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UPFRONT

Stomach Studies

Researchers examine how changes in the gut – including those that result from posture – affect drug bioavailability

Researchers at Johns Hopkins University and Johns Hopkins School of Medicine have been delving into the complicated workings of the stomach – specifically, trying to gain a better understanding of what happens after we swallow a pill (1).

"The stomach is a very complex chemical and biomechanical environment. The rate at which an orally ingested pill will dissolve within is a function of many factors, including what we have eaten, our posture, and whether we have some condition that impairs our stomach function," explains Rajat Mittal, co-author of the study and a professor of mechanical engineering at Johns Hopkins University.

According to Mittal, current methods for testing oral pill dissolution employ a device that does not accurately mimic what happens in the stomach. To address that limitation, Mittal and colleagues spent three years developing an in-silico stomach simulator – aptly named StomachSim – that leans on earlier models of cardiovascular flow, as well as on the anatomy and morphology of the stomach.

"Using StomachSim, we realized that we could easily model the effect of posture on drug dissolution and gain some interesting results," says Mittal. In fact, the research team discovered that changes in posture can affect the release of the API from the pill into the duodenum by 83 percent – one of the most surprising outcomes of the work. "Our results showed that the effect of posture may even be larger than that of gastroparesis – a condition in which the grinding movement of the stomach is impaired."

The tool requires immense amounts of computing time; each simulation must be run in parallel on thousands of computer processors for over a week. Nevertheless, the team see promise in their work and are now exploring other applications for StomachSim; for example, understanding how the stomach processes different foods, researching stomach conditions associated with diabetes and enteric infections, or even adapting the model to guide gastric surgery.

With plenty of past experience working on the biomechanics and fluid dynamics associated with other organ systems, Mittal had come to recognize a dearth of bioengineering research into the stomach. "The work on drug dissolution was, in some sense, an easy entry point into this arena compared with modeling the digestion of food," he explains.

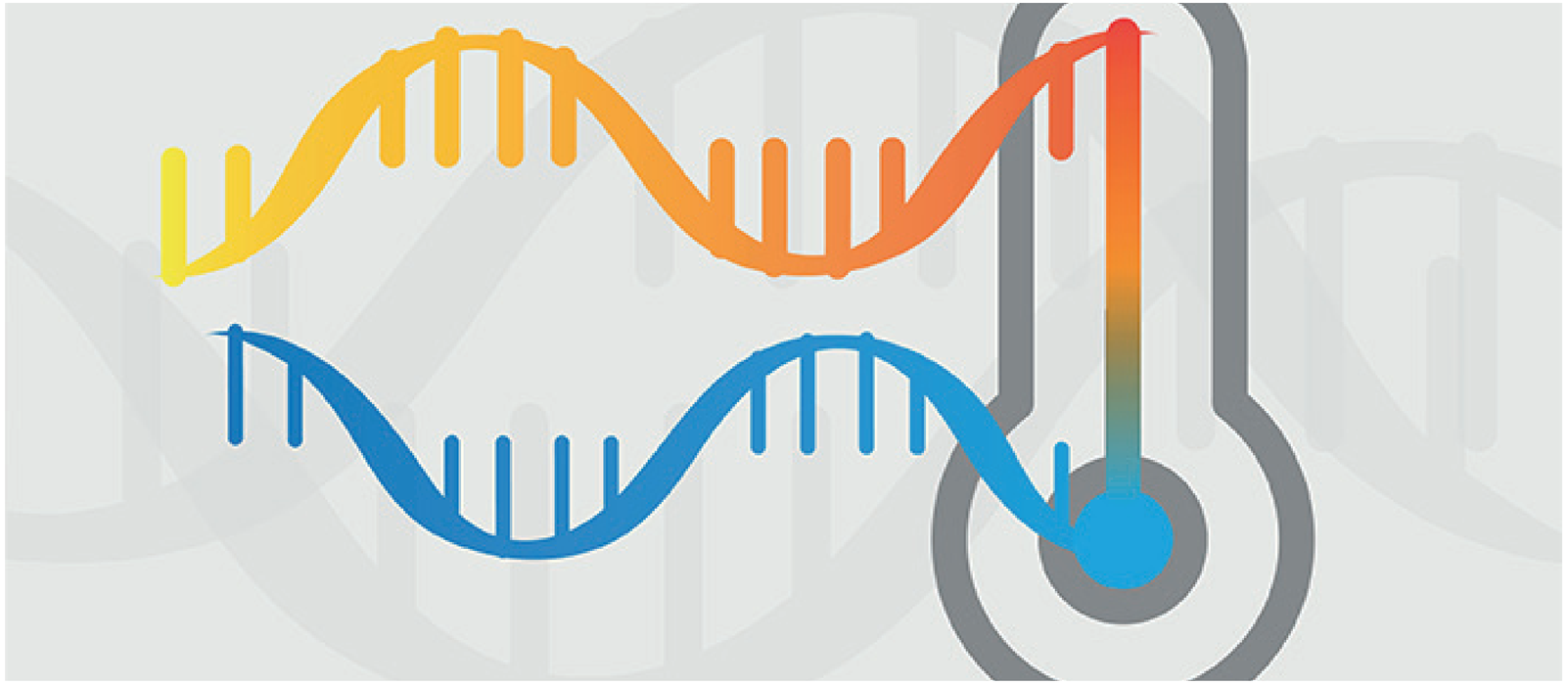
Reference

1. JH Lee et al., Physics of Fluid, 34 (2022). DOI: 10.1063/5.0096877.

UPFRONT

Goodbye, Moisture. Hello, mRNA

Could dry vaccines help improve global access to the latest vaccine technologies?



If you mention mRNA vaccines and therapeutics in any industry circle, you'll be hard-pressed to find a person who doesn't have an opinion. Many companies are exploring how they can be applied to treat a broad range of diseases – and there is a sense of understandable excitement in the air.

But it's not all plain sailing with mRNA. Stability issues could easily hamper attempts to ensure equitable access; in some cases, these therapies require temperatures as low as -70 °C, creating obvious limitations in how far they can be transported and where they can be stored.

However, one team claims it is inching closer to resolving the problem. RISE – a national research institute in Sweden – is working with the Karolinska Institutet, the production unit Vecura at Karolinska University Hospital, and NorthX Biologics on new processes that would create mRNA medicines able to withstand 4 °C or higher. RISE's new project, NucleoDry, will focus on "dry" mRNA vaccines.

