

Vaccine Characterization Using Advanced Technology

Mass spectrometry offers the potential for an unprecedented understanding of vaccines and why they fail.

Steven Becht, Xuelin Gu, Xiaoya Ding

Abstract:

The development of safe, effective, and affordable vaccines has become a global effort due to its vast impact on overall world health conditions. In this paper, a brief overview of vaccine characterization techniques, especially in the area of high-resolution mass spectrometry, is presented. It is highly conceivable that the proper use of advanced technologies such as high-resolution mass spectrometry and nuclear magnetic resonance, along with the appropriate chemical and physical property evaluations, will yield tremendous in-depth scientific understanding for the characterization of vaccines in various stages of vaccine development. In addition, this approach can potentially be more efficient and effective for supporting vaccine research and development.

New initiatives, strategic planning, and guidance from the World Health Organization (WHO), the US Food and Drug Administration, the European Union and other regulatory bodies, through collaborations with industry and academia, have resulted in an increasing level of scrutiny of biologically based pharmaceuticals such as vaccines.¹⁻¹⁴ The Draft FDA Guidance for Industry, “Characterization and Qualification of Cell Substrates and Other Biologi-

cal Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Disease,” indicates a trend in this direction. Characterizing these drug products is becoming increasingly important in bringing a new vaccine to the market, since this information helps to define the vaccines’ safety and efficacy. Modern mass spectrometric



cGMP Laboratory of PPD

Xiaoya Ding, PhD, is the director of scientific and technical affairs at the cGMP Laboratory of PPD, 608.827.9400, Xiaoya.Ding@madison.ppd.com. At the same company, **Steven Becht, PhD**, is a research scientist and **Xuelin Gu, PhD**, is a laboratory manager.

(MS) methods for characterizing vaccines provide many advantages relative to the more classical characterization techniques that have been historically employed. Additionally, this technology has applications in all phases of vaccine development.

The development of safe, efficacious, and affordable vaccines is the main focus of multiple government agencies, the WHO, industry, academia, health care providers, the public, and philanthropic organizations such as the Bill and Melinda Gates Foundation. Grants funded by the National Institutes of Health (NIH) and the Centers for Disease Control, along with pharmaceutical industry research, begin the process of developing potential vaccines. The pharmaceutical industry, with oversight from the FDA, moves these vaccines from early development to clinical trials. The involvement of the public begins with these trials and continues throughout the useful lifespan of the vaccine. Health care providers and the public provide vital feedback regarding vaccine effectiveness and complications that are then used to improve the vaccines further.

Background

In general, vaccines are a very heterogeneous group of preventative medicines with an increasingly wide variety of adjuvants used in their formulation. Some of these adjuvant components can cause unwanted side reactions in the vaccinated individual that in some cases can result in serious complications. These complications are generally monitored as part of the potency and toxicity testing of vaccines in animal or other cell-based models, and are the surveillance responsibilities of health care providers once the vaccines are released. As greater characterization of vaccines becomes more prevalent, it

may be possible to connect structural changes in the vaccine components with lost potency and increased toxicity issues. This, in turn, may provide a better understanding of how certain vaccines function and interact with the immune system. Information gained in this area will undoubtedly improve the effectiveness and safety of future vaccines above today's already high standards.

Vaccines can be broken down into three major categories: live vaccines, killed or attenuated vaccines, and component vaccines. The third type, the component vaccines, are generally the most easily characterized of the three. They usually consist of a relatively small number of immunogenic molecules and an adjuvant system, which is often well defined. The other two vaccine types include complex biological components such as attenuated or killed viruses and intact bacteria or multiple bacterial components. The characterization of these vaccines typically focuses on the adjuvants used to improve effectiveness. Advances in proteomics make the characterization of even these difficult vaccines more manageable.

Vaccine Characterization

Vaccine Characterization Using Classical Methods

The classical methods of vaccine characterization rely on the study of physical-chemical properties using methods such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), pH, various stress conditions (agitation, freeze-thaw, etc.) based on particulate formation, and methods of quantitating protein content as well as elemental composition. While these methods are capable of determining whether or not the end product is consistent with previous batches, they are unable to detect small changes that can

result in a vaccine with reduced or even lost preventative characteristics. Not all changes to the structure of the vaccine components have physical consequences, but many of them result in reduced vaccine performance. Most of these techniques lack sensitivity when it comes to detecting small changes in the structure of the vaccine components that can cause them to fail during use. Some changes can cause severe side reactions even in small quantities.

