A Quick and Simple Polarographic Method for Aluminum Measurement in Recombinant Hepatitis B Vaccine

Mahmoud Alebouyeh (PhD)
Department of Food and Drug, Laboratory Research Center, Ministry of Health and Medical Education, Tehran, Iran

Zahra Abedi (MSc)
Department of Analytical Chemistry, Biology Unit of Food and Drug Administration for Quality Control, Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran

Hossein Rastegar (PhD)
Department of Pharmacology, Health and Cosmetics Research Center, Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran

Hasan Bagheri (MSc)
Department of Analytical Chemistry, Faculty of Engineering, Islamic Azad University, Research and Sciences Branch, Saveh, Iran

Javad Vaez (MSc)
Department of Chemistry, Pasteur Institute of Iran, Karaj, Iran

Behrouz Akbari-Adergani (PhD)
Department of Analytical Chemistry, Food and Drug Laboratory Research Center, Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran

Corresponding Author: Behrouz Akbari-adergani

Email: analystchemist@yahoo.com

Tel: +98(21) 66496152

Address: Food and Drug Laboratory Research Center, Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran

ABSTRACT

Background and Objective: Aluminum salts are among the most common useful additive compounds in preparation of human and animal vaccines. Aluminum phosphate and aluminum hydroxide are two additives that show good immunoadjuvant effects with many antigens. Aluminum-containing vaccines lead to a better and longer immune response compared to adjuvant-lacking vaccines. The Chromogenic methods used for determination of aluminum amounts in manufacturing centers are time-consuming and requires some experienced technicians to obtain accurate results. This study aimed to design and validate a simple polarographic method to measure aluminum in recombinant hepatitis B vaccine.

Methods: In this study, the effects of temperature, pH, potential range and potential scan rate on the polarographic method of measuring aluminum in hepatitis B vaccine was evaluated and the optimal values for each of these factors were achieved.

Results: In order to measure aluminum, temperature of 60 °C and pH of 4.5 were found as the optimal values. Implementation of polarographic method in the potential range of -0.25 to 0.1 volts had a better signal.

Conclusion: Since the polarography method is more simple, accurate and faster than the chromogenic methods, it is suitable to be used for the measurement of aluminum in hepatitis B vaccine and it is recommended to be used in quality control laboratories for biological products.

Keywords: Adjuvant, Hepatitis B Vaccine, Polarography, Aluminum.

INTRODUCTION

Aluminum salts are among the most commonly used adjuvants in human and animal vaccine production. Aluminum phosphate and aluminum hydroxide have adequate immunoadjuvant effects on several antigens, and they have different physical and chemical properties which enable each of them to be used for a specific antigen. Hepatitis B virus surface antigen (HBsAg) has several antigenic epitopes binding to aluminum adjuvants through ligand switching mechanism (1-5). Aluminum adjuvant reduces toxicity of particular antigens such as pertussis, diphtheria and tetanus and increases the solubility of some vaccine components (6-8). Golni et al. in 1926 were the first to report the effects of aluminum in vaccine composition. Although a number of aluminum compounds such as aluminum silicate has been known as adjuvants, only limited aluminum compounds such as aluminum hydroxide can be practically used in vaccine production (9-11). Studies have shown that aluminum-containing vaccines can cause a better and longer immune response compared to vaccines that lack adjuvants (12-13). Emperical evidence indicates that excessive use of aluminum adjuvants can lead to serious immunological disorders such as increased risk of autoimmune diseases and long-term brain impairment in humans (14-18). To protect the recombinant vaccines, Aluminum hydroxide (3%) is added to the formulation. This compound is a white, opaque, odorless solution which is highly soluble in HCl. The allowed limit for aluminum in the recombinant hepatitis B vaccine and HBsAg is 0.35-0.65 mg and 20 mg, respectively. Measuring the amount of aluminum in vaccines is extremely essential because its high levels in the vaccine can cause some side effects such as deposition in the kidneys. Therefore, controlling the concentration of aluminum and other heavy metals in various biological, pharmaceutical and environmental products has been the subject of many recent studies (19-21). Since vaccines are generally injected to children and healthy individuals for prevention of infectious diseases, the method for determining the level of this substance in the vaccines should be accurate. Electro-chemical methods, especially voltammetry have high capability in controlling biological products due to benefits such as short analyze time, optimal detection limit, use of simple and inexpensive materials, lack of organic solvents and high selectivity. There are other reports on controlling other metal ions with various electrochemical methods and mainly the voltammetry method (22-25). The aim of this applied research was to design and validate a quick and simple polarographic method for controlling the aluminum ion contents in recombinant hepatitis B vaccine.

