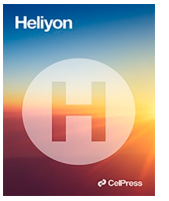
Heliyon 9 (2023) e18800

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Physicochemical properties and adsorption state of aluminum adjuvants with different processes in vaccines

Cunpei Bo [1], Xiaoli Wei [1], Xue Wang, Wenheng Ji, Huan Yang, Yuxiu Zhao, Hui Wang [*]

Beijing Institute of Biological Products Company Limited, Beijing 100176, China

ARTICLE INFO

Keywords:
Aluminum adjuvants
Particle size
Zeta potential
Adsorption state

ABSTRACT

Aluminum salts are by far the most widely used adjuvants for human vaccines, showing acceptable safety and efficacy. Previous studies have shown that each aluminum adjuvant have different charges and morphologies, but whether the manufacturing and production processes affects the physicochemical properties of aluminum adjuvant has not yet been reported. In this study, we explored the physical and chemical properties of different aluminum adjuvants and Hib, sIPV antigens through particle size, zeta potential and morphological characteristics. The adsorption rate and efficacy were also investigated. The results showed that the preparation process had an impact on the physical and chemical properties of aluminum adjuvants, including differences in the particle size , zeta potential and morphological structure. Hib vaccine had larger particle size than sIPV vaccine with different aluminum adjuvants in the process of vaccine preparation. In addition, by measuring the adsorption rate, increasing the concentration of phosphate or Aluminum phosphate (AP) can improve the adsorption rate of Hib, but Aluminium hydroxide (AH) and amorphous aluminum hydroxyphosphate sulfate (AAHS) adjuvants are not affected. *In vivo* result showed that increasing the adsorption rate of Hib could enhance the Hib-IgG antibody titers. In conclusion, this study provides a reference for the application of adjuvants in vaccines by studying the physicochemical properties and adsorption conditions of different aluminum adjuvants and antigens.

1. Introduction

Vaccination is one of the main measures for prevention and control of human infectious diseases. Because the immunogenicity of some antigens is poor, adjuvants are usually needed to improve the level and duration of vaccine induced protection [1]. In 1926, Glenny et al. first showed that insoluble aluminum salt precipitation can enhance the immunogenicity of diphtheria toxoid [2]. Since then, aluminum adjuvants have often been used as immune enhancers in human vaccine formulations. The three aluminum adjuvants commonly used in licensed vaccines are aluminum hydroxide (AH), aluminum phosphate (AP), and amorphous aluminum hydroxyphosphate sulfate (AAHS) [3]. After the antigen absorbed by aluminum adjuvant enters human body, the antigen will be released slowly. At the same time, it can stimulate the process of phagocytosis of phagocytes, antigen presentation, cytokine secretion, the synergy between T cells and B cells, and the humoral immune response [4,5]. That is the reason why aluminum adjuvant could

* Corresponding author.
E-mail address: wanghui_bsy@163.com (H. Wang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2023.e18800
Received 31 October 2022; Received in revised form 17 July 2023; Accepted 27 July 2023
Available online 28 July 2023

improve the antibody response and enhance the immunogenicity of the antigen. However, aluminum adjuvants cannot participate in cellular immunity and cannot be used in some vaccines, such as influenza vaccine [6].

AH adjuvant is chemically crystalline aluminum oxyhydroxide AlO(OH), with the shape of fibers [7]. AP adjuvant is chemically amorphous Al $(OH)_x(PO_4)_y$, with the shape of spherical. AAHS, which is proprietary to Merck, forms an amorphous mesh and carries approximately zero charge at neutral pH. The point of zero charge (PZC) of AH adjuvant is 11.4, and the PZC of AP adjuvant is usually between 5.0 and 5.5. However, AAHS adjuvant PZC were between AH and AP [8,9]. At physiological pH value, AH adjuvant has a positive charge, which can generate electrostatic attraction with acidic isoelectric point antigen [1]. The AP adjuvant has an negative charge, attracting with the antigen of alkaline isoelectric point. This electrostatic attraction between antigen and adjuvant is the main adsorption mechanism, and sodium chloride ion strength will affect the adsorption level of antigen [9]. Ligand exchange is the strongest adsorption between antigen and adjuvant, which is generated by the exchange of phosphate groups on antigen and hydroxy groups on the surface of aluminum-containing adjuvant [10]. In addition, other attractive forces, such as hydrogen bonding, hydrophobic interactions, and van der Waals forces may also contribute to this process [11,12]. Understanding the physical properties of adjuvants is important for improving the adsorption of adjuvants and clarifying the mechanism of combination of antigens and adjuvants.

Haemophilus influenzae type B (Hib) is one of the common causes of pneumonia, meningitis and other serious infectious diseases in children [13]. Because the immunogenicity of Hib alone is not strong, it needs to be adsorbed by carrier to enhance its immunogenicity [1]. The World Health Organization (WHO) recommends that Hib conjugate vaccine be included in all routine infant immunization plans [14]. Hib conjugate vaccine has four main carriers: diphtheria toxoid protein based conjugate vaccine (Polyribosyl-ribitol-phosphate-D, PRP-D), weak diphtheria flavone CRM197 based conjugate vaccine (Hb OC), group B meningococcal membrane protein (PRP-OMP), and tetanus toxoid based conjugate vaccine (PRP-T). At present, tetanus toxoid is mainly used as carrier (Hib-TT) [15,16]. Poliovirus is one of the most harmful viruses to human health, which can cause symptoms such as delayed nerve palsy and poliomyelitis [17]. It can be divided into type 1, 2 and 3 according to its antigenicity [18,19]. The inactivated polio virus vaccine (IPV) content of D-antigen which is a key index for determining the efficacy of IPV in vitro, which is consistent with its immune efficacy in human body.

