Review Article

Thermostable Vaccines: Past, Present and Future Perspectives

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ABSTRACT:

Vaccines stability has a major role in the success of immunization programs and saves millions of lives every year. To stabilize vaccines cold chains are developed for storage and transport, as efficiency of vaccines is hampered if they are not kept under proper temperature. Aluminum is used for making vaccine thermostable. The development of vaccine formulation is a critical part of overall development cycle of approving, testing and producing new vaccines. However, Liquid vaccine formulation is still preferred over dry formulation because of ease in using, packaging and manufacturing. Other approaches have been used to make vaccine thermostable. This study demonstrates those processes, used to develop thermo-sensitive vaccines into thermostable vaccine and also describes vaccine formulation designing and use of heat shock protein including mHSP70 and mHSP65 to generate innate and adaptive immune response.

Key words:

Cold chain, Aluminum, Thermostable vaccine, Vaccine formulation design, Heat shock protein.

Introduction:

Every year millions of lives are saved through vaccination and the success of immunization program depends upon vaccine stability. As vaccines are thermally labile, so, from production to dispensing, vaccines must be stored at temperature between 2–8 °C all times in order to maintain efficiency [1]. Thermal sensitivity is the critical factor that impact on product potency and its quality and to maintain vaccine stability cold chain have been established but due to temperature excursion, stability of vaccines in cold chain is highly affected. The reason behind improper functioning of cold chain include, lack of fuel to operate equipment, outdated or improperly maintained refrigerator, poor compliance with cold chain procedure that results in temperature fluctuation in cold chain [2]. The thermal sensitivity impact on the distribution of vaccine worldwide and concern to the philanthropic organizations, government Institutions, health authorities and vaccine industry attempting to enhance the distribution of vaccines [3]. To maintain the vaccine's thermostability, aluminum as an adjuvant has been used but further approaches are being implemented because of aluminum neurotoxicity effect and potential to cause autoimmune disorders in human.

For the stability evaluation of vaccines, World Health Organization (WHO) set guidelines [4] which provide framework for storage condition and shelf life and are used to investigate those factors that alter vaccine stability [5]. To estimate shelf life several methods exits. These methods are described in International Conference of Harmonization (ICH) [6] based on statistical modeling, non-linear and linear regression through pool ability test. In order to improve ICH procedure the application of quintile regression, batch effects and mixed model tolerance interval methods was proposed [7, 8].

Previously, with a low moisture content (typically<3) the stability of live attenuated vaccines has been enhanced by lyophilization to develop dry powder [9]. However these vaccines require cold chain storage. Aluminum salt

adjuvant is present in subunit vaccines and cannot be lyophilized because of failure to maintain vaccine immunogenicity and particle size. Recently, drying processes such as vacuum-foam drying, spray-freeze drying, super-critical fluid drying and spray drying have been applied to improve versatility of the final formulation as well as thermostability of vaccines [10-12].

The development of vaccine formulation has a critical role in overall development cycle of approving, testing and producing vaccines. Vaccine formulation is the conversion of vaccine antigen into medicines. The development of vaccine formulation from immunogenic discovery to a usable vaccine include stability development-indicating assays including potency secondly, characterization of chemical and physical antigenic component thirdly optimization and evaluation of administration route and adjuvants and Design formulation to enhance the candidate vaccine's stability, immunogenic potential and shelf life. The main focus on the development of vaccines formulation is to increase the potency by utilization of vaccine adjuvants which not only promote immune response but also sheer the humoral and cellular immune response in the desired direction.

Overview of available Vaccines

The stability parameters and composition of commercially available vaccines has shown in table-1. These vaccines are summarized according to pharmaceutical point of view i.e., vaccine type, formulation of vaccine, heat sensitivity, shelf life and addition of adjuvant. Live attenuated vaccines (LAV) do not contain adjuvant but during storage and distribution, they are more heat sensitive in order potency loss. These types of vaccines contain weakened germs that replicate in-vivo (as shown in Table 1). Adjuvant is added in non-replicating vaccines to increase the immune response. Such types of vaccines include carbohydrate antigen and purified subunit protein, inactivated bacteria and viruses and recombinant subunit protein antigen. Adjuvant is required to protective immunity, as these vaccines are unable to replicate in-vivo. Generally subunit and in-activated vaccines developed as liquid formulation and are more stable however, during storage and distributions, such vaccines can be freeze sensitive to potency loss.

