

Review on Effect of the Chemical Composition of some Pharmaceutical Vaccines and their Biochemical Efficacy with Chemical Techniques

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Abstract

Chemical compounds are fundamental components in the formulation of medicines and vaccines, playing a critical role in ensuring their safety, stability, and effectiveness. Vaccines contain a combination of active and inactive ingredients, including weakened or inactivated viruses or bacteria, toxins, preservatives, stabilizers, adjuvants, antibiotics, and minerals. The active ingredient is an antigen, which stimulates the body's immune system to recognize and defend against specific infectious diseases. Adjuvants, such as aluminum salts, are commonly added to enhance the immune response and improve antibody production. Other components, including gelatin and antibiotics, help maintain vaccine stability and prevent microbial contamination during manufacturing and storage. The chemical composition of vaccines is carefully evaluated using a range of analytical techniques to ensure quality, purity, and consistency. Among these, molecular spectroscopy is widely used to identify and quantify small-molecule components, preservatives, adjuvants, and trace impurities. This technique provides detailed molecular information, making it indispensable for the characterization and quality control of complex vaccine formulations. Vaccines are broadly classified into live attenuated and inactivated vaccines. Live attenuated vaccines contain weakened forms of viruses or bacteria that retain their ability to stimulate a strong and long-lasting immune response without causing disease in healthy individuals. In contrast, inactivated vaccines contain killed pathogens or their components and generally require booster doses to maintain immunity. Although live attenuated vaccines are highly effective, they may not be suitable for individuals with weakened immune systems. Overall, understanding the chemical composition and analytical evaluation of vaccines is essential for ensuring their efficacy, safety, and successful prevention of infectious diseases.

Keywords: Vaccines, chemical composition, molecular spectroscopy, adjuvants, live attenuated vaccines, inactivated vaccines, analytical techniques.

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INTRODUCTION

The incorporation of chemical compounds into specific laboratory-developed vaccines is a fundamental aspect of vaccine formulation and development. This intricate, multi-step process requires thorough assessment of every ingredient included in the vaccine composition. The overall safety, effectiveness, and stability of a vaccine are influenced not only by the active antigen but also by supplementary components such as adjuvants, preservatives, and stabilizers. Comprehensive analysis and testing of these constituents are vital to ensure compliance with the strict quality, safety, and regulatory requirements necessary for vaccine

approval and widespread distribution [1]. This article discusses the various analytical methods used to test drug components during vaccine development, providing valuable insights for professionals in the biopharmaceutical industry. The rapid development of new chemotherapeutic therapies using live biological or genetic components and gene therapy mechanisms is sparking discussions across all sectors of healthcare regarding the safe handling of these drugs and the adoption of standardized measures to minimize occupational exposure risks. The application of principles for the classification and handling of hazardous drugs, pharmacovigilance, and collaboration among experts in biosafety, epidemiology and infectious diseases, infection prevention, and virology is an effective means of developing a unified policy, strategies, and a systematic set of standards for determining the appropriate precautions required for therapeutic drugs, live attenuated viruses, or viral vaccines [2].

The Chemical Composition of Vaccines

Toxic vaccines contain inactivated toxins from pathogenic bacteria. Conjugated vaccines are made from a mixture of pathogenic sugar molecules and toxic proteins, as the sugars themselves do not elicit a strong enough immune response. Vaccines are made using bacteria or yeast cells to produce multiple copies of specific molecules from the pathogen. But in addition to all these components, vaccines also contain other substances, scientifically known as excipients [3]. Excipients include preservatives, stabilizers, and adjuvants that enhance the effectiveness of the vaccines. While many vaccines contain potent active ingredients sufficient to stimulate our immune system to produce antibodies against the disease, some require a little extra help to be effective. Adjuvants are compounds that stimulate a strong immune response, thus improving vaccine effectiveness. Examples of adjuvants include Minerals, oils, and aluminum, which is used in vaccines in the form of aluminum salts. A variety of vaccines, including many routine childhood vaccines, contain aluminum because scientists have found that this adjuvant increases antibody production [4].