The studies of adjuvant can provide a basis for vaccine researchers to select appropriate adjuvants and accelerate the drug development, especially combined vaccines. Combined vaccines contain a variety of antigens, such as recombinant proteins, inactivated viruses, polysaccharides, etc. Because the appropriate adjuvants for each antigen are different, it is necessary to comprehensively consider the characteristics of each adjuvant to improve combined vaccines development.

In this study, a variety of aluminum adjuvants and vaccines were prepared and characterized physicochemical properties and immune efficacy. It was found that AH, AAHS and AP were different in particle size, zeta potential, and morphology. And aluminum adjuvants prepared using different processes also differ. Compared with AP, AH and AAHS can adsorb more antigens. The adsorption rate of AP adjuvant to Hib antigen increases with the increase of phosphate and adjuvant concentration. And high Hib adsorption rate can enhance the efficacy *in vivo*. These results indicate that the nature of vaccine components and the mechanism of adjuvants adsorption need to be fully studied to provide references for different vaccine design and product.

2. Materials and methods

2.1. Mice

All the mice (NIH, 17–19 g) were purchased from SPF (Beijing) Lab Animals Technology Co., Ltd., Beijing, China, and maintained in a specific pathogen-free (SPF) environment at Beijing Laboratory Animal Research Center.

2.2. Antigens and aluminum adjuvants

Hib was produced by Lanzhou Institute of Biological Products Co., Ltd (LIBP) of Sinopharm. And sIPV was produced by Beijing Institute of Biological Products Co., Ltd (BIBP) of Sinopharm. AH1 was purchased from Croda (Danmark, 21645-51-2). AP was purchased from InvivoGen (Danmark, 7784-30-7). AH2 and AAHS were produced by Beijing Institute of Biological Products Co., Ltd (BIBP) of Sinopharm. D-antigen ELISA kit was obtained from Beijing Institute of Biological Products Co., Ltd (BIBP) of Sinopharm.

2.3. Vaccine suspensions preparation

The final concentration of Hib was 20 μg/mL, and that of sIPV type 1/2/3 were 30/90/90 DU/mL, respectively. The final bulk was prepared by adding 1.3 mg/mL AH or 1.22 mg/mL AP as the adjuvant unless otherwise specified. Mix the suspensions using a magnetic stirrer. The adsorption rate of the samples was measured after being stored at 4 °C for 24 h.

2.4. Determination of particles size and zeta potential

The particle size and zeta potential of adjuvants and related vaccines were determined to delineate the physical characteristics and stability. The zeta potential of adjuvants and vaccines were determined by Zetasizer Nano ZS90 (Malvern, UK). The particle size was performed using Mastersizer 2000 (Malvern, UK).

2.5. Electron microscopy sample preparation

SEM and TEM were used to observe the structure of samples. For the scanning electron microscope (SEM), stick the conductive adhesive on the sample table and stick the clean thin copper sheet on the conductive adhesive. Drop the liquid sample on the copper sheet, and wait for the solvent to completely volatilize, and then spray the gold and observe by Gemini SEM 300 (ZEISS, Germany). For the transmission electron microscope (TEM), dilute the sample by 1:10. Drop the diluted sample on the copper mesh, and dry it completely before loading for observation using JEM-2100 Plus (JEOL, Japan).

2.6. Hib adsorption rate assay

Add 5 mL 0.1% ferric chloride hydrochloric acid solution to the test solution and then add 0.4 mL of lichenol ethanol solution. After water bath for 5 min at 100 °C., the mixtures were transferred into the ice bath and was measured the absorbance at the wavelength of 670 nm. The concentration of ribose reference solution was used to perform linear regression on its corresponding absorbance, and the linear regression equation was obtained. Substitute the absorbance of the test solution into the linear regression equation to calculate the ribose content of the test solution.

2.7. D-antigen ELISA assays

A sandwich enzyme-linked immunosorbent assay (ELISA) was used to quantify D-antigen units (DU) of the inactivated polio vaccine. ELISA plates coated with anti-polio antibodies were obtained from Beijing Institute of Biological Products Co., LTD. 100 μL vaccine and supernatant were added to the plate, respectively. Plates were kept at 37 °C for 1 h and then washed 5 times using the wash solution. And then 100 μL corresponding working solutions were added to the plates. Plates were incubated at 37 °C for 20min. After washing the plates 5 times, the chromogenic solution was added to the plates and the plates were kept at 37 °C. After 10mins, the

