's, there still seems much to learn about the precise nature and structure of such products. In 2021, Brader[15] reported on the location of mRNA within LNP's revealing information about the LNP structure, nanoheterogeneity, and microenvironment.

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Assessing Market Entry: What to Consider When Evaluating UK, US or EU Market Entry First

Leeanne Baker
Managing Director and Quality & Regulatory Consultant
IMed Consultancy

The world of healthcare and technology are undergoing a period of rapid transformation especially when it comes to evolving regulatory compliance requirements for medical devices. On one hand advancements in medical technology, ranging from AI to robotics, and on the other the development and deployment of innovative devices, have brought forth immense opportunities for improving patient care and revolutionizing the healthcare industry. As we have seen, global regulators are responding to a growing need to improve patient safety. All these regulations in fact aim to safeguard patient safety, ensure device effectiveness, and maintain ethical standards throughout the product lifecycle, from design and manufacturing to post-market surveillance (PMS). As a result, achieving regulatory compliance for medical devices has become increasingly complex.

Europe and the UK, for example, are experiencing unprecedented regulatory shake-ups in the form of the introduction of the new EU MDR and IVDR and the Future UK Regulatory System. To add to this climate of uncertainty there have been various extensions for Medical Devices Regulation (EU) 2017/745 (MDR) and the In Vitro Diagnostic Medical Devices Regulation (EU) 2017/746 (IVDR) implementation, which medical device manufacturers have had to keep on top of.

The rewards for businesses that face these challenges head on however are significant as the global medical devices market size was valued at $512.29B in 2022 and is projected to grow from $536.12B in 2023 to $799.67B by 2030, with a CAGR of 5.9% over the period.[1] Only a deep understanding of the changing regulatory landscape, meticulous planning, robust quality management systems, and effective risk assessment strategies can help manufacturers truly get to grips with complex standards, guidelines and national regulations to ensure seamless market entry or expansion into new territories.

Opportunities and Risks of Launching in the US First

At first glance, the U.S. medical device market immediately reveals two opposing poles: on the one hand the FDA is traditionally seen as a particularly strict regulator, on the other the market size of the US is enough to whet any medical device manufacturer's appetite.

The US market was in fact valued at USD $176.7B in 2020 according to Grandview Research, with imaging diagnostics and orthopaedics leading the fray.[2]

While the route for a novel device, where the manufacturer is not able to demonstrate similarity to an existing legally marketed device, may indeed be deservedly perceived as particularly arduous, the majority of medium risk devices typically require the submission of a 510(k) registration. This is a premarket submission made to FDA to demonstrate that the device to be marketed is as safe and effective as an already legally marketed device therefore not raising any questions on safety and effectiveness. The result is that products for which an equivalent device with the same intended use can be found are relatively simple to place on the market in the US.

Although the medical device manufacturer needs to ensure their QMS meets FDA requirements (21 CFR Part 820), overall regulatory approval fees are also generally seen as lower than in the EU for product assessment. Another requirement is the establishment of registration and finding a US Agent (21 CFR Part 807).

As we move into the cons of entering the US market first, even though the process is simpler and cost-effective when a predicate can be found, the latter is not always an easy task. Gaining access to all the information about the predicate, when this has been manufactured by another company, may prove difficult. Information will be restricted to that in the public domain (e.g., the equivalent device 510(k) summary, manufacturer websites, publications, IFU, brochures and public complaints data and critical technical information will be hard to come by. In addition to this drawback, the manufacturer must accurately prove the device's substantial equivalence when preparing the 510(k) submission, in accordance with the publicly available FDA guidelines. To do this they should rely on their development documentation.

On a positive note, the manufacturer can expect the FDA to follow specific response timelines that favor planning and commercialization objectives. Furthermore, demonstrating substantial equivalence via the 510(k) submission can sometimes obviate the need for a clinical investigation, thus reducing time-to-market and overall costs. A favorable outcome will result in the manufacturer receiving a confirmation letter, affirming the device's substantial equivalence. Conversely, if the manufacturer receives a Not Substantially Equivalent (NSE) letter, they must initiate the process anew, either by identifying a new predicate device or exploring an alternative submission type.

Understanding the FDA's procedural and cultural expectations is vital for successful engagement. While manufacturers with a suitable predicate experience a relatively straightforward approval process, innovative devices might need to navigate a more intricate path, known as *De Novo*. In cases where a manufacturer is unable to identify a suitable predicate, it may indicate that there are currently no similar devices cleared in the US market. Alternatively, similar devices might exist, but the search terms used failed to yield pertinent results. Before embarking on a more intricate and costly submission route, manufacturers should consider seeking the guidance of an expert consultant who can aid them in the search for a suitable predicate device and explore available market access options in the USA.

Opting for EU Market Entry as a First Route

While the US is the biggest world market with 43.5% share of medical device sales, the European Union trade bloc follows closely with 24.5%.[3] Its largest five markets, Germany, France, the United Kingdom, Italy, and Spain offer solutions for many disease areas with in vitro diagnostics (IVD) representing the largest sub-sector of devices, followed by cardiology. The clinical need the medical device is addressing and whether it is relevant to the target geography is in fact a key element in evaluating the potential of a target first market for product launch.

Compared with the 510(k), the journey towards approval for a medical device in the EU is quite complex and lengthy. The body of evidence that the manufacturer needs to prepare to launch a medical device on the EU market is in fact much more extensive and needs to be supported by the creation of a technical file dossier. In addition to this, it is necessary to engage with a Notified Body (NB) to certify the device, evaluate their QMS, review their technical documentation and assess their clinical evidence.

There have been notable NB capacity concerns with the number of certified NBs climbing slowly and risking a significant slow-down. In October 2022 the 38 existing Notified Bodies had issued 1,990 of the 8,120 applications received according to BSI, leaving a worrying majority of devices still to transition to the EU MDR by May 26, 2024.[4]

Many NBs are not accepting new manufacturers, or requiring lengthy pre-assessment wait periods to begin the technical assessments needed for approval impacting the time to market of products and, critically, access to sometimes life-saving devices for patients. In particular, NB bandwidth is occupied with this transition making it difficult for businesses wanting to propose new, innovative products or more simply to make product changes or reclassify devices to find an NB willing to take them on.

Like elsewhere, in the EU the device's risk classification plays a pivotal role in determining the financial resources, workforce, and time required to bring a product to market. However, due to the prevailing uncertainties and the limited capacity of Notified Bodies (NB), an increasing number of businesses are opting to either abstain from or postpone entering the European Union market.

Furthermore, aside from the linguistic diversity that entails investments in specific labelling and marketing material translations for each country, there are also variations in regional regulations that need to be taken into consideration. For instance, Germany enforces stringent regulations on medical device pricing. Pricing decisions may also be influenced by regional, provincial, and other governing bodies, depending on where the medical device will be sold.

It is important to note, however, that the EU Commission has recognized the existing issues and is actively working to enhance the appeal of the EU market and safeguard patients from potential risks associated with a shortage of new, innovative, and existing medical devices. As a result, several measures are being developed to simplify

the transition from earlier regulations to the EU MDR/IVDR. One of these measures involves extending deadlines for the implementation of new regulations, which eases the burden on products already certified under older regulations.

Simultaneously, the availability of the EUDAMED database is providing businesses and the public with greater access to information, promoting innovation. However, this accessibility also means that competitors can access more data related to manufacturers' devices.

It is important to note that while obtaining CE Marking may be more time-consuming and slightly more complex, it remains one of the most widely recognized certifications in the world. It often holds more weight than FDA approval and is especially effective in expediting market entry in regions such as the Middle East, Africa, and former Commonwealth nations. Therefore, manufacturers looking to expand rapidly and export their products globally would be wise to consider obtaining EU certification before pursuing other certifications.

The Promise of the UK: A Fast-Track for Innovation

The UK's medical device market may be smaller in comparison to the broader EU and US ones, yet it still holds a significant position on the global stage. This is due not only to the absence of substantial language barriers for US manufacturers and many other global producers who have adopted English as the universal language of business, but also to the appeal of the National Health Service (NHS). The NHS is perceived as presenting a "one provider/one payer" model, and its four-year tender framework provides ample opportunities for manufacturers aiming to engage with a single, major buyer.

Currently, however, the UK is still operating a more mature regulatory system than the EU MDR, where risk classes are generally lower, especially for AI and Software devices as well as certain IVD products. Moreover, the UK is increasingly establishing itself as an attractive market for innovative and specialized products. This is in direct contrast to the EU, where Notified Bodies are under strain and are hesitant to take on new products, and to the US, where the approval pathways for innovative and high-risk products tend to be more intricate. The Medical Technology Strategy plan published in February 2023 highlights this new tole: *"MedTech is a vitally important industry for the UK economy, representing over half of all life sciences employment, with businesses situated across the UK and contributing billions of pounds to the economy. As a country we are known for world-leading scientific research and development capabilities, and the UK health and care system is globally recognised as a successful and trusted health system, making the UK a major player on the global healthcare stage."*[5]

This environment is a fertile breeding ground for innovation, especially for emerging medical device manufacturers. Notably, there's a strong emphasis on innovative software devices, which have climbed the regulatory priority ladder, opening up the possibility of novel, swifter routes to market.

On the regulatory front, the shift to the new UK Medical Device Regulation (MDR) is in full motion, and the pace at which new requirements are emerging may catch many medical device manufacturers off guard. To accommodate the diverse requirements of advancing cutting-edge technologies, the UK is also exploring the recognition of approvals from other countries to expedite market access.

For medical device manufacturers contemplating their initial market entry, there isn't of course a single straightforward answer. However, fully grasping the merits and drawbacks of the existing regulatory systems in major markets can provide valuable context for the decision-making process. Undoubtedly, factors like device maturity, therapeutic domain, and classification will influence the choice of the market with the highest potential for success. Nevertheless, embarking on this journey without the guidance and expertise of experienced professionals can entail unwarranted risks, especially within today's dynamic and intricate regulatory environment.

Enlisting the support of international regulatory specialists not only aids in navigating the intricacies of specific markets, efficiently managing the expectations of regulatory authorities, but also facilitates the identification of commonalities among the regulatory requirements of different regions. This, in turn, assists in structuring a well-informed and strategic market entry approach.

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Author Biography

Leeanne Baker, *the founder of IMed Consultancy, guides the team with her extensive experience in Global Medical Device Regulation and Quality projects, supported by a solid grounding in industry. A highly regarded Regulatory and Quality professional and Senior Consultant, Leeanne can also relate to business challenges bringing a solid commercial understanding to the process of launching a medical device into a new market.*

Transforming Signal Detection with Real-World Data

Elizabeth Smalley

Director of Product Management, Data & Analytics
ArisGlobal

In a 2022 life sciences industry report,[1] 70% of biopharmaceutical professionals indicated a maturity gap in the efficiency of safety signal analytics processing. Transforming signal detection, as well as signal validation, in ever smarter ways promises to close that gap, boosting pharmacovigilance (PV).

And not before time. Safety signal monitoring has barely changed in 70 years, from when the practice was first introduced in response to the international thalidomide tragedy - after GPs began to observe an increase in babies being born with disabilities including the shortening and absence of limbs, and a correlation was discovered with their mothers having taken thalidomide during pregnancy.[2] Systematic monitoring of adverse drug reactions was one of the formal regulatory responses to the tragedy which affected more than 10,000 babies around the world.

Since then, other than adapting paper-based manual reporting with some form of electronic equivalent, very little has progressed in the discipline of signal detection and analysis. Outside of the clinical environment, with its inherent limitations (as well as being highly controlled, trials' safety monitoring cannot touch the vast variety of human genomes that a drug will encounter post-market), adverse event monitoring relies too heavily on patient and clinician reporting. And, still today, too many adverse events go unreported (up to 95% in the worst cases).[3]

Reducing Delay and Signal 'Noise'

Even once submitted, Individual Case Study Reports (ICSRs) take time to process before they are used in signal detection. Analyses of medical literature, another PV channel, also inevitably involve a time delay, while scouring of online forums yields too much noise.

With smart, sophisticated analytics technology that can filter for causal and sensitivity to substantially reduce signal 'noise' (with 40%+ more accuracy than traditional signal detection methods, as demonstrated in extensive studies conducted by ArisGlobal),[4] professionals will be able to distil precise adverse event insights directly using robust real-world data from electronic medical records and healthcare claims, boosting drug safety and driving new efficiency for developers. Access to this data is being democratized, thanks to strategic partnerships designed to empower drug developers and their Safety teams with timely, reliable feeds combined with smart, intuitive analytics technology.

Moving signal detection closer to the patient will help address gaps and lag time in adverse event reporting, reducing marketing authorization holder (MAH) risk. Indeed, the benefits will be widely felt right across the healthcare ecosystem – by patients, drug development companies, regulators, and clinicians.

