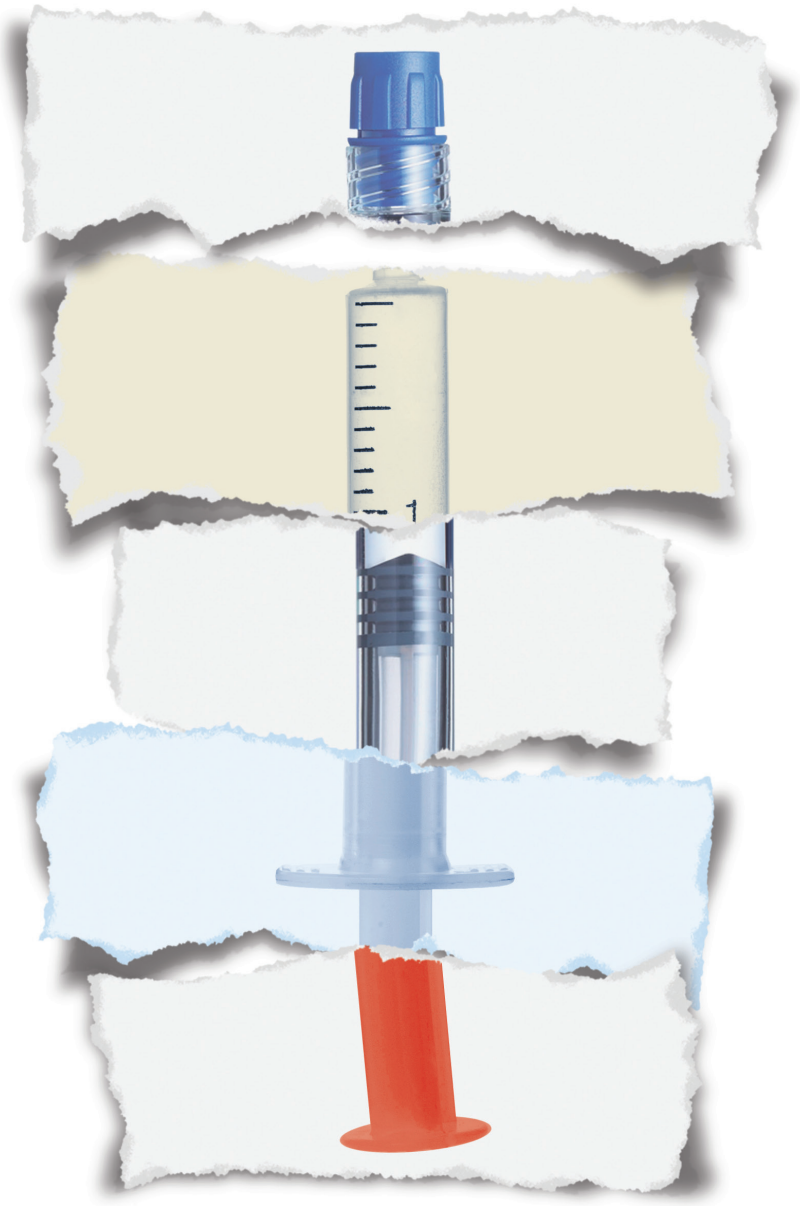
Putting the Pieces Together

Are the Components in Your Prefilled Syringes Compatible?

Wendy Saffell-Clemmer, Director, Research, Baxter Biopharma Solutions LLC, Baxter Healthcare



Prefilled syringes offers potential cost savings for high-value, complex, biologic products, as minimal overfill volume is needed in the primary container compared to vials ***(1).*** While a prefilled syringe offers many advantages for biologic products, manufacturers must carefully evaluate the potential impact of a prefilled syringe on product quality by conducting laboratory studies prior to selecting the final components. Particularly, when it comes to silicone oil and tungsten residues.

Generally, a prefilled syringe ready for administration consists of a barrel, piston, plunger, plunger rod, needle, and needle cover, such as a tip cap or a needle shield. Type I glass remains the primary material for syringe barrels, but new options in plastic syringes are gaining popularity, including cyclic olefin polymer (COP)and cyclic olefin copolymer (COC) ***(1).*** Multiple polymeric formulations are available for the elastomeric components, specifically the plunger and tip cap. All syringe barrels, with one exception, require the application of silicone oil to allow the plunger stopper to glide smoothly during use. Syringes may be provided with a staked needle—a needle embedded in the syringe tip—or with a luer slip or luer lock to be connected to a needle or other device for administration. During the manufacture of most glass syringe barrels, a tungsten probe is used to form the fluid path in the tip of the syringe, potentially leaving residual tungsten oxide vapor and tungsten particles.

