Research Article

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The Effect of Aluminum Nanoparticles on Vaccine-dosed Rabbits Investigated Using Cyclic Voltammetry

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Abstract

This study investigates the effect of aluminum nanoparticles (AL NPs) on vaccines (e.g., poliomyelitis and bacille Calmette–Guérin (BCG) vaccines). Cyclic voltammetry was used to determine the extent of its effect on the blood composition using an *in vivo* study on blood samples obtained from rabbits injected with poliomyelitic virus and BCG vaccines across a period of four weeks. The oxidation current peaks of the vaccines in the rabbit blood samples were enhanced with increasing doses of AL NPs. AL NPs acted as oxidative reagents to the rabbit blood components.

Keywords: cyclic voltammetry; blood; aluminum nanoparticles (AL NPs); vaccines

Introduction

This study investigates the effect of drugs on rabbit blood components using cyclic voltammetry [1–6]. An adjuvant is a component that enhances the immune response to the vaccine. Adjuvants allow for smaller doses of the vaccine. Aluminum (AL) has been used as an adjuvant in vaccines for hepatitis A, hepatitis B, diphtheria, tetanus, *Haemophilus influenza* type B, and pneumococcal, since its effect was first discovered in 1926. However, AL is not used in live viral vaccines such as measles, mumps, rubella, varicella, or rotavirus vaccines [7].

The vulnerability of respiratory mucosa to the invasion of airborne pathogens, such as SARS, MERS, and avian viruses that can potentially cause life-threatening epidemics and pandemics, underscores the importance of developing a pulmonary vaccine adjuvant delivery system (VADS). Although AL adjuvants are commonly used, they are not suitable for the delivery of pulmonary vaccines owing to their side effects. In contrast, 30-nm AL nanoparticles (NPs) are capable of acting as a suitable inhaled immunization for VADS to elicit widespread antigen immunity [8].

Vaccines have a significant impact on the fight against infectious diseases. However, the development of effective vaccines for many infectious diseases has been elusive. In many cases, the failure to invent vaccines can be attributed to the inability of the vaccine candidate to elicit appropriate immune responses. Therefore, comparing the characteristics of different NP systems for the delivery of subunit vaccines and evaluating the potential of these delivery systems in the

development of novel vaccines against a range of pathogens are necessary [9].

NP-based vaccines could serve as the candidates against several viral infections for their potential to enhance broader preventive efficacy under field conditions. Recent developments in NP-based vaccines against viral pathogens have highlighted how an NP-based vaccine delivery system stimulates innate and adaptive immune responses, resulting in diverse levels of protective efficacy [10].

Many vaccines against COVID-19 use nanoparticles to protect the antigen cargo (both proteins and nucleic acids), which ultimately increases its immunogenicity and efficacy. The characterization of these nanomedicines is challenging given their intrinsic complexity and requires the use of multidisciplinary techniques and competencies [11].

Hence, the aim of this study is to investigate the effect of AL NPs on blood and in different vaccines by the oxidation–reduction reaction using cyclic voltammetry.

Experimental

Materials

Alum ($KAl(SO_4)_2 \cdot 12H_2O$) used in this study was obtained from a Chinese company. The inactivated poliomyelitis vaccine was acquired from Bilthoven Biologicals (India). Further, bacille Calmette–Guérin (BCG), diphtheria-tetanus-pertussis, *Haemophilus* type B conjugate vaccines were obtained from Shameerpet, India. Blood samples were from rabbits' hearts after they were injected with different vaccines and different doses of AL NPs.

Preparation and characterization of AL NPs

The lyophilization method was used to convert the micro alum to nanoparticles using the lyophilizer shown in Fig. 1. Spectroscopic methods, such as field emission scanning electron microscopy (FE-SEM), atomic force microscopy (AFM), and transmission electron microscopy (TEM), were employed to characterize the conversion of micro alum to nanoparticles, as shown in Figs. 2–4, respectively. The results of FE-SEM analysis elucidating the morphology and dimension of the alum NPs are shown in Fig. 2. The image reveals that the AL NPs

Fig. 1 Lyophilization instrument.