TGA and DSC are used to analyze the denaturing point of the vaccine protein or nucleotide. These tests generally give indirect indications of changes in the vaccine with time and stress. Changes in protein sequence or modifications can substantially affect denaturing kinetics, but these techniques provide no way to correlate these changes with actual changes in the structure of the molecule. Appearance and pH are used to monitor major changes in the composition of the vaccines and are relatively insensitive to these changes. Other physical characteristics that affect vaccine function include particle size and particle size distribution. Clumping of the vaccine antigen can degrade the function of the vaccine and can cause unwanted side effects. Specific tests for quantitating proteins or oligonucleotides, such as elemental analysis and total protein content (bicinchoninic acid, or BCA) tests, can provide vital quality control data for troubleshooting manufacturing problems, but they are of limited value in analyzing degradation of the vaccines since elemental composition changes from degradants represent only a small percentage of the overall elemental composition. In addition, most protein degradants will still be identified as proteins in a total protein analysis. The conditions used for these assays also break up clumped proteins or

oligonucleotides and are insensitive to most changes caused by minor structural modifications of these molecules.

Vaccine Characterization Using Mass Spectrometry Technologies

Various mass spectrometry (MS) technologies are readily available today for use in vaccine characterization to assist in understanding vaccine properties and functions for all phases of vaccine development processes from vaccine discovery, development, formulation, manufacturing, stability, QC, and release, to post-market surveillance. ICP-MS, GC-MS, HPLC-MS, and ESI-Q-ToF-MS are a few of the MS technologies that can be used extensively in vaccine characterization.

ICP-MS is used to qualitatively and quantitatively measure toxic metals that can be introduced into a vaccine by the manufacturing process and can have severe negative effects when the vaccine goes into widespread use. The ICP-MS technique oxidizes all of the organic components in the vaccine to CO₂, leaving behind all of the metal ions to be analyzed by either hot or cold plasma mass spectrometry. The results obtained from ICP-MS analysis can provide some insights into loss of metal co-factors and other groups.

GC-MS and HPLC-MS are used to determine the molecular weight of the various vaccine components. These technologies can provide a means of determining if a change in the molecular structure has occurred, but generally cannot localize it. Improvements in the sensitivity of mass spectrometers and the increasing mass accuracy of the instruments have improved the capabilities of these tests to the point where they can identify even minor changes in the vaccine components. Localizing these modifications relies on another feature of

the mass spectrometer. By placing a collision cell between two mass analyzers, additional structural information can be obtained. In the case of proteins and oligonucleotides, these fragmentation events occur in a predictable manner that allows interpretation of even very complex spectra. With instruments possessing a high mass accuracy, localization of modifications to the protein can be accomplished. Many software packages currently available can automate this process substantially.

Electrospray ionization (ESI) has significantly increased the size of proteins and biopolymers that can be detected by most mass spectrometers, and provides a

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means to accurately characterize component vaccines. This is accomplished by generating multiple charge states by ESI and using deconvolution software to analyze the spectra. This technology added a separation capability, through HPLC, to the analysis, allowing vaccine components to be separated online before mass spectrometric analysis. With the advent of high-resolution mass spectrometers such as the Quadrupole–Time of Flight (Q–ToF) and highly sensitive MS instruments, such as the Quadrupole–Ion Trap (Q–Trap), characterization of vaccines has entered a new phase. Minor modifications to the immunogenic protein or nucleic acid can now be detected and a better understanding can be achieved of what causes vaccines to fail as they age. These new techniques have not replaced

older cell-based potency assays, however, because the mechanisms connecting these structural modifications to functional differences have not been fully established. Many modifications have no effect on the efficacy of the vaccine, but modifications to the sections of the protein or nucleic acid that interact with the host immune system can change or eliminate the ability of the immune system to recognize the epitopes and thus elicit an immune response. By combining these data with the results of potency and toxicity studies, a new picture may be developed of the changes in the vaccines that cause these failures.

New advances in analytical technology have also provided a means to characterize these vaccines to a degree that had not previously been possible. Older techniques such as gel electrophoresis (one and two dimensional) and affinity chromatography are being augmented by high-resolution, high-accuracy mass spectrometric methods as well as the newly evolved field of protein NMR. The mass spectrometric methods offer the ability to identify even very minor structural changes in the protein or lipid components of the vaccine with a high degree of accuracy. The NMR methods provide powerful conformational data regarding biopolymers present in the vaccines. These changes in the structure of vaccine components may have previously gone undetected using the classical techniques of vaccine characterization.

High resolution mass spectrometry provides the ability to better characterize the changes in the immunogenic protein or nucleotide that result in changes in physical properties. Q–ToF can provide detailed sequence information about these nucleotides and proteins to a level that the physical techniques cannot. Through the use of enzymatic digestions,

even large proteins can be broken down into manageable fragments for sequencing. The peptide bonds between amino acids and the bonds in the phosphate backbone of oligonucleotides are the weak points in the structures and fragment predictably during MS–MS analysis. The high-mass accuracy of the mass spectrometers allows unambiguous assignment of sequences based on molecular weight data and can be combined with MS–MS analysis when the molecular weight data provides more than one possibility. When dealing with known or simple vaccines, this process is fairly straightforward.