Proactive, hypothesis-free signal detection along with improved signal strength is shown to reduce false positives and detection signals earlier. The incorporation of real-world data, meanwhile, means signals are detected even faster and with impressive precision - the equivalent of a thermometer quantifying the progression of an illness, or a financial credit score objectively assessing an individual's economic health/risk, enabling robust new protocols and better overall outcomes.

These developments couldn't have come at a better time. Up to now, an element of industry inertia has curbed proactive innovation in safety signal detection; typically, compliance has been the primary driver of the measures implemented.

The recent pandemic prompted swifter and more continuous vigilance as advanced vaccines were rolled out with speed, and across huge swaths of the global population. Regulators meanwhile have led the

way in breakthrough innovations for signal detection and validation. Examples include EMA's adoption of DARWIN EU, a platform to generate real-world evidence (RWE) to support the decision-making of EMA scientific committees and national competent authorities in EU Member States throughout regulatory processes. Sentinel, FDA's national electronic system, meanwhile, is transforming the way researchers monitor the safety of FDA-regulated medical products (here real-world data is used for validating signals, though not for detection).

Large Language Models Pave the Way for AI

To keep pace with accelerating change and new waves of innovation in Life Sciences, drug developers have little choice but to ramp up their signal detection and analysis capabilities now. At a practical level, a combination of technology advances and more readily available real-world data is paving the way for much more robust and responsive safety vigilance.

At an artificial intelligence (AI) level, 'large language models' (next-generation neural networks) are transforming the precision with which Safety teams can distil insights from vast data sets, quickly learning and progressively honing their knowledge of what to look out for and what to discount.

The technology is so intuitive to use that Safety teams have less need for the intervention of epidemiologists or data retrieval experts, now being able to perform a deeper level of causal analysis themselves. Large language models (LLMs) are priming the pharma industry to easily embrace all kinds of AI, something that was not true even three years ago. As a result, we can expect to see extensive adoption of proactive signal detection in record time.

Provided there is an appropriate interface, and that the right data preparations have been made so that Safety teams cannot be misled by the findings, Safety professionals can perform their own investigations on the fly, in a highly repeatable way. This is ultimately much more efficient and responsive than requesting a one-off, hypothesis-based study which, as well as being labor-intensive, requires that the query parameters are known up front.

Beyond Speed and Accuracy

The benefits of proactive signal detection, via AI-sharpened analysis of extensive and robust real-world data, in conjunction with ICSRs, are broader than simply faster speed and greater accuracy.

Firstly, as correlations are detected earlier and with improved precision, drug developers will be in a position to spend more time on higher-value activities including innovation in drug discovery, and on delivering safer drugs to patients, sooner.

Secondly, safety-based communications will become much more targeted. Instead of stating generically that a drug may increase the risk of heart attack, the advice can specify that this risk applies specifically to women between the ages of 30 and 60 who have a pre-existing heart condition, for instance - in the context of a very specific phenotype, in other words.

Developing the Commercial Potential of a Drug

Beyond safety and compliance applications, the same mathematical modelling used in adverse event monitoring also supports signal detection in drug repurposing, potentially presenting new commercial opportunities to drug developers as previously unknown and unexpected positive correlations are discovered. (A signal is *any previously unknown information* about the causality of a drug and event; it needn't be a negative outcome.) In the context of a benefit-risk profile, this is an opportunity to focus as much on the benefit as on the risk profile, and to grow the commercial potential of a drug. Safety has an unprecedented opportunity to shine as a strategic partner to the business, rather than merely a cost centre that exists to contain risk.

Next-generation signal detection is emerging as an exciting field to watch and leading pharma companies are already exploring the potential to boost patient health and safety and focus more on drug repurposing.

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Author Biography

Elizabeth Smalley *is the Director of Product Management, Data & Analytics at ArisGlobal. With over 15 years of experience across data analytics, Elizabeth has a passion for exploring the intersection of data science and human reasoning, with experience bringing AI-powered software to market to drive safety and clinical outcomes for patients and clinicians. In her current role, she leads the teams managing the Data Platform, LifeSphere Clarity and LifeSphere Signals, and Risk Management product lines. www.arisglobal.com, esmalley@arisglobal.com*

VENDOR VIEWPOINT

Quality by Design: Unlocking Precision in Pharma Formulation

Dr. Parizad Elchidana
Principal Technical Consultant
ACG

In the ever-evolving pharmaceutical landscape, Quality by Design (QbD) ensures development of genuinely robust formulations.

Underpinned by universal risk-management and scientific principles, QbD's systematic approach enhances product quality and predictability. For pharmaceutical professionals, embracing it has become a strategic necessity.

Implementing QbD successfully in formulation development ensures products have predetermined quality attributes and much-reduced variability. It also informs a far deeper understanding of the process, and of the relationships between raw materials, process parameters, and Critical Quality Attributes (CQAs). This not only ensures regulatory compliance, but also streamlines the development process, saving time and resources.

QbD Essentials

Product Design

QbD starts with clear definition of the Target Product Profile (TPP) and the Quality Target Product Profile (QTPP). This involves specifying the desired attributes of the final product – including its safety, efficacy and quality characteristics.

Process Design

With the product attributes defined, attention shifts to developing a manufacturing process that can consistently achieve the desired product quality. This involves identifying Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) that can affect the product.

Process Performance

QbD requires a thorough understanding of the relationships between various factors, and how they can affect the process. Design of Experiments (DoE) is often employed to explore these relationships systematically, and helps to establish a 'Design Space' in which proven processes combine to ensure product quality.

Product Performance: This element of QbD focuses on monitoring and assessing a product's Critical Quality Attributes (CQAs) throughout the manufacturing process using PAT. Real-time monitoring allows for proactive adjustments, ensuring the final product consistently meets the predefined quality standards.

The importance of QbD in pharmaceutical formulation development cannot be overstated. It shifts the traditional focus from post-production testing to a proactive, design-based approach. By embedding quality into every stage of formulation development, QbD reduces variability, enhances process understanding, and provides a structured methodology for risk management. In a field where precision and predictability are paramount, QbD emerges as a strategic imperative, aligning seamlessly with the principles of pharmaceutical development excellence.

QbD Advantages: Enhancing Product and Process Design

Enhanced Process Understanding

QbD encourages a joined-up understanding of the formulation's critical aspects, from raw materials to the manufacturing process. This depth of comprehension minimizes uncertainties and unexpected variations, resulting in a more robust and reliable process.

Reduced Variability

The systematic nature of QbD – especially in identifying and controlling critical parameters early in the development process – means far less variability. This not only ensures consistent product quality, but also minimises the risk of batch failures.

Streamlined Regulatory Compliance

Aligning QbD with regulatory expectations means a smoother regulatory approval process. The emphasis on scientific understanding and risk management resonates with current Good Manufacturing Practices (cGMP) and other regulatory guidelines.

Cost Savings

QbD minimises the need for extensive post-production testing and reworking. This reduces the number of batch failures and rejections significantly, thereby saving time and money.

Faster Time to Market

The proactive nature of QbD not only enhances process understanding, but also accelerates the development and manufacturing processes, enabling pharmaceutical companies to get high-quality products to market much faster.

QbD principles apply across all stages of pharmaceutical research and manufacturing:

- **Research and Development**

  QbD guides the selection of formulation components, the design of manufacturing processes, establishment of in-process controls, analytical method development and design of clinical protocols. It ensures that potential issues are identified and addressed early in the development cycle, preventing delays and setbacks.

- **Quality Control**

  QbD provides a framework for quality-control teams to monitor critical parameters during production. Real-time monitoring allows for immediate corrective actions, ensuring the production of consistently high-quality pharmaceuticals.

- **Production and Operations**

  QbD facilitates process optimisation and scale-up in production and operations. Establishing a design space supports efficient transfer of processes from development to production, minimising the risk of unexpected variations.

Implementing QbD in pharmaceutical formulation development demands a structured and collaborative approach. Here are the key steps for basic product design and formulation development:

Define Objectives: Clearly articulate the TPP and QTPP to align the team with the project's goals.

Risk Assessment: Identify CMAs and CPPs through a thorough risk assessment using tools such as Failure Mode and Effects Analysis (FMEA).

Design of Experiments (DoE): Utilize DoE to systematically explore the impact of various factors on product quality and process performance.

Establish Design Space: Based on the results of DoE, define a design space that allows for flexibility in manufacturing, while maintaining product quality.

Real-Time Monitoring: Implement real-time monitoring and control strategies to ensure continuous assessment of critical parameters during production.

Control Strategy: Develop a robust control strategy that includes in-process controls, testing, and monitoring to ensure consistent product quality.

Documentation and Training: Maintain thorough documentation of the QbD process and provide ongoing training to ensure all team members are aligned with QbD principles.

Strategies for successful QbD implementation

Collaborative Cross-Functional Approach: Encourage collaboration between formulation-development scientists, R&D teams, quality-control experts, and production and operations teams. A cross-functional approach ensures diverse complementary expertise contributes to the QbD process.

Continuous Improvement Culture: Incorporate feedback from across manufacturing processes to refine and optimise the design space. This iterative approach ensures the agility to adapt to evolving challenges.

Thorough Documentation: Maintain detailed documentation of the QbD process, from initial formulation design to manufacturing. This is crucial for regulatory compliance and internal knowledge-sharing.

As we continue to explore the frontiers of pharmaceutical formulation, QbD is an essential tool for successfully navigating the complexities and uncertainties inherent in drug development. QbD isn't just a regulatory necessity – it's also a powerful tool for transforming pharmaceutical formulation development. In the pursuit of excellence, integrating QbD principles can achieve clear differentiation in a highly competitive pharmaceutical landscape. Pharmaceutical professionals can achieve excellence and push the boundaries of possibility in providing high-quality, super-reliable, and genuinely innovative pharmaceutical formulations.

Endotoxin Testing and RMMs - How to Improve Manufacturing Agility and Increase Efficiency

Meg Provenzano
Product Manager, Bio-Detection

Briana Nuñez
Microbiology Technical Specialist

Veolia Water Technologies & Solutions, Sievers Instruments

Time matters. Everyone in pharmaceutical manufacturing is busy, making it imperative to find better monitoring tools. In particular, bioburden monitoring and bacterial endotoxin testing (BET) are areas in need of improvement. These contaminants are high risk in any pharmaceutical manufacturing site and should be monitored as quickly and easily as possible. However, these tests are traditionally time-consuming and require significant hands-on time.

Whether it's performing tests, managing investigations, or signing off on paperwork, manufacturers need to be able to provide bioburden and endotoxin data quickly to manage the quality of products, raw materials, and in-process samples - all while keeping the process under control.

Now, with advances in instrumentation, bioburden and endotoxin testing are significantly faster and easier, improving the effectiveness of a monitoring program in alignment with EU GMP Annex 1. The revised Annex 1 urges sterile manufacturers to consider implementation of innovative technologies such as Rapid Microbiological Methods (RMMs) to improve the effectiveness of microbial monitoring and quality of final product.

Along with the benefit of enhanced monitoring, implementation of new tools such as the Sievers Soleil Rapid Bioburden Analyzer and Eclipse BET Platform can provide efficiency gains from data integrity, ease of use, and fewer human errors to impact the final product.

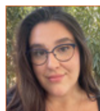
How can microbial testing be faster and easier?

With the use of rapid micro methods and simplified testing, you can improve control and have a better understanding of your entire process. Efficiency is achieved using innovative technologies that make testing faster, also requiring less hands-on time and fewer pipetting steps. As described in recent revisions to Annex 1 which went into effect in August 2023, there are benefits of implementing rapid methods to improve contamination control strategies. Faster and more efficient methods for bioburden and endotoxin testing will increase the protection of products and environments, and areas such as personnel, equipment, facilities and materials all offer opportunities to introduce tools for easier process monitoring.

Rapid bioburden methods

Traditional methods for microbial testing take days. These tests not only delay the production process, but lack the ability to provide real-time information to drive ongoing decision-making. The Sievers Soleil Rapid Bioburden Analyzer provides results that correlate to plate counts in less than 45 minutes. This is achieved using unique viability stains in conjunction with ultra-sensitive optics to provide users with results that correlate to traditional methods.

