ANALYSIS AND CONTROL OF PARTICLES IN BIOPHARMACEUTICALS

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Biologics-based products are playing an increasingly important role in the healthcare professionals’ armoury in the fight against disease, both in prevention and treatment. In this growing market, managing product contamination from intrinsic and extrinsic particles, and protecting patients from the associated risk, will become increasingly important.

According to an IMS report in 2013, biologics will make up a fifth of the pharma market by 2017, and biologics represent about a third of the current late stage pipeline. However, many biologics are less stable than small molecule drugs, and need to be stored and transported carefully and in controlled conditions to ensure a long shelf life. This can restrict their use, particularly in environments where the temperatures are high or fluctuating, or where products need to be transported over long distances, and it is difficult to maintain a consistent cold chain. One of the key challenges faced by companies developing and manufacturing biologics is the control of particles in the final formulation, whether these are extrinsic (extraneous particles introduced during manufacturing) or intrinsic (arising from the container closure, or aggregation of protein API that develop during the product’s life cycle (see Table 1)).

Protein aggregates generally occur as a result of even slight changes in the conformation of the therapeutic protein, caused by variation in the temperature, or interactions with the interface between the air and liquid, or the surfaces of the container. These conformational changes can trigger aggregation creating particles held together reversibly or irreversibly. Once the proteins have started to associate, these create a catalyst for further aggregation, leading to formation of even larger particles. Particle sizes, whether intrinsic or extrinsic, vary in size, from visible particles that are larger than 100 µm, down through sub-visible particles to oligomeric structures.

Because of the range of sources, manufacturers need to screen the products for particles at every stage of manufacturing, both alone and in combination with any delivery systems or devices. It is also important to look at the particle levels and types within and between batches, especially if there are any changes in the process, however slight. The products will also need to be monitored over time to check whether the numbers or the types of particle are changing.

THE IMPACT OF PARTICLES

Particles in biologics can trigger immune responses in patients, with the effects ranging from just an inconvenience to a severe or even life-threatening reaction. Because of this potential impact on patients, the regulatory authorities require information on the levels of particles, and evidence of the limitation, control and identification of any product-related impurities.

Particles can form during storage and transport, triggered by changes in temperature, or agitation and vibration. This limits the type of storage required for biologics, and shortens the potential shelf life of the product, which ideally needs to be two years or more. Both of these will have an impact on the biologic’s cost and profitability, and the size of its market, and therefore on the company’s ability to recoup its investment in R&D.

CHARACTERISATION: WHAT KIND OF PARTICLES AND WHERE DO THEY COME FROM?

Particles exist everywhere, even in the cleanest of environments. Before looking at control, it is important to know the kinds of particles involved and where they have come from.

The particles could be extrinsic contaminants introduced into the therapeutic during the manufacturing process and normal use, or intrinsic particles arising from the container closure, or aggregation of protein API that develop during the product’s life cycle (see Table 1). Protein aggregates generally occur as a result of even slight changes in the conformation of the therapeutic protein, caused by variation in the temperature, or interactions with the interface be-
THE NEXT STEP: PARTICLE CONTROL

Once companies have found evidence of particle formation, the next step is to focus on particle control, in order to protect patients, improve product shelf life, and meet the needs of the regulators. If there are any changes in particle type or development during manufacturing or storage, the regulators will require evidence of further characterisation, clearance and control.

It is important to find the issues causing particle formation as early as possible during the development of the drug and its manufacturing process. This is because it is much easier to make changes at earlier stages of development, for example at the sequence, expression and purification or the formulation steps (see Figure 1). Any significant formulation or process changes made at a later stage could be harder to control and have a larger impact on the cost of development.

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**TABLE 1: INTRINSIC AND EXTRINSIC PARTICLES**

<table>
<thead>
<tr>
<th>EXTRINSIC PARTICLES</th>
<th>INTRINSIC PARTICLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibres – shed from clothing, filters or packaging</td>
<td>Protein aggregates – formed during manufacturing or storage as a result of:</td>
</tr>
<tr>
<td>Dust – from the environment</td>
<td>• Protein-protein interactions</td>
</tr>
<tr>
<td></td>
<td>• Protein-air/liquid interface interactions</td>
</tr>
<tr>
<td></td>
<td>• Protein-container interactions</td>
</tr>
<tr>
<td></td>
<td>• Protein-contaminant interactions</td>
</tr>
<tr>
<td>Silicone oil – used to lubricate moving parts in devices, such as syringe barrels; introduced during drug administration</td>
<td>Fragments of glass, plastic or rubber – shed during manufacturing or packaging, including shreds of rubber from stoppers and shards of plastic or glass from vials or devices</td>
</tr>
</tbody>
</table>

