Accelerated stability studies of Otto Flu Plus Vac under the influence of stress conditions

Aqsa Afzal, Muhammad Danish Mehmood, Huma Anwar Ul-Haq, Muhammad Ismail

Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan
Dept. of Microbiology, Ottoman Pharma (Immuno Division), Lahore, Pakistan

ABSTRACT

Real time and accelerated stability testing plays a vital role in determining the integrity of a vaccine during shelf life under controlled environmental storage conditions. Currently, vaccines have been manufactured utilizing both conventional and allied technology however, conservation of immunogen strength and stability during transportation and storage under specified thermal conditions are the pre requisite for its in vivo potency. Therefore, current study was undertaken to evaluate the potency of such vaccines following manufacturer recommendation under the influence of artificially induced stress conditions. Total of 96, company retained reference samples of Otto Flu Plus vaccine were analyzed under real time and accelerated stability testing for its physiochemical properties and serological potency. In an attempt, vaccine samples were kept at 2-8°C for 15 months. A second group of samples were analyzed when stored at 4 to 20°C for 6 months. Total of 30 broilers were injected with vaccine at 25th day of age. Seroconversion was evaluated by haemagglutination inhibition assay at 28-day post vaccination. It was revealed that Otto Flu Plus vaccine stored for 15 months at 2-8°C showed optimum results (p>0.05) as compared to the vaccine stored at 20°C for 6 months (p<0.05).

It is concluded that Otto Flu Plus vaccine stored at 2-8°C showed intact emulsion integrity and induced protective antibody titer for 15 months as compare to the vaccine stored at 20°C for 6 months. The results suggest that the respective vaccine is safe to use at 2-8°C for 15 months without any deleterious effect on immunity.

© 2019 Published by Innovative Publication. This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by/4.0/)

1. Introduction

Biological preparations are thermolabile products and unstable during storage. This fluctuation may lead to a decrease in safety and efficacy of these products. During the preparation of a vaccine, there may be some proteins and other macromolecules that can be sensitive to heat, humidity, light and other environmental conditions or it may interact with packing material or other products used in vaccine. The chemical reactions like solvolysis, oxidati on, reduction, racemization etc. that occur in pharmaceutical products may lead to formation of degradation of products, loss of potency of active pharmaceuticals ingredients (API), loss of excipient activity like antimicrobial preservative action and antioxidants etc.1 After determining these relationships, enhancing the stability from production to administration to patient is an important part of vaccine preparation. As time passes since production, the reduction in potency may occur gradually. The stability of a pharmaceutical product can also be affected because of microbial changes like growth of microorganisms in non-sterile products and changes in preservative efficacy.2

Stability may be defined as the capability of a formulation in a specific container/closure to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications.3 Fluctuations
in handling and storage conditions may exert temperature stresses that leads to significant changes in stability profile. The stability standards of a vaccine must be calculated analytically through testing. The handling and storage conditions be illustrated to ensure the minimum levels of potency, identity and purity continue to be met over the stated shelf life of a vaccine. The development of cold chain requirements has become a new approach to deal with temperature sensitivity of vaccines. Thus, stability testing evaluates the effect of environmental factors on the quality of a drug substance or a formulated product which is utilized for prediction of its shelf life, determine proper storage conditions and suggest labelling instructions. Moreover, the data generated during stability testing is an important requirement for regulatory approval of a drug or formulation.4

The modern vaccine formulation development path from the discovery of an immunogen to a usable vaccine includes: (1) physical and chemical characterization of the antigenic component, (2) development of stability-indicating assays including potency, (3) evaluation and optimization of the route of administration and adjuvants (in both animal models and in clinical trials), and (4) formulation design to maximize the candidate vaccine’s (antigen and adjuvant) stability, shelf life and immunogenic potential. A major focus of vaccine formulation development, in many cases, is the enhancement of potency through the use of vaccine adjuvants, since many candidate immunogens fail to transfer from the laboratory to the patient due to suboptimal efficacy in humans. One key approach to increase the success rate for new vaccine candidates is thus to ensure the appropriate formulation in the presence of conventional and/or novel adjuvants. The purpose of this study is to hike perception about the scientific and technical challenges confronting to successfully formulate and stabilize different types of vaccines, both in terms of stability of antigens, adjuvants and their complexes.

