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Preparation of Aluminum Hydroxide by Precipitation Method for Vaccine Adjuvant Application

Etik Mardliyati*, Damai Ria Setyawati*, Sabar Pambudi*, Suryandaru**, Retno Kusumaningrum***, M. Ikhlasul Amal****
**Center for Pharmaceutical and Medical Technology, Agency for The Assessment and Application of Technology, Indonesia*
Email: etik.mardliyati@bppt.go.id
*** Department of Industrial Engineering, Nahdlatul Ulama University, Indonesia*
Email: surya@nano.or.id
**** Nano Canter Indonesia*
Email: anink.kusuma@gmail.com
*****Development and Application Unit for Biocompatible Implant Materials in Orthophaedics, Indonesian Institute of Sciences, Indonesia*
Email: muha137@lipi.go.id

ABSTRACT
Aluminum hydroxide is commonly used as vaccine adjuvant. The adjuvant activity of aluminum hydroxide is considered to depend on its particle size. In order to obtain aluminum hydroxide with high adjuvant activity, we synthesized aluminum hydroxide at various particle sizes and investigate the effect of particle size on protein adsorption property. Aluminum hydroxide was synthesized by precipitation method at various agitation rates. The aluminum hydroxide product synthesis was identified by FTIR. Both and found peak on 595, 1634 and 3331 cm^{-1} that contributed to aluminum hydroxide. The size of aluminum hydroxide particles highly depends on agitation rate, in which higher agitation produce smaller particles. Particle size analyzer resulted 3422, 305 and 279 nm respectively for 200, 5000 and 10000 rpm agitation rate. The adsorption of protein using BSA as a model protein on aluminum hydroxide particles was carried out by mixing the particles in suspension with the protein in solution. The protein adsorption of BSA to the aluminum hydroxide nanoparticles was reach 90%.
Keywords: Adjuvant, Aluminum hydroxide, nanoparticles

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I. INTRODUCTION

The vaccine is a type of product or ingredient used to produce the immune system from various types of diseases. This vaccine may contain biological products and parts of viruses or bacteria, or viruses or live bacteria that have been attenuated. That matter, it can stimulate the body immune (Plotkins, 2013). Vaccine was found for first time in 1796 by Edward Jenner when studied a daily worker who contracted smallpox (Riedel, 2005). The most fundamental benefit of vaccines is as a preventive measure of infectious diseases. Not only that, the benefits of vaccines as well as the best defense and protection against infections and serious diseases. Vaccinations may be given in the form of injections, oral, or aerosols (inhaled substances).

The vaccine-forming component generally consists of various substances, including antigen, adjuvant, stabilizer, and preservative. The vaccine itself undergoes type updates on every generation. In the early generations, vaccines are developed in live attenuated or attenuated viruses (ex. polio vaccine, MMR), or viruses that are switched off (ex rabies vaccine). In the new generation of vaccines, many use DNA or recombinant proteins (ex hepatitis B vaccine). The recombinant protein-based vaccine is safer because it is not infectious, but has a low immunogenicity level. Therefore, the use of adjuvant in this protein-based vaccine formulation becomes very important in order to obtain a vaccine with a high level of effectiveness (Awate et al, 2013).

Adjuvant has a very important role in the preparation of recombinant protein-based vaccines in stimulating the immune response, since recombinant proteins have low levels of immunogenesis. There are several different ways how adjuvants can improve the immune response : (1) adjuvants can increase the immunogenicity of weak antigens; (2) enhance the speed and duration of the immune response; (3) modulate antibody avidity, specificity, isotype or subclass distribution; (4) stimulate cell mediated immunity (CMI), (5)

promote the induction of mucosal immunity; (6) enhance immune responses in immunologically immature, or senescent individuals; (7) decrease the dose of antigen in the vaccine and reduce costs; or (8) help to overcome antigen competition in combination vaccines (Hagan et. al., 2001). The mechanisms of action of most adjuvants still remain only poorly understood till now.

The new adjuvant formulation is generally a combination of several adjuvants into one formulation. Two or more adjuvants with different mechanisms of action are combined to enhance the potential and type of immune response to the vaccine antigen. For example, alum salts can be combined with other adjuvants such as lipid A to increase their immunogenicity. Another example is algamulin which is a combination of inulin and alum that can increase the absorption capacity and increase the ability to stimulate Th2 response (Petrovsky and Aguilar, 2004).One of material which widely use as vaccines adjuvant is aluminum hydroxide. Adjuvant containing aluminum has been approved by the US Food and Drug Administration (FDA) for use by humans (Li et al., 2014). Adjuvant-based aluminum used in vaccines is aluminum hydroxide and aluminum phosphate. In the mastery of Alum adjuvant production technology, there are 2 main problems that must be solved, namely the particle size distribution and adsorption ability, where both of these matter greatly determine the stimulation of immune response. First, the adjuvant aluminum hydroxide is generally prepared by adding an alkaline solution to the aluminum salt to form a fine precipitate of aluminum oxyhydroxide. Generally the particle size of the aluminum hydroxide particulate system manufacturing process is heterogeneous because it is susceptible to agglomeration in water-based preparations. This is very different from the characteristics of Alhydrogel® products whose particle size is relatively homogeneous (He et al., 2015). Second, the ability of adjuvant aluminum adsorption to protein / antigen is greatly influenced by particle size and uniformity. Some researchers report that particles of smaller size exhibit higher adsorption speed and adsorption capacity, as a result of increased surface area of particles

Aluminum hydroxide is the most commonly used chemical as adjuvant and is an amphoteric compound with an isoelectric point 11.4 (He et al., 2015). The primary particles of aluminum hydroxide adjuvant are fibers with average dimension of 4.5 x 2.2 x 10 nm. The nanometer dimensions of the primary particles give rise to a surface area of approximately 514 m^2/g (Johnston, et al., 2002).