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**FIGURE 1: POTENTIAL ROUTES FOR AGGREGATION & CONTROL**

**SEQUENCE**
- Control through Sequence design: Technologies available for evaluation of aggregation propensity
- Free thiols

**EXPRESSION & PURIFICATION**
- Low pH hold
- Filtration / column selection
- Include in-process aggregate analysis

**FORMULATION**
- Inadequate formulation design: Ensure aggregation assessed upon agitation and freeze / thaw

**CHARACTERISATION**
- Include continued sub-visible particle testing as part of characterisation & comparability studies
- Reformulate?

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**STABILITY STUDIES**
- Measure particle trends
- Characterise any particles generated
- Reformulate?

**RELEASE**
- Route of administration: Assess with in-use studies
- Reformulate?

**DRUG PRODUCT FILL**
- Thawing may show particles – ensure before and after tests performed
- Filter before fill
- Reformulate?

**SHIPMENTS**
- Agitation of liquids
- Ensure shipment studies and excursions studies completed: alternative condition
- Reformulate?
and on the drug launch timeline, in turn delaying the time to market.

As Figure 1 shows, there are a range of stages where control can be implemented, and the orange text highlights where the problems are likely to arise. The main route to particle control is through formulation.

At the early product research phases, various aggregation prediction software are available that allow the product development scientists to identify sections of the primary sequence that could increase the risk of aggregation – as an example, any free thiols could increase the risk of covalent (irreversible) binding. Upfront early evaluation of the protein primary sequence can significantly reduce the propensity of the protein candidate to aggregate. In addition, during process development, there are stages that are required that can also impact the product and increase aggregation, e.g. pH reduction for virus inactivation and UF/DF filtration. All these processes that are required for effective purification can have significant effects on the integrity of the product and hence can trigger aggregation. Therefore, it is important to carefully monitor the levels of aggregation and subvisible particles levels during process development to ensure any problems are recognised early on so that can be controlled and effectively monitored through routine analysis.

Formulation development is one of the most vital steps in creating an environment that stabilises the conformation of the protein API. As part of early phase product development it is critical to implement preformulation screening as early as possible, this can even be implemented at the clone selection stage. Once the process has been locked down, and the product enters the clinic, it is still important to monitor the number and types of particles present, as events such as batch scale up and manufacturing site changes can affect the product produced. Therefore it is critical subvisible particles are continued to be monitored during characterisation and comparability studies.

Shipment of the product is also another stage in the process where aggregation levels can be significantly increased as a result of agitation and the potential for freeze/thaw, and analysis at this stage should include replication of shipping conditions, including fluctuations in temperature, pressure and movement. The filling stage also has potential to introduce extrinsic particles such as fragments of stoppers or vials. Because of this, the process needs to include a filtering step before and after filling to check the types and origins of any particles. If this does highlight any issues, then processes will have to be evaluated, or alternative packaging used, which could cause significant extra costs and delays. Once the product is packaged in its final form, ready for launch, in-use studies can then assess whether the route of administration is likely to introduce particulates, such as silicone oils or shards from delivery devices, with analyses both before and after administration. Finally, stability studies are essential to monitor particle levels and types of particles that may develop over time.

**CASE STUDY: CONTROLLING PARTICLES THROUGH FORMULATION**

The case study described below discusses an IgG1 monoclonal antibody candidate that had successfully progressed through drug development, but was shown to exhibit significant aggregation when shipped and upon freeze/thaw. In order to control the levels of aggregation it was decided to reformulate, creating a product that would be more stable to shipment and storage at lower temperatures. Significant time and material constraints were encountered, making this a more challenging project and required incorporation of high throughput technologies to achieve.

The characteristics of the original formulation:

- IgG1
- pI 9.6
- ~150 kDa
- formulated in 20 mM PO4, 125 mM NaCl, pH 7

SGS M-Scan put a plan in place, beginning with preformulation characterisation to establish the degradation pathway for the molecule and its specific issues, followed by a pH and excipient screen to find the optimal formulation.