2. Materials and Methods

The current research has been conducted in mutual consent of the university of Lahore and Ottoman Pharma, Lahore. The whole research was executed in the research and development (R&D) department of Ottoman Pharma, Lahore. In pharmaceutical industry samples of each batch of every product are retained at specified temperature up to one year of its date of expiry and humidity for future reference. The focus of this study is to utilize these retained expired samples for the long lasting stability of Otto Flu Plus Vaccine.

2.1. Source of vaccine

Total number of five retained samples was collected from the retained samples in refrigerator placed in QC department of Ottoman Pharma. Each vial was properly labeled and stored at 4°C. The details of each sample are given below:

2.2. Source of broilers

Total of 25- day old broilers were purchased from hatchery of Big Bird poultry breeders and shifted to the clean and fumigated experimental animal house of Ottoman Pharma. All the birds were offered with feed and water ad libitum under same environmental conditions.

2.3. Experimental design

2.3.1. Real time stability testing

Total number of five retained samples was collected from the retained samples refrigerator placed in QC department. Each vial was analyzed at different time period of 3 months, 6 months, 9 months, 12 months and 15 months. Each vial was analyzed for its physicochemical properties (Table 1).

2.3.2. Accelerated stability testing

Five vials of 300 ml of Otto Flu Plus oil based vaccine were further transferred into sterile vials in such a way that every vial has 100 ml of vaccine. Each of the vials was properly labeled according to master sheet (Table 2). Master Vial of 300 ml was de-sealed in sterile bio safety cabinet and transferred to vials containing 100 ml each. Each vial was transferred to incubator pre-set at different temperature as 4°C, 8°C, 12°C, 16°C and 20°C. Vaccine was evaluated for its physicochemical stability such as density, viscosity, pH, particle distribution, stability and in vivo determination of anti-Influenza HI antibody titer after storage for 2 months, 4 months and 6 months. The details of each vaccine are given in (Table 2).

2.3.3. Efficacy testing

Total of 30 birds were divided into four groups each containing 4 birds. The birds were marked with specific color and immunized with respective vaccine at different time interval. The blood of each bird of every group was collected from wing vein at 28-day post vaccination. The serum was extracted and subjected for anti-Influenza HI antibody titer.

2.3.4. Haemagglutination inhibition titer

U-shaped bottom microtitre plates of 96 well were labelled appropriately. 50 µl of normal saline was dispensed in 1st row of 96 well plate upto 12th well with the help of microtitre pipette. 50 µl of antigen was added to the first well of appropriately numbered column. 2-fold serial dilution were made by transferring 50ss µl serum from first well of numbered columns to successive wells. Added 50 µl of 4HA virus antigen in each of the well upto 11th well and incubated for 30 minutes at 37°C. 50 µl of 1% washed RBC were added to all wells. Plates were gently tapped and
kept at 37 °C for 30 minutes.

2.3.5. Statistical analysis

The data obtained in the study was analyzed by mean standard deviation and subsequently through repeated measure analysis of variables (ANOVA) using SPSS version 21.

3. Results

3.1. Effect of accelerated temperature on viscosity at different time interval

Otto Flu Plus vaccine was evaluated for change in physiochemical properties. This evaluation was done after storage at 4°C, 8°C, 12°C, 16°C and 20°C for 75, 90 and 120 days.

Otto Flu Plus vaccine stored at 4°C for 75, 90 and 120 days showed mean viscosity values of 37.6±0.43, 37.6±0.23, and 37.5±0.31 mpa/sec respectively (Figure 2, Table 3). The vaccine stored at 8°C showed mean viscosity values of 35.8±0.13, 35.5±0.35 and 35.6±0.31 mpa/sec (Figure 3, Table 3). At 12°C showed mean viscosity values of 31.8±0.13, 31.5±0.85 and 31.0±0.70 mpa/sec viscosity (Figure 4, Table 3). At 16°C showed mean viscosity values of 28.3±0.78, 27.3±0.77 and 25.1±0.71 mpa/sec viscosity respectively (Figure 5, Table 3). And the vaccine stored at 20°C for 75, 90 and 120 days showed mean viscosity values of 20.8±0.15, 17.6±0.18 and 15.5±0.32 mpa/sec viscosity respectively (Figure 6, Table 3).