"A dry vaccine can be one of two things. It's either a formulation where water has been removed through freeze-drying or it can be a dry formulation," explains Randi Nordström, Researcher in Formulation Development at RISE. "The former definition typically includes drugs that can be resuspended and injected [...] The latter includes more humble drug formats, such as oral tablets. We're focused on the first kind. [mRNA vaccines] are highly complex products chiefly because of their lipid nanoparticle structure, which incorporates both water and mRNA."

There are two phases to the NucleoDry project. The first phase will explore drying technologies for mRNA vaccine formulations. The second phase will see the researchers building early phase development infrastructure capable of taking an API candidate through formulation development, upscaling, GMP adaption, and early phase clinical trials.

"These ambitions will require hard work, expertise, and a bit of luck; as with all research projects, there is no guarantee of success," says Nordström. "But the Swedish research scene is teeming with talent and we're working with an experienced team. One of our collaborators, Matti Sällberg, a group leader at Karolinska Institute, Sweden, is working on developing mRNA strains. NorthX Biologics is well-versed in large-scale GMP manufacturing of APIs for biologics and small molecule drugs, and RISE itself has a dedicated formulation unit for the development of pharmaceutical formulations for pre-clinical and clinical work. If successful, dry mRNA vaccine formulations could make storage and handling cheaper, and increase the availability of pharmaceuticals around the world."

IN MY VIEW

You Shouldn't Need a Spoonful of Sugar

If companies truly aim to achieve patient-centricity, they must embrace the importance of taste masking

Let's be honest: the bitter taste that is present in many solid and dispersible tablets can be enough to put people off taking the drug at the required dose. There are many methods and technologies that can be used to improve the taste of medicine but, even in 2022, the patient experience often seems to come as an afterthought – and aftertaste.

For oral dosages that are soluble or require the patient to keep them in the mouth for a prolonged period, the bitterness of the API can be overwhelming (note that we are programmed as humans to be sensitive to bitter tastes as it indicates that something may be toxic).

Certain patients, especially geriatric and pediatric, often find it difficult to swallow solid tablets and capsules whole, meaning liquid and soluble formulations are a preferred delivery method. Unsurprisingly, taste for these products is key to patient compliance. We should also be aware that children are often more sensitive to bitterness than adults – and also far more likely to refuse medication! Should we care about the young and old? Well, around 34 percent of the world population is either aged under 14 or over 65 – that's a lot of people who could benefit from drug formulations that are easy to swallow and not bitter to taste.

You may already know that there are many ways that bitterness can be masked. The API can be coated, sweeteners or flavors can be used, resins and polymers can be added to the formulation… Each approach either overpowers the bitter flavor, reduces contact with taste buds, or delays the release of the API. With all of these options available, is it not surprising that so many formulators are still wedded to their traditional delivery formats?

So you're sold on taste masking. What's the best approach? Well, it really depends on the API used, the degree of bitterness, the final dosage form, manufacturing method, and the target patient population. The addition of sweeteners and flavors may not be suitable for certain patients, such as diabetics, as increased sugar intake can raise their blood sugar level, so film coating and the use of polymers and resins may be a more suitable approach.

Ion exchange resins (IER) – insoluble polymers that contain acidic or basic functional groups – are increasingly popular but not at all new to the industry; in fact, they've been used for many years to control the release of APIs. By binding an ionic API to an oppositely charged polymer – the IER – to generate insoluble "resinates," the API is not released into the mouth, and is masking its bitter taste. It later will release the API in the gastrointestinal track to produce its therapeutic effect. And as well as facilitating delayed release, they can also be used to create fast dissolving formulations, such as dispersible and orally disintegrating tablets where the API comes into direct contact with the taste receptors in the mouth but masks the bitter taste.

I'm passionate about treating taste as an important factor in the development of new drugs – especially when so many of the world's medicines take an oral dose form and we see a continued rise in aging populations. Do we really want higher rates of noncompliance, hindered therapeutic management, and unhappy patients? There is a clear market for liquid and soluble formulations with efficient taste masking – and it will improve patient outcomes.

By Amie Gehris, Technical Service Manager, DuPont Water Solutions

"What's the best approach? Well, it really depends on the API used, the degree of bitterness, the final dosage form, manufacturing method, and the target patient population."

IN MY VIEW

Complexity and its Questions

Pipelines are changing, and chemical complexity is on the rise. What can pharma manufacturers do about it?

Some people believe that the days of small molecule drugs are over – that pharma is now all about biologics and novel modalities, such as RNA and cell therapies. But this is far from reality. In 2021, the small molecule R&D pipeline was around 4 percent larger than it was the year before, with a record 8000 candidates in development. Interestingly, the increase is slightly skewed towards the earlier phases, with 5 percent growth for preclinical and phase I.

But complexity in all its forms is also increasing within the small molecule drug pipeline. Growing numbers of novel APIs are deemed highly potent. Some are used as drugs in their own right; others are used to make the linker payload component of an antibody–drug conjugate. Either way, their biological activity even at very low doses means that they must be carefully handled to ensure operator and environmental safety, which adds an additional layer of complexity.

Many new small molecule drugs have complex chemical structures, such as multiple chiral centers or tricky functional groups, which also pose manufacturing challenges. Opting for the synthetic route might demand reactions that require challenging reagents or conditions, such as very low temperatures.

Redesigning the synthetic route is sometimes an option. For example, while working on phase I API development for a potential sickle cell disease treatment, one company found that a bromide intermediate in the original route was unstable, requiring low temperatures and complicated purification. Installing and qualifying the new cryogenic equipment would have taken at least six months. Even then, the bromide's purity was only about 80 percent, which would have produced a low yield of below 60 percent when making the final API. By replacing bromide with chloride, the company fixed the problem; the modified intermediate fitted seamlessly into the route and was more stable. With 97 percent purity, the API yield was increased to 77 percent in the next step, using a simple isolation. And because no cryogenic step was needed, the six-month equipment delay was avoided.