MATERIAL AND METHODS

Various samples of recombinant hepatitis B vaccine with different production series were obtained from the Pasteur Institute of Karaj, Iran. Specifications and optional parameters used in the Polarography system for aluminum measurement are shown in table 1. First, 20 ml of deionized water was poured into a 100ml beaker and then 2 ml of prepared buffer was added. The container was placed on magnetic stirrer to achieve a well-uniformed solution. In order to prepare the buffer solution, 10ml of 30% sodium hydroxide was mixed with 11.4ml of 100% glacial acetic acid and then filled up to 100 ml with distilled water. PH of the solution was measured and its First, 20 ml of deionized water was poured into a 100ml beaker and then 2 ml of prepared buffer was added. The container was placed on magnetic stirrer to achieve a well-uniformed solution. In order to prepare the buffer solution, 10ml of 30% sodium hydroxide was mixed with 11.4ml of 100% glacial acetic acid and then filled up to 100 ml with distilled water. PH of the solution was measured and its amount was evaluated and optimized using acetic acid or sodium hydroxide solution in the range of 3 to 5.5. The obtained solution was evaluated in a controlled temperature ranging between 30 - 80 °C in the polarographic vessel, in accordance with Table 1 schedule. Each of the samples and standards were tested for three times directly and twice in the form of standard addition. After 80 seconds, 120 µl of Calcon solution was added to the contents of the vessel. After measuring the electrolyte flow as a control, 25 µl of the vaccine sample at 100 µg/ml concentration was added to the container. After three readings of the sample flow, 45 µl of 10 µg/ml standard solution, for twice was added to the contents of the vessel.

Upon completion of the program in the polarography system, the current variation curve was plotted based on potential and the sample's aluminum concentration was calculated. Each milliliter of hepatitis vaccine contained 20 µg HBsAg, 0.5 mg aluminum ion in the form of aluminum hydroxide and 0.05 mg thiomersal, shown in table 2-4 To calculate the percentage recovery, two concentration levels of 70 and 130 µg/ml were selected and all previously performed test stages were performed on the 100 µg/ml sample in a similar manner. For the 70 µg/ml sample and 130 µg/ml sample, 70 and 130 standards were used, respectively, to be added to the hepatitis B vaccine-containing vials. Three different concentrations (70, 100, 130 µg/ml) and a solution from each of them was prepared in three consecutive days and polarography was conducted three times per day. Finally, the obtained data in the three days was analyzed using Excel by calculating the mean, standard deviation, variation coefficient and the percentage of relative standard deviation (Tables 2 to 5).

RESULTS

To determine the optimum temperature for the polarographic vessel, 30 to 80 °C range was used according to the method section. Based on the recorded currents, the optimum temperature was found as 60 °C (Figure 1). To evaluate the effect of pH on the polarographic system response, different sample solutions were prepared in the range of 3 to 5.5 pH. After recording the flow rate changes in proportion to pH of the sample , shown in Figure 2, the optimum pH value (5.4) was reported.