***Meg Provenzano** is the Product Manager for Sievers Bio-Detection Instruments at Veolia Water Technologies & Solutions. She has over ten years of experience in the bacterial endotoxin testing industry and has held several positions in Quality Control, Technical Support, and Product Management. Prior to joining Veolia, Meg was a Product Manager with Charles River Laboratories. She is customer-centric and enjoys hands-on problem solving, whether for technical issues, assay assistance, or software. Meg holds a BS in Marine Science and Biology from Coastal Carolina University where she focused on Bottlenose Dolphin population research.*



***Briana Nuñez** is a Microbiology Technical Specialist for the Sievers Instruments product line, part of Veolia Water Technologies & Solutions. She was previously the Lead Researcher within the Sievers Instruments Microbiology Center of Excellence in Tucson, Arizona. Briana has participated in research and development of Rapid Microbiological Methods (RMMs) for bioburden analysis and is responsible for supporting the implementation of RMMs and automated endotoxin testing. Briana also plans and conducts laboratory experiments, provides product training, and assists in customer support. Briana holds a B. from State University of New York at Purchase College in Biology.*

Advantages include:

- Make timely data-driven decisions about the manufacturing process to reduce risk, increase cost savings, and enable greater confidence when releasing products
- Perform testing in a laboratory or at-line throughout manufacturing to monitor contamination control processes within water systems, cleaning validation, environmental monitoring, raw materials, and drug substances
- Easily perform testing with only three pipetting steps
- Remove the bioburden testing bottleneck in product release

More efficient endotoxin testing

Traditional endotoxin assays are time consuming, error prone, and difficult to perform. They also rely heavily on resources. Historical options for BET include traditional 96-well microplate assays that are manual and not ergonomically friendly. Automation with robots can be prone to errors using multiple softwares to control the instruments and still require many liquid handling steps. In recent years, recombinant technologies have gained popularity, however they are not considered compendial globally. They are also still prone to user error, with manual pipetting steps. In addition, these various methods leave room for improvement in terms of speed, footprint, hands-on time, complexity of validation, sustainability, and compliance.

The Sievers Eclipse microfluidic endotoxin testing system enables users to set up fully compliant assays in less than ten minutes with a 90% reduction in Limulus amoebocyte lysate (LAL) reagent and only 55µL of sample. With this technology, BET assays are simple, easy and compedial and can be performed with your choice of commercially available kinetic chromogenic reagent. Assays are remarkably easier, as you don't need to make your own standard curve - it's already preloaded on the microplate along with positive product controls (PPCs).

Advantages include:

- Faster turnaround times, minimal training, and improved sustainability
- Less analyst hands on time and less chance of manual errors due to 5-10 minute setup time with 89% fewer pipetting steps than 96-well plate
- Easily train production technicians and analysts on simpler, faster endotoxin testing procedures
- Intuitive, highly customizable software with full 21 CFR Part 11 compliance capabilities
- Reduced cold room storage - store microplates at room temperature and reduce the number of reagents needed to store in 2-8°C
- Increased sustainability - reduce LAL usage (1mL LAL per plate)

What can be gained from greater manufacturing agility?

A combination of rapid micro methods and microfluidic technology can lean out the bioburden and endotoxin testing processes within your facility. This allows you to get results quickly and make faster, actionable decisions to mitigate risks and increase efficiencies throughout manufacturing. The goal is to increase your manufacturing agility and reduce delays in production.

When designing and implementing a contamination control strategy (CCS), it all starts with a culture of quality. A robust CCS can help you decrease risks for out-of-specification (OOS) investigations and reduce cost and unnecessary use of resources. A focus on quality keeps process control at the forefront to ensure product standards are met. This means deviations are quickly identified and addressed. New and innovative technologies allow for this proactive approach by making monitoring of processes and products easier and more reliable. These technologies also mitigate risks such as time to results, risk to the business, and risk to the process.

Conductivity, total organic carbon, endotoxin and bioburden are four parameters that are critical to test in pharmaceutical water systems. To improve process control and understanding, and to achieve compliance, manufacturers are encouraged to use technologies that help track and trend data, make real-time decisions, and optimize uptime with fewer OOS investigations. Manufacturers of sterile products are subject to special requirements in order to minimize risks of microbial, particulate, and endotoxin or pyrogen contamination. The following areas should be considered: personnel, facilities, utilities, equipment, processes, and materials.

Process analytical technologies (PAT) are used as part of methodology to design, analyze, and control the manufacturing process. The ultimate goal of PAT is to ensure the quality and safety of products and processes, with benefits including improvements in product quality, shorter manufacturing times, and faster release of products.

Simplified technologies to make timely, data-driven decisions

Technology that is easier to use is a time saver, and this can be amplified with easier validations and purpose-built software. Simplification of consumables, reagents, and other accessories will also lead to efficiency gains throughout the manufacturing process. For bioburden testing, reducing time to results from days to less than an hour offers significant agility to make important decisions and maximize efficiency and quality. In particular, correlation to plate counts is critical to have the confidence you need to make these decisions. For endotoxin testing, setting up assays in less than ten minutes using a simple platform enables analysts to eliminate the majority of hands-on time, thus reducing errors and retests. Efficiency gains are coupled with improvements in sustainability using 90% less lysate and simplifying reagent use and storage.

Today's pharmaceutical manufacturing environment requires agility, efficiency, flexibility, and quality. To meet these needs, innovative instrumentation and PAT can be implemented to improve process control and increase speed. With faster microbial detection, you can make timely, data-driven decisions and quickly take action to control contamination events and reduce risk.

FACILITY TOUR

Microbiologics

Expanding Infectious Disease Testing

Microbiologics Increases BSL-3 Lab Space to Meet Demand for Pathogen Testing Services

As scientific research into new treatments continues to grow, the need to effectively test and contain infectious agents is increasingly becoming a top priority. Biosafety Levels (BSL) have been established to rank pathogen risk from the lowest risk, BSL-1, to the highest risk, BSL-4. A significant amount of research is currently being done with pathogens ranked as BSL-3. The BSL-3 risk group includes pathogens such as SARS-CoV-2 (COVID-19) and *Mycobacterium tuberculosis* (MTB).

BSL-3 represents a pivotal juncture in the biosafety continuum, introducing heightened measures to address the challenges posed by infectious agents of moderate risk. Unlike the introductory confines of BSL-1 and the controlled environment of BSL-2, BSL-3 laboratories are designed to handle pathogens that can cause serious disease through inhalation, but for which effective treatments may exist.

Microbiologics, a leading infectious disease contract research organization (CRO), recently announced an expansion of their Biosafety Level 3 laboratory spaces at the company's Global Virology Center in San Diego, California. The increased BSL-3 space is the first phase of a broader expansion of the San Diego location, signifying Microbiologics' commitment to supporting their partners and sharing their expertise in virology and microbiology. Phase one focuses on increasing capacity for BSL-3 infectious agents, including high-throughput virology assay services as well as the production and inactivation of viral stocks for research use.

Facility Overview

Microbiologics' mission is to create a safer, healthier world by providing the highest quality biomaterials. They take pride in helping their partners and customers across the globe create confidence in science. The Microbiologics team brings a consultative approach to each engagement, and they collaborate with each customer to build a custom

program for their specific needs. This expansion allows Microbiologics to further pursue their mission, ensuring they are prepared to continue growing and supporting their partners' needs as a leading CRO.

Microbiologics acquired and renovated a 43,000 square foot building near its original Global Virology Center in the Mira Mesa biotechnology area of San Diego. The new facility features multiple BSL-3 laboratory spaces spanning over 2,000 square feet, including a designated campaign suite for sensitive projects. BSL-3 laboratories are equipped with unidirectional air flow, biosafety cabinets, and antechambers to allow proper donning of personal protective equipment, including respirators.

"Demand for our BSL-3 services continues to grow, and limited laboratory space was soon going to constrain our ability to support the industry," says Microbiologics Vice President of Research and Development, Brian Beck, Ph. D. "The new San Diego facility allows us to grow alongside our partners. We aim to be the approachable experts our partners can rely on throughout the drug development process," Beck added.

Many times, commercial BSL-3 facilities lack the space to execute multiple projects or to work with more than one pathogen at a time. In total, Microbiologics' new BSL-3 laboratories are 4X the size of the company's previous BSL-3 space in San Diego. They also have additional BSL-3 certified laboratory space at their World Headquarters in St. Cloud, Minnesota. The company will be further expanding their offering to include pathogens from the CDC and USDA Federal Select Agents and Toxins Program in the future.

Safety Measures

As BSL-3 research involves high-risk pathogens, testing is performed in highly specialized laboratories under negative pressure, stricter PPE, and extensive administrative controls based on risk assessments that are performed for each pathogen and type of work.

Common BSL-3 safety controls include:

- The use of full-body PPE (Tyvek suits/coveralls), including goggles and double layer of gloves
- The use of respirators to protect against possible respiratory exposure
- Sustained directional airflow (negative pressure) to draw air into the laboratory from clean areas toward potentially contaminated areas before being exhausted through HEPA filters (exhaust air cannot be recirculated back into the lab)
- Self-closing set of locking doors with access away from general building corridors
- Extensive security protocols and redundancies. Access to a BSL-3 laboratory is restricted and always controlled.
- Laboratory safety manual, risk assessments, and SOPs that designate how the work is performed safely

Full Range of Testing Services

The Microbiologics team is highly experienced with BSL-3 pathogens. They have deep expertise with respiratory agents, including SARS-CoV-2 and MTB, as well as other emergent threats like Avian Influenza and Mpox. They also support research for HIV and mosquito-borne pathogens such as West Nile Virus and Chikungunya. With multiple BSL-3 laboratories, a variety of high-throughput antiviral testing and custom manufacturing services are available for any BSL-3 agent.

Antiviral Testing Services

Antiviral testing methods evaluate if a compound has any effects on the virus' ability to cause an infection. "The same way we know if a cell is infected, we can also observe the lack of infection if an antiviral is added to the cells," says Beck. "To do this, we mix the virus with the inhibitor (drug or antibody) and test if the virus is neutralized and no longer infects the cells. We have many different assays in which to do this depending on the virus system being used. For example, for influenza viruses, there are several standard assays to assess impact of a vaccine or drug on different virus components and we perform those assays to give the client a full scope of the impact of their drug or vaccine."

Since the start of the COVID-19 pandemic, Microbiologics has been at the forefront of providing SARS-CoV-2 biomaterials and assay services to support researchers and developers. They continuously source and produce the most relevant variants for monitoring immunological responses to vaccines and therapies, measuring PCR signal detection, and performing other critical research. With a highly specialized team and ample BSL-3 laboratory space, Microbiologics provides high-titer inactivated SARS-CoV-2 viral stocks and high-throughput custom assay services. Antiviral testing is done using advanced 3D cell system models, and studies can be performed to look for escaped mutants with a customer's antiviral or therapeutic.

To stay current with the emergence of new viral strains, Microbiologics readily obtains any new variants or strains as they are available from biospecimen depositories to maintain an up-to-date virus inventory. Modern molecular biology techniques are used to create custom mutations in viruses that are identified by partners to test if the virus is becoming resistant to treatment.

Antibacterial and Antifungal Testing Services

Microbiologics has deep expertise in designing and executing antimicrobial drug screening programs that lay the groundwork for seamless regulatory submission. "We offer a wide range of standard and custom antimicrobial assays, advanced microbial identification, extraction and preservation, as well as strategic consulting services," says Beck. "Our partners also gain access to our extensive biobank comprising more than 10,000 clinically important microbial isolates to test compounds against."

Mycobacterium tuberculosis (MTB) is responsible for more deaths worldwide than any other infectious agent with 10.6 million new cases

and 1.6 million deaths in 2022. In support of the ongoing battle against MTB, Microbiologics is ready to assist with primary and secondary profiling for the research and development of new MTB treatments. The company has validated broth microdilution susceptibility testing of MTB using custom frozen panels. These panels can be made to suit customer screening needs with any number of investigational compounds alongside relevant comparators. A variety of MTB strains are available for primary profiling, including susceptible and resistant isolates.

Viral Stock Production

Microbiologics has developed and optimized virus stock production methods for multiple BSL-3 pathogens and has a method to inactivate virus allowing others to work with the material under more accessible conditions. They employ numerous control measures to ensure consistency and quality of their product. Their Global Virology Center in San Diego has received ISO 13485 certification for its BSL-2 and BSL-3 viral stock production methods. A panel of assays is performed to provide full characterization of the viral stocks they produce, ensuring their quality.

Assay Development

Developing assays for antiviral, antibacterial, and antifungal testing is crucial. Microbiologics' approach to assay development is to ensure accuracy, sensitivity, and reproducibility of results.

"We have a proven record of success in navigating the anti-infective drug development process from discovery and development through clinical research and FDA review," says Beck. "We offer standard and custom virology and microbiology assays and biomaterial services including advanced sequencing, propagation and reverse genetics. Our team is skilled in a variety of cell-culture based assays for the assessment of anti-infective compounds."

Current Market Perspective and Future Plans

The demand for accurate and reproducible antiviral, antibacterial, and antifungal testing services is expanding. Microbiologics is continuously reviewing their capabilities and assessing market needs to ensure they will be able to effectively support their partners long-term. "Infectious diseases caused by viruses and bacteria continue to pose significant threats to public health," says Beck. "Accurate testing is essential for diagnosing infections, determining the appropriate treatment, and tracking the spread of diseases. Our team is highly trained and flexible, allowing us to work with unique compounds, testing procedures, or pathogens that can be difficult to grow or test."