Making the molecule is not the only challenge, however. A substantial – and growing – proportion of developmental drugs nowadays have poor solubility, with the knock-on effect of poor bioavailability. Some active molecules are so insoluble that they are commonly described as "brick dust" compounds. Solid form services experts can help improve the solubility of even these most insoluble compounds, enabling the creation of effective dosage forms with decent bioavailability. Sometimes, a more soluble stable polymorph, a salt form, or even a cocrystal can be found. Other times, smaller particles (via micronization) can help. Amorphous solid dispersions (often achieved via spray drying) are another common strategy. This latter process converts the API into a high-energy amorphous form, usually in combination with a performance-improving polymer. In my view, finding the best option is as much an art as it is a science.

Formulators responsible for designing the dosage forms may add further complexity with a wish list of essential properties. An inhaled drug, for example, will require a tight distribution of the optimal sized particles, which may be challenging to achieve.

In short, increased complexity and challenges go hand in hand. And smaller companies may not have the necessary in-house skills and capabilities to bring complex formulations to the market. Even large companies may need assistance from a niche specialist.

Responding to demand, CDMOs have invested in technology and capacity to enable these complex molecules to be made and modified effectively. Phase-specific, streamlined offerings provide the necessary flexibility for new chemistries to be incorporated seamlessly into a process

stream. Many CDMOs can now make and formulate highly potent APIs at more than one site. Some CDMOs are also putting a big focus on solid form services and their ability to overcome solubility issues.

When working with complex molecules and chemistries (especially where the prior art may be limited), you may need to accommodate changes to processes – and that requires flexibility and agility. The sooner a particular challenge is addressed, the less likely it is to cause a major delay in the development timeline. In fact, by integrating multiple technologies and teams into a single workflow, the timeline can often be accelerated.

As an example of the effectiveness of an integrated team working to solve complex chemistry problems, we recently worked on the development and kilo-scale manufacture for a phase I asset. The route was convoluted, with eight steps and an overall yield of just 14 percent – but the timeline for the delivery of the first batch was just six months! Our team in China optimized each of the eight steps in the process, while groups in Florida and Switzerland worked on particle engineering and API encapsulation. The result? A scalable kilo lab process delivered about 3 kg of the API with an overall yield of 29 percent inside the six-month deadline.

We dare say you'll agree that time is of the essence in drug development projects. And we hope you'll agree that using experts to solve tricky problems is key to getting complex small molecules over the finish line.

By Charles Johnson, Senior Director, Commercial Development, Lonza Small Molecules

FEATURE

Protect the Virus!

Viral vectors can enable innovative medical treatments, but only if we address the unstable elephant in the room with a comprehensive formulation development plan

By Gideon Kersten, Daniel Weinbuch, Tim Menzen and Andrea Hawe, all at Coriolis Pharma

Viable viruses are an important group of biopharmaceuticals – and likely the oldest. As early as the 16th century, the practice of variolation was applied in India to combat smallpox. Dried pus from pustules from smallpox patients was administered to the skin of healthy people, unknowingly using viruses as medicines. This primitive and dangerous form of vaccination with a crude preparation of live smallpox virus provided some protection against smallpox. At the turn of the 18th century, Edward Jenner described the potential of less dangerous cowpox material to protect against smallpox. A century later, Louis Pasteur pioneered attenuation of infectious agents, including viruses, to use them as vaccines. All this is remarkable, when you consider that viruses were not discovered until the 1930s, when the development of filters allowed us to isolate viruses and the invention of the electron microscope finally allowed us to visualize them.

Since the 1990s, the use of viruses as gene delivery vehicles has taken off; hundreds of clinical trials have been performed and several dozen gene therapy products are now approved – and many of them use viral vectors. These can be applied directly to the patient (in vivo gene therapy) or used to transfect cells outside the body of the patient (ex vivo) before being returned to the patient.

A third therapeutic application of viruses is tumor targeting. Oncolytic viruses are made native or modified to specifically infect and destroy tumor cells and/or to stimulate anti-tumor immune responses. Several such products have been marketed since the first therapy, Rigvir, was approved in 2004 in Latvia, and numerous clinical trials are ongoing.

The origins of virus instability

As with all complex biological systems, viruses are intrinsically unstable. The loss of viability observed for viruses can be caused by:

1. Protein deterioration that prevents the binding of the virus to the receptor and/or destabilizes the protein capsid in the case of non-enveloped viruses. For instance, after a short treatment of poliovirus or vaccine at 56oC, the structure of the capsid changes, resulting in virus-like particles that can no longer bind to the receptor.
2. Damage to genetic material (DNA or RNA). RNA is particularly prone to hydrolysis in the presence of water, and at elevated temperatures it may lose the critical secondary structure of its regulatory elements.
3. Damage to the lipid membrane in enveloped viruses. For instance, the stability of retroviruses depends on the composition of the viral membrane, and therefore on the type of production cell line used (1).
4. A combination of all the above. For example, the viral genome is protected not only by a proteinaceous capsid and/or a lipid envelope, but may also contribute to the structural integrity of the virus (2). Therefore, the size of the genome of a viral vector should not be very different from the native genome. Also, the manner in which the DNA is packaged – dense or less dense – has an impact on the viral vector's stability. Higher 'DNA pressure' may result in less stable virus and, as a result, lower infectivity (3).

The relative contribution of these factors to virus destabilization during processing (for example, freezing or drying) and storage is not well understood (4). But it is probably safe to say that viruses can and will deteriorate in any number of ways.

The development of stable virus formulations intended for human application first requires a clear target product profile (TPP) that defines, among other things, the route of administration, dosing, and primary packaging. Second, scientists must establish a set of stability-indicating and phase-appropriate analytical methods to identify and monitor critical degradation pathways and prove activity of the virus. Third, the laboratory infrastructure and analytical methods need to fulfill certain biosafety regulations for virus-based medicinal products; often a biosafety level (BSL) 2 is required. Last, but certainly not least, scientific expertise and prior knowledge in developing virus formulations and setting up analytical methods is extremely beneficial.

Liquid formulations – keep it cold!

The poor stability of viruses is the reason why the majority of currently licensed virus-based products are stored as frozen liquids at -20 °C or even lower temperatures. A few – Zolgensma is one example – can be stored at 2–8 °C for around 14 days. Oral polio vaccine is reasonably stable at 2–8 °C, but for storage periods exceeding 6 months, -20 oC or lower is advised.