3.2. Effect of accelerated temperature on pH at different time interval

Otto Flu Plus vaccine stored at 4°C for 75, 90 and 120 days showed mean pH values of 6.68±0.10, 6.66±0.11 and 6.62±0.14 respectively (Figure 2, Table 3). The vaccine stored at 8°C showed mean pH values of 6.68±0.10, 6.66±0.11 and 6.62±0.14 (Figure 3, Table 3). At 12°C showed mean pH values of 6.58±0.19, 6.54±0.11 and 6.54±0.13 (Figure 4, Table 3). At 16°C showed mean pH values of 6.62±0.19, 6.50±0.70 and 6.46±0.54 (Figure 5, Table 3). Moreover, the vaccine stored at 20°C for 75, 90 and 120 days showed mean pH values of 6.64±0.89, 6.50±0.12 and 6.48±0.13 respectively (Figure 6, Table 3).

3.3. Effect of accelerated temperature on Density at different time interval

Otto Flu Plus vaccine stored at 4°C for 75, 90 and 120 days showed mean density values of 0.91±0.01, 0.91±0.01 and 0.90±0.01 respectively (Figure 2, Table 3). The vaccine stored at 8°C showed mean density values of 0.91±0.00, 0.90±0.00 and 0.90±0.01 (Figure 3, Table 3). At 12°C showed mean density values of 0.91±0.00, 0.90±0.00 and 0.90±0.01 (Figure 4, Table 3). At 16°C for 75, 90 and 120 days showed mean density values of 0.89±0.01, 0.89±0.01 and 0.89±0.01 (Figure 5, Table 3). In addition, the vaccine stored at 20°C for 75, 90 and 120 days showed mean density values of 0.89±0.01, 0.87±0.01 and 0.87±0.01 respectively (Figure 6, Table 3).

3.4. Effect of accelerated temperature on stability at different time interval

Otto Flu Plus vaccine stored at 4°C for 75, 90 and 120 days showed mean stability values of 4.00±0.00, 4.00±0.00 and 4.00±0.00 respectively (Figure 2, Table 3). The vaccine stored at 8°C showed mean stability values of 4.00±0.00, 4.00±0.00 and 4.00±0.00 (Figure 3, Table 3). At 12°C showed mean stability values of 4.00±0.00, 4.00±0.00 and 3.20±1.09 (Figure 4, Table 3). At 16°C showed mean stability values of 3.60±0.89, 3.20±1.09 and 2.80±1.09 (Figure 5, Table 3). And the vaccine stored at 20°C for 75, 90 and 120 days showed mean stability values of 2.40±0.89, 2.40±0.89 and 2.00±0.00 respectively (Figure 6, Table 3).

3.5. Effect of accelerated temperature on particle distribution at different time interval

Otto Flu Plus vaccine stored at 4°C for 75, 90 and 120 days showed mean particle distribution values of 6.00±0.00, 6.00±0.00 and 6.00±0.00 respectively (Figure 2, Table 3). The vaccine stored at 8°C showed mean particle distribution values of 6.00±0.00, 6.00±0.00 and 6.00±0.00 (Figure 3, Table 3). At 12°C showed mean particle distribution values of 6.00±0.00, 6.00±0.00 and 4.80±1.64 (Figure 4, Table 3). At 16°C showed mean particle distribution values of 5.40±1.34, 4.80±1.64 and 4V0±1.64 (Figure 5Table 3). Moreover, the vaccine stored at 20°C for 75, 90 and 120 days showed mean particle distribution values of 3.60±1.34, 3.60±1.34 and 3.00±0.00 respectively (Figure 6, Table 3).