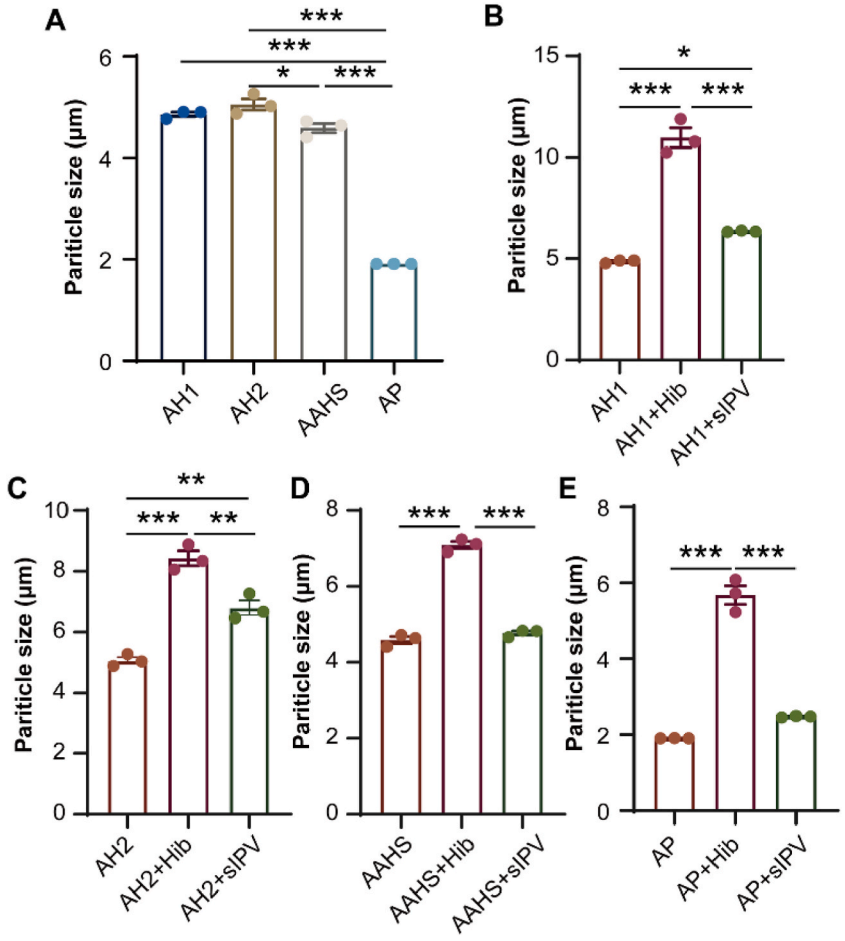


Fig. 1. Particle size of aluminum adjuvants and vaccines. (A) Particle size of four kinds of aluminum adjuvant. (B–E) Particle size of four kinds of aluminum adjuvant-containing vaccines.

stopping solution was added to each well. The absorbance was read by a microplate reader at 450/630 nm wavelength. Adsorption rate = (antigen content of vaccine - antigen content of supernatant)/antigen content of vaccine x 100%.

2.8. Animal immunity

Normal saline was used as the negative control and the Pasteur Hib vaccine was used as the positive control. 10 μg/mL of Hib polysaccharide conjugate vaccines with different adsorption rates were injected subcutaneously through the abdomen. Mice were inoculated twice on the 1st and 14th days. On the 28th day, serum was collected to determine Hib titer.

2.9. Hib titer determination

The plate was coated with PRP tyrosine hydrochloride and incubated at 2–8 °C for 18–24 h. Wash the plate for 5 times. Add sealing solution and keep the plate at 37 °C for 1 h. After washing 5 times, add 100 μL serum sample to the plate. Keep the plate at 37 °C for 1 h and wash 5 times with detergent. Then add 1:1000 diluted enzyme labeled antibody to the plate. Keep the plate at 37 °C. After 30 min, add chromogenic solution to the plate and keep the plate at room temperature. After 10 min, add stopping solution. Use a microplate reader to read the absorbance at 450/630 nm wavelength. Calculate cut off value and the Hib antibody titer.

2.10. Statistical analysis

All results are represented as the means ± SEM. Multiple comparisons among three or more groups were performed using one-way ANOVA. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ were defined as statistically significant.

3. Results

3.1. Size of particles

In order to evaluate the sizes of four aluminum adjuvants and related vaccines, suspensions were prepared and the sizes of particles were analyzed. The suspensions were homogeneous. The results showed that the particle sizes of AH using two different processes were 4.86 μm, 5.06 μm, while that of AAHS and AP were 4.59 μm and 1.91 μm, respectively (Fig. 1A). We found that the particle size of AP was significantly smaller than that of AH and AAHS ($p < 0.001$), indicating that the particle size of various aluminum adjuvants was different based on their processing varies (Fig. 1A).

Previous studies have found that sIPV antigens were spherical particles of about 30∼40 nm [20,21]. When aluminum adjuvant combined with Hib or sIPV, the particle size of all vaccines increased compared with the corresponding aluminum adjuvant. Among these vaccines, the combinations of AH1 and Hib had the largest particle size, which was 10.97 μm (Fig. 1B). The particle size of the mixture of aluminum adjuvant and Hib was significantly larger than that of aluminum adjuvant and sIPV ($p < 0.01$) (Fig. 1B–E). This result suggests that the particle size changes after binding with antigen, and the changes are different after binding with different antigens. This may be explained by the fact that the particle size of Hib-TT is larger than that of sIPV, or Hib combines with adjuvant to produce an irregular shape, resulting in larger particle size than that of vaccines containing sIPV.

3.2. Zeta potential analysis of aluminum adjuvants

Zeta potential is a measure of the strength of mutual repulsion or attraction between particles and an indicator of the stability of colloidal dispersions [22]. According to zeta potential, it can be judged whether there is a binding mode of electrostatic adsorption between adjuvant and antigen [23]. The zeta potentials of different aluminum adjuvants and related vaccine samples are shown in

Table 1
Zeta potential and pH of antigens, adjuvants and vaccines.

Group	Zeta Potential(mV)	Conductivity (mS/cm)	pH
Hib	−18.57 ± 0.86	1.58	6.35
sIPV	−0.71 ± 0.44	2.42	6.48
AH1	11.80 ± 0.70	0.03	6.13
AH2	7.94 ± 0.50	0.51	6.33
AAHS	7.85 ± 0.14	2.28	6.35
AP	−24.70 ± 1.15	0.12	6.91
Hib + AH1	7.87 ± 0.11	1.63	6.03
sIPV + AH1	3.89 ± 0.18	2.31	6.24
Hib + AH2	19.9 ± 0.46	0.53	6.25
sIPV + AH2	29.73 ± 1.04	0.8	6.22
Hib + AAHS	6.04 ± 0.37	1.88	6.38
sIPV + AAHS	8.99 ± 0.37	2.52	6.43
Hib + AP	−28.47 ± 1.17	0.41	6.74
sIPV + AP	−21.23 ± 1.06	0.57	6.61

Table 1.

