In this paper, Dr Daniele Zuccato, R&D Department, Dr Andrea Sardella, R&D Engineer, and Dr Alberto Chillon, R&D Department, all of Nuova, Stevanato Group, and Dr Emanuel Guadagnino, Scientific Advisor to Stevanato Group, describe the development of their method for the analysis of tungsten levels in glass prefilled syringes at sub-ppb levels, and detail the method itself.

**INTRODUCTION**

Prefilled Syringes (PFS) are recognised worldwide as the most appropriate container for the delivery of new biopharmaceutical drugs such as recombinant proteins, monoclonal antibodies (MAb) and other bio molecules. These complex molecules require the highest possible level of inertness from all different parts of the PFS system that may otherwise have a marked impact on the protein products over their shelf life. Container closure integrity, surface state of the glass barrel after silicone oil treatment, extractables and leachables from the whole system are critical factors to be kept under strict control.

For this reason the concept of chemical inertness of a PFS system must be reconsidered, as the glass barrel is subject to a specific transformation process to form the cone and the flange, the wall surface is modified by the silicone-oil treatment and the cone by needle gluing. Extractables & leachables from any component or material may impair the integrity and therapeutic efficiency of biopharmaceuticals, meaning that they must be substantially minimised.

**THE TUNGSTEN ISSUE**

This paper is focused on the development of an analytical procedure intended to estimate the tungsten contamination level in a glass syringe. Recent studies have shown that soluble tungsten polyanions may cause the precipitation and/or aggregation of biomolecules such as MAb to form visible particles. It is also reported that...
exposure of proteins to increasing concentrations of tungsten polyanions in acidic pH strongly favours aggregation and may induce reduced activity and increased immunogenicity.1,3,4

The native borosilicate glass does not contain tungsten, arising from the contamination of the raw materials for instance, in a significant amount likely to induce protein aggregation. On the other hand it is well known that during the cone formation of glass syringes a tungsten containing pin is kept in contact with the glass tip to form the bore where the needle shall be accommodated in a later stage. The use of pins at elevated temperatures favours the formation of tungsten oxides (WO3 and WO4) which evaporate and interact with the outermost glass surface of the funnel to form soluble sodium tungstate polyanions that become easily extractable. Figures 1 and 2 are scanning electron micrographs of the inner surface of the funnel, the white spots represent deposits of sodium tungstate, as confirmed by punctual EDS analysis (results not shown here).

Several experiments have been carried out to estimate the maximum permissible concentration of tungsten which is deemed not to cause an adverse effect to biopharmaceuticals. It was found that the typical tungsten concentration which may cause a detrimental interaction with proteins is about one order of magnitude higher than the one which is found in PFS systems5 and that 500 ppb is a reasonable estimate of the mean tungsten concentration that is extractable from glass syringes.5

**CHALLENGES IN DEVELOPING A RELIABLE METHOD FOR TUNGSTEN ANALYSIS**

From a regulatory point of view, a worldwide established procedure for the extraction and detection of tungsten from a PFS system does not exist.

The lack of a commonly established method results in a confusing market situation: each pharmaceutical company established its own acceptable limit for tungsten release and a proprietary extraction method to verify the compliance with its own specifications.

This uncertainty also generated an unclear situation from the syringe producers’ point of view, for each customer has its own in-house method and compliance with its own specifications.

SGLab, the research laboratory of Nuova Ompi – Stevanato Group, has developed a reliable method for the measurement of tungsten, based on a trial and error approach.

The following factors soon appeared to be critical: ensuring the wettability of the funnel area by an appropriate filling procedure; selecting the most appropriate extraction conditions; and developing a digestion procedure that could guarantee complete tungsten extraction, possibly in one single run.

**FUNNEL WETTABILITY**

The tungsten salts are mostly present on the syringe funnel, the small gap which is purposely created by a tungsten pin where the needle will be assembled.4 This area is typically in contact with pharmaceutical formulation during the vacuum plunger placement process, exposing the solution to the highest possible concentration of tungsten. From an extraction method perspective, this means that it is mandatory to force an intimate contact between the extraction solution and this small area of the syringe.

In principle any operation, either manual or automatic, that guarantees the full contact between the contaminated area and the extracting solution may be used. At SGLab, a manual procedure is used. The effectiveness of the procedure for the complete wetting of the small funnel area has been verified with a colouring solution. Figure 3 shows that an intimate contact between the extraction liquid and the contaminated area has been achieved.

**EXTRACTION METHODS**

Several extraction procedures were tested using two different approaches: a) dissolution of the outmost inner surface of the funnel by a mixture HF/HCl; and b) extraction by water of the soluble tungsten salts.

The surface attack by a cold mixture of HF/HCl is well described in the literature6,7 and at room temperature is known to produce the dissolution of glass layers about 1 μm thick per minute of contact. This method is well reproducible, goes to completion within ten minutes and no further extractions are required, but regulations concerning the safe use of HF in the lab means that this procedure is not recommended in most laboratories.

Comparable results were obtained using a two-stage water extraction at 75°C for 1h in a ultrasonic bath. During the second run the extraction of tungsten was always less than 10% of the total amount extracted during the first run.