Beck continues, "We strive to challenge ourselves to think ahead even of our partners. With the expansion planned, we are being purposeful in earmarking space to be available when and where our partners require our support for developing new technologies and treatments. Since we support custom requests, we have a tremendous amount of flexibility, more than most CROs. Our team brings a consultative approach to each engagement, and we collaborate with our partners to build a custom program for their specific needs. This means that we have a long history of taking on challenges and learning alongside our customers."

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Enhancing Compliance with EU GMP Annex 1 Requirements

Anna Cluet

Pharmaceutical Consultant and Senior Auditor
Rephine

In August 2023, the revised EU GMP Annex 1 requirements related to the Manufacture of Sterile Medicinal Products came into force, with implications for contamination control strategies and, by extension, for plant facilities, equipment, manufacturing processes and Quality systems.

The updated requirements are designed to increase confidence in these products. Now that they are live, the expectations of auditors have adapted accordingly which will have a bearing on audits and their findings from this point on.

In a previous article some months ago, we detailed the confirmed new requirements under Annex 1, in particular relating to a fit-for-purpose Contamination Control Strategy (CCS), and associated policy, to ensure that consistent standards are upheld, end to end, across manufacturing operations.

It is here that many manufacturers have struggled, with some of the main issues (and remedial advice) set out below.

Inadvertent Broadening of Scope

Although the focus of Annex 1 is very clearly on microbial, particulate and pyrogen contamination, some companies have inadvertently broadened their Policy scope to cover product residue (as per Annex 15 of EU GMP). This in turn introduces the concept of cross-contamination – e.g., if the same equipment is being used for different products. Although this is important, it is not within the scope of Annex 1 and is a distraction here.

Lack of Detailed Gap Analysis

We've also seen policy documents drawn up at a too high and theoretical level. In many cases, companies have drawn up a document which merely explains the current control measures and monitoring plans - without evaluating their level of compliance and any gaps with the expectations of the new Annex 1. Without a detailed gap analysis, these companies risk not fulfilling the new measures that are required to control contamination risks, especially in Class A or Class B production areas. The whole point of Annex 1 was to force pharma manufacturers to review their contamination controls, and make, document, and measure targeted improvements.

Failure to Take a Holistic View of Risk

A further issue involves the links between the various different contamination controls. Here, too many companies are failing to provide a holistic overview of potential risks which is essential to achieve the required sterility assurance level.

Evaluating each component individually can mean that companies fail to take into account interdependencies between and with other systems, processes, and considerations. The expectation under Annex 1 is that Contamination Control Strategies consider facilities/equipment, utilities such as water management, and people-related risk controls as a whole, because any weakness in the chain could compromise all other measures.

Ongoing Review

As we have noted previously, devising the Contamination Control Strategy, associated Policy and identified measures is and should not be viewed as a one-time event. To remain effective and contain risk over time, provisions must be periodically reviewed – and once a year may not be sufficient. It is the determined risk that should dictate how often measures and readings should be reviewed. If there is deemed to be a medium risk level, for instance, reviews and evaluation of raw data must be conducted at more regular intervals. If the evaluated data shows a negative trend, meanwhile, the risk level will need to be modified and the data evaluated more frequently still.

To check that water microbial content remains within safe limits not just in the cooler months but also throughout the summer when temperatures can soar in southern Europe, for instance, adapted control measures may be needed (the capacity to control the

temperature of water, or to increase the sanitization process during peak temperatures), along with increased frequency of sampling and analysis.

Similarly, training those working in Class A and Class B facilities to understand human contamination risks won't mitigate eventualities such as holiday cover when other employees or temporary staff may be covering that work.

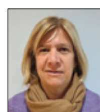
Closing the Gaps

Bearing in mind some of the shortcomings we have encountered with companies' CCSs and associated policy implementations relating to existing (rather than new) products/facilities, we have developed the following specific guidance to close the identified gaps:

1. Objective/scope: e.g., include/don't include product residue contamination in the policy
2. Compile all controls and monitoring systems
3. Gap assessment of the existing control measures and monitoring systems versus the requirements of the new Annex 1 (see table below for example scenarios):
    - Check the following:
    - » People
    - » Facilities, equipment, utilities
    - » Process
        - Close/open
        - Cleaning methods
        - Disinfection, sterilization methods
    - » Product and container closure
    - » Raw materials
    - » Sterility Assurance performance metrics
    - » Ongoing evaluation
4. Correct gaps identified in the gap analysis
5. Implement new control measures/actions
6. Close CCS policy
7. Review periodically, on an ongoing basis.

It is likely that guidance and recommendations will continue to undergo refinement as inspectors work through the process of offering their insights and feedback on the ongoing CCS measures implemented by manufacturers. This iterative process aims to ensure that companies can strategically allocate their resources and budgets to areas that will yield the most significant positive outcomes for product sterility and quality assurance.

Author Biography



Anna Cluet *is a Pharmaceutical Consultant and Senior Auditor at Rephine. She has been a qualified GMP auditor since 2005. She studied Chemical Engineering at the Instituto Químico de Sarrià and Industrial Engineering at Ramon Llull University, both in Spain.*

Contact: anna.cluet@rephine.com, www.rephine.com

Table 1. Gap analysis – example scenarios

Item	Current Measure	Reference (Annex 1)	Identified Gap & Resolution
Facilities	Filling machine (class A), people	4.30	No smoke test to verify homogeneous of the air- Non speed measure on the working position Unidirectional airflow systems should provide a homogeneous air speed in a range of 0.36 – 0.54 m/s (guidance value) at the working position, unless otherwise scientifically justified in the CCS. Airflow visualization studies should correlate with the air speed measurement.
	Differential pressure monitoring, recorded once per day No alarm system	4.16	Install visual and sound alarm
HVAC	Definition of number and location of sample points	4.28	Assessment to identify critical points that must be sampled and monitored
	Enter to class B from to a non-classified area	4.12	Personnel enter Grade B area from unclassified change room Grade C change room Grade B change room, which does not comply with "increasing cleanliness" defined in Annex 1 article 4.12.
	A list with the materials approved to enter to grade A or B is not in place	4.12	Material airlocks: used for materials and equipment transfer. Only materials and equipment that have been included on an approved list and assessed during validation of the transfer process should be transferred into the grade A or grade B areas via an airlock or pass-through hatches.
	Separation between personnel and material flows	4.12	No gap
Equipment	Some indirect contact parts are not sterilized	5.5	For aseptic processes, direct and indirect product contact parts should be sterilized. Direct product contact parts are those that the product passes through, such as filling needles or pumps. Indirect product contact parts are equipment parts that do not contact the product, but may come into contact with other sterilized surfaces, the sterility of which is critical to the overall product sterility (e.g., sterilized items such as stopper bowls and guides, and sterilized components).
Utilities	Monitoring plan does not include non-condensable gases, dryness value (dryness fraction) and superheat	6.17	Other aspects of the quality of pure steam used for sterilization should be assessed periodically against validated parameters. These parameters should include the following (unless otherwise justified): non-condensable gases, dryness value (dryness fraction) and superheat
	Exit and return of the loop is sample and analyzed twice per week	6.13	Ensure that at least one representative sample is included daily for the water used for manufacturing processes.

Expectations for Microbial Environmental Monitoring Investigations for Sterile Manufacturing Critical Areas

Angel L. Salaman -Byron

Janssen Biotech Inc.

Abstract: Microbial environmental monitoring (EM) is a semi-quantitative assessment that is extensively limited by method, sample size, technical variables, and biological variables. EM action level or out of trend excursions should be investigated and the analysis should be thoroughly documented. This article reviews the expectations for environmental monitoring excursion investigations for critical sterile manufacturing areas.

Keywords: Environmental Monitoring, Microbial contamination, investigation, cleanroom, environmental control, Grade A, microbial limit.

Introduction

ISO 14644-1 (2015) establishes the classification of air cleanliness specifically in terms of concentration of airborne particles in cleanrooms and clean zones.[1] The viable (microbial) and non-viable air particles limits (i.e., levels) shall be assessed to ensure the engineering controls, administration procedures, and aseptic behavior maintain the required cleanliness of the cleanroom.[2-4] Environmental Monitoring program (EMP) is a system to plan, organize and implement all the activities to achieve and maintain the required levels of air and surface cleanliness in the manufacturing areas.[2] The intent is to manufacture aseptic pharmaceutical products at a high quality, by foreseeing deterioration of environments in manufacturing areas, preventing bad influence on the quality of products, and performing appropriate cleanliness control through a proper monitoring of the manufacturing environment. An EMP should provide accurate and reliable information of the manufacturing environment to demonstrate against action and alert limits that the manufacturing environment process is safe.[2] Microbial EM action limit excursions and alert limit adverse trends should be documented, verified, and investigated.[5-10]

The real purpose of a microbiological EMP is to confirm environmental conditions, but the industry has been addressed out of EMP action limit excursions investigation at the same level and expectations as Out-Of-Specifications (OOS) investigations. OOS investigations are triggered by sample test results performed on a bulk product, final product, manufacturing raw materials and/or manufacturing equipment.[11,12] While microbial EM results are obtained from manufacturing cleanroom air or non-product contact surfaces such as walls, floor, personnel and, workbench.

This article reviews the reasonable expectations from environmental monitoring excursions investigations in critical aseptic process areas.

Out Of Specification Concept

An item specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria for raw materials, packaging components, labeling, testing, and performance specifications which are numerical limits, ranges, or other test criteria. The tests are described for the item filed with the relevant Regulatory Authority. According to International Council on Harmonization (ICH), Q6A (2000) Specifications "Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances" "…it establishes a set of criteria to which a drug substance or drug product should conform to be considered acceptable for its intended use".[13] FDA defines (OOS) as "all test results that fall outside the specifications or acceptance criteria established in drug applications, drug master files (DMFs), official compendia, or by the manufacturer".[11] Based on this definition, "OOS" is not an applicable concept for environmental monitoring results unless the test includes assaying components of the product (i.e., medical devices, sterile surgical instruments, etc.). Specifications are part of a total control strategy for the drug, pharmaceutical article, or medical device to ensure quality, consistency, and adherence to Good Manufacturing Practices, "e.g., suitable facilities, a validated manufacturing process, validated test

procedure, raw material testing, in-process testing, stability testing, etc. If any of the specification requirement is not met, an Out-Of-Specification (OOS) occurs.[11,12]

The term OOS was established in the Barr decision (1993) and started to identify the procedures and criteria to be used for the production and release of drug products. Differences in definition by different country regulations and changing regulatory opinions since the ruling have led to confusion and indecision when handling microbiological contamination investigations. The intent of many companies to satisfy regulatory expectations lead to an improper use of the term and overwhelmed processes which is what has happened with environmental monitoring of cleanrooms.[14]

Out of Trend (OOT) is generally a concept for when a result (i.e., product test data, for example product stability result) does not follow the expected results, either in comparison with other product batches or with respect to previous results collected.[11] Test results of starting materials and in-process samples may also yield out of trend data. OOT reveals that there may be a problem with the analysis or the production process. The result is not necessarily an OOS but does not look like a typical data point.[12]

A single result or several results that do not follow the expected trend, for a particular batch or series of batches, either in comparison with historical data is considered an Out of Trend (OOT) result. A product's adverse trend must be monitored closely. Additional time points may be required before the next scheduled time point to further confirm the trend. When a trend is regarded as adverse it is important to determine if the reason for the possible OOT is regarded to a planned change, for example, change in raw material supplier, manufacturing process, new equipment and/or equipment component.[11] Once identified as OOT, it is determined whether the adverse trend is isolated to one batch or is affecting many batches. In either case, an investigation must be initiated. A detailed investigation must document the review and assessment of the test data, the statistical models chosen, and follow-up actions as required. For example, the registered specifications may require review to ensure that the established limits are set at an appropriate level or if changes are required because of the investigation. Actions may also include but not limited to requesting an investigation to determine process, formulation or testing changes, new analyst, equipment or instrument changes, or deviations associated with the batch.[11,12]

Therefore, the purpose of the OOS and OOT investigations is to determine the cause of the non-compliance result or adverse trend. The root cause of the out-of-compliance result is used to determine the corrective action. The expectancy is avoiding the re-occurrence of the event.[11]

Microbial Environmental Monitoring Investigations

21 Code of Federal Regulations (CFR) Part 211.42 establish the requirement for "A system for monitoring environmental conditions".[15] FDA Aseptic Processing Guidance states:

"This program provides meaningful information on the quality of the aseptic processing environment (e.g., when a given batch is being manufactured) as well as environmental trends of ancillary clean areas".