The results showed that the isoelectric points of various aluminum adjuvants were different. At pH 6–7, the AH and AAHS adjuvants were positively charged (11.80 ± 0.70 mV, 7.94 ± 0.50 mV and 7.85 ± 0.14 mV), while the AP adjuvants had opposite charge (−24.70 ± 1.15 mV). The zeta potential of Hib and sIPV were −18.57 ± 0.86 mV and −0.71 ± 0.44 mV, respectively. When antigens were mixed with adjuvants, the suspension showed the same charge as the adjuvants. When Hib antigens were mixed with AP, the suspension also showed the same charge as Hib and AP. This indicates that in addition to electrostatic adsorption, there are other adsorption mechanisms when Hib or sIPV mixed with AP adjuvants, such as ligand exchange, hydrogen bonding, hydrophobic interactions, and van der Waals forces.

3.3. Morphology analysis of aluminum adjuvants

Electron microscopy has been used to evaluate the structural characteristics of aluminum adjuvants [24]. But the structures of aluminum adjuvants prepared using different processes remain unclear. Here, we characterized four kinds of aluminum adjuvants using SEM and TEM. SEM microscopy indicated that AH1 have a fibrous structure (Fig. 2A). The structure of AH2 was fibers connected into a mesh. (Fig. 2B), while AAHS and AP showed spherical agglomerate (Fig. 2C–D). The diameter of AAHS was larger than AP, which was consistent with the particle size results.

TEM images corresponded to the results of SEM and the particle size. TEM results showed that AH1 was fibrous (Fig. 3A). AH2 had the shape of net (Fig. 3B). AAHS and AP appeared spherical particles (Fig. 3C). Compared to AP, AH and AAHS were characterized by its irregular shape and interparticle connections, which may be the reason why AH and AAHS had larger particle size (Fig. 3D). Although the particle sizes of AH adjuvants were similar, the morphologic characters were different. This illustrates that the preparation process can affect the particle size, shape and density of aluminum adjuvants. Morphology may be responsible for the different adsorption capacities. AH adjuvant may combine more antigens due to larger surface area.

3.4. Morphology analysis of aluminum-related vaccines

To further investigate the morphological changes of adjuvant after combining with antigen, TEM was performed. Observation results revealed that the fibrous structure of the adjuvant became dense after Hib was combined with AH1, which may be the cause of the large particle size (10.97 μm) (Fig. 4A). Antigen particles were observed on the surface of the adjuvant when Hib was combined with AH2, AAHS and AP, respectively (Fig. 4B–D). We also found that sIPV-related vaccines had small particle sizes and less aggregation than Hib-related vaccines (Fig. 4E–H). AH1 became dispersed and spherical antigen particles were attached to the adjuvant evenly (Fig. 4E). The mesh of AH2 became small (Fig. 4F), and the sIPV antigen was coated on the surface of the AAHS (Fig. 4G). AP combined with sIPV was still observed spherical structure (Fig. 4H). The antigen is bound to the adjuvant surface, and the interaction between them does not form a new structure. These results suggest that the types of adjuvants and antigens, the mechanism in which adjuvants bind to antigens can affect morphology. The binding of Hib to AH results in large particle size and tight structure, whereas sIPV binds loosely to AH adjuvants. This may be due to the stronger force between Hib and AH that allowed AH to adsorb more Hib, or Hib changed the microenvironment of AH to make AH bind closely.

3.5. Adsorption of aluminum adjuvants to Hib and sIPV

In order to further investigate whether the characteristics of adjuvant will affect the adsorption of antigen, we explored the

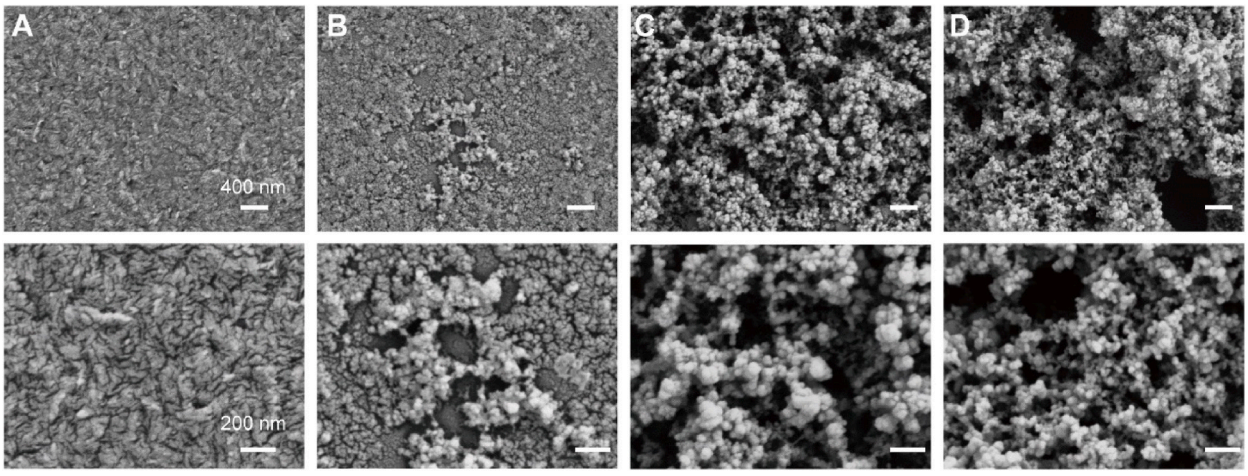


Fig. 2. SEM morphology of aluminum adjuvants. (A–B) SEM morphology of AH1 and AH2. (C) SEM morphology of AAHS. (D) SEM morphology of AP. Scale bar: 400 nm (up) and 200 nm (down).