Aluminum is a naturally occurring metal with many uses besides its adjuvant properties. Cans, foil, and some window frames contain aluminum. In addition to aluminum, vaccines also contain gelatin, a stabilizing agent used in some vaccines to protect the active ingredient. Gelatin is usually derived from pigs and is highly processed. Other stabilizers include sweetener sorbitol, sugar molecules, sucrose, and lactose. Antibiotics are also used in the production of vaccines against some viruses to prevent bacterial contamination [5].

The term "chemical vaccine" often refers to two distinct types: vaccines developed using chemicals to neutralize toxins (such as toxoid vaccines for diphtheria and tetanus), or newer cancer vaccines that use mRNA technology to stimulate immunity. More recently, personalized vaccines have been developed that are injected directly into tumors, where the tumor is analyzed and a specific vaccine is developed to train the immune system to attack it [6].

Chemically Manufactured Vaccines

Some types of vaccines are prepared from pharmaceutical chemical compounds with a known pharmaceutical composition that includes active groups that are effective in the type of disease for which they are used, such as glutaraldehyde and formaldehyde. These are chemical substances used to stop the activity of toxins from viruses and bacteria in some vaccines [7]. These chemical substances are toxic in large quantities. There are differences between live and inactivated vaccines. An inactivated or killed vaccine is a concentrated substance combined with an oil suspension or aluminum hydroxide after being inactivated with formalin or beta-proprionate. It provides high immunity, especially after starting with live vaccines. It must be administered by injection only. It often contains 2-3 different disease variants in a single vaccine. Workers in chemical laboratories, especially those handling biological or medical samples (biochemical and analytical laboratories), are considered a risk group, and it is recommended that they be offered a range of vaccinations to ensure their safety [8]. Some of these vaccines contain biochemical substances to prevent and reduce the risk of dangerous diseases [9].

Characteristics of Chemically Synthetic Vaccines

Some may be alarmed by the idea of synthetic chemical adjuvants in vaccines, but in reality, they are present in extremely small quantities. A typical vaccine dose contains no more than 0.2 milligrams of aluminum, less than the weight of a grain of sand. There is no evidence that these currently used adjuvants cause side effects. However, the primary motivation behind adding these chemical adjuvants was to enhance vaccine safety. In the 1970s, John Wilson, a pediatric neurologist, addressed the Royal Society of Medicine, falsely blaming the pertussis vaccine for brain damage in 36 children. This suggested that adding chemical adjuvants to some vaccines could lead to adverse health effects. The news was picked up by the press, quickly escalating into a full-blown scandal that dominated media attention. Years later, pertussis vaccination rates in the UK plummeted to less than half, and some countries stopped offering the vaccine altogether. But this accusation proved unfounded. Many people had received the vaccine over decades without any reported harm or side effects, aside from some minor, immediate effects like fever [10]. The scandal spurred scientists to search for new ways to manufacture vaccines. Most vaccines previously were made from live microorganisms that had been weakened in some way to make them less harmful or less capable of causing disease, but which still helped the body recognize pathogens. Some vaccines contained whole, killed microorganisms, as in the pertussis (whooping cough) vaccine, which is usually given in conjunction with the tetanus and diphtheria vaccines. If a person is immunocompromised, they may still contract the very disease the vaccine is intended to protect against. Consequently, many vaccines contain inactivated forms of the disease-causing agent, such as whole killed bacteria or viruses. However, the majority of modern vaccines are acellular and do not include the complete pathogen. Instead, they are produced using chemically synthesized components derived from the pathogen, including specific proteins or carbohydrate molecules. These molecular fragments are recognized by the immune system as foreign substances, thereby triggering a protective immune response [11].

The Biochemical Mechanism of Vaccines

When we receive a vaccine, the virus stimulates our immune system to release a cascade of antibodies that protect us from future pathogens. But a quick look at the ingredients in common vaccines reveals a long list of other components, whose roles may not be immediately obvious. Why do some vaccines contain aluminum? There are both active and inactive ingredients that make their way into vaccines, revealing their role in protecting us from infectious diseases. These adjuvants, added to vaccines, have become increasingly important, particularly because vaccines do not elicit a stronger immune response in those most vulnerable to infection. For example, the flu vaccine was observed to be only 58 percent effective in preventing complications in people over 65, compared to 77.6 percent effectiveness in younger age groups [12].