There is a misconception in the industry that a robust microbial EM program can detect nearly all aerobic bacteria and fungi present within environmentally controlled areas. Any manufacturing process where personnel are required, the recovery of microorganisms at some level is inevitable.[5-10,16] Other common sources of microbial contamination include raw materials, the air, and inanimate items such as surfaces and water. Microorganisms may be transferred directly (e.g., touching a surface with a contaminated object) or indirectly (e.g., distributed through the air). There is no single microbial medium or practical combination of media and incubation conditions that can reliably cultivate all possible microbes that may occur.[8,9] In fact, the amount of air and surfaces routinely sampled within process cleanrooms is extremely small compared with the total volume of air supplied and the surface area present.[5,7,8] In addition, the lack of precision of enumeration methods and the endogenous variability of biological results is characteristic of bioburden recovery testing. Harmonized pharmacopeia "Microbiological Examination of Non-Sterile Products (Total Viable Aerobic Count)" recognizes the inherent limitations of the enumeration methods and allows a test value exceeding the monograph limit (or acceptance criterion) by not more than a factor of five.[18-20] Similarly, USP <51>, "Antimicrobial Effectiveness Tests," notes that variations in test values may exist when multiple samples are collected over time and allows count variability in logarithmic intervals (0.5 $\log^{10}$) for selected results.[21] In the case of microbiological assays, the USP prefers the use of averages because of the innate variability of the biological test system.

Microorganisms are not distributed evenly in the air and surfaces which leading to more variability. In fact, any microbiological EM sampling plan cannot prove the absence of microbial contamination, even when no microbial contamination is recovered. The absence of growth on a microbiological sample means only that growth was not detected; it does not mean that the environment is free of contamination.[16,17] Microorganisms' recovery methods rely on the appearance of visible colonies containing one or more clonal bacterial cell strains. The establishment of A Microbial Environmental Monitoring Program (EMP) alert and action limits relies on the ability of microorganisms to make colony forming units.[17] Therefore, the microbial EMP is considered a semi quantitative exercise as a complete quantification of microorganisms is not possible.[5-10]

Every EM excursion shall be investigated initially to discount an assignable cause of Laboratory Error. Therefore, the first phase of such an investigation includes an assessment of the accuracy of the laboratory's data. The manufacturing side must have an EM event plan to make the investigative process consistent. The plan shall provide a clear differentiation between Alert Limits and Action Limits, guidance for the identification of organisms, list of the items to be evaluated, EM historical data and excursions analysis to identify adverse trends and/or similar occurrences. Finally, the EM event plan will provide guidance on notification to production, resampling and following up testing requirements, and timely closure of the investigation. The Microbial EM investigation outcome will be

evaluated to assess the quality of the product batch or batches and the evaluation documented. Considering that "*Monitoring or testing alone does not give assurance of sterility* [Annex 1 (2022) section 2.2.], Annex 1 (2022) say *"Where aseptic operations are performed monitoring should be frequent using methods such as settle plates, volumetric air and surface sampling (e.g., swabs and contact plates). Sampling methods used in operation should not interfere with zone protection. Results from monitoring should be considered when reviewing batch documentation for finished product release. Surfaces and personnel should be monitored after critical operations. Additional microbiological monitoring is also required outside production operations, e.g., after validation of systems, cleaning and sanitization."*[3]

Discussion

As stated before, the scope of OOS has gone beyond the ICH definition and has been applied to more than just product specifications. Regulatory bodies have clear expectations for the total particulate and microbiological control levels in aseptic processing and aseptic preparation environments. However, different aseptic processes have different levels of risk relating to biocontamination. For example, there are more contamination risks when the product or ingredients are opened and exposed in a Grade A/ISO 5 than those that were added through closed transfers. In 'closed systems' the risks are limited to the sterile interfaces and aseptic connections/disconnections. Eudralex Annex 1 (2022) guidelines for clean-areas classification, in a Class-A environment recommend limits for microbial contamination of 0 CFU/m^3 in air sample, 0 CFU/4 hours in settle plates (diameter 90 mm) and, 0 CFU/plate in contact plates (diameter 55 mm).[3] Sharp and coworkers (2010) reported that ISO 5/Grade A zones (clean and correctly designed with unidirectional airflow) at rest conditions, shown zero counts of ≥ 0.5 μm and ≥ 5.0 μm particles, i.e., particles were absent at all; and in operation, there were zero counts, even when an operator in a cleanroom was shaking a hand slightly at a distance of approximately 10 cm aside the sampling probe at the same level (height).[22] The evidence and documentation around an isolate excursion in Grade A would not provide enough information to determine a root cause as Grade A data is mostly 0 CFU. Therefore, many samples (and a large volume of air or surface testing) would be required to demonstrate an adverse trend or a recurrent breach in environmental controls. In fact, a more meaningful interpretation can be made by evaluating the portion of samples that yield any growth.[17] The outcome of an investigation would be expected to identify at least the most probable cause if the actual cause cannot be fully identified. Only when data are collected that relate time or location to multiple microbiological observations a meaningful conclusion can be drawn. Similarly, when a single EM excursion occurs, it should be noted and adequately catalogued to enable a comparison when other anomalies occur. This type of analysis is suited for atypical isolates (qualitative data), so rational data storage and retrieval systems are needed to enable this system to work. Statistic Environmental Monitoring data systems coupled with artificial intelligence software may offer an excellent tool to determine adverse trends.

There is also a misconception about entitled Grade A classified areas in low bioburden manufacturing (e.g., Biologics). The Grade A EM limits requirements would be difficult to meet where human intervention is needed. Therefore, a high number of investigations may occur in the absence of a thorough assessment of potential microbial contamination risks. The Grade A area is a zone for sterile product manufacturing. It is widely dedicated to high-risk sterile operations such as fill/finish (i.e., sterile filling of vials and syringes), stopper bowls, open ampoules, vials, and making aseptic connections. The expectation of zero contamination at all Grade A locations during every aseptic processing operation is likely unrealistic in the presence of human intervention. In practice low bioburden manufacturing should not have Grade A areas. Grade A requirements could be applied for processes in unidirectional air flow units within ISO 7 areas for cell-culture process, for example[23] to mitigate microbial contamination to the product (e.g., cell culture).

Grade A microbial in-process excursion is considered the worst case for microbial contamination, as the presence of contamination was detected. It is a breach in the environmental control that occurred within the critical zone where the product and components were exposed to the environment. For this reason, every Grade A microbial excursion must be promoted and investigated. For example, a group of five test results with 1 CFU each one may have more significance (i.e., possibly indicating an adverse trend) in a Grade A area than a single 5 CFU result out of five samples (i.e., one result is 5 CFUs while other 4 samples are 0 CFUs).

Microbiological EM limits must be reasonable in terms of the capability of the recovery method. This leads directly to the question of the linear range of plate counts. USP relies heavily on the established scientific literature in its discussion of this range of countable colonies on a plate to note that colonies have a lower limit of quantification of approximately 25 colonies per plate.[24,25] This is opposed to the level of less than one CFU per plate.[3] EM alert and action levels between 1-10 CFU range is of questionable accuracy.[26,27] In that instance, it is highly suggested the verification of plates by a second technician and document the outcome.

Because of the relative rarity of microbial EM action limit events in Grade A, the investigation of EM action limit excursions often proves to be identified as a likely preventable event. For example, the loss of glove integrity or the accidental introduction of material into a closed Restricted Access Barrier Systems (RABS) or Isolator that has not been previously decontaminated are among the most common root causes of microbial contamination excursions.

Finally, viable limit excursions in ISO 7 or ISO 8 aseptic manufacturing supporting areas at rest conditions (i.e., Grade C and Grade D) are less likely to be amenable. Investigations should be addressed to identify adverse impact to critical processes with higher quality air areas. Support areas are less likely to be a thread to the manufacturing environment but may contribute to increase the ingress of undesirable contamination in critical areas. Therefore, the impact assessment of the event must be addressed about the possible adverse impact on critical areas and/or aseptic manufacturer processes. It is not suggested to consider CFU-count-based alert

and action levels as *one-size-fits-all* to assess environmental control conditions. The risk to the manufacturing process or product must be considered case-by-case. For Grade C and Grade D an evaluation of contamination recovery rates in addition to EM limits will help to understand the excursion event around the EM event.

Engineering Controls

Airborne microbial contamination in isolators has always been an exceptionally rare event, and this is true even of the flexible wall turbulent airflow isolators used in sterility testing. As isolators eliminate direct contact between human operators and products, any aseptic manipulations within the isolator are made with half-suits or glove ports which allow the manipulation within the isolator. The greatest risk of contamination in isolators has been thought to arise from glove tears, separations, or pinhole leaks.[26,27] Sterilization-in-place processes decontaminate other isolator surfaces with steam and chemical treatments to prevent microbial growth. Isolator systems are either "open" or "closed." Microbiological sampling of surfaces that have been decontaminated with Vapor-Phase Hydrogen Peroxide (VPHP) is unlikely to be positive. RABS are a type of sterile processing environment for non-sterile and sterile manufacturing. RABS are built inside ISO 5-7 clean rooms. They provide ISO 5 unidirectional air inside the barrier and prevent contamination with an air overspill system from within the barrier. Open RABS have specialized barrier openings to enable human intervention. Closed RABS do not allow human intervention and operate with the same operator restrictions as isolators. Closed RABS operate with positive or negative pressure, like isolator systems. Sterile items are manipulated in RABS using glove ports. Materials are transferred aseptically without opening the system. A RABS, like other regulated cleanrooms, requires decontamination before use. RABS designs are less capable than isolators relative to their ability to exclude microorganisms. Some activities require the operator to access the interior of RABS, increasing the likelihood of the contamination associated with the aseptic intervention. On the other hand the use of RABS requires process items to be sterilized remotely, transferred to the RABS, aseptically installed, and set for operation. This represents a further risk of microbial contamination that cannot be avoided.[28]

Real-time viable particle detectors have been added to Isolators and RABS.[29] Real-time viable detectors use optical techniques to determine particle viability on a particle-by-particle basis. Real-time viable particle detectors must differentiate between viable particles and non-viable particles. False positive results occur when non-viable particles are classified as viable particles. False positives can occur due to the non-specific nature of the laser induced fluorescence (LIF) technique. Non-viable particles such as pollens, skin flakes, and paper dust have fluorescence properties and create optical signals that must be addressed during instrument design. Typically, higher sensitivities result in higher levels of false positives. These false positive results are known as "noise". For example, if Grade A is placed on a result of 1 cfu it is highly recommended to perform a data analysis for establishing deviations that are not random perturbations of the system. If the investigation reveals a real-time viable particle detector issue, the data shall show an unusual pattern (from the baseline) even if they were considered "noise". There, the search for assignable causes of non-random data should be emphasized for use in identifying needs for process improvement.

The use of Vertical Horizontal Unidirectional Air Units, Biological Safety Cabinets and Gloveboxes increase the likelihood of microbial contamination. Aseptic manipulations in those units do not have the protections from the operator like isolator and RABS. Most processes would need assistance that should be located nearby to transfer material in and out the Grade A area. All these transfers are considered interventions and increase the likelihood of contamination even more. Materials must be sanitized thoroughly prior to entering the aseptic perimeter. The aseptic perimeter should be disinfected before and after processes. Batches most be worked one at a time. Because the in-process EM sampling is handled by the operator, the possibility of contamination may occur leading to false positive results during every step on the process including the delivery of samples to the QC laboratory. Therefore, Aseptic Behavior is key.[23] The following items are recommended to evaluate the production room and primary and secondary engineering controls.

- Classified Room (Refer to Annex 1):
  - Is the area visually clean?
  - Is there peeling paint, chipped drywall, acoustic ceiling tiles with cutout holes, rusty stainless steel or other breaches in the walls or ceilings?
  - Is the aseptic workbench organized as instructed on site procedures?
  - Is there any new equipment additions, room layout changes and/or any other process changes that may not have been evaluated for air flow?
  - Are return vent(s) unclean or presence of foreign material.
  - Are HEPA filters caulked around each perimeter to seal them to the support frame.
  - Are classified areas used for other activities not essential for manufacturing?
  - Are access doors and path thrus functioning and closing as intended?
- Engineering Controls (Refer to Annex 1). In addition:
  - Is the HVAC of the Isolator/RABS unit on 24/7 or is there a shutdown period?
  - If there is an "off" period, is there a cleaning prior to start of the unit?
  - Are system alarms reviewed for loss of pressure, are pressure differentials maintained?
  - Was a visual inspection performed? Is there presence of dirt, soils, debris, leaks or condensation?

- » Turn on the system: Is there any unusual vibration or noise from the HEPA filter or equipment?
- » Is there any issue with the Grade A area during the process? Are there any issues that occurred during the process around the Grade A area?
- » Was there any intervention during the process? Was the intervention documented and described?
- » Was a smoke study performed and evaluated within the unit?
- » Include the advice of outside expert on the matter.
- » Determine a microbial contamination risk-reduction plan.
- » Include an effectivity check.