As freezing may cause dramatic changes in ionic strength, osmolarity, and pH, the sensitivity of viruses to these effects should be investigated during formulation development, making it possible to select suitable conditions with respect to pH, buffers, and excipients. In addition, the effects of final storage temperature, freezing speed, thawing procedure, and so on should be determined experimentally. Knowing the physical state of a frozen solution as a function of temperature is important. Phase separation and other inhomogeneities in the matrix may occur during freezing. At moderately low temperatures such as -20 oC, solutions may not be completely frozen, leaving room for molecular mobility and chemical deterioration. Crystallization events and freeze concentration of excipients during freezing can damage the virus. Differential scanning calorimetry (DSC) reveals some of these effects and can help to select optimal freezing and storage conditions. In general, fast freezing rates are beneficial because they promote the

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Andrea Hawe, Chief Scientific Officer at Coriolis Pharma

formation of amorphous glasses instead of crystallized solids. In the case of enveloped viruses, fast freezing may avoid formation of lipid membrane damaging ice crystals (4).

Your selection of buffer and cryoprotectant is particularly important. Phosphate-based buffers may induce very considerable pH shifts of several pH units during freezing because of separate crystallization of the buffer salts. The main cryoprotectant groups are sugars, sugar alcohols, and alcohols.

The development of effective formulations often has a highly empirical nature. It is all but impossible to reliably predict the optimal compositions or concentrations of stabilizing excipients. The number of variables is large (consider type and concentration of excipients, freezing rate, thawing rate, combined effects of excipients, and so on), making it hard to perform extensive screenings that cover all aspects.

Therefore, a systematic and stepwise approach is highly recommended to generate a scientific understanding and to de-risk the development: starting with a pH/buffer screening, followed by an excipient screen with a selected cryoprotectants and other stabilizers, followed by an optimization phase in which, for instance, different excipient concentrations are tested. One complication with frozen liquid formulations is that accelerated stability studies are intrinsically impossible. Time-consuming real-time stability studies (apart from repeated freeze-thawing) are therefore the only way to assess stability. Despite these challenges, having experience in formulation of different viruses is beneficial to the setup of a scientifically sound and knowhow-driven formulation development approach. Moreover, multi-disciplinary teams of formulation scientists, analytical specialists, and virologists can increase success rates considerably.

The lyophilized "solution"

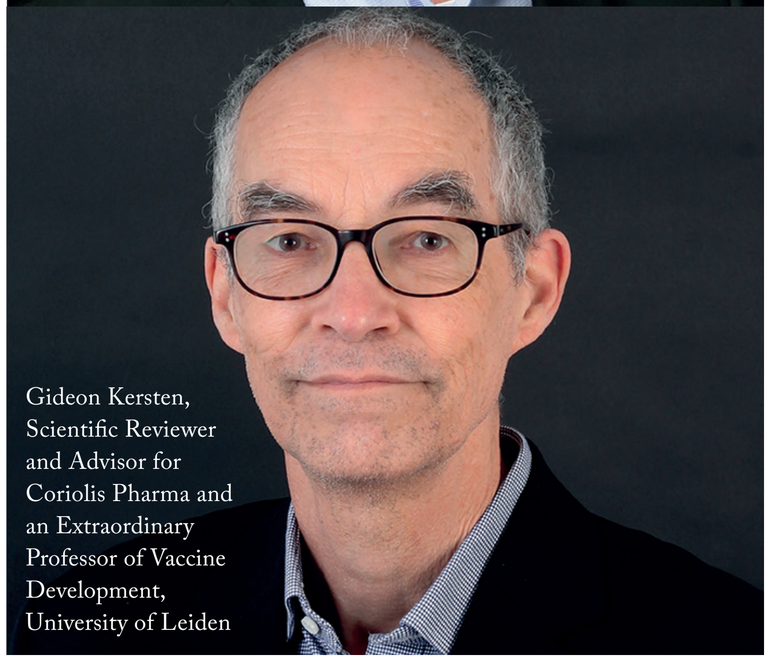
Supply chains with sub-zero temperatures are not always feasible. If frozen liquid formulations will not work, lyophilization can be used to stabilize the virus – indeed, this process is used for most live attenuated viral vaccines, including vaccines against measles, yellow fever, and rabies. However, it is important to design a formulation that protects the virus against potentially harmful events during freezing and drying. Typically, excipients with cryoprotecting and lyoprotecting activity must be present. Efficient lyoprotectants, such as sucrose and trehalose, are good water substitutes, which maintains conformation of viral proteins in the dried state. Lyoprotectants also contribute to a high glass transition temperature (Tg) of the lyophilized material, which is advantageous for storage. When the product is stored at temperatures above the Tg, the glassy state of the freeze-dried matrix becomes more rubbery, causing increased molecular mobility. In addition, recrystallization of amorphous excipients may occur, which may damage virus particles. Note that even small amounts of residual water will reduce the Tg significantly, rendering the product less stable. In short, it is important to keep water content low and to optimize the lyophilization process accordingly. In fact, the lyophilization process (the unit operation) must be developed and aligned with the formulation development process (the composition). Freezing rate, drying temperatures and pressures, and the application of controlled nucleation to reduce inter-vial differences in freezing rate all need to be considered.

Analyzing virus quality and stability

To assess the effect of formulation and storage conditions on product quality and virus stability, you'll need appropriate analytical assays, which can be categorized as functional, semi-functional, and non-functional (see Table 1). Functional assays measure the potency of the virus, such as its ability to transfect cells or the immunogenicity of a live viral vaccine in experimental animals. These methods are usually time consuming, expensive, and not sufficiently accurate, which means they are less suitable for formulation screening purposes.

Alternative stability indicating assays for viruses are available. Infectivity assays are particularly important because they are semi-

Daniel Weinbuch, Business Development Manager for Coriolis Pharma



Gideon Kersten, Scientific Reviewer and Advisor for Coriolis Pharma and an Extraordinary Professor of Vaccine Development, University of Leiden

functional but less labor-intensive than functional assays. In general, the ability of viruses to infect cells is determined by measuring cytopathic effects (CPE) in host cells incubated with dilution series of the virus. Depending on the virus and the host cells, CPE can range from barely affected host cells to complete cell lysis. Readouts will differ depending on the type of CPE and may include direct microscopic visual assessment of the cells, immune fluorescence in a FACS, or fluorescent focus assays.