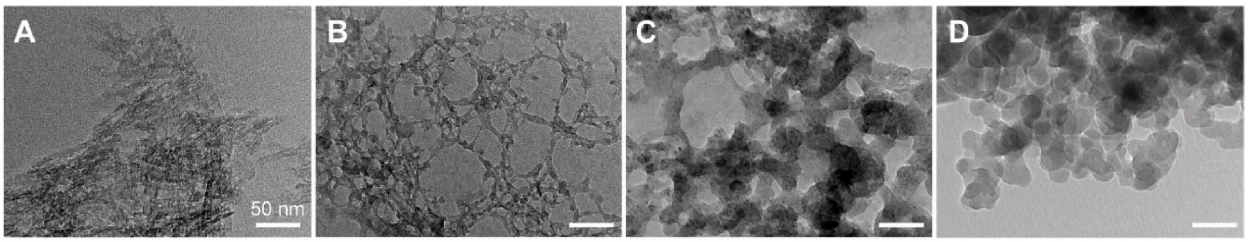


Fig. 3. Structure of aluminum adjuvants. (A–B) TEM morphology of AH1 and AH2. (C) TEM morphology of AAHS. (D) TEM morphology of AP. Scale bar: 50 nm.

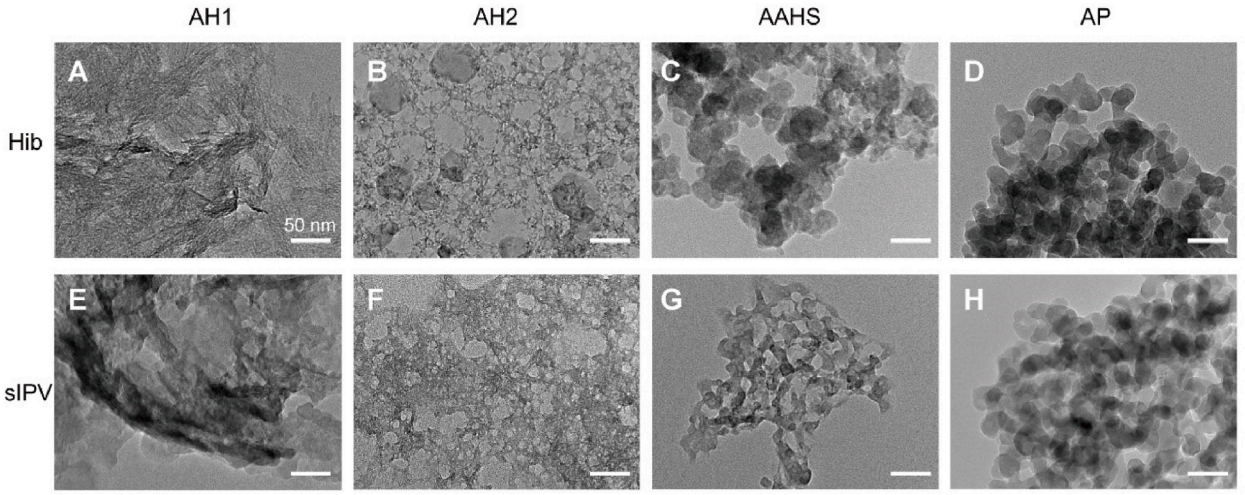


Fig. 4. Structure of aluminum adjuvants combined with antigens. (A–D) TEM images of aluminum adjuvants combined with Hib. (E–H) TEM images of aluminum adjuvants combined with sIPV. Scale bar: 50 nm.

influence of aluminum adjuvant on the adsorption capacity of Hib and sIPV antigen under different conditions. We found that the adsorption capacity of AH1 to the four antigens selected in this study was not affected by the concentration of PBS (pH = 6.0) (Fig. 5A). The adsorption capacity of AP on three types of sIPV is not affected by phosphate concentration (Fig. 5B). With the increase of PBS (pH = 6.0) concentration (from 0.75 mM to 3 mM), the Hib adsorbed on AP increased (from 63.11% to 86.19%) (Fig. 5B). Phosphate ions and sodium chloride ions in the buffer may be factors that affect the adsorption rate.

The concentration of adjuvant also affected antigen adsorption rate. We found that the adsorption rate of aluminum adjuvant to the four antigens was 100% under three AH1 concentrations (1.3 mg/mL, 2.6 mg/mL, 3.9 mg/mL) (Fig. 5C). The adsorption of AP to Hib increased (from 74.1% to 100%) with the increase of AP concentration (from 1.3 mg/mL to 3.9 mg/mL). The adsorption rates of sIPV type 3 were 53%, 54%, and 51%, respectively (Fig. 5D). The free phosphate in the AP may affect the adsorption of the AP to the antigen. The particle size and electron microscope results suggest that AH1 has a significantly larger surface area than AP and can adsorb more antigens.

