New method to quantify redispersion potential of Al salts in vaccines

PHARMACEUTICAL

Introduction

Adjuvants such as aluminium salt (Alum) are commonly added to vaccines to enhance their immune responses. These adjuvants can aggregate and then settle over time due to their electrical charges. The resulting sediment can be more or less compact and difficult to redisperse depending on the strength of the bonds between particles. If such phenomena occur with storage time, the problem arises of knowing whether:

- the injected dose remains the same (Do all the active ingredients pass through the needle of the syringe despite large and compact aggregates?)
- or is the therapeutic efficacy and so the immunogenicity reduced (masked antigen in the aggregate does not get injected).

In this note, we propose a rapid evaluation method (less than 30 minutes) of the sediment redispersibility.

KEY **BENEFITS**

FAST

NO DILUTION

SENSITIVE

Reference

Investigation of the Sedimentation Behavior of Aluminum Phosphate: Influence of pH, Ionic Strength, and Model Antigens; Kevin Muthurania and al.; Pharmaceutical Research and Development; Pfizer Inc.; JOURNAL OF PHARMACEUTICAL SCIENCES 104:3770–3781, 2015).

Reminder on the technique

Turbiscan® technology, based on Static Multiple Light Scattering, consists on sending a light source (880 nm) on a sample and acquiring backscattered (BS) and transmitted (T) signal all over the height of a sample.

By repeating this measurement over time at adapted frequency, the instrument enables to monitor physical stability.

The signal is directly linked to the particle concentration (φ) and size (d) according to the Mie theory knowing refractive index of continuous ($n_{Ã}$) and dispersed phase ($n_{Ą}$): $BS = f(\varphi, d_p, n_f)$

Materials & Method

To prevent the loss of immunogenicity, the solution is subjected to a controlled flocculation (by varying pH or ionic strength). This will result in weakly-bonded particles that form a loose floc and produce a low-density sediment (large amount of water entrapped) easy to redisperse.

*Conventional method: **SVR** (Sedimentation Volume Ratio)*

The conventional method to determine the flocculation amount of vaccines is to measure the height of the sediment (SVR) upon the completion of suspension settling after 24 hours. SVR is the ratio of the height of the settled sediment to that of the initial suspension. A larger value (> 0.1) of SVR should translate to better suspendability /redispersability as the sediment is less compact.

This ratio can be measured with Turbiscan from backscattering evolution as shown on the figure below.

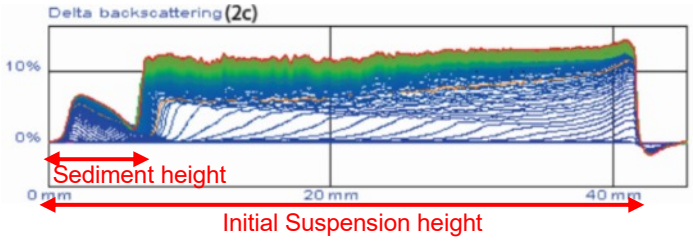

Figure 1: Delta Backscattering data for SVR measurement after 24 hours measurement

New method: S_{onset} (Settling Onset Time)

Although SVR method is very reliable, it is still too long for quality control or routine. The Settling Onset Time (S_{onset}) appears to be more convenient because of its quick response (less than 30 min).
S_{onset} corresponds to the time to reach 50% of the clarification of the suspension (50% of maximum amount of light transmitted through the sample). S_{onset} is determined from the transmission signal by determining the time for which clarification area at 50% of transmission gets higher than few mm as shown on the figure below.

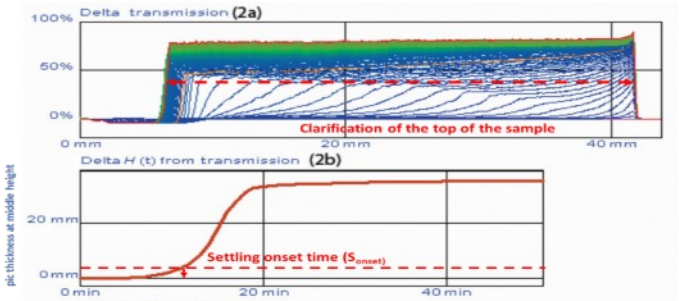

Figure 2: (a) Delta transmission, (b) clarification layer at 50% of maximum transmission level

In order to validate the S_{onset} as a new method of screening, the relationship between SVR at 24 hours and S_{onset} was determined with suspensions of $AlPO_4$ at different pH (3 to 9), ionic strength (0 to 1000 mM NaCl) with or without model antigen (BSA, lysozyme).
Turbiscan measurements were carried out using 20mL of suspension in a flat-bottom cylindrical glass vial. Each data set was collected for a period of 24 h. The settling onset time (S_{onset}) after 30 minutes and the sedimentation volume ratio (SVR) after 24 hours for each sample were obtained from the transmission and the backscattering data, respectively, as described above.

Results: S_{onset} and SVR relation

The results (figure 3) show a 2-slope curve depending on whether the system is flocculated (steep slope) or deflocculated (gentle slope).

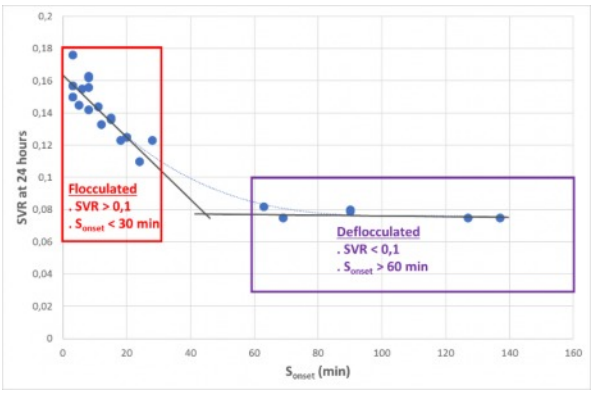

Figure 3. relationship between Settling Onset time (Sonset) and Sediment Volume Ratio (SVR) at 24 hours

1. When the **suspension is deflocculated** (example: low pH and without NaCl), the $AlPO_4$ particles remain as discrete units (<3µm) resulting in slow sedimentation rate (S_{onset} > 60min), thereby preventing the entrapment of solvent within the sediment. **It tends to compact to a hard cake and is difficult to redisperse. It is reflected by a low value of SVR (<0,1).**

2. In a **flocculated suspension** (medium to high pH or/and high ionic strength), the loose structure of the flocs is difficult to preserve in the sediment that contains a significant amount of entrapped water. **The volume of final sediment is relatively large - reflected by larger SVR (> 0,1)**. The sediment rate is high (S_{onset} < 30 min) and is strongly dependent on the flocculation level (steep slope).

Controlling these properties guarantees a good redispersion of the sediment and by extension its immunogenicity. The lowest the S_{onset}, the most powerful the therapeutic efficiency.

CONCLUSION

Turbiscan^{LAB} can help to design a properly flocculated Alum-containing formulation. By varying the formulation pH, ionic strength, and the quantity of antigens, it is possible to identify ideal conditions that favor a flocculated system. The use of the settling characterization methodology described above (SVR and S_{onset}) facilitates the decision. The S_{onset} and SVR data from the Turbiscan analysis identified the transition zone between the flocculated and deflocculated states of $AlPO_4$ formulations. Finally, because **the analytical time is much shorter, the S_{onset}** values can replace the SVR data (a widely used parameter for characterizing suspensions) being more adapted for formulation screening activities.

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