Infectivity assays, although very relevant, are still time consuming and lacking in accuracy. Instead, quantification of gene copy numbers by PCR is often used. This method does not measure viable viruses, but the number of viral genome copies. This may or may not correlate with infectivity.

A third group of assays are non-functional characterization methods. Despite the somewhat unappealing name, these assays can provide detailed information about the structural integrity of viral particles. Assays belonging to this category determine physico-chemical properties, such as particle size measurements by light scattering techniques, size exclusion chromatography, analytical ultracentrifugation, and AF4 (5). If necessary, viral components, such as proteins and nucleic acids, can be analyzed by electrophoresis, HPLC, and spectroscopic methods. The advantage of many of these techniques is that they are high throughput and generally more accurate and sensitive compared with the functional methods. The onset of viral aggregation or loss of virus – for example, due to adsorption – during a stability study is in some cases detected earlier than a statistically significant loss of virus titer. In this way, a more accurate ranking of formulations is possible, which in turn allows for rational selection of the best formulations.

Over the course of virus formulation development, a combination of functional, semi-functional, and non-functional assays is not only recommended – it is required.

We can do better

Formulation development of viral products is still highly empirical and, in our view, often not performed adequately. In that respect, one could argue that not much has changed since the 16th century's variolations! The air-dried material from those times, high in potentially stabilizing impurities, may have been as stable (at least for a short period of time) and effective as some of today's virus formulations.

In many cases, there is room for improvement – particularly when aiming for a high quality product with long-term stability. To achieve this goal, you should not rely on off-the-shelf formulations for the virus of interest and expect them to result in an acceptable stability profile. Viruses are highly sensitive, and – depending on the type of virus – different stability challenges may arise. To obtain a stable product, you must perform dedicated virus-specific formulation development from initial screenings to formulation optimization (including the proper set of analytical methods).

Scientific knowledge about factors influencing virus stability is growing – and so is our collective ability to overcome virus instabilities with science-driven formulation development. In the years ahead, we should be using expert know-how and applying a range of formulation approaches, including lyophilization, to obtain stable and phase-appropriate virus formulations.

REFERENCES AVAILABLE ONLINE

Category of assay	Name of assay	Target information
Functional (potency)	Cell transduction	Ability to deliver gene to cells
	Immunogenicity	Ability to induce an immune response
	Oncolytic potency	Ability to kill tumor cells
Semi-functional (potency -indicating)	Virus titration	Viable virus
	PCR	Genome copy number
Non-functional (quality indicating)	Analytical ultracentrifugation	Particle size of fragments, intact virus and small aggregates, sedimentation coefficient
	Chromatography (SEC, ion exchange)	Size, purity
	FFF-MALS	Particle size, amount, aggregation
	Immunoassay (ELISA)	Amount of viral antigen
	Dynamic light scattering	Virus size, sub-visible aggregates, virus fragments
	Nanoparticle Tracking Analysis	Particle size and particle concentration
	Backgrounded membrane imaging	Subvisible particles
	Electron microscopy	Particle morphology, size
	Flow imaging microscopy	Subvisible particles

Table 1. Examples of analytical techniques for characterization of viruses (5). ELISA: enzyme-linked immunosorbent assay; FFF-MALS: Field-flow fractionation with multi-angle light scattering detection; PCR: polymerase chain reaction; SEC: size exclusion chromatography.

DEPARTMENT

Small but Mighty

With numerous applications for small molecules – from improving solubility and bioavailability to making formulations suitable for inhalation – micronization is a versatile technique. But how does it work and what challenges does it pose?

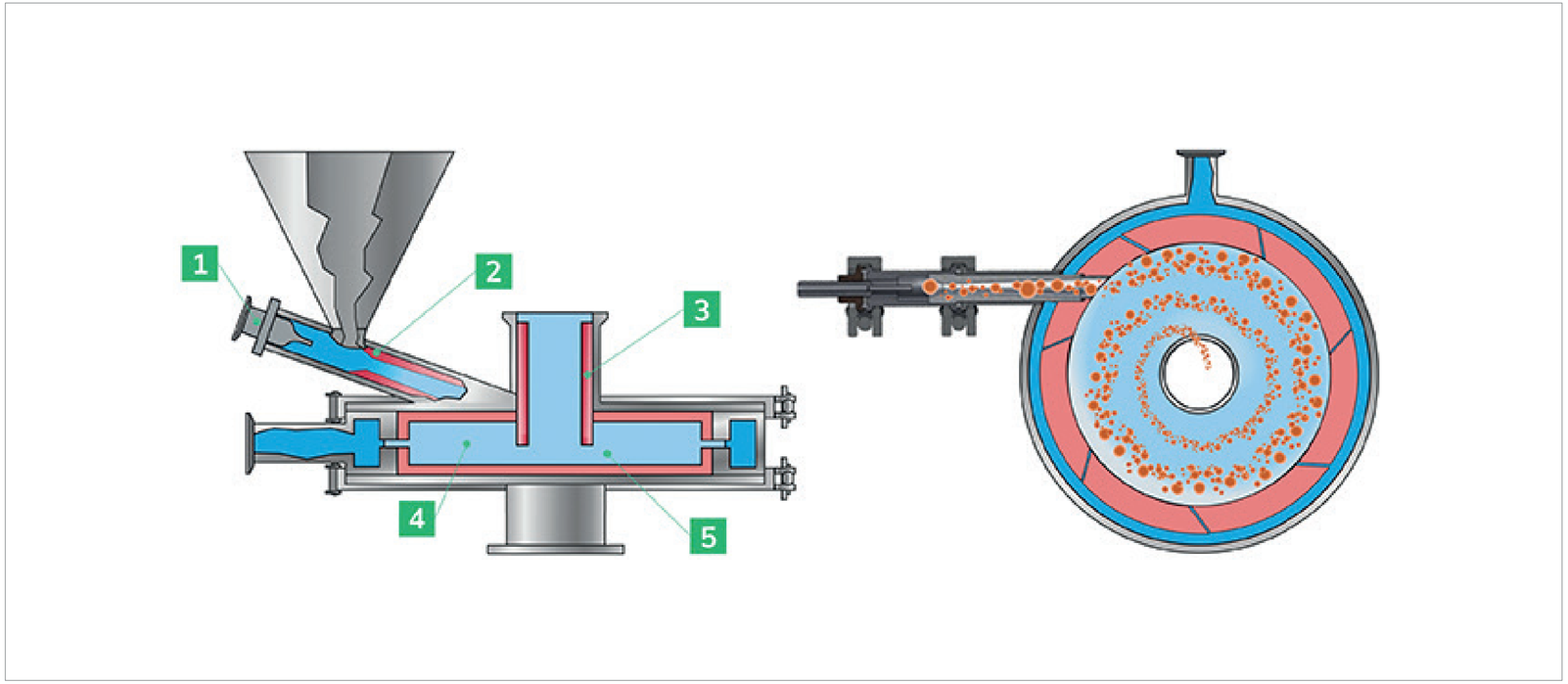


Figure 1: Spiral Jet Mill schema. 1 - Injector nozzle; 2 - Venturi tube; 3 - Upper classification pipe; 4 - Micronization chamber; 5 - Bottom classification pipe.