Table 1: Detail of Otto Flu Plus Vac retained samples used in current study

| S.No. | Sample ID   | Quantity | Manufacture Date | Expiry Date |
|-------|-------------|----------|------------------|-------------|
| 1     | FQ-235-OB   | 300 µl   | 15-06-17         | 15-03-18    |
| 2     | GQ-238-OB   | 300 µl   | 22-07-17         | 22-04-18    |
| 3     | BR-252-OB   | 300 µl   | 09-02-18         | 09-08-18    |
| 4     | PBR-15-OP   | 300 µl   | 17-02-18         | 17-08-18    |
| 5     | DR-269-OB   | 300 µl   | 09-04-18         | 09-10-18    |
### Table 2: Master data sheet of labels

| Sample no | Label | Temperature | Shelf Life |
|-----------|-------|-------------|------------|
| Otto.F 1/Vial 1 | Otto.F1V1/4°C/2M | 4°C | 2 |
| Otto.F 2/Vial 1 | Otto.F2V1/4°C/4M | 4°C | 4 |
| Otto.F 3/Vial 1 | Otto.F3V1/4°C/6M | 4°C | 6 |
| Otto.F 4/Vial 2 | Otto.F4V2/8°C/2M | 8°C | 2 |
| Otto.F 5/Vial 2 | Otto.F5V2/8°C/4M | 8°C | 4 |
| Otto.F 6/Vial 2 | Otto.F6V2/8°C/6M | 8°C | 6 |
| Otto.F 7/Vial 3 | Otto.F7V3/12°C/2M | 12°C | 2 |
| Otto.F 8/Vial 3 | Otto.F8V3/12°C/4M | 12°C | 4 |
| Otto.F 9/Vial 3 | Otto.F9V3/12°C/6M | 12°C | 6 |
| Otto.F 10/Vial 4 | Otto.F10V4/16°C/2M | 16°C | 2 |
| Otto.F 11/Vial 4 | Otto.F11V4/16°C/4M | 16°C | 4 |
| Otto.F 12/Vial 4 | Otto.F12V4/16°C/6M | 16°C | 6 |
| Otto.F 13/Vial 5 | Otto.F13V5/20°C/2M | 20°C | 2 |
| Otto.F 14/Vial 5 | Otto.F14V5/20°C/4M | 20°C | 4 |
| Otto.F 15/Vial 5 | Otto.F15V5/20°C/6M | 20°C | 6 |

3.6. Effect of accelerated temperature on serological potency of Otto Flu plus vaccine

Otto Flu Plus vaccine stored at 4°C for 75, 90 and 120 days displayed 38.40±14.31, 38.40±14.31 and 38.40±14.31 mean anti-AIHI antibody titer 28 days’ post vaccination respectively. The vaccine stored at 8°C displayed 44.80±17.52, 44.80±17.52 and 32.00±0.00 mean anti-AIHI antibody titer 28 days’ post vaccination. At 12°C displayed 44.80±17.52, 44.80±17.52 and 32.00±0.01 mean anti-AIHI antibody titer 28 days’ post vaccination. At 16°C displayed 30.40±21.46, 22.40±8.76 and 17.60±8.76 mean anti-AIHI antibody titer 28 days’ post vaccination. And the vaccine stored at 20°C for 75, 90 and 120 days displayed 30.40±21.47, 14.40±3.577 and 12.80±4.38 mean anti-AIHI antibody titer 28 days’ post vaccination respectively. (Figure 7Table 3)

3.7. Effect of storage conditions on Otto Flu Plus vaccine

Otto Flu Plus vaccine (FQ-235-OB) stored at 2°C for 15 months showed milky white appearance with 36.46±1.10, 6.57±0.05, 0.89±0.001, 6.00±0.00, 6.00±0.00 and 1μm mean standard values of color, Viscosity, pH, density, stability, Particle distribution and particle size respectively.
The vaccine stored at 4°C for 15 months showed milky white appearance with 37.61±0.04, 6.57±0.08, 0.90±0.00, 6.00±0.00, 6.00±0.00 and 1 μm mean standard values of color, Viscosity, pH, density, stability, Particle distribution and particle size respectively. (Figure 8, Table 4). The vaccine stored at 8°C for 15 months showed milky white appearance with 35.07±2.08, 6.46±0.01, 0.89±0.00, 4.76±0.09, 5.0±1.03 and 1 μm mean standard values of color, Viscosity, pH, density, stability, Particle distribution and particle size respectively. (Figure 9, Table 4).