Table 1- Specifications and optional parameters in the polarography system

Working electrode	Mercury emitting electrode
Stirrer speed	rpm2000
Mode	Differential Pulse
Drop size	7
Purge time	300 s
Addition purge time	180 s
Deposition potential	-350 mV
Deposition time	30 s
Equilibration time	10 s
Pulse amplitude	50 mV
Start potential	100 mV
End potential	-250 mV
Voltage step	6mV
Voltage step time	0/4 s
Sweep rate	15 mV/s
Peak potential	-420 mV

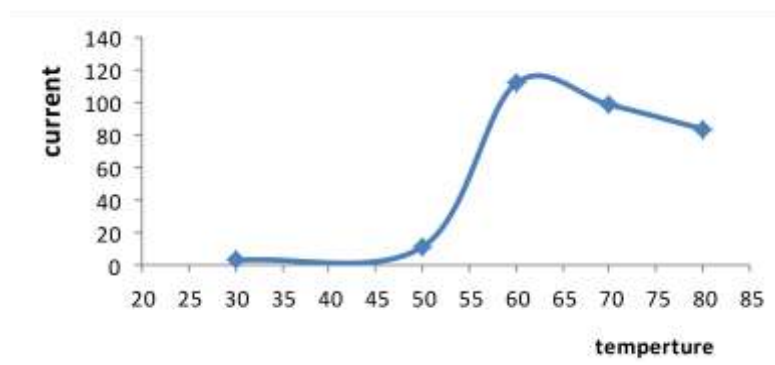**Figure 1- Current changes according to the temperature of the tested solutions**

Figure 3 represents the voltammogram of measured aluminum concentration in hepatitis B vaccine at three different concentrations of 70, 100 and 130 ppm. As it is shown, the increased aluminum concentration leads to the increased flow rate. A good matching of voltammogram pairs indicates the high accuracy of the system in optimal conditions. In order to evaluate the accuracy of measurements, the concentration preparation was conducted in the actual vaccine environment by standard addition at three levels for each vaccine-containing vial. Figure 4 shows the changes in the current based on the final concentration of the standard addition. The line across the obtained points on the curve until its intersection with the concentration axis was used to determine the concentration of sample.

The results of tests to determine the concentration of aluminum by polarographic method at three different concentrations during a day and in three consecutive days on vaccine (1), vaccine (2) and vaccine (3) are presented in Table 2. The intra-assay test results for sample 1 had 0.014, 0.019 and 0.008 coefficients of variation with variation range of 0.011. Based on Table 2, the intra-assay changes for sample 2 are 0.015, 0.014 and 0.008, respectively with variation range of 0.007. For sample 3, the coefficients of variation in the intra-assay test at three different concentrations are 0.016, 0.015 and 0.009, respectively with variation range of 0.007. Overall, having a coefficient of variation of less than 0.02, with variation range of less than 0.01, represents a favorable validation of the proposed method for the measurement of aluminum in hepatitis B vaccine samples. On the other hand, as the results show in Table 2, in concentration level of 70 ppm for sample 1, 2 and 3, the coefficient of variations of intra-assay tests was 0.014, 0.015 and 0.016, respectively, with fluctuation rate of 0.002. These values for concentration of 100 ppm were 0.019, 0.014 and 0.015, respectively with fluctuation of 0.005. Values of 0.008, 0.008 and 0.009 were for the changes at concentration of 130 ppm with variation range of 0.001. Given the coefficient of variation of less than 0.02 and its variation range of less than 0.005, it can be concluded that the **19/ Alebouyeh and colleagues** method has an acceptable accuracy.