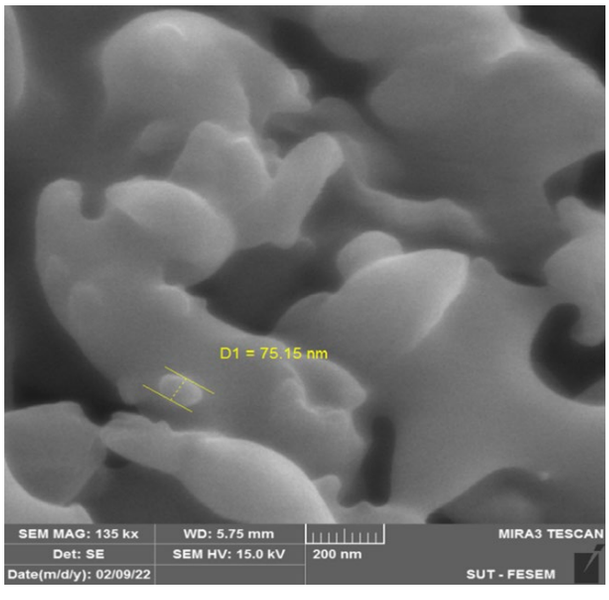

Fig. 2 FE-SEM image of the AL NPs.

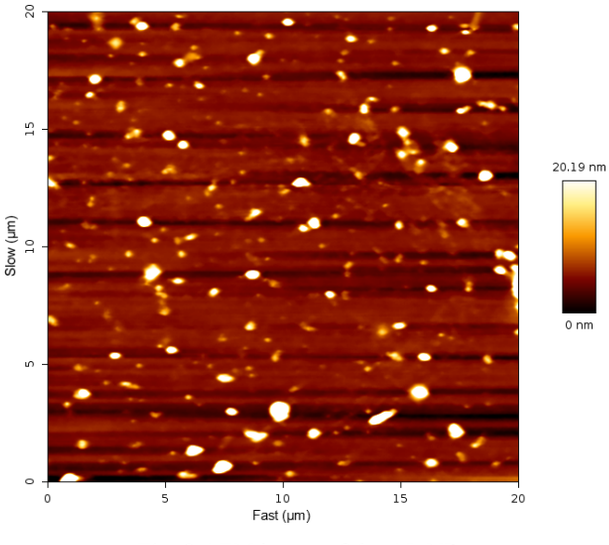

Fig. 3 AFM image of the AL NPs.

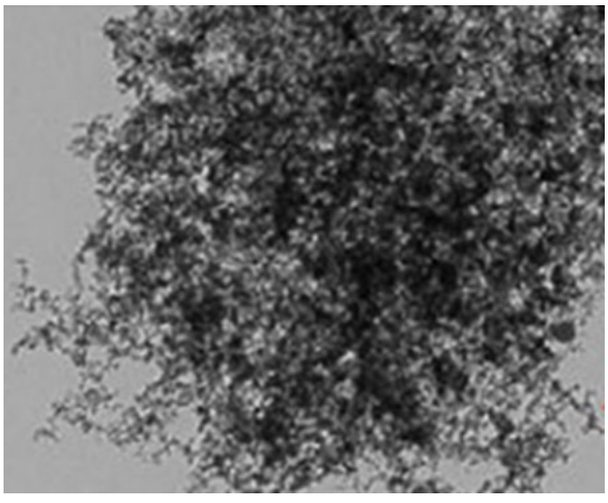

Fig. 4 TEM image of the AL NPs.

had a dimension of approximately 75.15 nm [12, 13]. In addition, the spherical form of AL NPs was observed in the AFM analysis, as shown in Fig. 3.