When analyzing more complex vaccines, such as those containing complex adjuvants or multiple immunogenic components, the situation becomes less clear. With these types of vaccines, the discriminatory power of the mass spectrometer is of great aid. HPLC–UV analysis of these complex mixtures often results in multiple components co-eluting. Due to the heterogeneous composition of these types of vaccines, this is often unavoidable. The mass spectrometer offers an additional level of separation through its mass sensitive or selective detector. Even co-eluting components can be resolved through mass spectra.

These techniques have been success-

fully used, for example, to determine phospholipid composition and to characterize proteins in complex vaccines. Phospholipids contain polar head groups that contain both phosphates, which respond strongly in negative ion mode, and amine containing groups that respond well in positive ion mode electrospray. One common adjuvant is lecithin. This material is composed of a number of related phospholipids that can be separated by HPLC and identified by their mass spectra. Proteins such as the B subunit of cholera toxin can be characterized in much the same way as protein-based component vaccines. Aluminum-based adjuvants can be detected through the use of ICP-MS, and other amine-based adjuvants are easily detected through positive mode electrospray mass spectrometry. An overall fingerprint of a vaccine can be constructed using mass spectrometry data combined with HPLC retention time data. This fingerprint can then be used to monitor changes in the vaccine from batch to batch or as the vaccine ages, or to characterize reference standards during the process of standards development, evaluation, and further improvement. This is the centerpiece in the field of developing effective (novel), affordable, and safe vaccine formulations. These, in turn, will aid the global development and further refine-

Table 1. A summary of various vaccine characterization technologies

Technology	Advantages	Disadvantages
Physical	Fast and non-selective	Lack of sensitivity and/or specificity
Chemical	Moderate sensitivity/selectivity	Lack of unknown identification power
Routine MS	Two-dimensional selectivity, moderate specificity	Lack of specificity to some degree, especially for large-molecule analysis
Advanced MS	High mass accuracy/specificity, which simplifies unknown identification using techniques such as peptide mapping/sequencing/fingerprinting	Not readily available because of relatively high upfront equipment investment

ment of good manufacturing practices for vaccines and development of global policies and guidelines.

Small peptide antigens are normally conjugated to a large carrier protein to increase immunogenicity. The number of small peptides per carrier protein in the conjugation product (i.e., the epitope density) is important for vaccine efficacy. Quantitation methods for epitope density are needed for vaccine production control and stability determination. Amino acid analysis can be used to determine the vaccine epitope density. After conjugating a small peptide to a larger carrier protein, the amino acid composition will be changed. The epitope density can then be measured by comparing the amino acid compositions of the unconjugated carrier protein and the vaccine peptide conjugated carrier protein. This method can only determine the mean epitope density, not the distribution of the epitope density.

Mass spectrometry can be used to measure the epitope density by determining the molecular mass difference between the unconjugated carrier protein and the vaccine peptide conjugated carrier protein. Both the mean and the distribution of epitope density can be determined by mass spectrometry, but the method accuracy and precision are not as good as with amino acid analysis due to the heterogeneous properties of the carrier protein and the conjugation. These two methodologies are complementary to each other and it is recommended to use amino acid analysis to measure the mean epitope density and mass spectrometric method to measure the epitope density distribution. The epitope density also can be measured with enzyme linked immunoassay (ELISA) by using the anti-vaccine peptide antibody. Since the variation in ELISA method is normally significantly larger than in physical-

chemical methodology, this method is not commonly used to measure the epitope density. Instead, it is used to measure the vaccine activity.

Conclusion

A summary of various vaccine characterization technologies are presented in Table 1. As can be seen, advanced mass spectrometry as a tool has applications in all phases of vaccine development. The ability to characterize a vaccine to the levels now allowed by modern mass spectrometers offers the potential for an unprecedented understanding of vaccines and why they fail. Classical characterization techniques are often used as a screening method to identify which aged or stressed vaccines need further investigation by mass spectrometry. All phases of vaccine development, from initial characterization to troubleshooting of the manufacturing process, and all the way up to release testing of the final vaccine product, can benefit from these new technologies. They may even be used to monitor the stability of vaccine materials once they have entered the marketplace. It may eventually become possible to characterize the changes that make vaccines fail on a structural level, based on data collected through the analysis of de-graded vaccines by mass spectrometry. Mass spectrometry also provides a means to verify the more classical techniques, and can be used as an adjunct to these methods to provide a deeper understanding of how vaccines function. This can lead to improvements that will make vaccines even safer and even more reliable and affordable. ★

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