**Sample treatment and preformulation characterisation**

To mimic the problem, SGS recreated conditions equivalent to the worst-case scenario possible under which the product was likely to be exposed, including 24 hours of agitation at ambient

**TABLE 2: PREFORMULATION CHARACTERISATION TECHNIQUES**

- Visual appearance
- SEC (size exclusion chromatography)
- SE-UPLC (size exclusion ultra-performance liquid chromatography)
- SV-AUC (sedimentation velocity analytical ultracentrifugation)
- DLS (dynamic light scattering)
- LO (light obscuration)
- Microflow imaging
- Particle counts
- Intrinsic fluorescence
- DSC (differential scanning calorimetry)
temperature and three cycles of temperature variations from -20°C to 40°C. The degraded protein was then characterised and compared with the original (control) protein using a variety of different techniques (see Table 2).

Preformulation characterisation demonstrated that although though there were no apparent charge-based degradation changes to the molecule, significant disulphide-bridge scrambling had occurred and structural conformational changes were evident. In addition, there was a significant increase in the number of subvisible particles over 2.2 µm.

pH and excipient screening

The next step was to carry out a pH screen and excipient screen, selecting from a range of buffers, salts and excipients. The aim was to create a formulation that would be the most stable to agitation and freeze/thaw degradation.

The pH screen used buffer solutions and pH values from 3.5 to 7.5, and exposed the samples to agitation and three F/T cycles, monitored using SE-UPLC and DLS. This process found an optimal pH and buffer ion of 25 mM succinate, pH 6.5 containing 125 mM NaCl.

The next step was to design a formulation based on these findings, using a variety of excipients at various concentrations for a selection of amino acids: His, Pro, Glu, Gly, Arg, (0-67 mM) and the surfactants Tween 20 and Poloxamer (0.01-0.1%), along with 2% trehalose using a Design of Experiment approach (DOE). Screening involved using SE-UPLC and DLS. At all stages, SGS had to keep aware of the time taken and sample volume used, as both of these were limiting factors.

Lead candidate selection and analysis

The team selected four lead candidates (see Table 3), and these then went through a more complex process of screening to find the optimal formulation for the monoclonal antibody.

These next steps included predictive analysis of the undegraded material using intrinsic fluorescence and DSC to assess the candidate’s thermal and conformational stability, and to check the counts for particles greater than 2 µm. Exposing the material to temperatures between 20°C and 100°C allowed the team to determine the formulation that provided the most stability, by evaluating the most thermally stable formulation. Ranking the formulations based on these findings revealed R25 to have the best results based on conformational analysis and particle counts (see Figure 2).

IN SUMMARY

While the risk of protein aggregation will differ from product to product, it is an issue that needs to be considered for all biologics. It is critical to investigate any particulate / aggregation issues early on in any biologic’s development in order to minimise the risk of problems being encountered at a later stage in the product development life cycle that could significantly affect costs and time to market launch.

To deal with this issue, traditional screening tools, such as SEC and DSC, remain useful methods in formulation screening. It is also critical, however, to use a wide range of imaging and analysis tools to make sure that all sizes and types of particles are included, from larger aggregates down to the subvisible level. This ensures that all relevant degradation pathways are evaluated, and nothing significant is missed.

TABLE 3: LEAD CANDIDATE SELECTION

<table>
<thead>
<tr>
<th></th>
<th>HIS</th>
<th>PRO</th>
<th>GLU</th>
<th>GLY</th>
<th>ARG</th>
<th>TWEEN 20</th>
<th>POLOXAMER 188</th>
</tr>
</thead>
<tbody>
<tr>
<td>R25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>67 mM</td>
<td>67 mM</td>
<td>0.01%</td>
<td>0.05%</td>
</tr>
<tr>
<td>R25B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>67 mM</td>
<td>67 mM</td>
<td>0.06%</td>
<td>-</td>
</tr>
<tr>
<td>R30</td>
<td>-</td>
<td>67 mM</td>
<td>22 mM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1%</td>
</tr>
<tr>
<td>R38</td>
<td>-</td>
<td>67 mM</td>
<td>22 mM</td>
<td>-</td>
<td>-</td>
<td>0.1%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

25 mM succinate, 125 mM NaCl, 67 mM Gly, 67 mM Arg, 0.01% Tween 20, 0.05% Poloxamer 188, pH 6.5
Because of cost and time constraints, analysis of particles and aggregates may have to be completed in a short period, using only small volumes of sample. This means that developers and manufacturers should consider highly sensitive and high-throughput analytical techniques. Particles in biological therapeutics will remain a significant issue because of the potential risk to patients, as well as the possible impact on product stability and shelf life. Because of this, manufacturers are required to evaluate the levels of particulate matter in the products throughout the product life cycle. This includes looking at the types and numbers of particles, and monitoring and predicting their levels throughout the lifespan of the product, as well as focusing on effective formulation and controlled process design to minimize any issues.