Concerns have already been raised about the effectiveness of the COVID-19 vaccine in stimulating an immune response in the elderly, especially since those over 80 are hundreds of times more likely to die from the disease than those under 50. With the world's aging population, we may be in dire need of a new generation of adjuvants that promise to improve the effectiveness of modern vaccines. Experiments are underway to utilize flagellin, a protein found in the flagella of bacteria such as Salmonella. Scientists sometimes obtain bacterial flagella by isolating them from the cells, but recently they have been working to develop them in genetically modified cells. Although flagellin has not yet been approved for use in a human vaccine, the results of trials so far have been promising. When a person is infected with bacteria, the protein binds to receptors on the surface of immune cells, triggering signals that alert other immune cells to gather around the infected cells and produce antibodies [13]. This is what scientists expect will happen when flagellin is added to a vaccine; it will attract the attention of the immune system, allowing the vaccine to do its job. Some have also suggested adding bacterial envelopes, called "ghost bacteria," by cutting open bacterial cells, such as E. coli cells, and obtaining their outer cell membranes. Like squalene-based adjuvants, these bacterial envelopes stimulate cells to produce signals to other immune cells, improving the vaccine's chances of finding them [14].

The Effect of Adding Chemical Compounds to Some Vaccines

Aluminum is one of the earliest adjuvants added to vaccines. In 1926, shortly after Ramon discovered that nutrients added to vaccines stimulated an immune response, Alexander Glenny, a British immunologist, was conducting experiments to purify the toxins produced by diphtheria bacteria so they would dissolve slowly in the body. Glenny hypothesized that if they remained at the injection site for a longer period, they would stimulate a stronger immune response. Glenny began adding aluminum salts, which were probably the first substance he encountered on a laboratory shelf. When he injected pig squirts with a diphtheria vaccine to which aluminum salts had been added, he observed that it stimulated a significantly stronger immune response compared to a vaccine without the added aluminum salts [15].

Aluminum is still added to vaccines today in the form of salts, such as aluminum hydroxide (used as an antacid), aluminum phosphate (used in dental cement), and potassium aluminum sulfate, which is sometimes added to baking powder. Glenny theorized that aluminum salts boost immunity by binding to the vaccine's main component, which resembles the pathogen, and slowly releasing it from the vaccine. This allowed the immune system to prepare and respond, resulting in stronger immunity against the disease. However, this idea is now outdated, and newer, more complex explanations have emerged. One such explanation is that the effectiveness of aluminum salts stems from their toxicity, as they stimulate infected cells to release uric acid, which triggers an immune response upon detecting injury or damage. Immune cells then gather at the site of the injury and produce antibodies. Another explanation came from a study led by Richard Flavel at Yale University. Flavel genetically modified mice to lack the Nalp3 gene, which codes for a protein, and injected them with a vaccine containing aluminum. He observed that the immune response in these mice was barely noticeable. This indicates that the aluminum in vaccines relies on activating the Nalp3 receptor, which triggers signals to stimulate the immune system and generate a stronger response [16].

This is perhaps the primary reason for the effectiveness of adjuvants, in all their various forms and mechanisms of action. These adjuvants draw the attention of the immune system, helping it recognize the pathogen mimicked by the vaccine and release the appropriate antibodies to fight it should it attack the body in the future. For example, squalene, an oil extracted from shark liver and a key component of the adjuvant MF59, has been added to seasonal flu vaccines and is being tested for use in vaccines against COVID-19. This has sparked considerable controversy, as distributing the vaccine to the entire world's population could lead to the killing of 250,000 endangered sharks. This adjuvant works by stimulating neighboring cells to release chemokines, small proteins that regulate the immune response. The chemokines attract immune cells that engulf the vaccine, including the pathogenic part that it is supposed to protect the body from, and transport it to the lymph nodes, which expel the pathogens from the body and help identify the infection [17].