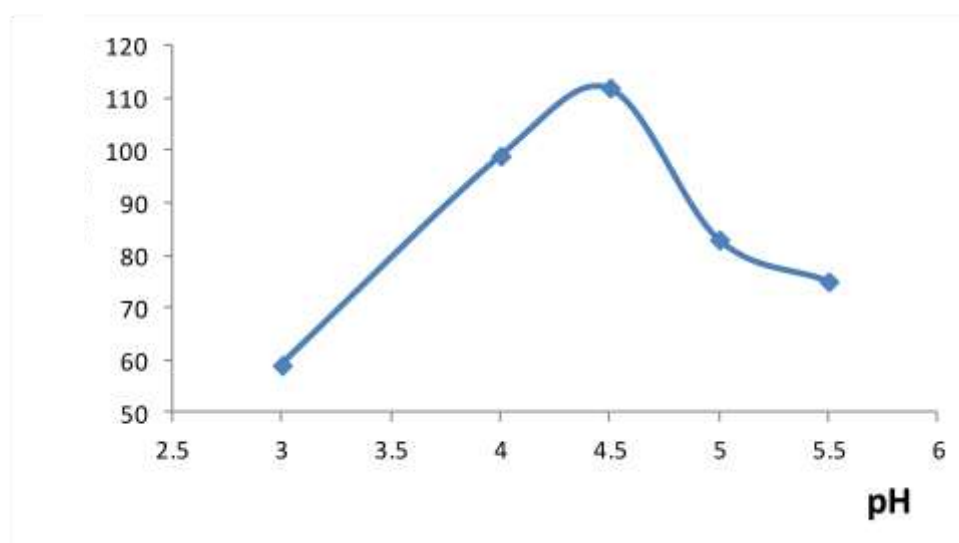**Figure 2- Flow rate (current) changes according to the pH of the tested solution**

Figure 3- voltammogram of measured aluminum concentration in hepatitis B vaccine at three different concentrations of 70, 100 and 130 ppm
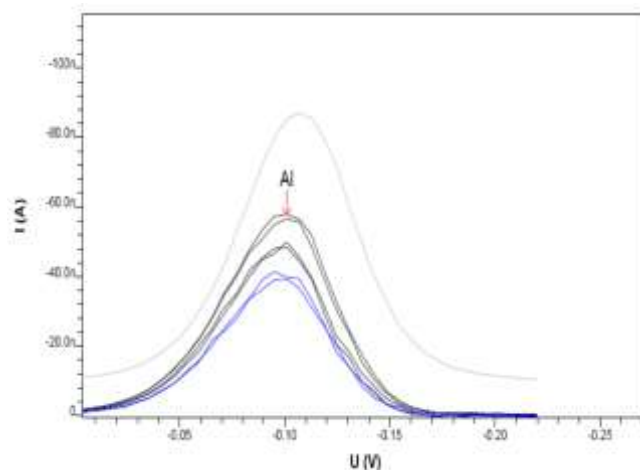

Figure 4- standard addition calibration curve of current changes based on aluminum concentration
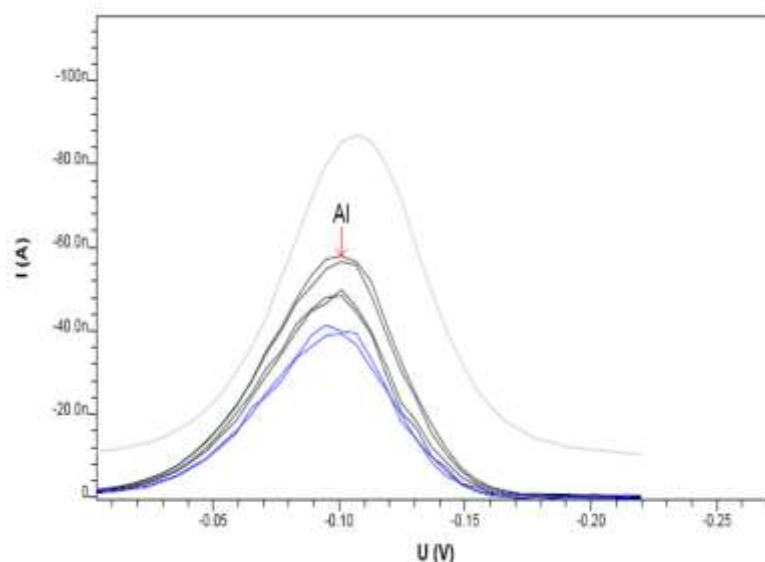

Table 2- Results of validation experiments for measurement of aluminum concentration in vaccine samples 1, 2 and 3