By Salvatore Mercuri, head of Research and Development at Lonza's site in Monteggio, Switzerland

Micronization reduces the particle size of APIs down to a few micrometers to enhance bioavailability. The technique is often performed using a jet mill; a compressed gas source expands at the outlet of a series of grinding nozzles, accelerating API particles in a spiral vortex flow path within the grinding chamber of the jet mill. Then, comminution, caused by particle-on-particle collisions, starts taking place for the particles in the flow field. And each particle is subject to centrifugal and drag forces: large particles are dragged to the outside of the milling chamber via centrifugal force while small particles migrate to the center mill outlet. From there, the smaller particles, dispersed into the gas stream, are discharged into a cyclone filter and progressively collected for use.

The jet milling process is controlled by three operating parameters: material feeding rate, feeding pressure, and grinding pressures. At a constant feeding rate, the increase in the grinding pressure leads to increased particle size reduction. The process is also thermally stable – with the heat produced by a jet mill dispersed by adiabatic cooling as the gas expands – compared with a mechanical mill that generates heat without dispersing it.

Micronization can be used for particles that need to be reduced in size to improve solubility, dissolution rate, or processing. It is also required for dry powder inhaler (DPI) applications, which have specific size requirements for lung and central airway delivery. Recently, these formulations have seen significant growth due to the increasing number of patients diagnosed with respiratory illnesses, such as asthma and COPD. Additionally, the lung's absorptive capacity continues to be explored as an attractive delivery point for both local and systemic applications. The ideal spherical equivalent diameter for an inhaled API is usually a Dv50 of 3 µm (meaning 50 percent of the product is composed of particles under 3 µm). Particles designed to target areas deeper into the lung have to be even smaller.

Beyond inhaled drug products, micronization can also be used for many other types of treatments including oral medications, topical creams, suspensions, and eye drops.

Although any solid material – even diamonds – can be micronized in theory, the efficiency of the process and the extent of size reduction can be affected by a material's chemical and physical characteristics. Plastic materials or those with a high level of water and/or solvents may result in low or negligible size reduction. Materials with a low

"Optimizing working conditions is a sustainable and efficient way to ensure that these challenges are minimized."

melting point and/or a tendency to adhesion or crust formation can be addressed with the appropriate engineering solution, such as cryogenic conditions, product contact material, and special geometric adaptation of the milling chamber.

What challenges does micronization pose?

First, the properties of the micronized material are different from those of non-micronized material; the increased surface area can result in poor flow and cause electrostatic charge to build up. Inadequate flow can cause handling problems in downstream processes, which means that excipients (such as glidants, binders, and lubricants) may be required to improve flow properties. Material can also become more hygroscopic, so storage conditions may require humidity control.

Given the higher specific energy required to reach the desired particle size distribution, there is also a risk that phase changes or side effects could occur, such as conversion to a different crystalline polymorph, dehydration or desolvation, or production of amorphous content.

To avoid these challenges, the solid state of the material needs to be well characterized, meaning the appropriate techniques, such as X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), dynamic vapor sorption (DVS) or Brunauer-Emmett-Teller (BET) surface area analysis, need to be employed to highlight differences in the material before and after micronization. For example, amorphous

content may require additional processing to obtain a stable product prior to formulation. Oftentimes, surface amorphous content rapidly converts back to the crystalline phase under ambient conditions, causing product aggregation or agglomeration, and thus leading to a particle size increase. Short-term physical stability studies can help assess any conversions.

Optimizing working conditions is a sustainable and efficient way to ensure that these challenges are minimized. Here, I offer some best practices:

1. Identify all possible critical process parameters, including the variability of the raw material size, water content specifications, and knowledge of solid-state changes.
2. Conduct a stress test and a short stability study to assess how the energy applied will affect the solid state of the API, as well as its impact on contingent changes on post-micronization stability, such as particle size increases. It could take around one week and should be factored into the development timeline.
3. Perform at least one confirmation run. During the trial duration, ensure that you are assessing the different variables in the process by implementing intensive sampling protocols to help fortify the results you have gathered.

Going further

Particle size reduction technologies – such as micronization – are well-known manufacturing processes with an established track record. They are highly flexible, have the ability to be scaled up, are good for substances with poor thermal stability and are easily applicable to different chemical properties. However, the technique requires a number of resources, including experts and well-equipped facilities. Emerging companies may not have access to these resources or a team with commercial expertise, and even larger companies may not have the appropriate resources. And that's why most companies choose strategic partners, who can be especially helpful under accelerated timelines with the goal of scaling up.

What is the future of micronization? Although it is an established technique, I think there is still room for improvement. Advances in computing power may help us develop more accurate models to predict gas flow or better study particle behavior. In addition, improved design, automation, and AI could be applied to make the process more predictive and reliable.

DEPARTMENT

Lipid Nanoparticles: From Little League to the Majors

As lipid nanoparticles have evolved, the number of potential applications has grown to include not only small molecule drugs and vaccine delivery, but also gene therapies and more. But what does the future hold?

By Bowen Tian, Senior Applications Specialist at Particle Works

The COVID-19 pandemic brought lipid nanoparticles (LNPs) to the fore, creating a surge in interest as the pharma sector exploited their benefits for mRNA vaccine delivery. But they have been around for much longer. LNPs are based on well-established liposome technology developed after British scientist Alec D Bangham first discovered liposomes in the 1960s. LNPs are formed by adding ionizable or cationic lipids to liposomes to allow the encapsulation of negatively charged oligonucleotides – such as RNA and DNA – through electrostatic interactions. The lipid shell then serves to protect the encapsulated genetic material against premature degradation in vivo until it reaches the target cells.

