



Precipitation of Allergenic Extracts

Occasionally, physicians and other health care practitioners who use allergenic extracts notice precipitation in those extracts. What is precipitation? Is it dangerous for patients? Why does it occur? How can it be prevented? In this newsletter, we explore the issue of precipitation with the objective of helping you to become more knowledgeable about the nature of allergenic extracts.

What is Precipitate?

Precipitation is the formation of a solid in a solution during a chemical reaction. When the reaction occurs, the solid formed is called the precipitate, and the remaining liquid is called the supernate.

What Conditions Cause Precipitation?

Mechanical shock – sudden shocks of saturated solutions are known to cause the solute to precipitate.

pH shock – a solution that interacts with the vial or stopper may rise or fall in pH, causing precipitation.

Temperature shock – a large rise in temperature, or several warm/cold cycles (in and out of the refrigerator) may cause precipitation.

Excipients and other components of the solution, such as phenol, may react with the vial or stopper or with the air in the head space of the vial and cause a brown discoloration and/or precipitation.

Suspended particles may begin to stick together, forming complexes that become larger and larger until they drop out of solution and become visible. The cause may be attraction of opposite charges of the particles at a molecular level or Van der Waal interactions, but in the end, these complexes fall out of solution.

A common misconception is that precipitation is the result of microbial contamination. While a precipitated allergenic extract *could* be contaminated with bacteria, microbes are not the cause of precipitation, and are not the precipitate. Some people have equated a cloudy allergenic extract with a contaminated vial of growth medium such as Tryptic Soy Broth. Testing has shown that the growth medium must contain millions of microorganisms per mL to become visually cloudy $>(10^6)$. Although a cloudy allergenic extract may look the same, the conclusion is not correct.

Is Precipitation Dangerous for Patients?

The treatment of patients with allergenic extracts in the form of immunotherapy has existed for many years. In the past, physicians were familiar with seeing precipitate, or particles falling out of solution and collecting on the bottom of vials of allergenic extract. Common practice for many physicians was to continue to use the extract based on their experience with this phenomenon.

However that began to change around the year 2000 when the issue of precipitation became a regulatory concern. The impetus for this was a question of safety. Now the question was asked of manufacturers, what is precipitation? Is it dangerous to patient safety? Does it indicate that the product is contaminated with bacteria?

Allermed has tested the sterility of multiple precipitated allergenic extracts and has found no correlation between precipitation and sterility. Also, allergenic extracts are preserved with either phenol, glycerin or both. (Allermed's extracts are preserved with 0.4% phenol and 50% glycerin). These bacteriostatic agents present in the allergenic extract make it extremely unlikely that a single organism or small number of organisms could ever proliferate to such a high concentration.



Finally, refrigeration of allergenic extracts does not provide a suitable environment for such an occurrence. Most of the common bacteria found in the environment grow best when incubated between 20°C and 35°C, but not at 2°C - 8°C, the labeled storage temperature for allergenic extracts.

The Manufacturing Process

One of the first steps in manufacturing an allergenic extract is to place the solid allergen in extraction fluid. The allergenic portion is extracted into fluid (supernatant), and the solid material is removed by centrifugation and/or filtration. At this point the supernatant is allowed to sit in the refrigerator at 1°C to 5°C during a period of cold storage. Some extracts have been noted at this stage to routinely precipitate.

After this step, the product is filtered through successively smaller pore-sized filters to remove the particles. This results in a solution of materials that do not precipitate from the solution, but remain in solution or suspension. Think of this as a saturated liquid¹. Depending upon the extract, some materials dissolve into solution, while others remain in suspension due to their small size (smaller than the largest pore in the smallest pore-sized filter).

Most extracts that have completed the manufacturing process remain in a dissolved or suspended state unless some physical condition changes.

How can Precipitation of Allergenic Extracts be Prevented?

There is more than one answer to this question. It is known that glycerinated allergenic extracts are more stable than aqueous extracts. Glycerin acts as a stabilizer for proteins, keeping them in solution even under some of the adverse conditions noted above, such as temperature or pH shock. Aqueous short ragweed for example, will almost always precipitate over time. So using glycerinated extracts will reduce the number of precipitated allergenic extracts that you experience.

In general *the higher the protein concentration of the allergenic extract, the more likely it is to precipitate*. The higher protein allergenic extracts are more commonly extracts of plant pollens. As with all rules, there are exceptions. It has been noted that highly dilute (aqueous) allergenic extracts (i.e. 1:100 w/v or higher) also tend to precipitate. It is to your advantage, therefore, to purchase and compound allergenic extracts preserved with 50% glycerin.

After receipt, keep allergenic extracts between 2°C and 8°C at all times, even during use. This will help prevent precipitation caused by repeated changes caused by moving the vial from room temperature to refrigerated temperature and back. During processing, allergenic extracts are exposed to several temperature cycles for processing and visual inspection. They are relatively stable, but the more temperature changes that occur, the higher the risk for precipitation. Be careful to avoid extremes caused by summer heat or winter freezing.

As part of the production process of allergenic extracts, Allermed performs a 100% visual inspection of each product manufactured and if precipitation is found, a product is rejected (not released for sale). An additional visual check is performed on each vial before it leaves Allermed's facility to ensure that a precipitate has not formed during cold storage at Allermed prior to shipment. Any vial found to contain a precipitate will not be shipped from Allermed.

In Summary

Office staff should be aware that precipitation is a known phenomenon of allergenic extracts. They should be trained to look at each vial prior to use for a sediment on the bottom of the vial. If precipitate is found, the vial of allergenic extract should not be used. Report the incident to the manufacturer and return the precipitated vial for

¹Saturation refers to the ability of a liquid (solvent) to hold as much of a solute as possible at a given temperature and pressure.



examination and testing. If precipitation is found in allergenic extract purchased from Allerved, it will be replaced without charge. Take precautions to prevent precipitation from occurring, such as maintaining the products at the labeled temperature.

As always, if you or your staff have any questions about Allerved's products, techniques or supplies related to the aseptic manufacture of allergenic extracts, please feel free to contact us by email at info@allerved.com, or at (800) 221-2748. Our technical support staff will be happy to assist you.