Cyclic voltammetric setup

A cyclic voltmeter (NuVant Systems Inc., USA) was used to obtain the redox current peaks. The voltmeter consisted of a potentiostat was connected to three electrodes: the working electrode (GCE, glassy carbon electrode), reference electrode (Ag/AgCl electrode), and counter electrode (platinum wire) in a 15-mL quartz cell, as shown in Fig. 5. Furthermore, the potentiostat was connected to a personal computer, as shown in Fig. 6, to analyze the cyclic voltammography results [14, 15].

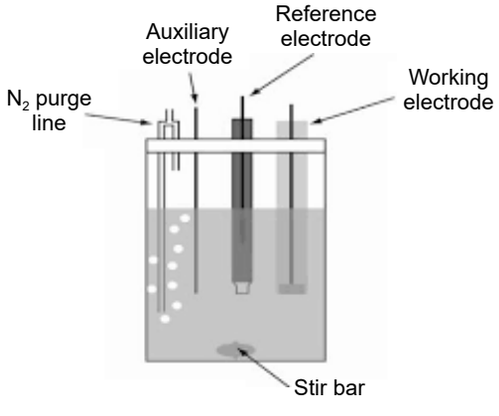

Fig. 5 Schematic diagram of a cyclic voltametric cell.

Preparation of rabbits for examination

This study was conducted over a period of four weeks. Five-month-old healthy male Balbo (white colored) rabbits (average mass: 2–3 kg) were selected for this study. The rabbits were categorized into five groups; the first group was the control group without any injections, and the remaining four groups were injected with 0.5 and 1.0 mL of 0.1 mmol/L AL NPs, poliomyelitis, and BCG vaccines, respectively [16, 17].

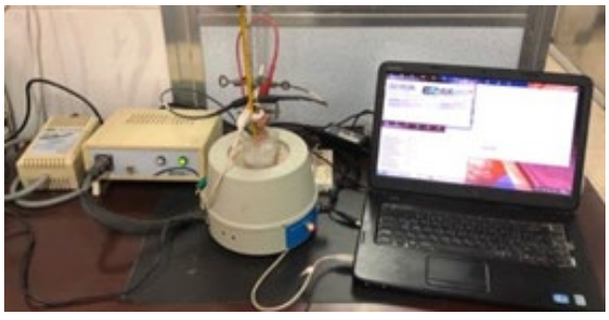

Fig. 6 Experimental setup for cyclic voltammetry.

Results and Discussion

Blood samples were obtained weekly from the rabbits injected with 0.1 mmol/L AL NPs, poliomyelitis, and BCG vaccines for the duration of this study. The rabbit blood was examined using *in vivo* cyclic voltammetry to determine its oxidation–reduction current peak and to understand the effects of these reagents on it [18, 19].

Effect of different doses of AL NPs on rabbit blood samples

Figure 7 shows the cyclic voltammogram of the blood of the rabbits injected with 1 mL and 0.5 mL of 0.1 mmol/L AL NPs, which affected the oxidation–reduction peak currents at potentials of 1.0 and –0.5 V, respectively. The oxidation current peak was enhanced with increasing nanoparticle concentration. This trend was also observed for the reduction current peak. As shown in Table 1, the oxidation current peak increased from 35 to 45.8 μA during four weeks after the rabbits were injected with 1.0 mL of 0.1 mmol/L AL NPs [20].

Figure 8 illustrates the relationships between the oxidation–reduction peak currents against different doses of AL NPs during the four-week period. Straight lines were derived from the relationships between the redox peaks and the different doses of AL NPs, as shown by the following equations:

$$\text{Oxidation peak}: y = 3.32x + 31.7 \text{ with a sensitivity of } R^2 = 0.929\,7 \quad (1)$$

$$\text{Reduction peak}: y = -3.61x - 23.55 \text{ with a sensitivity of } R^2 = 0.874\,8 \quad (2)$$