Chemical or Biological Preparation of the Vaccine

One common method of vaccine production involves using the disease-causing virus or bacterium, or a closely related microorganism, and then inactivating or killing it through physical or chemical treatments such as heat, radiation, or chemicals [18].

Another approach involves the use of ultrasound, which can support several stages of vaccine manufacturing. The first step in vaccine production is the preparation of the antigen, and the method used depends on the type of pathogen involved. Viruses are typically cultivated either in primary cell systems, such as chicken eggs for influenza vaccines, or in continuous cell lines like cultured human cells for hepatitis A vaccines. Bacteria, on the other hand, are generally grown in bioreactors, as in the production of *Haemophilus influenzae* type b vaccines. In addition, recombinant proteins originating from viruses or bacteria can be produced in cultures of yeast, bacterial systems, or other cell lines. Once the antigen has been generated, it must be separated from the host cells in which it was produced. In some cases, the virus may simply need to be inactivated without extensive purification. Recombinant proteins, however, usually require additional downstream processing steps such as ultrafiltration and

column chromatography. Finally, depending on the formulation of the vaccine, other components such as adjuvants, stabilizers, and preservatives are incorporated to improve its effectiveness and stability [19].

Adjuvants are added to strengthen the body's immune response to the vaccine antigen, while stabilizers and preservatives help maintain the vaccine's quality and extend its shelf life. Ultrasound can be employed at multiple stages during vaccine production. Since it is a non-thermal technique, it minimizes the risk of heat-induced degradation of sensitive biological materials. One of its major roles in vaccine manufacturing is the dispersion of antigens. For a vaccine formulation to remain stable, antigens such as protein molecules or cell fragments must be evenly distributed within suspensions, polymer matrices, or lipid coatings. Sonication has long been recognized as an effective method for producing fine dispersions in pharmaceutical manufacturing, making it a well-established tool in modern vaccine development. Aluminum-based adjuvants, which are widely used in vaccine formulations, are composed of extremely small particles that can be readily incorporated into the final product. To ensure effective interaction between antigens and adjuvants, the antigen must be uniformly distributed throughout the aluminum-containing formulation. Ultrasonic dispersion facilitates this process by promoting a homogeneous mixing of antigens and adjuvants [20].

Chemicals Added to Vaccines

Vaccines generally include one or more adjuvants to strengthen the immune response generated against the antigen. Ultrasound plays an important role in this process by dispersing and homogenizing adjuvant microfibrils, thereby improving the binding of proteins to their surfaces. Among the various adjuvant systems used in vaccine formulation, emulsion-based adjuvants are particularly common. These may be prepared in several forms, including oil-in-water (O/W), water-in-oil (W/O), water-in-oil-in-water (W/O/W), and protein-stabilized emulsions. In addition to adjuvants, preservatives are incorporated into vaccines to protect them from bacterial and fungal contamination during production and storage. Such preservatives may be introduced at different stages of vaccine manufacturing. The use of ultrasonic homogenization enables more uniform and controlled mixing and dispersion of these components, making it a valuable technique for the development of more effective vaccine formulations [21].

Another important application of ultrasound in vaccine and pharmaceutical production is degassing prior to packaging. Before filling and final packaging, vaccine preparations and liquid formulations such as suspensions, solutions, and emulsions must often undergo deaeration to remove dissolved or trapped gases. During this process, gas bubbles such as oxygen and carbon dioxide are eliminated from the liquid medium. Ultrasound facilitates the release and flotation of these entrapped gas bubbles, allowing them to rise to the liquid surface because of their increased buoyancy. The efficiency of bubble removal can be further improved by applying a mild vacuum to the sonication vessel during the degassing process.

Ultrasonic degassing is a fast and straightforward method for removing dissolved or entrapped gases from aqueous suspensions. Vaccines remain one of the most effective strategies for controlling and preventing infectious diseases, including the recent global outbreak caused by the novel coronavirus. A clear understanding of vaccine action is important for evaluating their protective role and supporting informed vaccination decisions. In general, vaccines function by training the immune system to identify and retain memory of a particular pathogen, such as a virus or bacterium, without causing the disease itself. As a result, the body becomes prepared to generate a rapid and effective immune response upon future exposure to the actual infectious agent [22].