3.8. Effect of storage conditions on serological potency of Otto Flu Plus vaccine

Otto Flu Plus vaccine stored at 2°C for 3, 6, 9, 12 and 15 months displayed 38.40±14.31, 38.40±14.31, 38.40±14.31, 38.40±14.30 and 38.40±11V8 mean anti-AIHI antibody titer 28 days’ post vaccination respectively (Figure 10, Table 4). The vaccine stored at 4°C displayed 34.65±4.3, 34.65±4.4, 34.65±4.3, 34.65±4.4 and 34.65±4.5 mean anti-AIHI antibody titer 28 days’ post vaccination (Figure 10, Table 4). At 8°C displayed 32.00±1.54, 32.00±1.51, 32.00±1.50, 32.00±1.48 and 33.00±1.42 mean anti-AIHI antibody titer 28 days’ post vaccination respectively (Figure 11, Table 4).

4. Discussion

Vaccination is considered as one of the strongest public health goals during the 20th century which reduces morbidity and mortality from a number of vaccine-preventable diseases. There are many types of routinely used vaccines including live attenuated, killed or inactivated, subunit and subunit-conjugated vaccine. Live attenuated organism often need complex formulations and careful handling as sometimes they are fragile organisms that need to be kept in a state in which they can replicate in order to stimulate immunity. So, they are lyophilized during manufacturing to maintain this viability. However, inactivated and subunit vaccines are more stable to the thermal stress and are able to stimulate the immune response efficiently. Inactivated vaccines are usually associated with adjuvants particularly mineral oil which encapsulates the antigen creating defense line against unfavorable conditions. However, Aluminium Based adjuvants are susceptible to freeze-thaw damage. As these are biologicals and sensitive to both heat and cold environment, therefore need to be maintained within a properly organized range of temperature referred as “cold
Table 3: Effect of accelerated temperature on the stability of Otto Flu Plus vaccine at different time period

| Temperature | Physiochemical Characteristics n=5 | Seroconversion (HI Units) 28 day post vaccination |
|-------------|-----------------------------------|-----------------------------------------------|
|             | Viscosity | pH       | Density | Stability | Particle Distribution |                             |
|             | M±SD      | M±SD    | M±SD    | M±SD      | M±SD                  |                             |
| 4°C         | M±SD      | M±SD    | M±SD    | M±SD      | M±SD                  |                             |
| 2 months    | M±SD      | M±SD    | M±SD    | M±SD      | M±SD                  |                             |
| 8°C         | 35.8±0.13 | 6.64±0.11 | 0.90±0.00 | 4.00±0.00 | 6.00±0.00             | 44.80±17.52                 |
| 12°C        | 31.8±0.13 | 6.58±0.19 | 0.90±0.00 | 4.00±0.00 | 6.00±0.00             | 44.80±17.52                 |
| 16°C        | 28.3±0.78 | 6.62±0.19 | 0.89±0.01 | 3.60±0.89 | 5.40±1.34             | 30.40±21.46                 |
| 20°C        | 20.8±0.15 | 6.64±0.89 | 0.89±0.01 | 2.40±0.89 | 3.60±1.34             | 30.40±21.47                 |
| Total       | 30.8±6.10 | 6.63±1.37 | 0.90±0.01 | 3.60±0.81 | 5.40±1.22             | 37.76±18.26                 |
| 12°C        | 31.5±0.85 | 6.54±0.11 | 0.90±0.00 | 4.00±0.00 | 6.00±0.00             | 44.80±17.52                 |
| 16°C        | 27.3±0.77 | 6.50±0.70 | 0.89±0.01 | 3.20±1.09 | 4.80±1.64             | 22.40±8.763                 |
| 20°C        | 17.6±0.18 | 6.50±0.12 | 0.87±0.01 | 2.40±0.89 | 3.60±1.34             | 14.40±3.577                 |
| Total       | 29.9±7.24 | 6.58±0.13 | 0.89±0.01 | 3.52±0.87 | 5.20±1.30             | 32.96±17.63                 |
| 4°C         | 37.5±0.31 | 6.62±0.14 | 0.90±0.01 | 4.00±0.00 | 6.00±0.00             | 38.40±14.31                 |
| 8°C         | 35.6±0.31 | 6.68±0.83 | 0.90±0.01 | 4.00±0.00 | 6.00±0.00             | 38.40±14.31                 |
| 12°C        | 31.0±0.70 | 6.54±0.13 | 0.90±0.01 | 3.20±1.09 | 4.80±1.64             | 32.00±0.00                  |
| 16°C        | 25.1±0.71 | 6.46±0.54 | 0.89±0.01 | 2.80±1.09 | 4.00±1.64             | 17.60±8.76                  |
| 20°C        | 15.5±0.32 | 6.48±0.13 | 0.87±0.01 | 2.00±0.00 | 3.00±0.00             | 12.80±4.38                  |
| Total       | 28.9±8.12 | 6.55±0.13 | 0.89±0.01 | 3.20±1.00 | 4.80±1.50             | 26.56±12.15                 |