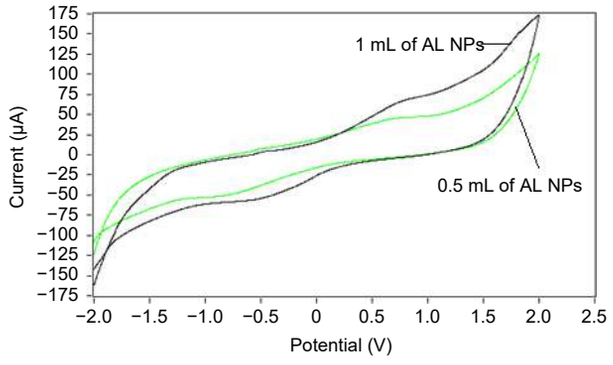

Fig. 7 Cyclic voltammogram of rabbit blood samples with different injected doses of AL NPs using GCE as the working electrode vs. Ag/AgCl as reference electrode at 0.1 V·s^{-1}.

Table 1 Oxidation–reduction current peaks of blood samples injected with 1.0 mL of 0.1 mmol/L AL NPs recorded weekly

Week No.	I_{pa} (μA)	I_{pc} (μA)
1	35	−25.6
2	39	-32.5
3	40	−35.8
4	45.8	−36.5

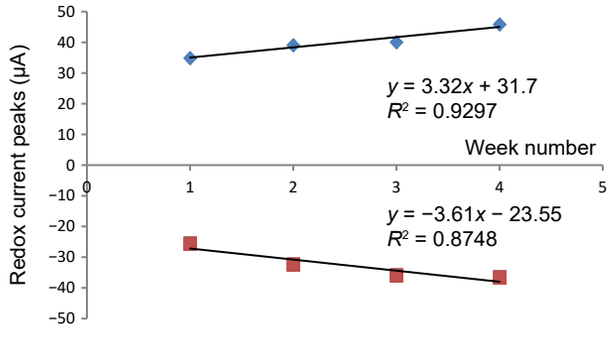

Fig. 8 Relationship between the oxidation–reduction peak currents for rabbit blood samples injected weekly with 1 mL of AL NPs.

The experiment was repeated, but doses of 0.5 mL of 0.1 mmol/L AL NPs were injected into the rabbits weekly instead of a four-week period. Figure 7 illustrates the effect of different doses of AL NPs on the redox peak currents of the cyclic voltammogram. The peaks were enhanced between the two doses of AL NPs (0.5 and 1 mL). The results listed in Table 2 were consistent with that observed in Fig. 9, where the following equations for high resolution straight lines of the oxidation–reduction peak currents of AL NPs were obtained:

$$\text{Oxidation peak}: y = 6.12x + 16.1 \text{ with a sensitivity of } R^2 = 0.8676 \quad (3)$$

$$\text{Reduction peak}: y = -3.15x - 24.6 \text{ with a sensitivity of } R^2 = 0.9132 \quad (4)$$

Table 2 Oxidation–reduction peak currents of the rabbit blood samples with 0.5 mL of 0.1 mmol/L Al NPs injected weekly

Week No.	I_{pa} (μA)	I_{pc} (μA)
1	24	−28
2	27.7	−29.7
3	30.5	−35.7
4	43.5	−36.5

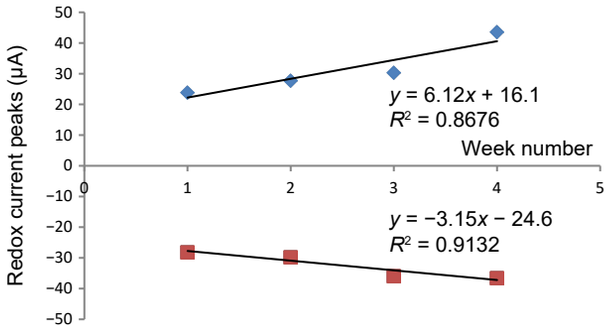

Fig. 9 Relationship between the oxidation–reduction peak currents for rabbit blood samples with 0.5 mL of AL NPs injected weekly.