After 30 years of effort, the FDA finally approved the first liposomal drug – Doxil – in 1995. A few years later, surface conjugation of antibodies became a popular area of liposome research, as the large biomolecules were able to bind to specific receptors on cancer cells. However, antibody-targeted liposome delivery systems failed to improve therapeutic effect compared with Doxil in clinical trials. More recently, the first liposomal gene product for siRNA delivery, Onpattro, was approved in 2018, making it possible to treat liver disease by intravenous administration of LNPs carrying therapeutic genetic material.

Liposomes and LNPs have many proven advantages as drug delivery systems, as they greatly reduce the likelihood of drug-associated cardiotoxicity – a key benefit Doxil is known for – and offer the ability to preferentially target the tissue of interest. We've already covered their unprecedented success in vaccine delivery; it's widely known that several COVID-19 vaccines – such as those from Moderna and Pfizer-BioNTech – use LNPs to deliver mRNA into cells, where it is released to produce proteins that aid in counteracting the SARS-CoV-2 virus.

However, one of the most exciting areas for the future of LNPs is gene therapy, where they are proving their value as non-viral delivery vectors. Traditional viral vectors have a limited gene delivery capacity, typically fewer than 10 kilobase pairs (10,000 base pairs) and require a complicated engineering and manufacturing process. They also often stimulate an unwanted immune response within the body – for example, inflammation and organ failure – and have the potential to cause mutagenesis by inserting their own genes into the genome of the target cells. Non-viral vectors, such as LNPs, have emerged as a way of overcoming all of these problems, removing the limitation on the size of the gene you can deliver and reducing the risk of adverse effects. As work with LNPs continues to increase across the industry, there are two areas that warrant close consideration: production processes and drug delivery methods.

The production problem

LNPs are formed through a self-assembly process that is very difficult to control and scale up, incurring substantial financial costs that can be unviable for many biotech companies. As a result, much effort has been poured into improving LNP-mRNA complex formation through more precise mixing of lipids and mRNA to increase the consistency

of the process. An example of this is a widely applied ethanol injection method, where a syringe is used to inject lipids suspended in ethanol drop-by-drop into aqueous mRNA solutions. T-mixing is another common option, and is mostly used in large-scale LNP synthesis.

However, neither of these batch production methods can generate truly monodisperse LNP-mRNA nanoparticles, and they can lack batch-to-batch consistency in terms of particle size, composition, and morphology. Additionally, the shearing forces applied to the particles by these techniques could accelerate degradation and rupture the LNP-mRNA nanoparticles. The result is that most LNP-mRNA treatments are unnecessarily costly with low therapeutic efficiency. In fact, numerous studies have shown that less than four percent of LNP-mRNA nanoparticles are capable of effectively releasing mRNA intracellularly in cell culture, and this is believed to be even lower when administered into the muscle (1, 2).

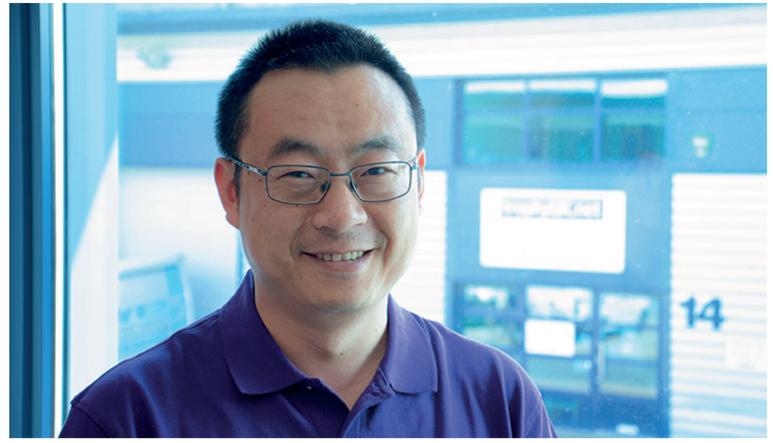
Microfluidic devices could provide advantages compared with ethanol injection and T-mixing, as they maintain consistent conditions for LNP self-assembly and offer greater process control. This approach makes it easier to determine the formulation that works best in terms of both maximizing therapeutic effects and minimizing side effects. Unlike traditional production methods, microfluidic systems rely on fluids flowing into a microfluidic chip from separate channels, meeting at the junction and being actively mixed in a reproducible manner to form nanoparticles. Depending on the fluidic chip design, this allows precise mixing of fluids down to single microliter volumes, and continuous operation, rather than batch production. In addition, an optimized microfluidic set-up generates highly monodisperse LNP-mRNA nanoparticles, with improved homogeneity across the population in terms of lipid composition, mRNA payload, and morphology (3). These particles also show greater mRNA loading efficiency, which has the dual benefits of reducing wastage of expensive materials, and potentially allowing significantly reduced doses.

On top of this, microfluidic workflows are well suited to automation and high-throughput manufacture, making it possible to produce more vaccine or therapeutic drug within a short space of time – for example, when under time pressure during a pandemic. Automated, high-throughput methodologies are also generally more cost-effective than batch techniques, which tend to be more labor and time intensive.

Ongoing developments in delivery

LNPs containing therapeutic genetic material or other APIs can be delivered to the target tissue either systemically or topically. Systemic delivery is typically achieved by intravenous administration, such as in the treatment of liver disease. Once the LNPs reach the organ of interest, they must penetrate the tissue and be taken upby the target cell type, for instance, through receptor-mediated endocytosis. The LNP then disintegrates, the therapeutic is released and, after endosomal escape, it begins acting on target pathways. At present, the mechanism of endosomal escape is not yet fully understood, and its efficiency is thought to be very low. The particles' protein corona may have a large influence on the performance and capacity to target the tissue of interest, but the underlying mechanisms of this are still under investigation. These big unknowns have sparked a significant amount of research into how to design LNPs for optimal performance, ensuring efficient endosomal escape and preferential accumulation in the target cell type. There has also been a recent focus on LNP-based delivery mechanisms that are capable of crossing the blood-brain barrier.