The formula been set for the calculation of stability and particle distribution is based on the following parameters:
Stability: 0= Unstable, 2= Partial stable, 4= Stable

Fig. 12: Microscopic vision of vaccine’s stability at different temperatures (a; 2°C b; 4°C c; 8°C d; 12°C e; 16°C f; 20°C)
Table 4: Effect of real time study on the stability of the Otto Flu Plus vaccine at different time period

| Temperature | Sample ID     | Physiochemical Characteristics | Seroconversion 28 day post vaccination |
|-------------|---------------|---------------------------------|----------------------------------------|
|             |               | Mean standard Deviation n=3      |                                        |
|             |               | Color | Viscosity | pH       | Density | Stability | Particle Distribution | Particle Size | M±SD            |
| 3           | FQ-235-OB     | Milky White | 37.52±0.08 | 6.66±0.15 | 0.90±0.00 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 38.40±14.31 |
| 6           | GQ-238-OB     | Milky White | 37.62±0.05 | 6.60±0.10 | 0.90±0.00 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 38.40±14.31 |
| 9           | BR-252-OB     | Milky White | 36.38±0.06 | 6.53±0.05 | 0.90±0.00 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 38.40±14.31 |
| 12          | PBR-15-OP     | Milky White | 35.64±0.09 | 6.53±0.05 | 0.89±0.00 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 38.40±14.30 |
| 15          | DR-269-OB     | Milky White | 35.15±0.03 | 6.53±0.05 | 0.87±0.00 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 38.40±11V8  |
| Total       |               | Milky White | 36.46±1.10 | 6.65±0.03 | 0.90±0.005 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 38.40±0.00 |
| 4°C         | FQ-235-OB     | Milky White | 37.65±0.03 | 6.70±0.10 | 0.90±0.00 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 34.65±4.3   |
| 6           | GQ-238-OB     | Milky White | 36.60±0.08 | 6.60±0.10 | 0.90±0.01 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 34.65±4.4   |
| 9           | BR-252-OB     | Milky White | 37.64±0.02 | 6.53±0.05 | 0.90±0.01 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 34.65±4.3   |
| 12          | PBR-15-OP     | Milky White | 37.64±0.01 | 6.53±0.05 | 0.90±0.00 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 34.65±4.4   |
| 15          | DR-269-OB     | Milky White | 37.55±0.03 | 6.50±0.10 | 0.90±0.00 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 34.65±4.5   |
| Total       |               | Milky White | 37.61±0.04 | 6.54±0.80 | 0.90±0.00 | 6.00±0.00 | 6.00±0.00 | 1μm±0.00 | 34.65±0.00 |
| 3           | FQ-235-OB     | Milky White | 37.55±0.01 | 6.43±0.05 | 0.90±0.00 | 4.83±1.04 | 6.00±0.00 | 1μm±0.00 | 32.00±1.54  |
| 6           | GQ-238-OB     | Milky White | 35.72±0.01 | 6.56±0.05 | 0.89±0.01 | 4.83±1.04 | 6.00±0.00 | 1μm±0.00 | 32.00±1.51  |
| 9           | BR-252-OB     | Milky White | 35.17±0.01 | 6.30±0.26 | 0.90±0.01 | 4.83±1.04 | 6.00±0.00 | 1μm±0.00 | 32.00±1.50  |
| 12          | PBR-15-OP     | Milky White | 35.16±0.01 | 6.50±0.10 | 0.90±0.01 | 4.66±1.15 | 4.16±0.28 | 1μm±0.00 | 32.00±1.48  |
| 15          | DR-269-OB     | Milky White | 31.79±5.76 | 6.53±0.11 | 0.89±0.02 | 4.66±1.15 | 4.06±0.11 | 1μm±0.00 | 33.00±1.42  |
| Total       |               | Milky White | 35.07±2.08 | 6.46±0.10 | 0.89±5.47 | 4.76±0.09 | 5.24±1.03 | 1μm±0.00 | 32.2±0.44   |