The Human Factor

Operators, even when carefully and correctly gowned, continuously leach skin particles that may have microorganisms into the cleanroom environment.[5-10] Cleanroom operators, particularly those engaged in aseptic processing, must strive to maintain suitable environmental quality, and must work toward continuous improvement of personnel operations and environmental control. Japanese *"Guidance on the Manufacture of Sterile Pharmaceutical Products by Aseptic Processing" (2011) states that "Deviations from the action level specifications should be investigated for cause(s) prior to shipment of final products manufactured through the process where the deviation occurred, and corrective measures should be taken. The validity of corrective measures taken should be verified to confirm the recovery of acceptable environmental conditions, as needed. The recovery may be readily confirmed in some instances. For example, counting particulate matter, but not reproducible in other instances, such as with bacteria adherence to gowns. If the cause(s) cannot be traced, recovery should be established by general approaches including prohibition of personnel entry for a certain period, retraining of personnel, and reviewing assigned tasks."*[2]

Features to be considered during a microbial EM excursion investigation within Grade A areas would be the occurrence of unusually high number of colonies recovered, if this incident is isolated or can be correlated with other recoveries and the identity of the organism recovered. Excursions beyond approximately 15 CFU recovered from a single sample, whether airborne, surface or personnel should happen very infrequently in aseptic processing environments. However, when such occurrences do occur, they may be indicative of a significant loss of control, particularly when they occur within the ISO 5 critical zone near product and components. It is advised that any excursion >15 CFU should be the subject of a careful and thorough investigation (USP 1116). An investigation for an isolated single excursion, establishing a definitive cause probably will not be possible, and only general corrective measures can be considered. It is never wise to suggest a root cause for which there is no solid scientific evidence. Therefore, it is likely that any microbial EM investigations of an isolated viable action limit does not provide enough robust evidence that the cleaning and sanitization performance, gown usage or operator aseptic behavior is a definitive root cause of the event. Sub-optimal cleaning and sanitation, and process conditions are the most common contributor of Grade A events in the presence of operators. The most common inadvertent error in aseptic techniques is the unrecognized transfer of microorganisms with no aseptic technique error involved. The most likely source of the contaminated samples is touching contamination. However, the author recognize that contaminated samples can also result from airborne sources[30] Such events are rare or simply do not happen when the human presence is drastically reduced or removed from production.

In general, the fewer personnel involved in aseptic processing and monitoring, will reduce the risk of microbial contamination. In Grade A areas within an aseptic processing operation, the microbial recovery should be less than 1% of the EM samples.[6] The risk of microbial contamination during sterile product preparation would be practically non-existent were people not involved in the process. For technologies such as isolators or closed RABS, the recovery rate must always approach zero.[5] Robotic arms and robot systems remove human intervention during sterile compounding will reduce EM excursions dramatically.[31,32]

- Human Factor:
  - » What activities were being performed at the time the viable EM sample was being collected (material transfer, aseptic manipulation, etc.)? Was the product exposed at the time of the sampling?
  - » Oversight activities for Aseptic Behavior.
  - » Operator interview using a standardize checklist. Include observations in the investigation text.
  - » Determine and list potential "at-risk behavior". Connect action with consequence.
  - » Identify system errors that could result in repeat events but not acting upon them.
  - » Assess human factors or human error.
  - » Identify areas where the human error has been mitigated in previous investigations to minimize repeat events.
- Procedural Controls:
  - » Evaluate recent changes in manufacturing procedures; Is there personnel, material, or process flow change?
  - » Is there any recent change in gown supplier or material?
  - » Is there any outstanding number of personnel in the room at the time of the event?
  - » Review most recent gloved fingertip/out of room personnel microbial sample results.
  - » Review last Aseptic Process Simulation report.
  - » Review cleaning and sanitation procedures/documentation.

» Review cleaning and disinfection agents

» Review personnel qualifications

» Review environmental sampling procedures and bioburden recovery methods; confirm recovery methods are qualified.

Contamination Recovery Rate Analysis

USP <1116> defines Recovery Rates as *"...the rate at which environmental samples are found to contain any level of contamination. For example, an incident rate of 1% would mean that only 1% of the samples taken have any contamination regardless of colony number. "contamination recovery rate as the percentage of plates that show any microbial recovery irrespective of number of CFU. "*[6]

A microbial EM program should be able to detect changes in the microorganism's recovery rates that may be indicative of breaches in the state of control of the room or facility.[2-4] Microorganism's EM program results are compared against established action levels provided by United States Pharmacopeia (USP) and European Union Pharmacopeia (EU), current good manufacturing practice (cGMP), Food and Drug Administration (FDA) among other guidelines. Microbial EM levels should be threshold values to balance between an adequate control but without triggering toward unsafe environment conditions for manufacturing. Microbial EM programs are intended to assess the in-process environmental controls intended by design and cleaning/sanitization programs to maintain cleanliness conditions of the manufacturing-controlled environments. The reality is that Microbial EM programs are not validated processes and an EM action level excursion of the manufacturing environment is not a direct testing to assess product quality attributes. In fact, a microbial EM action level excursion is not considered an out-of-control incident of the manufacturing environment.[6] Microbial EM action and alert limits are not considered control measures. Therefore, tightening of the EM levels or increase EM sampling frequency are not considered corrective actions.

When contamination recovery rates or number of action limits events increase from an established norm a process for determining the possible source should be initiated.[34-36] For example, a group of five test results with 1 CFU represent 100% contamination rate while a single 5 CFU result out of five samples (i.e., one result is 5 CFUs while other four samples are 0 CFUs) would be 25% contamination rate.

EM investigations may differ depending on the criticality of process step, the quality attributes of the product, and area where the product is manufactured such as a cleanroom, Unidirectional Air Flow area, RABS, or Isolator. The investigation should include a review of area maintenance documentation; visual inspection of the compounding area, sanitization/decontamination documentation; the occurrence of non-routine events; the inherent physical or operational parameters, such as changes in environmental temperature and relative humidity; and the training status of personnel. Refer to PDA Technical Report No. 88, *Microbial Data Deviation Investigations in the Pharmaceutical Industry* has an excellent guidance for Microbial EM investigations.[34]

EM Adverse Trend investigations

Trending environmental monitoring (EM) data is a regulatory requirement.[5-10,16,38] Microbial EM data deviations or non-conformances periodical assessment for the presence of adverse trends may occur.[3] In addition, EM report on OOT, OOS, Corrective and Preventive Actions (CAPA) and effectivity checks. However, unlike chemical analytical deviations, all microbial non-conformances to include OOS and CAPA investigations, may require months to complete. Therefore, studies-based protocols and additional laboratory tests may be required for correction effectivity checks.

Adverse trend issues must be carefully considered. Variation in microbial counts is an expected phenomenon. The reliability of environmental results resides in validated methods and appropriate procedures that apply. Operator sampling technique variability as well as potential mishandling of samples and laboratory errors (i.e., some degree of subjectivity during colony counting) are unavoidable factors that will make it difficult to ascertain the determination of assignable cause. As stated above, it is unlikely that the cause of the Microbial EM excursion would be determined. Previous occurrences, sample type and location, proximity to the exposed product process and/or product contact surfaces, and other factors must be considered.

An isolated EM action limit excursion does not provide information about a possible control breach or does not identify a possible risk. It is only when data is pulled in the same framework and appropriate timeframe is it suitable to identify a potential risk. Therefore, Microbial EM alert and action level excursions must be evaluated every time new data is generated in an ongoing basis to identify runs of data that indicate a potential adverse trend, whereby an investigation is initiated if an adverse trend is identified. Once the adverse trends have been identified the environment must be monitored closely. Additional time points may be required before the next scheduled time point to further confirm the trend. The data analysis should determine if the adverse trend is isolated to one area/room or other adjacent areas/rooms. In either case, an investigation must be initiated.

Every EM program must include quantitative/qualitative assessment to identify an adverse trend. In case a potential mold adverse trend is confirmed based on site criteria, a full Quality investigation must be initiated to confirm such an adverse trend, identify the root cause, assess product quality impact, and define corrective actions to restore baseline values in the process/facility. Mold adverse trends do not necessarily indicate that product quality has been compromised but do indicate the need to identify a possible breach in environmental controls. A risk assessment should be performed to evaluate if the manufacturing process should be halted pending resolution of the issue and completion of a "return to service" plan. The EM plan must specify which actions (e.g., investigation and/or preventive measures) need to be taken in case of an adverse trend. However, mold isolation events below total microbial count alert levels may not require

specific actions or investigations given limited risk of these events. Nevertheless, a procedure should establish in what cases additional/special cleaning including sporicidal agents and, verify the absence of the mold in the impacted area/s (i.e., to verify effectiveness of cleaning procedures for removal of the organism) must be considered or performed.

A statistically robust set of rules is proposed for trending excursions in environmental monitoring data. These rules should be designed to minimize false alarms when the process is in control but signal quickly when the process goes out of control. An adverse trend is an early warning that the system is drifting from normal operating conditions. Prompt action may prevent further deterioration and avoid costly out-of-specification events. Adverse trends should be defined by site procedures and followed. Systems such as 98th percentile/95th percentile, provide a common ground to compare historical data even if they are not strictly associated. These percentiles were chosen because they are functional equivalents of control limits and warning limits used in statistical process control charting, which are set at three and two standard deviations above the mean, respectively. In addition, the USP <1116> recommended microbial recovery rates should also be implemented as trend metrics for microbial environmental monitoring of aseptic processing facilities. Occasional isolated alert level excursions may occur even if the process remains in a state of control. However, repeated alert level excursions occurring at a rate greater than 2.5% indicate the process is changing and the system is drifting from normal operating conditions. An adverse trend of alert level excursions should be investigated for root cause. It is critical to determine if an alert level excursion, at its onset, triggers an adverse trend. Rationale for choosing these rules must be justified.

EM trending must be performed for many reasons, including:

- Regulatory compliance
- Ensuring a state of control of the facility
- The ability to be proactive before a problem gets out of hand
- To provide a graphical representation of the data
- To determine any problem areas in the facility
- To determine if the cleaning and disinfection program is working as expected
- Monitoring the microbial flora of the facility and seasonal trends
- Providing a simpler means of communication of the EM data to management
- Identifying sources of microbial contamination.
- Establishing alert and action levels

Root Cause Analysis

The root cause analysis tool or methodology suggested to determine assignable/root cause should be the Fishbone Diagram, also called: cause-and-effect diagram or Ishikawa diagram. This cause analysis tool is considered one of the seven basic quality tools. The fishbone diagram identifies many possible causes for an effect or problem. It can be used to structure a brainstorming session. The major categories of causes suggested to assess during the investigation are the following:

- Methods: procedures, work instructions for process flow, cleaning and sanitation, gowning, aseptic behavior, others.
- Machines (equipment): stand alone or room engineering controls and/or other equipment used during the impacted process.
- People (manpower): interview outcome of manufacturing personnel and those who handle samples as applicable/training and qualifications. The use of a questionnaire and/or check list is highly recommended.
- Materials: materials used for the sampling.
- Measurement: system used to measure the non-conformance
- Environment: manufacturing environment assessment at the time of the sampling
- Mother nature: described as surrounding conditions around the manufacturing process that cannot be controlled and/or predicted.

Every category has multiple possible contributors to the event. The analysis will continue until the root cause(s) or possible root cause of the excursion have been identified. For each cause, supporting information must be provided and documented. Some areas would need some sort of questioning methods, such as the 5 Why's.

Conclusion

Microbial EMP is a semi-quantitative methodology limited by method, size, operator technique, and biological variables. The EMP ensures the manufacturing environment is within viable and non-viable counts requirements. There is a misconception that has led to an inappropriate use of microbial environmental monitoring results of critical areas in sterile manufacturing as a surrogate for product release criteria. This is especially troublesome when establishing alert or action levels at very low quantitative levels and assignable root cause is hard to be identified.

Microbial EM sample results are historically variable and depend on the method. However, any action level or out of trend should be defined and the analysis should be documented. There are different strategies that can be established by procedure to follow up alert and action limits excursions and adverse trending to assess the environment quality. Remember that sterile products do not consider manufacturing EM limits as a product specification.

Disclaimer

The scenarios discussed in this article were created by the author to establish his point of view. The views and opinions of the author expressed herein do not necessarily state or reflect those of Janssen Biotech Inc. and the Johnson and Johnson Family of companies.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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Sustainability in Pharmaceutical Packaging

Duncan Flack
Global Sustainability Leader
Honeywell Healthcare Packaging

The pharmaceutical packaging industry is facing increasing pressure to adopt more sustainable practices and to use more sustainable materials. This is in part due to the carbon footprint of pharmaceutical packaging, which is traditionally plastic based. One study estimates that the pharmaceutical industry is responsible for emitting around 52 million metric tons of CO2 annually.

Furthermore, consumers are becoming increasingly influential over the demand for sustainable products and materials. Approximately 70% of global consumers are willing to pay more for sustainable packaging. Similarly, a demand for a more environmentally friendly packaging is also rising in the pharmaceutical sector, with more patients and consumers considering sustainability factors when choosing products. For example, since April 2022, National Health Service (NHS) procurements have to include a minimum 10% net zero and social value weighting in the United Kingdom (UK).