Delivery can also be localized, being performed intramuscularly, transdermally or by inhalation using LNPs that have been aerosolized in a nebulizer. Inhalation is now one of the most widely pursued delivery route, with several clinical trials currently ongoing. This method delivers therapies directly to the lungs and can be used to treat severe diseases like cystic fibrosis, making it a particularly promising delivery technique. However, the biggest challenge for delivery by inhalation is that the vibrating mesh used in a nebulizer for aerosol formation may damage the LNP structure, causing early RNA



degradation and loss of biological function prior to reaching the cells of interest. There is, therefore, an ongoing need for a specially designed nebulizer capable of aerosolizing LNPs without causing damage.

Much left to learn

The community of liposome and LNP researchers remained extremely small for many years, but, unsurprisingly, it has grown exponentially since the COVID-19 pandemic. LNPs are clearly an exciting field of research, and they are generating a great deal of interest across multiple industries thanks to their versatility. RNA therapeutics in particular are causing a rapid revolution in medicine – and the field of gene therapies is booming. Almost any disease you care to name can potentially be treated using RNA-based gene therapies, and all the necessary technologies are already available to enable delivery via LNPs. Although this is a very challenging niche, novel genomic medicines would provide significant clinical benefits for a range of conditions, and offer sought-after solutions that could address currently unmet clinical needs. In short, there has never been a better time than now to work on LNPs for gene therapy.

REFERENCES AVAILABLE ONLINE

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SITTING DOWN WITH

The Formulation Fixer

Sitting Down With… Chris Moreton, Partner, FinnBrit Consulting

Chris Moreton has spent decades working as a formulation scientist and is a past chair of IPEC-Americas. Here, we find out more about his career and why formulation is such a fascinating area to pursue.

What inspired your interest in science?

My father, who was an organic chemist. He worked for ICI, a company that no longer exists. He worked there all of his working life apart from his military service in WW II. I was always interested in science, but my father's one regret was that I never really got on with organic chemistry! Originally, I went to undergraduate school to study biochemistry, but that changed for various reasons and I ended up studying pharmacy.

How did you get into industry?

On leaving pharmacy school, I first worked in the hospital service as a trainee pharmacist. I was, quite frankly, unimpressed! I was counting medicines and that was it – and I felt it would drive me mad! I decided to switch to industry. I got a job with a small CMO, which fortunately doesn't exist anymore, because it was a dump! (This was in the days before GMP was mandatory in the UK; the first GMP inspections in Britain didn't happen until the summer of 1972.) I stayed on at the company after completing my registration as a pharmacist. After my boss moved to another position, I was promoted to Chief Pharmacist. We received a letter from the Medicines Control Agency (now the MHRA) notifying us of our next GMP inspection, and referencing a previous letter that required the company to do certain things. I took the letter to the general manager, and asked to see the letter from the previous year. None of the action points in that letter had been addressed, but his thinking was that we'd gotten away with it until now, so why change? I handed the letter back to him, walked back to my office, and started looking for another job. That was not the type of company I wanted to work for.

After that, I worked in various companies, including Pfizer, where I stayed for a little more than seven years. After a total of nearly 15 years working in different companies, I went back to university for a master's in pharmaceutical analysis and a doctorate. Later, I got a job in the excipients industry. I've worked in a lot of different places and there are a lot of stories to tell! At one point, I was in charge of quality in an excipient and drug delivery company. I think that was probably a mistake on their part. I knew what was acceptable and what was not acceptable – and, when a line is drawn, I will not step over it. If you step over a line once, you will be asked to do it again and again. My boss was not very happy with me on at least one occasion when I failed a batch!

Why formulation?

I've always enjoyed formulation work. In fact, I'm also fascinated by it! I also had a knack for finding solutions, but not necessarily with the tools people wanted me to use. For example, some companies have set management and research tools. On more than one occasion, I found a solution to the problem that using the tool did not achieve!

In some cases, the tools worked well for synthetic chemistry, but not so well for pharmaceutics. In the early days of my career, there was also a lot we didn't know about formulation. There is still a lot we don't know. For example, two of the most commonly used excipients are magnesium stearate and microcrystalline cellulose and we still don't know nearly enough about how and why they work – despite the fact that they have been used for decades.

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Meeting the Rapidly Growing Demand for Parenteral Products

Alcami's vice president of business development for parenteral services, Mike Babics, shares his insights on parenteral market trends

Pharmaceutical and biotech companies worldwide have been increasingly focusing their program development and manufacturing in the anticipated primary markets, which in most cases are the US and EU. Consequently, Alcami and the other five most active global sterile drug product contract manufacturers based in the US and EU have been significantly investing to expand isolator-based fill-finish capacity for both vials (lyophilized and liquid) and syringes. These investments are crucial to meet the rapidly growing demand for parenteral products, and the intensifying regulatory expectations that all sterile manufacturing lines will utilize isolator technology to minimize potential risk to patient safety from the manufacturing process.

The five most active sterile drug product contract manufacturing organizations (CMOs), along with Alcami, each manufacture over 20 global commercial products for a variety of clients, not to mention dozens of clinical programs annually. Alcami alone averages helping clients launch two to three commercial products annually, across a variety of formats. All of the leading parenteral CMOs also have capabilities for liquid vials, lyophilized vials, and liquid pre-filled syringes.

As pharma and biotech portfolios increasingly focus on rare and ultra-rare indications, they are also experiencing pressure from accelerated timelines and expedited program reviews. To accelerate timelines, drug developers are purposefully seeking out contract development and manufacturing organization (CDMO) partners that offer extensive formulation and analytical method development, which can be rapidly transferred into non-GMP batches for toxicology material followed by GMP manufacturing.

These factors, along with industry consolidation due to mergers and acquisitions, have led to a smaller number of experienced CDMOs with substantial industry knowledge and the resulting regulatory track record of successfully supporting program sponsor filings of an NDA, ANDA, BLA, or PMA, any of which are subject to a PAI.

Key drivers for program sponsors in their CMO selection are comprehensive development and analytical capabilities, robust technical expertise, state-of-the-art technology, and the ability (and willingness) to collaborate with the client's CMC, analytical, and program management leadership to ensure the program meets timelines. Alcami has repeatedly found that an experienced and integrated CMO program management team allows for a seamless transition from formulation and analytical development through manufacturing and analytical release, which are of the utmost importance.

READ THE FULL INTERVIEW WITH MIKE BABICS