Type	Vaccines	Formulation of vaccine	Heat sensitivity	Shelf life	Adjuvant	Freeze sensitive
Live attenuated bacteria	Bacille Calmette-Guerin (BCG)	Lyo (ID, PC) Lyo (PC)	≥9days(54^{0}C) 48 h (23^{0}C)	≥2year N/A	None None	No yes
Conjugates polysaccharide-carrier	Pneumococcal (23-valent) Pneumococcal (13-valent) Pneumococcal (7-valent) H. Influenza	Liquid (IM, SC) Liquid (IM) Liquid (IM) Lyo (IM) Liquid (IM)	N/A 4days(40^{0}C) N/A N/A ≥5weeks(55^{0}C)	N/A 2 year 2 year 3 year 3 year	None Aluminum Aluminum None Aluminum	Yes Yes Yes Yes No

Subunit, purified bacterial Antigens	Meningococcal Typhoid Anthrax Tetanus toxoid adsorbed	Lyo (SC) Liquid(IM) Liquid(IM, SC) Liquid (IM)	6 weeks (60^{0}C) N/A N/A N/A	3 year 3 year 4 year N/A	None None Aluminum Aluminum	Yes Yes Yes Yes
Combined vaccine	Hepatitis A &B Diptheria/Hepatitis B Tetanus toxoid *H. influenza*, Hepatitis B	Liquid (IM) liquid (1M) liquid(1M) liquid (1M)	1week(37^{0}C) N/A N/A N/A	3year N/A 3 year N/A	Aluminum Aluminum Aluminum Aluminum	Yes Yes Yes Yes

Table 1: Types of vaccines, formulation and storage consideration of FDA approved bacterial vaccines [13-15]

Approaches used for Vaccine Thermo-Stability:

Researchers have made considerable effort for making thermo-sensitive vaccine into thermostable vaccine. Thermostable vaccine could be stored without any conformational changes at 45°C for 7 days by attaching *M. tuberculosis* epitope to a self-assembling fibril-forming peptide [16]. Similarly, thermal tolerance of malaria-protein vaccine was enhanced at 10–15 °C by modifying it through the introduction of 18 mutations [17]. Modifying viral vectors has also used for thermal stable vaccine. Stability of adenoviral vaccine formulations was enhanced by adding polyethylene glycol (PEG), sucrose and gold nano particles that could be stored at 37 °C for 10 days [18]. The immunogenicity of inactivated influenza vaccine was maintained at 60 °C for 4 months by encapsulating it in micro-needle patches [19]. By using methionine, gelatin and trehalose potency of attenuated salmonella enterica vaccine was preserve for 12 weeks at 37 °C. [20]. By using spray drying process meningitis, a protein-polysaccharide conjugate and recombinant hepatitis B vaccine become thermally stabilized [21]. Three drying methods; spray drying, freeze and foam drying also used to stabilize live attenuated influenza vaccine in sucrose containing excipients and found that most heat stable vaccine produced through foam drying process having shelf life 4.5 months at 37 °C[22]. Researchers have also modified vaccinia virus Ankara and maintained titer retention in adenovirus at 45 °C up to 6 months by drying them on glass fiber membranes and polypropylene using trehalose and sucrose [23].

Although the above mentioned studies have made great contribution and maintained the vaccines heat stable that do not depend upon cold chain. But the drawbacks were that large number of adjuvants required for formulations which increases vaccine product complexity as well as increase the cost. Moreover, for sample preparation these procedures are limited applicable as spray, foam and freeze drying processes required specialized equipment and the exposure of vaccines to extreme pressure or temperatures conditions [20].