3.6. Efficacy of AP to Hib

In order to improve the efficacy of the Hib vaccine, we prepared vaccines with different adsorption rates of AP on Hib according to the above study to explore the relationship between adsorption rate and efficacy. The result showed that the seroconversion rate of all samples was 100%. The geometric mean titers (Log10) of Hib antibody corresponding to AP adsorption rates of 0%, 30%, 50%, 60%, 80% and 100%, were 2.94, 3.27, 3.28, 3.60, 3.37 and 3.72, respectively (Fig. 6). With the increase of AP adsorption rate on Hib, the level of Hib antibody is also increased. There was a significant difference between the efficacy of 0% adsorption rate and 100% adsorption rate ($p < 0.05$). Moreover, the Hib antibody level of more than 30% adsorption rate was higher than that of the Hib positive control (3.00). These results suggested that complete adsorption of Hib antigen with aluminum adjuvant could improve vaccine efficacy compared with non-adsorption. According to the repository effect, when the vaccine was injected into mice, the antigen was continuously released from the aluminum adjuvant [25,26], which led to the increase of antigen concentration at the injection site and enhanced the uptake of antigen presenting cells [27].

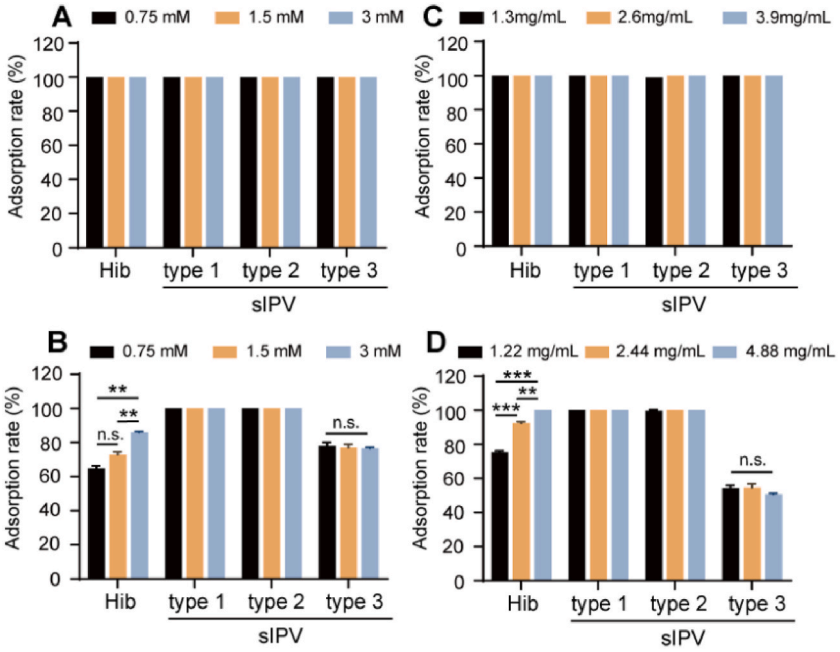


Fig. 5. Adsorption conditions of Hib and sIPV. (A, B) Effect of PBS concentration on the adsorption capacity of AH1 and AP. (C, D) Effects of different aluminum adjuvant concentrations on the adsorption capacity of AH1 and AP adjuvant.

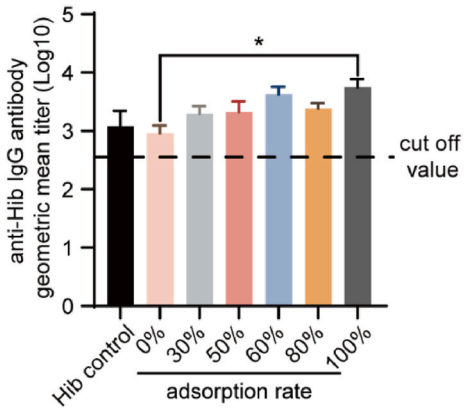


Fig. 6. Efficacy of Hib under different adsorption rates. Hib antibody geometric mean titer of Hib positive control group and adsorption rate from 0% to 100%.

4. Conclusion and discussion

Aluminum adjuvants have been used for nearly 100 years and have numerous advantages, including minimal reactogenicity, excellent safety profile, and low price [28]. In the early development of new vaccine products, it is necessary to understand the physical properties of the adjuvant used and their ability to adsorb antigens [29]. The three aluminum adjuvants commonly used in human vaccines are AH adjuvant, AAHS and AP adjuvant [9]. AH is prepared by mixing aluminum-containing solution with a base. AP is prepared by pumping aluminum chloride and sodium dihydrogen phosphate into a reaction container at a constant rate [30,31]. AAHS is similar to AP, but contains residual sulfate which was [$AlK(SO_4)_2$] used as the source material for AAHS produced. Due to different experimental conditions, temperatures, concentrations and even the addition speed of reagents, AH, AAHS, and AP from different sources may also have differences in physicochemical and biological properties [9,28]. However, there is currently no report in the peer-reviewed literature on a comparison of aluminum adjuvants from various process.

Here, we report the particle size, zeta potential, morphology of various aluminum adjuvants and adsorption rate for Hib and sIPV antigen. This study focused on the physicochemical properties of three main aluminum adjuvants: AH, AAHS, and AP. We found that the particle size of AP varies with preparation technology, and the average particle size of AH and AAHS are larger than that of AP. And

the charge and absolute value of zeta potential also change with the type of aluminum adjuvants and preparation processes.

It is reported that phosphate-containing antigens are more capable of binding to aluminum, and surface hydroxyl groups are obtained from AH. Unlike electrostatic adsorption, the ligand exchange of phosphate is not influenced by the relationship between the isoelectric point of antigen or aluminum-containing adjuvant and the pH value of vaccine [32]. Hib and sIPV have different characteristics. The polyribosyl-ribitol-phosphate, capsular polysaccharide of Hib, is highly negatively charged due to its phosphate groups [32,33]. The isoelectric points of sIPV type 1, 2 and 3 are 7.4, 7.2, and 6.3 [34]. However, the isoelectric points of AH, AAHS and AP are about 11.4, 7.0, and 5.0, respectively [35,36]. This property influences the surface microenvironment of different aluminum adjuvants and antigens, and further affects the combination mechanism between aluminum adjuvants and antigens. Complementary surface charges of the antigen and the aluminum adjuvants were expected to optimize conditions for antigen-adjuvant interactions [37,38]. Under vaccine pH, negatively charged Hib and positively charged AH exist electrostatic adsorption. While AP is negatively charged, Hib and AP still show high adsorption capacity. Considering Hib have phosphate groups, this indicated that binding mode of Hib and AP may be ligand exchange [32].