This shift is due to the growing awareness of the environmental impact of plastic pollution, the increasing demand for recyclable and compostable packaging materials, and the need to reduce the carbon footprint of pharmaceutical supply chains.

In response to these challenges, the pharmaceutical industry is developing new and innovative packaging solutions.

The Environmental Impact of Pharmaceutical Packaging

Steps are being taken by organizations and industries to improve the use of bio-sourced materials, recycled content, or where permitted, alternative compostable and sustainably sourced packaging materials. However, there remains a significant challenge surrounding the use of energy and resources in the production of most packaging materials and greenhouse gas emissions throughout the supply chain and subsequent transportation of packaging materials from manufacturing to the point of sale. Additionally, many packaging formats, whether sustainably derived and sourced or otherwise, do not have sufficient infrastructure or processes in place to mitigate the generation of waste at the end of life of the packaging.

Organizations such as the Circularity in Primary Pharmaceutical Packaging Accelerator (CiPPPA) have been assembled to combat these issues and are working to reduce pharmaceutical packaging waste and deliver aspects of circularity. Duncan Flack, Chairman of CiPPPA said

> "CiPPPA is a Not-for-Profit industry-wide, multi-stakeholder initiative with a mission of enabling companies, as well as members of the public, to recycle their primary pharmaceutical packaging, thereby contributing to the elimination of waste, whilst reducing greenhouse gas emissions. The initiative will achieve its ambitions by focusing on the packaging waste generated by blister packs, injector pens, and inhalers, and is being supported by major players across the Big Pharma and Healthcare sectors".

The Founding Principles – Reduce, Reuse, Recycle

Working within the founding principles of sustainability of "Reduce, Reuse, Recycle", one of the simplest ways of improving the sustainability of pharmaceutical packaging is to use less packaging.

Some materials can be thinned down, whilst other improvements can be achieved by choosing lightweight materials or switching from one packaging format to another.

For example, by packaging drugs in a thermoformed blister made of Honeywell's Aclar® instead of Cold Form Foil (CFF), the blister card can be reduced in size by up to 50%, while still delivering a high moisture barrier to protect medicines. Whilst blister cards are typically already very light weight, the consequential savings that are derived from smaller blisters, smaller cartons, smaller shippers, and the reduction in volume taken up in shipping containers, all represent changes that can be easily implemented, requiring less energy to transport, and enabling emissions reductions within pharmaceutical supply chains.

Despite various steps to reduce or reuse packaging, regulations and health concerns surrounding patient safety rightly remain utmost, and many items are still inherently single use by design. In such instances, recycling materials is often seen as one of the most effective ways to reduce the environmental impact of pharmaceutical packaging.

Recycling pharmaceutical packaging materials is far from straight forward, as traces of active pharmaceutical ingredients need to be considered within the recycling process and the energy requirements for the collection, sorting, collation, and recycling of the materials, whilst reducing the amount of waste sent to landfills or incineration, can still be inhibitive when looking at the footprint associated with the full product lifecycle.

Biodegradable and compostable materials are becoming increasingly popular in the packaging world, and in certain secondary or tertiary applications these materials which break down naturally in the environment without releasing pollutants or unwanted materials are gaining popularity.

Mono materials are single structure materials that can be easily recycled. Companies are increasingly looking to embrace these mono structures for low barrier purposes to help keep packaging materials out of landfills and ensure that they are either reused or recycled.

Further developments see pharmaceutical packaging gradually designed for easy recycling. Typical changes include using clear labelling that makes it easy to identify the materials used in the packaging and using standard packaging formats which allow recycling facilities to process the materials more easily.

At the Forefront of Innovative and Sustainable Packaging Solutions

Despite these advances and refined approaches, the fundamental requirement that pharmaceutical packaging must meet is the need to balance sustainable packaging whilst ensuring the safety and efficacy of medications. Pharmaceutical packaging must protect medications from contamination and degradation, and it must also be tamper-evident to prevent counterfeiting.

High barrier materials which enable degradation and contamination-free packaging require careful engineering and manufacturing and isn't always a cheap option. This means that high performance sustainable packaging materials are often more expensive than traditional materials, which can ultimately push up the cost of medications.

There are, however, several emerging opportunities associated with sustainable pharmaceutical packaging. As the industry responds to the increasing pressure from consumers and regulators to adopt more sustainable practices, demand for innovative and sustainable packaging solutions will also increase.

As governments set emissions reductions targets and more companies publicly disclose their Science Based Target Initiatives (SBTis), net zero goals, and carbon reduction programs, sustainable packaging and policies that support their market growth will play a key part in the achievement of these targets.

In the coming years, we are likely to see even more innovative and sustainable packaging solutions being developed. These solutions will reduce the quantity of packaging produced, challenge the sourcing of materials that are mined or derived from fossil fuels, and ultimately serve to reduce the environmental impact of pharmaceutical packaging. All while still ensuring the safety and efficacy of medications.

Revitalizing Quality Management Processes Starts with Eliminating Paper and Siloed Standalone Solutions

Kari Miller

Senior Director, Product Management, QMS
IQVIA

In today's life science industry, the adoption of digital technologies for greater efficiency and compliance has become commonly accepted, whether it is the patient using apps and wearables that report data to healthcare providers or data analytics for overall management, insights and trending. Quality management is no exception as teams embrace technologies to drive innovation and remove error-prone, labor-intensive paper-based systems or standalone solutions. Electronic quality management systems (eQMS) help instill quality principles into the overall culture of an organization, aiding in the achievement of harmonized policies, processes, and procedures. As a result, organizations are better positioned for growth, employee alignment and adherence to ever-changing regulatory policies. Those with paper-based systems will struggle to keep up with the rapid pace of change in regulatory requirements and the competitive edge technology provides other organizations.

Automating quality management operations brings better efficiency and consistency across a life science organization. The challenge in embracing a digital system is often as simple as individuals facing the reluctance to change from the traditional paper-based systems. So, even though paper-based systems have their own challenges and drawbacks, change is uncomfortable. These paper-based system issues include the need for greater manual administration, costly maintenance dependent on headcount and data errors due to how the information is collected, documented and then retrieved. Siloed systems have most of the same issues.

A digital eQMS brings a consistent methodology into the quality process, eliminating many of the prior challenges. It allows for flexibility for future innovations and accommodates current technologies, allowing necessary scalability, parallel processing and efficiency that paper processes are unable to provide. This singular, integrated environment manages and tracks QMS processes to simplify operations while also reducing risk and accelerating deployment. The shift to digital quality processes from paper will require operational, functional and departmental changes, in addition to cultural change.

Life science organizations that embrace comprehensive electronic quality solutions for true digital transformation and innovation require a change management process that integrates quality into the core culture. Engaging employees from the start through seeking individual insights will lead to better adoption. In the instance of one multinational life sciences manufacturer, they found that harmonization was essential as they built out their eQMS. This involved gathering input from stakeholders from across the various divisions and offices around the globe, ensuring that each employee's voice was captured and incorporating their input on important processes. This led to employees embracing the system. As components were tested and implemented, continuous feedback was gathered, and all employees had a sense of ownership in the initiative. Early and ongoing participation brought a sense of harmonization rather than a standardization mandate that IT or management pushed down.

When introducing digital eQMS to your organization, there are several common challenges to avoid throughout the deployment process. Outside of culture and adoption, the quality team must build a future-focused organization to process data in near real-time while also analyzing historical events to be proactive, preventative and predictive.

Five Pitfalls to Avoid When Digitizing Quality Processes

For a successful journey from paper to digital, or even from siloed to integrated processes, it is key that organizations do not look for

shortcuts – adaptation to the change takes time and planned change management. Organizations should work to avoid the following five dangers when implementing a digital eQMS.

1. **Lacking a detailed roadmap**

   Teams need to develop and follow a detailed roadmap to correctly implement a fully integrated eQMS. This roadmap must include process harmonization and configuration, data migration and integration, a focus on project management, training, validation, testing and go-live support. Capturing employee feedback through the implementation is essential to ensure everyone is engaged. By harmonizing rather than mandating standardization, adoption increases and quality is built into the culture of the organization through inclusion.

2. **Rebuilding paper-based systems or siloed processes as is within a digital eQMS**

   When attempting to quickly shift to digital processes, many organizations begin by automating current paper processes. This approach tends to weave many paper-based inefficiencies into the new digital procedures such as sequential processing. Siloed processes also build in inefficiencies by not leveraging opportunities to eliminate duplicate efforts.

3. **Ineffectively managing expectations and scope creep**

   Prior to the full shift, it is key to touch base with every department that is involved in the quality management system. Teams may see an opportunity to move their department into the new system without being included in the core project plan. Clear internal expectations for the process and the rollout plan can reduce the risk of scope creep or disagreements over what should be included in the system. It may be necessary to pause the process and regroup so that the scope of the project is clear, and all departments agree to the roadmap.

4. **Avoiding migration planning**

   Organizing and uploading current documentation and information are two integral steps in moving paper out of the quality. Avoiding internal conflict on what needs to be brought forward from paper-based systems is key to the success of migrating and integrating all data into the new system. Additionally, when bringing together multiple systems, there will be some aspects that will not carry over. While this can cause conflict, a solid plan with appropriate rationale for what will be included can deter from bringing outdated quality and compliance approaches into the future. These outdated approaches can limit the value of the new system and inhibit growth and innovation. As not all systems will be brought forward, not all legacy data needs to be shifted into the new system. Clear communication and planning will help to alleviate concerns over what will and will not migrate over.

5. **Failing to communicate openly and in detail**

   Successful eQMS implementation relies on communication between teams and stakeholders. Communication and resource management will be imperative as teams must keep the business functioning during the implementation process. Communication channels should be created from the start, along with formal processes for feedback and conflict resolution, to aid in achieving target dates. Ensuring all departments involved know how the organization is moving forward, what is expected from all parties and how to resolve roadblocks or conflict during implementation and validation is key to the success of the culminating rollout.

   Additionally, a lack of interest among leaders or poorly managed internal communications can cause adoption to lag. The impact of the changes must be understood and supported throughout the entire organization. Change control must be treated as an ongoing activity with processes in place to monitor the impact and effect of all outcomes.

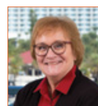
Successful Deployment Outlook

Moving to a digital eQMS is not as simple as it seems. It requires proper, detailed planning, a comprehensive roadmap and clear communication throughout the implementation process and beyond.

By harmonizing the processes, policies and procedures during the onset, these initiatives have a greater chance of achieving the anticipated improvements to operations, quality and patient outcomes.

It is time to embrace digital eQMS to drive the quality and safety of products and improve operations. Traditional paper-based methods may allow for compliance; however, they will very likely fall short regarding efficiency, productivity and continuous improvement. Successful eQMS implementations will improve operational efficiencies, increase revenue and patient satisfaction, reduce non-conformance in manufacturing and support continuous improvement. Furthermore, going digital allows for the use of technologies that will enable organizations to do more with less, solving business problems at a more rapid pace through improved detection, trending and decision support. Technology will allow for innovation to arise in ways that just cannot be supported by paper-based or disparate system implementations.

Author Biography



*As regulatory and product management leader for IQVIA Quality Compliance, **Kari Miller** is responsible for driving strategic product direction and delivering industry best practices and regulatory compliance solutions for quality management. She focuses specifically on translating market and industry requirements into industry-leading enterprise quality management solutions that meet the needs of the heavily regulated life sciences market. She is also responsible for the quality compliance product road map, product partner relationships, and overall product direction. Miller earned bachelor's degrees in business administration and psychology from Marian College, Fond du Lac, Wisconsin.*

Pittcon 2024

February 24-28 — San Diego, CA

So What is Pittcon?

- Pittcon is a dynamic, transnational conference and exposition on laboratory science, a venue for presenting the latest advances in analytical research and scientific instrumentation, and a platform for continuing education and science-enhancing opportunity.
- At Pittcon, our goal is to advance scientific endeavor through collaboration, bringing together a world of knowledge to impact, enrich, and inspire the future of science. Pittcon is a catalyst for the exchange of information, a showcase of the latest advances in laboratory science, and a venue for international connectivity.
- Proceeds from each and every Pittcon directly fund science education and outreach. Over 90% of Pittcon's net profit goes on to fund primary and secondary education, continuing education, scholarships, laboratory improvements, and outreach activities.
- Pittcon also offers networking opportunities, social events, and an environment that fosters knowledge and expands your network of scientific resources.