To achieve this, vaccines deliver harmless components of the pathogen into the body, such as specific proteins, inactivated organisms, or weakened forms of the virus. These components, referred to as antigens, stimulate the immune system and initiate the production of antibodies, which are specialized proteins capable of recognizing and neutralizing the targeted pathogen. In addition to antibody

generation, vaccines also stimulate other immune cells, particularly T cells, which are essential for regulating, coordinating, and strengthening the overall immune response [23].

Chemical Techniques Used to Test Vaccine Components

The analysis of different vaccine components requires a range of analytical methods, each tailored to identify and quantify specific constituents present in the formulation. Among these techniques, High-Performance Liquid Chromatography (HPLC) is one of the most commonly employed methods for assessing the purity and concentration of active pharmaceutical ingredients (APIs) in vaccines. It can also separate and quantify adjuvants and excipients, ensuring that each component meets the required specifications. Various detection methods, such as UV-Vis, fluorescence, or mass spectrometry (MS), can be combined with HPLC to enhance detection sensitivity and selectivity. Testing the chemical components of a vaccine, or any chemical preparation, is a crucial aspect of vaccine development, ensuring that every ingredient used in the formulation is safe, effective, and of the highest quality. By employing advanced analytical techniques, preparing precise samples, and adhering to regulatory standards, biopharmaceutical professionals can ensure that vaccines meet the necessary criteria for approval and distribution. As new technologies continue to emerge, the field of vaccine component testing will become increasingly sophisticated, enabling the development of safer vaccines [24].

Natural Vaccines

Traditional vaccines use mimicry of natural infection to stimulate the immune system to mount an immune response against the microbe being vaccinated. Vaccines use a whole (dead) or attenuated microbe, or a part of the microbe (protein, toxoid). When a person is injected with any of these forms, the immune system recognizes the vaccine components and develops immunity. The effectiveness of this immunity varies depending on the pathogen and the technology used, but it provides protection (which may be short-term, moderate, or even lifelong) against infection should the person be exposed to a real infection. In recent years, ideas have emerged regarding the use of DNA genetic material, and then the idea of using RNA as a vaccine has attracted many scientists, researchers, and vaccine manufacturers. The reason for this interest is the ease of manufacturing genetic material in the required sequence and the short time required for production. Furthermore, it eliminates the need to cultivate the virus itself or one of its components, which previously required large areas, a long time, and high costs [25]. Now, for example, a million doses of a COVID-19 vaccine can be produced in a container with a capacity of one to two liters.

For example, two vaccines, Pfizer and Moderna, are RNA-based vaccines that use a copy of a naturally occurring chemical called mRNA to trigger the body's immune response. When the immune response is activated, it protects the body from infection. According to a report on very well Health, the RNA is packaged in a similar way in both vaccines, which requires the use of polyethylene glycol, the chemical suspected of causing allergic reactions in a small number of patients who have had an allergic reaction to the Pfizer vaccine. The research confirmed that polyethylene glycol (PEG) is safe for use [26]. The Pfizer vaccine contains:

- Lipids or fats
- Potassium chloride
- Monobasic potassium phosphate
- Sodium chloride (salt)
- Dibasic sodium phosphate dihydrate
- Sucrose (sugar)

The Moderna vaccine contains similar components, such as:

- Single RNA (mRNA) encoding the coronavirus spike glycoprotein
- Lipids or fats, including: polyethylene glycol 2000, dimyristoyl glycerol, and cholesterol
- Tromethamine
- Tromethamine hydrochloride

- Acetic acid
- Sodium acetate
- Sucrose

The vaccine primarily contains salts and stabilizers in the form of sugars and lipids, which are not allergenic. It is worth noting that both vaccines are similar when it comes to the ingredients, but the key difference between the two is that the RNA package in the Moderna vaccine allows for storage in a regular refrigerator, compared to the ultra-cold freezers required to store the Pfizer vaccine [27].