Recorded concentration for aluminum solution (130 ppm)	Recorded concentration for aluminum solution (100 ppm)	Recorded concentration for aluminum solution (70 ppm)	Sample 1
128/5 - 128/8 - 129/8	105/6 - 105/3 - 106/5	73/5 - 76/7 - 74/6	Day 1
128/3 - 127/4 - 127/5	103/5 - 103/4 - 103/2	75/5 - 75/8 - 73/5	Day 2
130/2 - 130/0 - 128/0	101/4 - 103/9 -100/1	74/1 - 74/9 - 74/7	Day 3
128/7±1/0	103/6±2/0	74/8±1/0	Mean ± S.D (ppm)
0/008	0/019	0/014	Coefficient of variation (CV)
0/8	1/9	1/4	RSD %
			Sample 2
134/3 - 133/7 - 135/2	105/3 - 104/9 - 104/4	71/9 - 69/2 - 70/6	Day 1
134/9 - 132/5 - 134/2	101/0 - 106/2 - 105/2	69/0 - 68/3 - 69/3	Day 2
132/1 - 132/3 - 133/6	105/5 - 105/0 - 103/8	70/6 - 70/3 - 70/1	Day 3
133/6±1/1	104/5±1/5	69/9±1/0	Mean ± S.D (ppm)
0/008	0/014	0/015	Coefficient of variation (CV)
0/8	1/4	1/5	RSD %
			Sample 3
131/8 - 130/1 - 131/6	102/1 - 104/2 - 104/5	73/3 - 70/2 - 72/8	Day 1
134/0 - 132/7 - 130/8	104/7 - 101/5 - 105/5	71/6 - 71/2 - 70/6	Day 2
132/5 - 132/4 - 133/7	102/6 - 105/4 - 102/0	72/3 - 70/6 - 73/3	Day 3
132/1±1/2	103/6±1/5	71/7±1/2	Mean ± S.D (ppm)
0/009	0/015	0/016	Coefficient of variation (CV)
0/9	1/5	1/6	RSD %

DISCUSSION

In order to measure aluminum in different biological, food and environmental samples, guidelines and various test methods have been proposed in the literature, including graphite furnace atomic absorption, spectrophotometric methods with color indicators such as Eriochrome Cyanine R and ion chromatography methods (27-26). In reference chromogenic method introduced for aluminum measurement, Eriochrome Cyanine R was used as a complexing agent with aluminum. In this method, it is necessary to use an interference eliminator that increases test stages, as well as its costs. The use of these materials and accuracy of their post-test final disposal is absolutely essential due to the toxicity of reagents. Chromogenic method involveing the use of atomic absorption or emission device for reading the concentration of aluminum requires both expensive equipment and skilled staff. According to this method, ions such as Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+} and Fe^{3+} are among the most interfering factors (28). This method is usually applied for an aquatic environment with simple matrix, thus all these limitations highlight the need for an alternative method. In addition to chromogenic method, other mentioned methods are generally costly and time consuming with a lengthy sample preparation. It also sometimes requires some costly and sophisticated laboratory instruments demanding a group of skilled technitions. The polarographic method is more accurate compared to other traditional methods. The relative standard deviation was used as an indicator for the accuracy and reproducibility of the method and was fluctuating between 0.8 to 1.9 % for all three hepatitis vaccine samples. The amount of minimum to maximum difference for all three samples in multiple repetitions was 1.1% max (Table 2), representing the favorable accuracy of the proposed method for testing different samples. Given that the proposed voltammetry is more cost-effective in terms of time and using different solutions, it is recommended that the a forementioned method be used in laboratories for controlling the concentration of aluminum in hepatitis B vaccine. Our findings show that the polarographic method for the measurement of aluminum in hepatitis B vaccine requires specific conditions in terms of temperature, pH, potential range and potential scan rate. The optimal values for this test, which can be used in the form of potential difference with the mercury-emitting electrode, are the temperature of 60 °C, pH of 5.4, the potential range of -0.25 to 0.1 volts and the potential scan rate of 15 mVs.

CONCLUSION

The validity and accuracy of this method was assessed using Inter- and Intra-assay testing showing acceptable standard deviation values and the conformity with the pharmacopoeia standards.

ACKNOWLEDGMENT

CONFLICT OF INTEREST

There are no conflicts of interest.

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