According to He et al (2015), several factors affecting the response of adjuvant aluminum hydroxide are the adsorption rate, adsorption strength, size and uniformity of adjuvant particles, adjuvant dose and antigen properties. The particulate size of the vaccine carriers has significant effect on adjuvant activity. The optimal particulate size is about less than or equal to 200 nm (Kalkanidis et al., 2006). Based on a study by Li et al (2014), aluminum hydroxide nanoparticles measuring 112 nm showed better potential activity than a 9 μm aluminum hydroxide ion. In addition, adjuvants of aluminum hydroxide nanoparticles may also induce better antibody-specific responses than traditional adjuvant aluminum hydroxides in microparticle size. Adjuvant aluminum hydroxide nanoparticles also bind antigens extensively and increase the retrieval of antigens that have been adsorbed by antigen presenting cells (APCs). In addition, aluminum hydroxide nanoparticles can also reduce inflammation when injected.

Aluminum hydroxide can be obtained by reacting $AlCl_3$ solution with NaOH solution to form precipitate. The resulting precipitate is then synthesized and separated by sodium chloride using a desalting column (Li et al, 2014).

$$AlCl_{3\,(aq)} + 3\ NaOH_{(aq)} \rightarrow Al(OH)_{3\,(s)} + 3\ NaCl$$

Based on the research of Li et al (2014), the formation of $Al(OH)_3$ from a solution containing Al^{3+} depends on the function of NaOH and Al^{3+} concentration and its properties are observed using light scattering. A decrease in light scattering was seen at a concentration of 1.0-2.5 M NaOH and an increase in NaOH concentration to 7.0 M had no effect on particle light scattering. This indicates that there is a small or less formation of $Al(OH)_3$ (Hayrapetyan, et al, 2006). The other studies, resulted aluminium hydroxide in heterogeneous particles size and easy to aggregation in aqueous media (He et al., 2015) Furthermore, In this paper also described some of the main factors affecting the effects of adjuvant aluminum activity, such as adsorption speed, adsorption strength, particle uniformity, adjuvant dose and antigen characteristics. Recent research shows a close relationship between particle size and uniformity with the immuno-stimulatory effects of adjuvant aluminum.

II. MATERIALS AND METHOD

2.1 Materials:

Aluminium chloride hexahydrate (Merck), sodium hydroxide (Merck), potassium dihydrogen phosphate (Merck), di-potassium hydrogen phosphate (Merck), bovine serum albumin/BSA (Sigma-Aldrich), and Bradford reagent (Sigma-Aldrich) were obtained commercially.

2.2 Methods:

Aluminium hydroxide was synthesized by reacting aluminum chloride with sodium hydroxide in a solution. An equal volume of a 3.6 mg/ml $AlCl_3 \cdot 6H2O$ solution and a 0.04 M NaOH solution were added into a glass vial, and a small volume of 0.01 M NaOH was added to adjust the pH to 7.0. After 20 min of agitation at room temperature, particle suspension was decanted and washed with sterile water three times to remove sodium chloride.

The particles size distribution was determined using a Particle Size Analyzer (NanoPlus-3). The adsorption of protein (use BSA as a model protein) on aluminum hydroxide particles was carried out by mixing the particles in suspension with the protein in solution.

III. RESULTS AND DISCUSSION

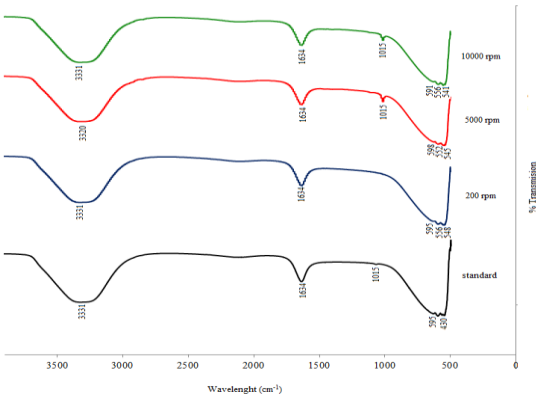


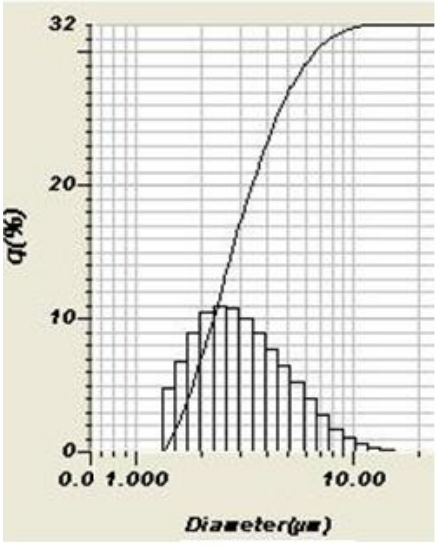
Figure 1. Pattern of FTIR aluminum hydroxide