Silicone oil and tungsten residues present new compatibility issues. Soluble tungsten residue was determined to be the root cause of unusually high levels of aggregation in clinical trial batches of epotetin alfa ***(2)*** and in an alfa helical protein formulation at ~ pH 4.0 ***(3).*** A study of precipitation of a monoclonal antibody (mAb) by tungsten demonstrated rapid coagulation by tungsten polyanions at pH 5.0 but concluded a lower risk for proteins formulated at pH greater than 6.0 since higher pH prevents formation of tungsten polyanions ***(4).*** Silicone oil has been demonstrated to cause aggregation of a variety of proteins, with the level of aggregation correlating to the amount of silicone oil present ***(5).*** USP <1787> Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections acknowledges that particle counts from silicone oil are intrinsic and vary, but recommends that the minimum amount needed to obtain proper syringe functionality be determined and used ***(6).***

Protein Aggregation Presents Potential Threat

Methodical laboratory studies must be included in prefilled syringe product development to assess the impact of tungsten and

Silicone oil and tungsten residues present new compatibility issues

silicone oil on product quality. Experimental designs to evaluate the effects of both tungsten and silicone oil on protein aggregation are described in the literature and can be adapted by a formulation development scientist to determine sensitivity of a product's formulation to the formation of protein aggregates.

Scientists working at West have evaluated protein sensitivity to tungstate ***(7).*** Proteins were incubated at concentrations of 0.1 to 1 mg/mL in microfuge tubes in 20 mM buffers at pH 4-7 with tungstate ranging from 1 to 100 ppm. Samples and a nonspiked control were incubated overnight and protein aggregation was measured by determining turbidity using measurement of absorbance at 350 nm as well as by Size Exclusion Chromatography (SEC-HPLC) following centrifugation to remove insoluble aggregates. Though not included in the West study, analysis of samples by a particle counting technique such as light obscuration or flow imaging could provide additional information regarding aggregate formation in response to tungsten exposure.

Flow Imaging Another Option

Another study described a general approach to understanding compatibility of a mAb formulation with silicone oil ***(8).*** In the experiments, a mAb formulation in a glass vial was spiked with a silicone oil/water emulsion and placed on accelerated and real-time stability and exposed to agitation stress. Samples were analyzed by the following test methods: concentration determined by UV-Vis absorbance at 240 nm, soluble aggregates assessed by SEC-HPLC, oxidation monitored using a product-specific reversed-phase method, and subvisible particles monitored using HIAC. Again, while not included in the published study procedure, use of a flow imaging technique would provide additional information on formation of subvisible particles.

The use of flow imaging provides particular benefit in the case of silicone oil/protein compatibility studies since the size range of silicone oil droplets may be similar to protein particles and result in high <10 µm particle counts in all spiked samples. The morphology of silicone oil is specific, however, with a spherical shape and characteristic appearance of a light center and increasing contrast toward the exterior of the sphere, allowing standard flow imaging software systems to identify and subtract silicone oil particles ***(9).***

A separate study evaluated multiple protein and mAb formulations in syringe barrels which were siliconized, uncoated, or coated with a proprietary coating, utilizing SEC-HPLC, Dynamic Light Scattering (DLS) and flow imaging ***(10).*** Syringes were placed on short-term stability, with some sets exposed to agitation. The study concluded that particle analysis by flow imaging was extremely valuable and detected differences that would not have been observed by SEC-HPLC. A significant increase in particle counts was observed in the samples stored in siliconized syringes that were subjected to agitation when compared to the unshaken samples.

The tungsten and silicone oil compatibility studies described form a framework for a general approach for the screening of protein and mAb formulations and selection of prefilled syringes. Sensitivity to tungsten should be evaluated through spiking studies in vials using 0 (control), 1, 10, and 100 ppm additions of sodium tungstate to the drug product formulation. Following overnight storage at refrigerated conditions samples should be compared using, at minimum, SEC-HPLC and particle analysis by flow imaging. To assess sensitivity to silicone oil, solution samples should be spiked with a silicone-emulsion to result in final silicone oil concentrations of 100, 200, and 500 µg/mL. Specific recommendations for storage duration cannot be concluded from prior studies described, however, agitation of the spiked samples should be included in the procedure.