This study also showed that 2.44 mg/mL AP (20 mM Aluminum) adsorbed about 18 μg/mL Hib, while 1.3 mg/mL (16 mM Aluminum) AH adsorbed 20 μg/mL Hib completely. Aluminum adjuvants maximum adsorption capacity differs. AH and AAHS with larger particle size can bind more antigens to the surface. However, it is not clear whether the adsorption capacity of adjuvant to antigen will affect the release and effect of antigen. Therefore, the optimal formulation of antigens adsorbed to adjuvants needs to be analyzed specifically. In addition to the study of the physicochemical properties of adjuvants and related vaccines, whether the adsorption capacity of aluminum adjuvants to antigens affects the immunogenicity of antigens needs to be further explored. Our study showed that the adsorption of Hib antigen on AP enhances the immune response, which may be caused by promoting phagocytosis and slowing the diffusion of antigen from the injection site. However, adsorption strength is also important because high-affinity interactions may interfere with the immune response [39].

Given the differences in the physicochemical properties of aluminum adjuvants under different preparation processes, different manufacturers need to have strict quality standards and process parameter design when producing aluminum adjuvants. During the preparation of adjuvant-based vaccines, sufficient adsorption process research (pH, ionic strength, buffer, adjuvant and antigen ratio, adsorption time and adsorption temperature) is required to ensure the optimal immune effect.

In conclusion, this investigation demonstrates that various aluminum adjuvants have different particle sizes, isoelectric points, and morphological characteristics, which may lead to differences in the affinity of adjuvants for antigens and vaccine efficacy. The broader research scope in the future should also include: (1) Study on the adsorption coefficient and adsorption strength of antigen by aluminum adjuvant with different processes; (2) Evaluation of antigen immune efficacy under different adsorption intensity conditions; (3) Exploring the effects of different adjuvants on the structure and stability of antigens after adsorption; (4) Optimization of thermal stability of aluminum adjuvant vaccines. Our results will contribute to the design of vaccines and optimize the adsorption and stability of antigens in vaccines.

Author contribution statement

Cunpei Bo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Xiaoli Wei; Xue Wang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Wenheng Ji: Performed the experiments; Wrote the paper.

Huan Yang: Analyzed and interpreted the data; Wrote the paper.

Yuxiu Zhao: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Hui Wang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank all participants who contributed to this work.

References

[1] G.L. Morefield, A rational, systematic approach for the development of vaccine formulations, AAPS J. 13 (2011) 191–200.

[2] N. Petrovsky, Novel human polysaccharide adjuvants with dual Th1 and Th2 potentiating activity, Vaccine 24 (Suppl 2) (2006). S2-26-9.