Aluminum as adjuvant:

Use of adjuvants and drying processes are the novel approaches to stabilize vaccines. Typically, adjuvants are added to boost protective immune response through vaccines. Beside this, vaccine formulation would have benefit regarding resistant to heat damage by; minimize vaccine wastage, less dependability on cold chain equipment and supplies and assist to ensure the potency of vaccine. More than seven decade, aluminum containing adjuvant have been used to boost the immune response by using vaccine

[24]. Aluminum is the most commonly used vaccine adjuvant but under its absence, exception of live attenuated vaccine, antigenic component of most vaccines cannot evoke the immune response [25]. Currently most vaccines cannot tolerate temperature fluctuations and are prepared and transported as refrigerated liquid suspension. So, cold chain is important to assure the efficiency as well as stability of vaccines. Several different approaches have been examined to improve vaccine stability.

Use of aluminum to elevate the suspension stability at high temperature:

Combinations of buffer with aluminum containing adjuvant not only govern the ionization state of antigen's amino acid but also the surface chemistry of adjuvant. The thermal stability of some antigen can compromise through adsorption [26]. The antigenic activity of Hepatitis B surface antigen (HBsAg) dependent on bulk pH for the adsorbed antigen in a phosphate-free buffer and the thermal stability of soluble antigen depend on pH. Thermal stability of adsorbed HBsAg across the bulk range of pH was improved by addition of phosphate (20-100mM) and examined. Exchange of phosphate with the aluminum hydroxide $Al(OH)_3$ adjuvant causes change in microenvironment pH near the adjuvant surface and the thermal stability was accelerated by lactate and histadine (pH 5.2). The effect of lactate and histidine in the mechanism of stabilization was not expound but it was assumed that the stability effect was due to competency of two group of excipients to swipe proton with HBsAg side chains [27].

Vaccine Formulation Designing:

Lyophilized Vaccine formulation:

Environmental stress i.e., at high temperature live attenuated bacterial and viral vaccines are very sensitive and to preserve potency level during distribution and storage, process of lyophilization is required (Table 2). Under specific temperature and decreased pressure, freeze dried (lyophilized) process involves removal of water through different steps i.e., sample freezing, sublimation of bulk water (primary drying) and desorption of bound water (secondary drying) [28]. Variety of stresses come across during lyophilization including phase separation, low temperature, change in ionic strength and pH and ice crystal formation among others which requires presence of stabilizing additives to minimize the risk associated with these stresses. To protect the vaccine during stability and drying process Excipients such as, amino acid, sucrose and protein are used.

PROCESS	VACCINES	STABILITY DURATION	TEMPERATURE
Spray drying	Measles BCG Hepatitis B	2 week 4 month >24 month	37^0C 25^0C 37^0C
Spray freeze	Influenza	12 week	40^0C
Foam drying	Live LaSota virus	21 day	37^0C

Table 2: Production of thermostable vaccines through drying process [29]

Lyophilized vaccines should be able to easily reconstitute and have uniform appearance. Residual moisture content, humidity, light, temperature and sealing atmosphere composition can effect on potency as well as stability of lyophilized vaccines [30]. Mixing of lyophilized vaccine powder with diluent refers as reconstitution and are designed to meet pH,

chemical and volume requirements of vaccines while some diluent not only solubilize freeze-dried powder but also maintain the sterility of the reconstituted vaccine. Reconstitute lyophilized vaccines has limited stability and therefore, these vaccines should be stored under proper conditions.

Liquid vaccine formulation:

Non-replicating vaccines are more stable and can be formulated as liquid solutions. Such types of liquid vaccines contain those recipients that assist in antigen stability and generally formulated to contain several excipients that maintain the antigen and preclude severe conformational changes that can cause to loss of potency. Stabilizing excipients play key role in developing a successfully stabilized vaccines [31]. Liquid vaccine formulation is still preferred over dry formulation because of ease in using, packaging and manufacturing. High throughput method are being developed for stabilization of vaccines through various mechanisms of excipients that stabilize proteins such as non-reducing sugars and polymers [32]

Heat shock protein:

Heat Shock Proteins (HSP) are most conserved proteins and are widely studied vaccine candidate that play critical role in maintaining cell homeostasis [33]. HSP serve as chaperon and prevent protein from misfolding as well as aggregation and help in recognition and binding to nascent polypeptide chain [34]. Most studied families of HSP are HSP90, HSP70 and HSP60 because of versatile functions in human immune system. In figure-1, Mycobacterial HSP70 (mHSP70) make a complex with antigenic peptides and activate antigen presenting cells that triggers secretion of pro-inflammatory cytokines, maturation of Dendric Cells (DCs), and representation Of chaperoned peptides to MHC II restricted CD4+ T cells and MHC I restricted CD8+ cells that integrates adaptive and innate immune responses [35].

