
Inactivating Virus Without Inactivating Your Product

by Pete Gagnon, Validated Biosystems

This column is in response to a plea that arose from someone finding that their low pH virus inactivation step also killed their product (an antibody). In this particular case, the results were catastrophic, causing total product inactivation. However, partial product inactivation following antiviral treatment is so common that it can almost be considered a given, and many people accept it without question. This is not as it should be. A loss of product activity more than likely reflects a change in product structure, which in turn implies the possibility that other important aspects of product function may have been altered -- for example pharmacokinetics or clearance. Besides affecting a product's biological function, important but nonspecific behavioral features may be affected as well. For example, partially inactivated products tend to exhibit much higher tendencies toward aggregation and truncated shelf life.

One option is to select an antiviral agent that doesn't affect product structure or function. While it is a good idea to screen more than one, there are limits. A superficial but real limit is the expense of validating antiviral effectivity for multiple methods. A more substantive limitation is that essentially all viral inactivation methods carry an inherent risk of product alteration or inactivation. This leads many people to default to acid inactivation since, if they have to tolerate product damage anyway, they might as well use the method that requires the least additional processing. Re-equilibrating pH after inactivation involves only titration. With other methods, there has to be a subsequent process step to remove the inactivation vector.

Given the logistic and economic motivation to employ low pH, are there ways to ameliorate its product-damaging effects without detracting from its efficacy? Fortunately

the answer is yes. Although some proteins have rigid structures, they are exceptions. Most undergo a considerable degree of conformational "flexing" in free solution. Published research with antibodies has shown that this flexing -- at low pH -- extends to the point where normally-buried hydrophobic residues come to the surface. The conformational changes accompanying this exposure are permanent. As a result of these alterations, antibody titer is depressed and effector functions are confounded. Corresponding changes occur in non-immunoglobulin proteins.

If a protein could be conformationally constrained, might it be possible to prevent, or at least reduce damage caused by exposure to low pH? Published data show this to be the case. Most antibodies are inactivated rapidly in free solution at pH 3.0. Measurable denaturation occurs within minutes. However, when bound to a cation exchanger, many antibodies are able to withstand pH 3.0 for hours, with no apparent loss of titer or other implication of conformational change.

If immobilization protects your product, why won't it protect viruses too? It could, to some degree. However, most proteins are very small compared to most lipid enveloped viruses. Proteins should therefore be constrained (ie., protected) to a much greater degree on a functionally 2-dimensional adsorptive surface. Viral particles, to the extent that they are adsorbed at all, should be immobilized by a relatively minor proportion of their surface, like a water-balloon glued to a wall. The remainder of an adsorbed particle body should have about the same vulnerability to the chromatography mobile phase as it would in free solution. There are no published studies on this point, but unpublished results are consistently favorable. They show

far better recovery of active protein, often with improved viral reduction. The improvement in viral reduction may result from some of the load washing through the column while the protein is bound.

Besides protecting your product, "solid phase" inactivation on cation exchangers offers several advantages. One is its potential breadth of applicability. Essentially all proteins bind to cation exchangers at the pH values customarily used for viral inactivation. In addition, many proteins can be loaded at a more moderate pH, and then the pH of the loaded column can be titrated down. After timed low pH exposure, the column can be retitrated back to a more moderate pH before the protein is eluted. This avoids any protein exposure to denaturing conditions while it is in a conformationally vulnerable state. Most current industrial chromatographs make this an easy process adjustment to automate. They also offer the validation benefit of producing hard copy documentation. Another benefit is that solid phase inactivation can be easily integrated into an existing (or developing) purification process -- and it can be integrated at any process step. Viral inactivation need not be a post-purification add-on.

The effectivity of solid phase inactivation can be enhanced by adding agents that further destabilize viral lipid envelopes. For example, you can add 2-4 M urea during low pH treatment without concern for protein stability. Urea is uncharged and won't interfere with protein binding to the exchanger. You can probably add 6-8 M since the protein is conformationally constrained (although this would require validation). Then after treatment, simply wash out the urea before elution. Instead of urea, you could enhance with a nonionic detergent or other nonionic agent, like ethylene glycol. These

enhancements are generally omitted from free solution low pH treatments because they add the complication of a subsequent removal step. Another mode of enhancement is to maintain flow of the inactivating solution through the column during treatment. As implied above, some viruses may not be adsorbed. If you can remove them from your product, instead of just inactivating them, it can only work to your advantage.

Solid phase inactivation can also be conducted with chromatography methods other than cation exchange. Immobilized metal affinity chromatography with ferric ions on iminodiacetic acid (IDA) supports low pH applications as effectively as cation exchange. It is likewise unaffected by uncharged additives, but also very tolerant of high salt concentrations. Copper, nickel, zinc, and cobalt on IDA do not support low pH application, but at alkaline pH they are compatible with a wide range of additive treatments, including the Solvent/Detergent (S/D) method of viral inactivation developed by the New York Blood Center. Protein A is also compatible with the S/D method in cases where the binding constant is very strong. Immunoaffinity applications are similarly compatible. Other combinations depend on your ingenuity. The requirements are that the inactivating agent(s) or conditions must not interact with or affect the column in a way that will interfere with protein binding, and neither can column interactions limit the ability of the inactivating agent(s) to affect virus.

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