The formula been set for the calculation of stability and particle distribution is based on the following parameters:
Stability: 0= Unstable, 2= Partial stable, 4= Stable
Particle distribution: 0= Unstable, 3= Partial stable, 6= Stable
Fig. 6: Effect of 20°C on physiochemical properties of vaccine

![Graph showing the effect of 20°C on physiochemical properties](image)

Fig. 7: Effect of storage conditions on serological potency of Otto Flu Plus Vaccine

![Graph showing the effect of storage conditions on serological potency](image)

Fig. 8: Effect of storage duration at 2°C on the stability of vaccine

![Graph showing the effect of storage duration at 2°C on the stability](image)

Fig. 9: Effect of storage duration at 4°C on the stability of vaccine

![Graph showing the effect of storage duration at 4°C on the stability](image)

Fig. 10: Effect of storage duration at 8°C on the stability of vaccine

![Graph showing the effect of storage duration at 8°C on the stability](image)

Fig. 11: Effect of storage duration on the serological potency of Otto-flu plus vaccine

![Graph showing the effect of storage duration on the serological potency](image)
Cold chain is also referred as “vaccine supply chain” or “immunization supply chain” that consists of a series of links that are designed to keep the vaccine in temperature ranges recommended by WHO, from the point of manufacture to the point of administration. Environmental stresses including inappropriate handling or reconstitution, excess agitation and exposure to light can result in loss of vaccine potency.

The thermal environment has disruptive effects on protein structure of antigen by changing the order of amino acids. In the current study, it was documented that viscosity of vaccine stored at 4°C and 8°C for 6 months did not show any significant difference (p>0.05). However, the viscosity of vaccine stored at 12°C, 16°C and 20°C for 6 months showed significant difference (p<0.05).

As the storage period increased to 15 months, it was observed that the viscosity and particle distribution of the vaccine stored at 2°C and 4°C for 15 months did not show any significant difference (p>0.05) as compared to the vaccine stored 8°C for 15 months (p<0.05). In contrast, Leonard stated in his study that the viscosities of oil emulsions decrease as temperature increases because high temperature makes the molecules of oil and emulsions to get higher energy from heat thus making them less viscous so the oil can flow easily. Goldwood & Deisberg reported that increase in temperature increase the mobility and settling rate of water droplet; making the interfacial films weakens and tension between the two phases. It reduces the viscosities of oil and increase in droplets collisions favoring coalescence. Thus, acceleration in process by heating helps to break the emulsion.

It was found that particle distribution of vaccine stored at 4°C, 8°C and 12°C for 6 months did not show any significant difference (p>0.05). However the particle distribution of the vaccine stored at 16°C and 20°C for 6 months showed significant difference (p<0.05). While the vaccine stored at 2°C and 4°C for 15 months showed significant difference (p>0.05) as compared to vaccine stored at 8°C for 15 months (p<0.05). Allison observed the diameter of polymer particles is not so effected by increasing temperature however, the lower particle size distribution is due to rapid particle nucleation caused by high temperature.