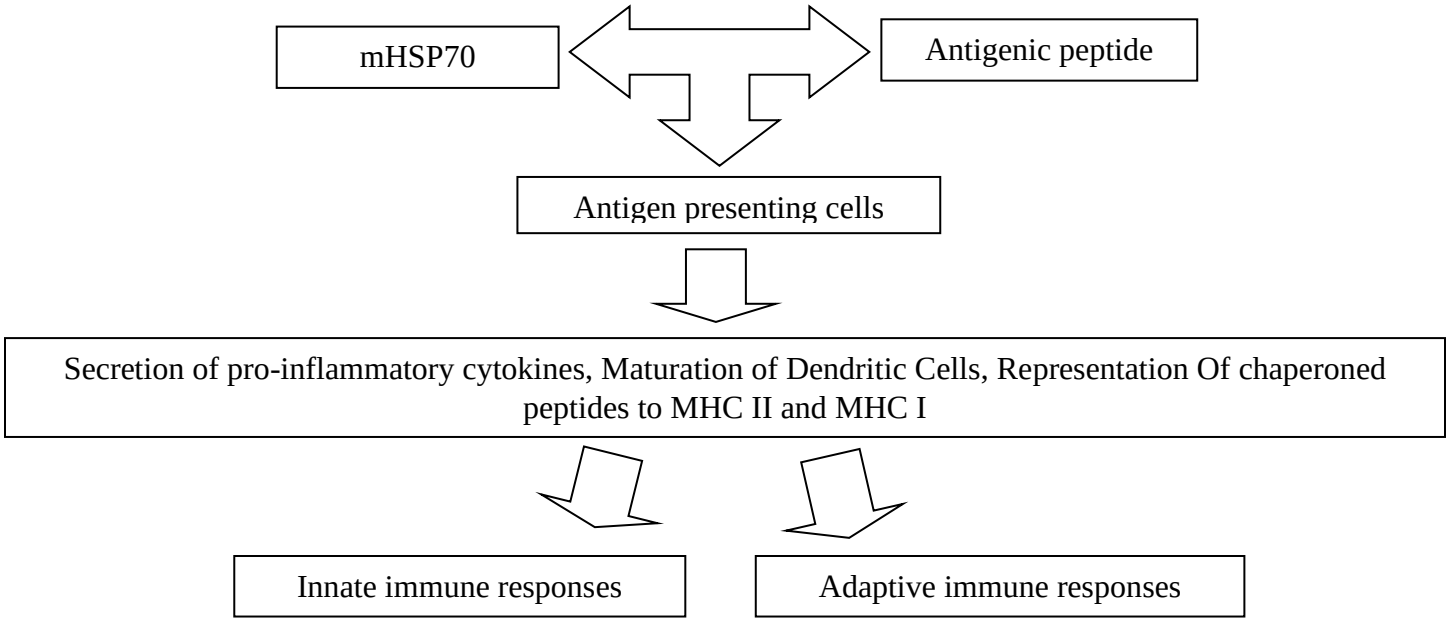

Figure 1: Mechanism to initiate innate and adaptive immune response through mHSP70

However, T-cell epitopes acts as carrier of antigen and make attractive vaccine candidates [36]. Besides that, specific anti-peptide antibodies response elicit through covalent complexes of mhsp65 which elicit antibodies and Cytotoxic T lymphocyte response. Thus, there are several opportunities to improve vaccine stability. For short term approaches, cold chain

technology is required to maintain the stability of vaccines, while in a long term point of view, excipients and vaccine formulation is used to stabilize the immunogenicity. By using combination of these approaches will not only enhance the stability rate and minimize the wastage of vaccines but will also play a major role in development of novel vaccines in future.

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