Once product suitability is understood, suitable prefilled syringe types, such as those with standard versus low or no siliconization, can be selected for further evaluation. Following hand filling of syringes with the formulated drug product, stability samples should be stored at standard and accelerated aging conditions, typically 4°C and 40°C, for a minimum of eight weeks. Agitation stress should also be included within the stability study panel of tests. At minimum the formulated product stored in each syringe should be evaluated by SEC-HPLC and flow imaging. The function of the syringe, in terms of the force required to initiate movement of the plunger and the force required to sustain the movement of the plunger, also known as the break force/glide force, should be determined throughout the stability study to ensure that the siliconization level selected is adequate for comfortable administration of the drug and/or meets requirements of any autoinjector system selected for use with the prefilled syringe.

Aggregation of biologic products resulting from incompatibility of tungsten residue and silicone oil is the greatest container compatibility risks when using a prefilled syringe. Risks can be identified through the simple laboratory studies suggested to assess the impact of components on product quality, particularly subvisible particle formation, and enable selection of appropriate prefilled syringe types for further evaluation. SEC-HPLC and flow imaging are the critical test methods needed for an understanding of tungsten and silicone oil's impact on protein aggregation. While

Article at a Glance

- Prefilled syringes for biologics can present compatibility issues
- Silicone oil and tungsten residues can result in aggregation
- SEC-HPLC and flow imaging critical to identifying component incompatibility

initial screening studies to understand individual sensitivities may be performed in vials, short-term stability should be conducted in candidate prefilled syringes to ensure that product quality is unaffected and that acceptable breakforce/glide force is obtained throughout stability. At minimum, SEC-HPLC and flow imaging should be performed throughout the stability study.

References

1. G. Sacha, Rogers, J.A., and Miller, R.L. "Pre-filled syringes: a review of the history, manufacturing and challenges." *Pharmaceutical Development and Technology* 20 (2015) 1–11.
2. Seidl, A., et al. "Tungsten-Induced Denaturation and Aggregation of Epoetin Alfa During Primary Packaging as a Cause of Immunogenicity." *Pharmaceutical Research* 29 (2012):1454–1467.
3. Liu, W. et al. "Root Cause Analysis of Tungsten-Induced Protein Aggregation in Pre-filled Syringes." *PDA Journal of Pharmaceutical Science and Technology* 64 (2010): 11–19.
4. Bee, J., et al. "Precipitation of a Monoclonal Antibody by Soluble Tungsten." *Journal of Pharmaceutical Sciences* 98 (2009): 3290–3301.
5. Jones, L., Kaufmann, A., and Middaugh, R. "Silicone Oil Induced Aggregation of Proteins." *Journal of Pharmaceutical Sciences* 94 (2005): 918–927.
6. USP 39 <1787> Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections
7. Waxman, L., Steeley, T., and Vilivalam, V. Development of an assay to determine protein sensitivity to tungsten and to investigate the effects of pH and Ionic strength on aggregation. West Pharma Scientific Poster.
8. Badkar, A., Wolf, A., Bohack, L., and Kolhe, P. "Development of Biotechnology Products in Pre-filled Syringes: Technical Considerations and Approaches." *AAPS Pharm Sci Tech* 12 (2011): 564–572.
9. Brown, L. "Using FlowCAM Pattern Recognition to Quantify Silicone Oil Droplets in Parenterals." Presented at CDER/FDA/IABS Conference on Protein Particles and Immunogenicity of Therapeutic Proteins: Filling in the Gaps in Risk Evaluation and Mitigation, November 2009, Bethesda, MD.
10. Majumdar, S. et al., "Evaluation of the Effect of Syringe Surfaces on Protein Formulations." *Journal of Pharmaceutical Sciences* 100 (2011): 2563–2573.

About the Author

Wendy Saffell-Clemmer is the Director of the Baxter Biopharma Solutions (BPS) R&D team, which offers contract development services for small volume liquid (vial and syringe) and lyophilized products, manufactured for external customers at both Baxter's Bloomington, Ind. and Halle, Germany facilities..