Exposition Dates

Monday, February 26: 9:00am – 5:00pm PST

Tuesday, February 27: 9:00am – 5:00pm PST

Wednesday, February 28: 9:00am – 3:00pm PST

Technical Program Dates

Sunday, February 25 – Wednesday, February 28

8:30am – 5:00pm PST

Short Courses Dates

Saturday, February 24 – Wednesday, February 28

8:30am – 5:00pm PST

What to Expect

Our goal is to advance scientific endeavor through collaboration, bringing together a world of knowledge to impact, enrich, and inspire the future of science. Pittcon is a catalyst for the exchange of information, a showcase of the latest advances in laboratory science, and a venue for international connectivity.

Exposition

Pittcon's transnational exposition gives you the opportunity to see the latest laboratory instrumentation, participate in demonstrations and product seminars, talk directly with technical experts, and find solutions to all your laboratory challenges.

Short Courses

With over 50 from which to choose, these courses cover significant topics in bioanalytics & life science, cannabis & psychedelics, environment & energy, instrumentation & nanoscience, pharmaceutical & biologic, professional development, and many more.

Technical Program

One of the foremost analytical chemistry conferences, Pittcon's technical program provides participants with direct access to the latest research and developments from top scientists and leading innovators found throughout the world.

Networking Sessions

These connectivity sessions provide a unique opportunity for attendees to meet other professionals with similar interests and backgrounds, working together to find solutions to a problem or collaborating to create novel laboratory approaches.

2024 Wallace H. Coulter Lecture

Sustaining Nanomaterials for Sensing Human Health and the Environment

Sunday, February 25, 5:00 PM PST

Presented by Omowunmi (Wumni) Sadik

BioSMART Center & New Jersey Institute of Technology

This presentation will describe the use of sustainable nanomaterials for understanding reaction mechanisms, small-scale synthesis, and biosensing. Sustainable nanotechnology is the research and development of nanomaterials with economic and societal benefits and little or no negative environmental impact. Hence there is a search for nano-synthetic methods that utilize sustainable materials without employing other reductants, capping, or dispersing agents. Notable developments include safer-by-design, electrosynthesis, sonochemistry, and the use of biomass, sugars, and flavonoid precursors.

Short Courses for Professional Development

With more than 50 topics to choose from, Pittcon's in-depth, professional quality Short Courses are a valuable, yet affordable opportunity for continuing education. Here are just a few of the topic areas to consider:

- Atomic Spectroscopy
- Biomedical Engineering
- Chemometrics
- Data Management and Data Analysis
- Environmental Analysis
- Gas Chromatography
- Homeland Defense
- Industrial Hygiene and Safety
- Life SciencesLiquid Chromatography
- Mass Spectrometry
- Microscopy
- Pharmaceutical Sciences
- Regulatory Compliance
- Sample Preparation
- Statistics
- Validation

Pittcon Tracks

One of the foremost analytical chemistry conferences, Pittcon's Conference programs provide participants with direct access to the latest research and developments from top scientists and innovators throughout the world.

Bioanalytics & Life Sciences

Biological molecules and xenobiotics (drugs, toxins) and their metabolites; study of biological systems; biosensors; forensic science and toxicology.

Cannabis & Psychedelics

Identification, quantitative measurement, extraction, and quality assurance of cannabis-based and psychedelic products.

Energy & Environmental

Environmental detection and monitoring; energy production and storage; sustainability, climate, and green chemistry; food science/safety and agriculture.

Food Science & Agriculture

Instrumentation, detection, and sensors; laboratory information systems, data analysis, and artificial intelligence; characterization and processing of nanomaterials; art and archeology.

Pharmaceutical & Biologic

Evaluating chemical composition and properties/activities of medicinal drugs and biologics; high-throughput screening and process control; drug discovery and design; personal care and consumer products.

Professional Development

Leadership and power/soft skills; career navigation, DEI (diversity, equity and inclusion), communication, and entrepreneurship; education and teaching and more.

Networking Sessions

Networking Sessions will run February 26, 27, and 28:

Networking Roundtables have undergone revitalization for Pittcon 2024! Workshops are now 90-minute sessions concentrating on practical applications. These roundtables provide an excellent opportunity to engage with a varied community of researchers tackling challenges in an open and dynamic setting. Participants can gain diverse insights and applications from individuals spanning undergraduate students, postdocs, industry leaders, seasoned scientists, as well as newcomers entering a laboratory.

What are the benefits of Joining a Networking Session?

- Increase awareness of a specific topic or issue
- Find solutions to a problem or brainstorm for new ideas
- Network with other professionals interested in and/or experienced with a topic
- Further yourself and your company's brand by sharing your knowledge and company's resources
- Face to face structured problem solving

An Interview with

Joseph Ehardt

Director Biologics, SGS North America, Inc.

What are the prevailing trends in biopharmaceuticals and biosimilars that are shaping the industry landscape today?

Biopharmaceuticals have greatly increased in the market year over year with current analysis indicating an increase in the range of 8-15% during the rest of the decade. As biopharmaceutical products are coming off patent, biosimilars are entering the market at an increasing pace, sometimes with multiple companies entering the market with the same drug.

As companies are able to decrease the cost of manufacturing of these drugs, and additional new drugs are released, the market for biosimilar products will continue to expand. This increases competition with the potential to drive prices down for the consumer.

There is also a trend in identifying new uses for drugs already on the market. Given the competitive landscape, this provides companies that are developing biosimilars with options to decide what treatment/s to target for the drug, creating a way to differentiate their product in the market.

In your opinion, what are the most pressing issues and concerns currently faced by the biopharmaceutical sector, and how are they influencing decision-making and strategy?

Speed to market is a significant focus for biosimilar developers. As a drug comes off patent, there are multiple companies that have a biosimilar ready for market. If a company is not first to market, this could dilute the company's market shares leading to decreased potential revenue.

The supply chain issues that we have seen since the start of the pandemic, such as longer lead times for raw materials and equipment, have a significant impact in the overall speed to market.

Another contributing factor affecting speed to market is the speed of testing and release of drugs. Whether companies are performing testing in-house or outsourcing to a contract lab, the timeline for testing biosimilar products has condensed due to the competitive nature of the market. Faster turnaround timelines for testing are critical for meeting tight development timelines.

All of these factors can influence a company to focus on being the first to market an affordable product that delivers as indicated.

Can you highlight any noteworthy recent developments or breakthroughs in biopharmaceutical research, manufacturing, or regulation that have the potential to significantly impact the industry?

The most influential development in the market is the use of artificial intelligence (AI). AI can scan multiple potential targets at once, resulting in a reduction in research and development, decreasing timeline and cost to expedite the drug's speed to market.

Looking ahead, what do you foresee as the most promising opportunities for growth and innovation in the biopharmaceutical and biosimilar space over the next few years?

Personalized medicine is targeted area of growth and innovation for biopharmaceuticals. As we learn more about specific genes affecting a disease, targeted gene therapy is an area of growth within the industry. CRISPR and siRNA gene therapy may have advantages to increase novel new drug development in the industry within the decade.

For the biosimilar market in particular, the opportunity to identify new uses for existing drugs could benefit the market and help to soften the competitive landscape by introducing multiple uses. An example of this is Semaglutide, a diabetes medication, that is now also prescribed for weight loss. As new usages are found, this will only increase market share for biological drugs.

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Pharmaceutical P.I.N. POINTS

Patent Innovation News

The purpose of this column is to highlight and summarize recent key patents in the pharmaceutical arena issued by the US Patent Office in August-September, 2023.

Neelam Sharma, MS,
Lakshmi Lavanya Kundurthy, BE and
Hemant N. Joshi, Ph.D., MBA*

Tara Innovations, LLC
www.tarainnovations.com,
**hemantjoshi@tarainnovations.com*

Abuse and Misuse Deterrent Transdermal Systems;

D.J. Enscore, F. Tagliaferri, S.P. Damon, A. Smith, J. C. Gaulding; Nutriband, Inc., USA; U.S. Patent # 11,759,431; September 19, 2023.

There are many drugs which have the potential for intentional abuse or accidental misuse. Many transdermal systems have a significant excess of the therapeutic agent to ensure that a therapeutically effective amount of drug is delivered to the patient. This patent describes a transdermal system, which deters abuse and misuse. One can abuse transdermal systems by placing in the buccal cavity, chewing the patch or extracting the drug. The inventors added an aversive or pungent agent, such as capsaicin, in the patch backing. The aversive agent shows a biphasic release profile comprising an immediate release followed by an extended-release profile.

High Concentration Antibody-Containing Lipid Formulation;

T. Morichika, D. Kameoka, Y. Imaeda, T. Maeda, O. B. Stauch; Chugai Seiyaku Kabushiki Kaisha, Japan and Hoffmann-La Roche, USA; U.S. Patent # 11,767,363; September 26, 2023.

In general, formulations containing antibodies are lyophilized and have high drug concentration. These formulations tend to be viscous. The inventors developed a formulation which inhibited the dimerization and deamidation during long-term storage. The formulation of humanized anti-IL-6 receptor antibody MRA contains 0.005 to 3% surfactant (polysorbate 80), histidine buffer, pH 6.0, 50 to 300 mM of arginine and 10 to 50 mM of methionine. The formulation produces less than 2.70% dimer after storage at 40°C.

Methods for Fat Reduction or Elimination of Lipid Droplets;

A.D. Widgerow and J.A. Garruto; Alastin Skincare, Inc., USA; U.S. Patent # 11,752,084; September 19, 2023.

The need for treatments effective at ameliorating skin laxity associated with body shaping and contouring procedures are rapidly growing. The peptide combinations of the embodiments can be employed in various types of topical formulations. The compositions for this comprise two different peptides: a dipeptide, tripeptide, or tetrapeptide in combination with a pentapeptide, hexapeptide or heptapeptide. These formulations improve skin barrier function. Formulations comprise niacinamide or hydroceramide and hydrogenated lecithin. Improved barrier functions are - protection against water loss, prevention of substances and bacteria penetrating into the body, and plumps skin by improved hydration. To facilitate application, the composition may be provided as an ointment, an oil, a lotion, a paste, a powder, a gel, or a cream.

Nicotine Tablet;
B.P. Nielsen and K.A. Nielsen; Fertin Pharma, Denmark; U.S. Patent # 11,738,016; August 29, 2023.

The invention relates to an orally disintegrating nicotine tablet for nicotine craving relief comprising a pressed powder formulation. The tablet disintegrates within a period of less than 60 seconds upon oral administration that helps to employ nicotine more efficiently. The faster release provides effective nicotine craving relief but also minimizes burning in the throat. Also, the tablet facilitates user compliance with instructions, such as not swallowing or spitting within a given time period from oral administration. The pressed powder formulation comprises an amount of nicotine, a pH regulating agent, at least one polyol, and a disintegrant.

Therapeutic Bandage;
L. Silbart and T.D. Nguyen; University of Connecticut, USA; U.S. Patent # 11,745,001; September 5, 2023.

Patent describes a therapeutic bandage that includes a matrix and an array of biodegradable micro-needles. The bandage matrix includes a hydration layer and a sequestration layer. The role of the hydration layer is to absorb foreign agents removed from a skin infection or skin condition. The sequestration layer, which includes an antibody and a dye, is configured to bind to the foreign agents. Each of the microneedles includes a first layer that encapsulates a first immunomodulatory compound and a second layer that encapsulates a second immunomodulatory compound. The array of microneedles is configured to guide foreign agents affected by the first immunomodulatory compound, the second immunomodulatory compound, or the first and second immunomodulatory compounds from one or more skin layers of a user to the bandage matrix. The immunomodulatory compound establishes a chemotactic gradient within the one or more skin layers to the bandage matrix such that the bandage matrix absorbs and captures the foreign agents.

Lipid Nanoparticle Compositions and Methods for mRNA Delivery;
B.C. Guild, F. DeRosa and M. Heartlein; Translate Bio, Inc., USA; U.S. Patent # 11,730,825; August 22, 2023.

Conventional DNA insertion techniques are commonly used to insert desired genetic information into host cells. But it has many deleterious effects too. The use of RNA is significantly safer, but is far less stable than DNA, especially when it reaches the cytoplasm of a cell where it gets exposed to degrading enzymes. The current patent describes the delivery of mRNA that encodes cytokines. The mRNA is encapsulated within PEG-modified cationic and non-cationic lipids such as DSPC, DOPE, etc.

Solid Dosage Form Production;
M.A. Alhnan and T.C. Okwuosa; University of Central Lancashire, Great Britain; U.S. Patent # 11,717,485; August 8, 2023.

Solid dosage forms are preferred mostly due to their ease of administration giving rise to better patient compliance, storability and transportability, and high stability. However, they are often more onerous to manufacture. The present disclosure utilizes 3-D printing technology, particularly fused filament fabrication (FFF) in conjunction with solid and/or liquid dispensers to produce solid dosage forms, such as pharmaceutical capsules. Such solid dosage forms have a shell, which is 3D printed, and a core, which is dispensed. The patent describes solid dosage forms obtainable by printing methods and apparatus, a package, a kit of parts, a computer for controlling the relevant printing process, a system for collecting data, and relevant blueprints for use in the printing of solid dosage forms.

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