In Figure 4, average inductively coupled plasma, optical emission spectroscopy (ICP-OES) results from the two methods are compared.

As expected, tungsten from HF/HCl is higher because the dissolution method guarantees the complete removal of tungsten that conversely is only partly extracted by water. As the coefficients of variation are comparable, it was concluded that both methods were able to reveal the presence of tungsten peaks that sometimes may occur due to an uncontrolled forming process. On the other hand pharmaceutical companies are conscious that the extraction ability of any buffer solution intended to go in contact with the funnel will never produce extraction values by far comparable with those obtainable by HF. This first screening, and subsequent discussions with pharmaceutical companies gave us confidence that the use of an aqueous solution at high temperature is sufficient to simulate the extractability produced by any parenteral preparation over its shelf-life.

<table>
<thead>
<tr>
<th></th>
<th>Dissolution (HF/HCl mixture)</th>
<th>Extraction (aqueous solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average (n=50) ppb W</td>
<td>440±128</td>
<td>321±90</td>
</tr>
<tr>
<td>% RSD</td>
<td>28</td>
<td>31</td>
</tr>
</tbody>
</table>

**Figure 4: Comparison of Tungsten ICP-OES results from different extraction methods.**
TUNGSTEN MEASUREMENT

Extraction solutions can be analysed by either ICP-OES (Figure 5) or inductively coupled plasma, mass spectrometry (ICP-MS) (Figure 6), depending on the expected tungsten concentration.

Tungsten detection limits by ICP-MS are typically approximately 0.1 ppb while the corresponding LOD by ICP-OES is two orders of magnitude higher (typically around 60 ppb). One of the major advantages of using ICP-MS is due to the smaller volume which is required to perform a single analysis. Syringes of 0.5 mL capacity can successfully be analysed one-by-one using ICP-MS, while ICP-OES would require extracts from several syringes to be combined to reach at least a 2 mL volume.

The increasing sensitivity of biomolecules to foreign contaminants combined with continuous improvements in the forming process over the years, has dramatically reduced tungsten levels in syringes. The ICP-MS technique is therefore the most eligible to measure tungsten and other contaminants at ppb and sub-ppb levels.

DESCRIPTION OF THE SGLAB METHOD

Syringes are filled with distilled water taking care that the funnel is completely wetted by the solution, plunger and needle shield are inserted manually. Syringes are placed in a metallic rack, heated up to 75°C and treated in an ultrasonic bath for one hour. After cooling at room temperature each syringe is washed with 1 mL of aqueous solution and then measured with both ICP-OES or ICP-MS, depending on the expected tungsten level. A second extraction is performed as a validation step to guarantee the completeness of each single extraction, whereas routine analyses are performed by one single extraction.

NUOVA OMPI SYRINGE SCREENING CURRENT PRODUCTION

The production of an EZ-fill™ syringe (sterile syringe, ready to be filled) requires a sequence of different stages: the bulk syringe production, the needle assembly, the washing + siliconisation + sterilisation finish.

The impact on the tungsten deposition due to each single stage was evaluated. Compared to the bulk, the staked needle syringe showed a considerable masking effect due to the reduced glass surface exposed to water extraction when the needle is in place (see Figure 7).

The washing step performed before the siliconisation is not sufficient to substantially reduce the tungsten content. In agreement with previous studies, results obtained from syringes before and after the washing step are nearly the same (see Figure 8).

Further research showed that three different tungsten concentrations can routinely be achieved depending on the final tuning of the process conditions. The standard production of 1mL long EZ-Fill syringes shows typical tungsten values below 1,000 ppb. The so-called “low tungsten” production that is obtained using a particular care on the cone forming step, shows typically tungsten values below 500 ppb. For pharmaceutical products which are known to be very sensitive to tungsten, the use of pins made of tungsten-free material can allow to reach tungsten levels well below 10 ppb (Figure 8).

CONCLUSION

The development of a procedure to be used as a rapid, reproducible method for the determination of extractable tungsten from a PFS system is described. Several methods were tested. The method proposed here simulates the extractability obtainable with parenteral preparations over their shelf life and shows high efficiency combined with a good reproducibility.

The ready availability of a wide selection of analytical instruments at SGLab allowed the systematic screening of the current syringe production at Nuova Ompi – Stevanato Group. As a follow-up a substantial improvement of the syringe-forming process was achieved, that is now capable of producing on an industrial scale batches of prefilled syringes compatible with the most sensitive biomolecules in the market.

A close collaboration between the pharmaceutical companies and the PFS producers is highly recommended in order to maintain the complete therapeutic efficiency of the new biomolecules in contact with the primary glass packaging.

REFERENCES


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**Figure 7:** Reduced exposed surface due to the needle’s presence.

**Figure 8:** Impact of the syringe-forming stages on the final extracted tungsten.

<table>
<thead>
<tr>
<th>Process type</th>
<th>Extracted W (in ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>&lt;1000</td>
</tr>
<tr>
<td>Low-tungsten</td>
<td>&lt; 500</td>
</tr>
<tr>
<td>Tungsten Free</td>
<td>&lt; 10</td>
</tr>
</tbody>
</table>

**Figure 9:** Typical tungsten extraction values of the current EZ-fill™ syringes process.

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