Chemical DNA extraction methods:

DNA extraction is the procedure used to isolate and purify DNA from biological samples through chemical or physical techniques. The process generally involves breaking down the cell and nuclear membranes to release the DNA, followed by the removal of unwanted components such as proteins, lipids, and other cellular impurities. In essence, DNA extraction consists of lysing the biological material to free the genetic material and then separating the DNA from contaminating substances to obtain a purified sample suitable for further analysis [28].

Steps of DNA Extraction

There are three main stages of DNA extraction, and the essential steps are generally the same in all extraction methods:

1. *Cell dissolution*: In this step, the cell and nuclear membranes are lysed to release the DNA.
2. *Precipitation*: Proteins, impurities, and other unwanted substances are removed from the sample.
3. *Purification*: This final step is performed to obtain a completely pure DNA sample ready for further use [29].

Chemical Common DNA Extraction Methods

Depending on the nature of the sample, DNA extraction methods may vary. For instance, the procedure used for isolating DNA from plant material is different from that used for blood samples. In the same way, the extraction of bacterial DNA also requires methods that differ from those applied to other biological materials. Therefore, specific DNA extraction techniques are selected according to the type of sample being analyzed. In general, DNA extraction methods are broadly classified into:

- Chemical (or solution-based) DNA extraction methods
- Solid-phase DNA extraction methods (physical methods)

In these methods, chemicals such as phenol, chloroform, CTAB, SDS, isoamyl alcohol, Tris-EDTA, and others are used in chemical-based DNA extraction. DNA Extraction from Inorganic Solvents This method relies on the use of inorganic solvents [30].

- K-Proteinase Method
- Salt-out Method
- DNA Extraction by Sodium Dodecyl Sulfate (SDS)
- DNA Extraction by Cetyltrimethylammonium Bromide (CTAB)
- Silica Gel-Based Techniques
- Physical or Solid-Film DNA Extraction Methods
- DNA Extraction from Paper
- Magnetic DNA Extraction

How to Extract DNA Using Liquid-Liquid Extraction

Liquid-liquid extraction is one of the most common methods for DNA extraction. In this method, solutions prepared with different chemical formulations are used for extraction and primarily depend on the preparation of the lysis solution. The lysis solution is prepared from one or two solvents, as it utilizes several different chemicals. Common chemicals used in liquid and liquid DNA extraction include phenol, chloroform, isoamyl alcohol, CTAB, SDS, Tris, EDTA, MgCl₂, and other detergents. These methods typically require centrifugation for separation.

Examples of liquid and liquid DNA extraction methods:

- Phenol, chloroform, and isoamyl alcohol DNA extraction
- DNA extraction using SDS
- DNA extraction using CTAB
- Phenol-chloroform DNA extraction

In this method, DNA is separated on the basis of its solubility in immiscible solutions. This approach is known as the liquid–liquid DNA extraction method, and the phenol–chloroform method is one of its common examples, as discussed earlier. It is also referred to as the phenol–chloroform–isoamyl alcohol (PCI) DNA extraction method. Although this technique is considered highly effective and yields DNA of good quality and quantity, it is generally not preferred because of the hazardous nature of phenol and chloroform. The major reagents used in this method include phenol, chloroform, isoamyl alcohol, and a buffer solution containing components such as EDTA, Tris, NaCl, MgCl₂, SDS, and other salts.

Principle of the method:

Phenol helps in the digestion of proteins, isoamyl alcohol aids in the separation of DNA, and chloroform reduces foaming between the interphases [31].

The major advantage of the PCI method is that it allows the extraction of DNA from almost all types of tissues, including animal, plant, and bacterial cells, and it can also be used for RNA isolation when combined with guanidine thiocyanate. However, as noted earlier, this method is considered hazardous because phenol is volatile and can cause burns, while chloroform may lead to dizziness or fainting. Therefore, the procedure requires proper training, careful preparation, and safe handling of the chemicals involved.

CONCLUSIONS

It is estimated that vaccines save between two and three million lives annually and help prevent chronic disability in others. While the exact proportion of adjuvants contributing to these achievements is unknown, they enhance vaccine effectiveness by stimulating an immune response and provide longer-lasting protection against disease compared to vaccines without adjuvants. Furthermore, some vaccines are simply not as effective in protecting certain age groups, such as the elderly, from disease without adjuvants.

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