Effect of different doses of poliomyelitis vaccine on rabbit blood samples

The second group of rabbits was injected with 0.2 mL of poliomyelitis vaccine throughout the four-week period. Figure 10 shows the cyclic voltammogram of the vaccine which contained AL NPs, which shows that the oxidation peak current was enhanced with increasing doses of the poliomyelitis vaccine. Furthermore, Table 3 confirms the values of both the oxidation and reduction peak currents. Notably, Fig. 11 shows an excellent relationship between the oxidation and reduction peak currents of the poliomyelitis vaccine, as shown by the following equations:

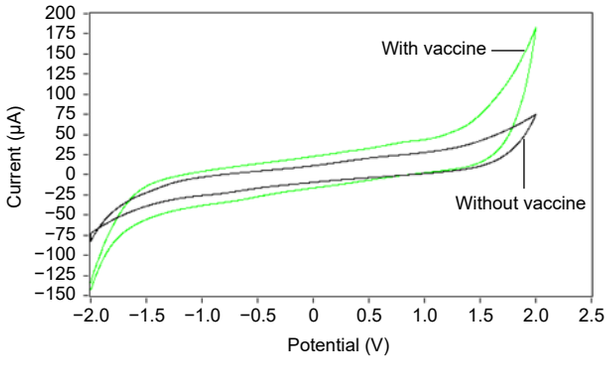

Fig. 10 Cyclic voltammogram of rabbit blood samples injected with poliomyelitis vaccine using GCE as working electrode versus Ag/AgCl as reference electrode at 0.1 V·s^{-1}.

Table 3 Oxidation–reduction peak currents of rabbit blood samples injected weekly with 0.2 mL of the poliomyelitis vaccine

Week No.	I_{pa} (μA)	I_{pc} (μA)
1	22.7	–23.2
2	29	–33.6
3	37.6	–35.8
4	40.9	–36

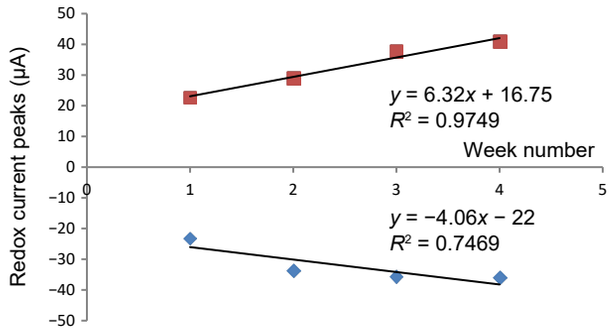

Fig. 11 Relationship between oxidation–reduction peak currents for rabbit blood samples with 0.2 mL of the poliomyelitis vaccine injected weekly.

Oxidation peak : $y = 6.32x + 16.75$ with a sensitivity of $R^2 = 0.974\,9$ (5)

Reduction peak : $y = -4.06x - 22$ with a sensitivity of $R^2 = 0.746\,9$ (6)

Effect of different doses of BCG vaccine on rabbit blood samples

The blood samples were investigated after vaccine injection using cyclic voltammetry, where the cyclic voltammogram showed a rise in the oxidation current peak, which can be attributed to AL NPs containing the vaccine, as shown in Fig. 12. Therefore, the BCG vaccine used for children BCG is considered an oxidizing agent for the blood components.

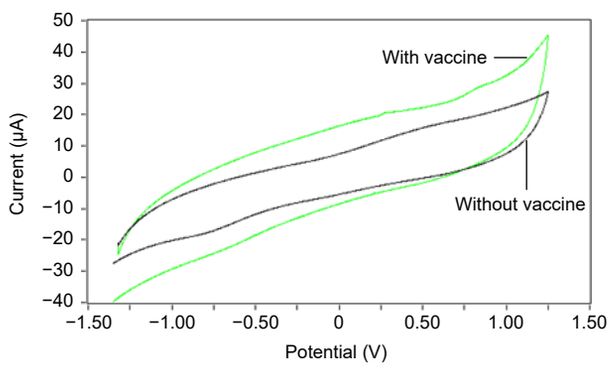

Fig. 12 Cyclic voltammogram of the rabbit blood sample injected with BCG vaccine using GCE as working electrode versus Ag/AgCl as reference electrode at 0.1 $V \cdot s^{-1}$.