Clenet stated that similar droplet size in microscopy is a helpful factor in maintaining emulsion stability. Large particles contain less interfacial surface per unit volume than small droplets. An emulsion having a uniform size distribution is more stable than one with a wider size distribution with the same average particle size.

While the pH, stability and density of vaccine stored at 2°C, 4°C and 8°C for 15 months did not show any significant difference (p>0.05). Moreover, pH of the vaccine did not show any significant difference when stored at 4°C, 8°C, 12°C, 16°C and 20°C for 6 months. The density and stability of vaccine stored at 4°C, 8°C and 12°C for 6 months did not show any significant difference (p>0.05) as compared to the vaccine stored at 16°C and 20°C for 6 months (p<0.05).

The Anti-AIHI antibody titer of the vaccine stored at 2°C, 4°C and 8°C for 15 months did not show any significant difference (p>0.05). The similar results were recorded for the vaccines stored at 4°C, 8°C, 12°C and 16°C for 6 months. While there is a significant difference observed in Anti-AIHI antibody titer for the vaccine stored at 20°C storage temperature for 6 months (p<0.05). Whereas, Quan concluded that heating of an oil emulsion displayed a significant difference in titer at 18 weeks but the difference did not remain significant at 24 weeks.

The results of the real time study concluded that Otto Flu Plus vaccine was stable at 2°C to 8°C when stored for 15 months. Whereas, in accelerated stability study the vaccine showed optimum result when stored at 4°C to 16°C for 6 months. There was least physiochemical deterioration at 16°C when stored for 6 months. Whereas, the significant deterioration in vaccine was recorded physically at temperature >12°C stored for 6 months. At 20°C the emulsion was broken and did not show any significant protective titer in experimental birds.

5. Source of funding

Nil

6. Conflict of interest

Nil

References

1. Carstensen JT, Rhodes CT. Drug Stability, Principles and Practices, Marcel Dekker, New York (2000). Clin Res Drug Reg Affairs. 1993;10:177–185.
2. Matthews RB. Regulatory Aspects of Stability Testing in Europe. Drug Dev Ind Pharm. 1999;25:831–856.
3. Kommanaboyina B, Rhodes CT. Trends in stability testing, with Emphasis on Stability During Distribution and Storage. Drug Dev Ind Pharm. 1999;25:857–867.
4. Singh S. Stability testing during product development in Jain NK. Pharmaceutical product development CBS Publisher and distributors. India. 2000p. 272–293.
5. Kumru OS, Joshi BS, Smith DE, Middaugh CR, Prusik T, Volkin BD. Vaccine instability in the cold chain: Mechanisms, analysis and formulation strategies. In Biologicals. 2014;42(5):237–259.
6. R R, Mandl CW, Black SD, Gregorio. Vaccines for the twenty-first century society. Nature, Reviews Immunol. 2011;11(12):865–872.
7. Wolfenden R, Lewis CA, Yuan Y. Temperature dependence of amino acid hydrophobicities. Proc Natl Acad Sci. 2015;112:7484–7488.
8. Leonard Y. Enhance the stability of inactivated Influenza vaccine encapsulated in dissolving microneedle patches. Pharm Res. 2016;33(4):868–8678.
9. Goldwood G, Deisberg S. The effect of cool water pack preparation on vaccine vial temperatures in refrigerators. Vaccine. 2018;36:128–133.
10. Allison LMC, Mann GF, Perkins FT, Zuckerman AJ. An accelerated stability test procedure for lyophilized measles Vaccines. J Biological Standardization. 1981;1:179–185.
11. Clenet D. Accurate prediction of vaccine stability under real storage conditions and during temperature excursions. Eur J Pharm Biopharmaceutics. 2018;125:76–84.
12. Quan FS, Li Z, Kim MC. Immunogenicity of low pH treated whole viral influenza vaccine. Virol. 2011;471(1):196–202.

Author biography

Aqsa Afzal Student
Muhammad Danish Mehmood Director Technicals

Cite this article: Afzal A, Danish Mehmood M, Anwar Ul-Haq H, Ismail M. Accelerated stability studies of Otto Flu Plus Vac under the influence of stress conditions. Indian J Microbiol Res 2019;6(4):284-293.