[3] M.J. Caulfield, L. Shi, S. Wang, B. Wang, T.W. Tobery, H. Mach, et al., Effect of alternative aluminum adjuvants on the absorption and immunogenicity of HPV16 L1 VLPs in mice, Hum. Vaccine 3 (2007) 139–145.
[4] B. Hansen, A. Sokolovska, H. HogenEsch, S.L. Hem, Relationship between the strength of antigen adsorption to an aluminum-containing adjuvant and the immune response, Vaccine 25 (2007) 6618–6624.
[5] A.C. Rimaniol, G. Gras, P. Clayette, In vitro interactions between macrophages and aluminum-containing adjuvants, Vaccine 25 (2007) 6784–6792.
[6] P. Traquina, M. Morandi, M. Contorni, G. Van Nest, MF59 adjuvant enhances the antibody response to recombinant hepatitis B surface antigen vaccine in primates, J. Inf. Dis. 174 (1996) 1168–1175.
[7] S. Shirodkar, R.L. Hutchinson, D.L. Perry, J.L. White, S.L. Hem, Aluminum compounds used as adjuvants in vaccines, Pharmaceut. Res. 7 (1990) 1282–1288.
[8] D. Jiang, C.T. Johnston, S.L. Hem, Using rate of acid neutralization to characterize aluminum phosphate adjuvant, Pharmaceut. Dev. Technol. 8 (2003) 349–356.
[9] H. HogenEsch, D.T. O'Hagan, C.B. Fox, Optimizing the utilization of aluminum adjuvants in vaccines: you might just get what you want, NPJ Vacc. 3 (2018) 51.
[10] I.Z. Romero Méndez, Y. Shi, H. HogenEsch, S.L. Hem, Potentiation of the immune response to non-adsorbed antigens by aluminum-containing adjuvants, Vaccine 25 (2007) 825–833.
[11] J.P. Goullé, P. Couvreur, L. Grangeot-Keros, About the alleged toxicity of aluminium-based adjuvants in vaccines: all published studies should be taken into account, Int. J. Pharm. 602 (2021), 120656.
[12] R. Danielsson, H. Eriksson, Aluminium adjuvants in vaccines - a way to modulate the immune response, Semin. Cell Dev. Biol. 115 (2021) 3–9.
[13] J.R. Gilsdorf, Hib vaccines: their impact on Haemophilus influenzae type b disease, J. Inf. Dis. 224 (2021). S321-s30.
[14] C. Cohen, A. von Gottberg, Hib combination vaccines: efficient and effective, Lancet Infect. Dis. 18 (2018) 700–701.
[15] M.R. Alderson, J.A. Welsch, K. Regan, L. Newhouse, N. Bhat, A.A. Marfin, Vaccines to prevent meningitis: historical perspectives and future directions, Microorganisms 9 (2021).
[16] M. Giufrè, E. Lindh, R. Cardines, P. Pezzotti, M. Cerquetti, Invasive Haemophilus influenzae type b (Hib) disease in children in Italy, after 20 years of routine use of conjugate Hib vaccines, Vaccine 38 (2020) 6533–6538.
[17] A. Irwin, New polio vaccine poised to get emergency WHO approval, Nature 587 (2020) 15–16.
[18] K.M. Thompson, D.A. Kalkowska, Reflections on Modeling Poliovirus Transmission and the Polio Eradication Endgame. Risk Analysis, vol. 41, an official publication of the Society for Risk Analysis, 2021, pp. 229–247.
[19] M.T. Yeh, E. Bujaki, P.T. Dolan, M. Smith, R. Wahid, J. Konz, et al., Engineering the live-attenuated polio vaccine to prevent reversion to virulence, Cell Host Microbe 27 (2020), 736-51.e8.
[20] W.J. Shin, D. Hara, F. Gbormittah, H. Chang, B.S. Chang, J.U. Jung, Development of thermostable lyophilized sabin inactivated poliovirus vaccine, mBio 9 (2018).
[21] Y. Huijuan, D. Xiaohu, L. Ze, C. Wei, Z. Jian, M. Lei, et al., Role of phenol red in the stabilization of the Sabin type 2 inactivated polio vaccine at various pH values, J. Med. Virol. 91 (2019) 22–30.
[22] J.D. Clogston, A.K. Patri, Zeta potential measurement, Methods Mol. Biol. 697 (2011) 63–70.
[23] P. Marrack, A.S. McKee, M.W. Munks, Towards an understanding of the adjuvant action of aluminium, Nat. Rev. Immunol. 9 (2009) 287–293.
[24] Y.J. Cheng, C.Y. Huang, H.M. Ho, M.H. Huang, Morphology and protein adsorption of aluminum phosphate and aluminum hydroxide and their potential catalytic function in the synthesis of polymeric emulsifiers, Colloids Surf. A Physicochem. Eng. Asp. 608 (2021), 125564.
[25] P. He, Y. Zou, Z. Hu, Advances in aluminum hydroxide-based adjuvant research and its mechanism, Hum. Vaccines Immunother. 11 (2015) 477–488.
[26] W. Matheis, A. Zott, M. Schwanig, The role of the adsorption process for production and control combined adsorbed vaccines, Vaccine 20 (2001) 67–73.
[27] H. HogenEsch, Mechanisms of stimulation of the immune response by aluminum adjuvants, Vaccine 20 (Suppl 3) (2002) S34–S39.
[28] E.B. Lindblad, Aluminium compounds for use in vaccines, Immunol. Cell Biol. 82 (2004) 497–505.
[29] D. Sacks, B. Baxter, B.C.V. Campbell, J.S. Carpenter, C. Cognard, D. Dippel, et al., Multisociety consensus quality improvement revised consensus statement for endovascular therapy of acute ischemic stroke, Int. J. Stroke : Off. J. Int. Stroke Soci. 13 (2018) 612–632.
[30] K.P. Yau, D.G. Schulze, C.T. Johnston, S.L. Hem, Aluminum hydroxide adjuvant produced under constant reactant concentration, J. Pharmaceut. Sci. 95 (2006) 1822–1833.
[31] L.S. Burrell, C.T. Johnston, D. Schulze, J. Klein, J.L. White, S.L. Hem, Aluminium phosphate adjuvants prepared by precipitation at constant pH. Part I: composition and structure, Vaccine 19 (2000) 275–281.
[32] S. Iyer, H. HogenEsch, S.L. Hem, Effect of the degree of phosphate substitution in aluminum hydroxide adjuvant on the adsorption of phosphorylated proteins, Pharmaceut. Dev. Technol. 8 (2003) 81–86.
[33] D. Tietz, Computer-assisted 2-D agarose electrophoresis of Haemophilus influenzae type B meningitis vaccines and analysis of polydisperse particle populations in the size range of viruses: a review, Electrophoresis 28 (2007) 512–524.
[34] Y.E. Thomassen, G. van Eikenhorst, L.A. van der Pol, W.A. Bakker, Isoelectric point determination of live polioviruses by capillary isoelectric focusing with whole column imaging detection, Anal. Chem. 85 (2013) 6089–6094.
[35] A. Langford, T. Horwitz, E. Adu-Gyamfi, C. Wiley, E. Holding, D. Zimmermann, et al., Impact of formulation and suspension properties on redispersion of aluminum-adjuvanted vaccines, J. Pharmaceut. Sci. 109 (2020) 1460–1466.
[36] J.V. Rinella, J.L. White, S.L. Hem, Effect of pH on the elution of model antigens from aluminum-containing adjuvants, J. Colloid Interface Sci. 205 (1998) 161–165.
[37] S.J. Seeber, J.L. White, S.L. Hem, Predicting the adsorption of proteins by aluminium-containing adjuvants, Vaccine 9 (1991) 201–203.
[38] P.M. Callahan, A.L. Shorter, S.L. Hem, The importance of surface charge in the optimization of antigen-adjuvant interactions, Pharmaceut. Res. 8 (1991) 851–858.
[39] H. Hogenesch, Mechanism of immunopotentiation and safety of aluminum adjuvants, Front. Immunol. 3 (2012) 406.