Table 4 shows the oxidation–reduction current peaks of the AL NPs in the BCG vaccine, which enhanced the oxidation peak of the rabbit blood sample. Figure 13 shows the enhanced redox peaks with increasing doses of the vaccine during the four week period, which are shown by the following equations:

Oxidation peak : $y = 9.55x + 7.4$ with a sensitivity of $R^2 = 0.987\,4$ (7)

Reduction peak : $y = -4.68x - 19.5$ with a sensitivity of $R^2 = 0.850\,8$ (8)

Table 4 Oxidation–reduction current peaks of rabbit blood samples with 0.2 mL of the BCG injected weekly

Week No.	I_{pa} (μA)	I_{pc} (μA)
1	15.9	–21.8
2	27.3	–31.8
3	37.6	–34.8
4	44.3	–36.4

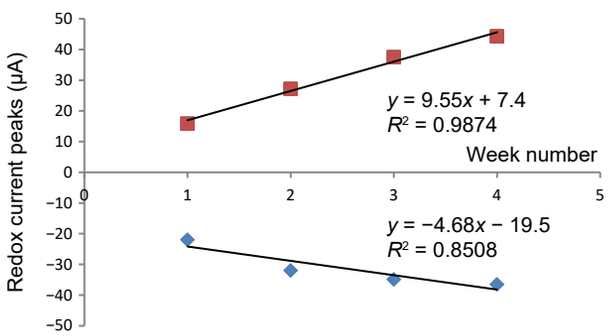

Fig. 13 Relationship between the oxidation–reduction current peaks for rabbit blood samples with 0.2 mL of the BCG vaccine injected weekly.

Reproducibility study

Working glass electrodes were used in this study to obtain results corresponding to the practical values. To confirm the reliability of the results, the cyclic voltammetry scans were repeated ten times [21, 22]. The relative standard deviation for the two oxidation and two reduction current peaks were calculated to be approximately ±1.5 and ±2.1, ±0.95, and ±0.88 μA, respectively. Figure 14 shows the cyclic voltammogram of AL NPs in the rabbit blood sample.

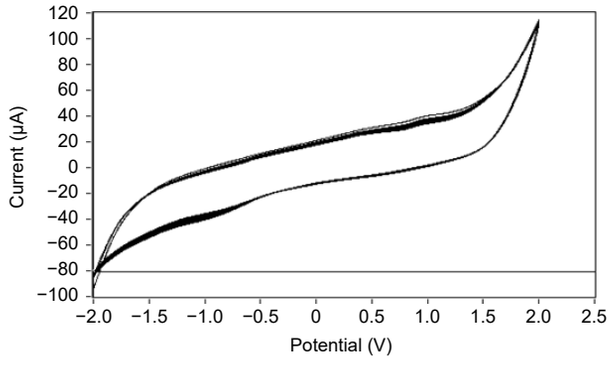

Fig. 14 Cyclic voltammogram of the rabbit blood sample injected with AL NPs repeated ten times using GCE as the working electrode and Ag/AgCl as the reference electrode at scan rate of 0.1 $V \cdot s^{-1}$.

Conclusion

The use of vaccines containing AL NPs leads to health problems for all vaccinated adults and children. The effect of AL NPs on rabbit blood samples that were injected with different doses of AL NPs and different types of vaccines was investigated. Cyclic voltammetry revealed that the oxidation current peaks were enhanced with increasing doses of the AL NPs. Notably, an oxidative effect was observed on the rabbit blood samples when injected with poliomyelitis and BCG vaccines.

CRediT Author Statement

Mohammed Abdul Hameed Younis: Conceptualization, investigation, methodology, project administration, supervision, visualization, writing (original draft), review, and editing. **Muhammed Mizher Radhi:** Data curation, writing, review, and editing. **Kalyani Chaudhari:** Data curation, writing, review, and editing. **Emad Abbas Jaffar Al-Mulla:** Data curation, writing, review, and editing.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

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