Aluminium hydroxide which synthesized in this research was characterized by FTIR and showed on Fig. 1. Based on the FTIR pattern, wavelength around 3200 cm^{-1} contributed to O-H bending from $Al(OH)_3$ and also visible in the fingerprint area on 1634 cm^{-1} as scissoring band of O-H. Vibration of Al-O-H and Al-O-Al bending absorption bands appears at 1220 to 1000 cm^{-1}. The other peak on 500 cm^{-1} may be contributed to metal oxide (Al-O) vibration. Variation on 200 rpm did not show peak on 1000 cm^{-1} it may be related with particle size of aluminum hydroxide product. All of aluminum hydroxide product synthesis was similar with aluminum hydroxide standard.

Particle Size Distribution (nm)	Agitation Rate		
	200	5000	10000
Range	1510-15172	172-1005	259-389
D10	1679	194	234
D50	2895	286	270
D90	5868	435	339
Mean	3422	305	279

Table 1. Particles size of aluminum hydroxide analysis by PSA

Particle size analysis using PSA is showed in Table 1. Procedure at 200 rpm given heterogneous particles size with range 1510 – 15172 nm and the mean particles size was 3422 nm. It is may be due to the aggregation of small particles to form larger particles. This assume is reinforced by optical microscope observation (Fig. 2) that showed the aggregation of particles. In order to produce homogeneous particles, a preliminary study has been conducted using high shear homogenizer (HSH) agitation. Particle size of aluminum hydroxide decreased from micrometer to nanometer by increasing agitation rate to 5000 rpm using high shear homogenizer. The particles size range were 172 – 1005 nm, and the mean particle size of 305 nm indicating that the resulting particles were smaller, but it was still heterogeneous because the larger range particles distribution and also optical micrograph on Fig. 3 showed the more various particles size (marked by the red line). Increasing the agitation rate to 10000 rpm did not significantly reduce particle size. The range particles distribution was on 259-389 nm and the mean particles size on 279 nm with homogenous the particle size distribution. Howerver, from optical microscope analysis, it can be seen that the aluminum hydroxide is the aggregate form of small-scale primary particles, in which they are could be easily broken down by homogenization. This is indicating that agitation rate on 10000 rpm could synthesize nanoparticles aluminum hydroxide but these particles were unstable and easy to form aggregation.



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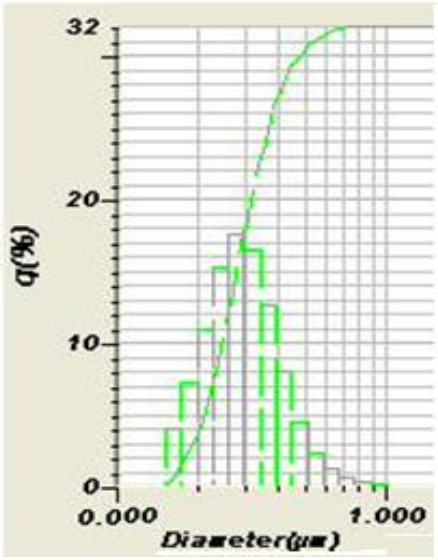

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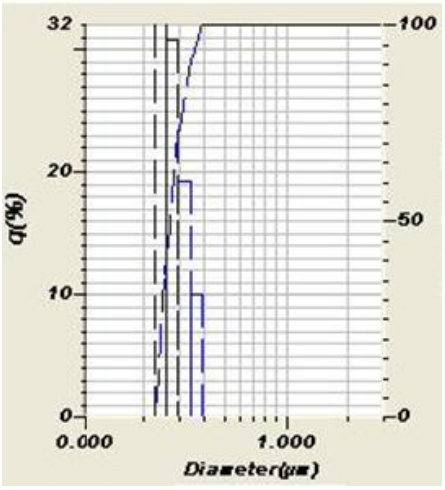

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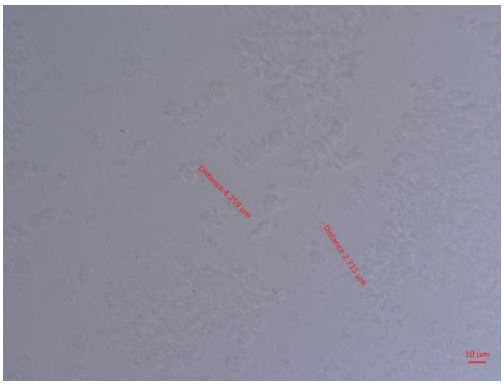
Figure 2. Particle Size Distribution of Aluminum Hydroxide Prepared



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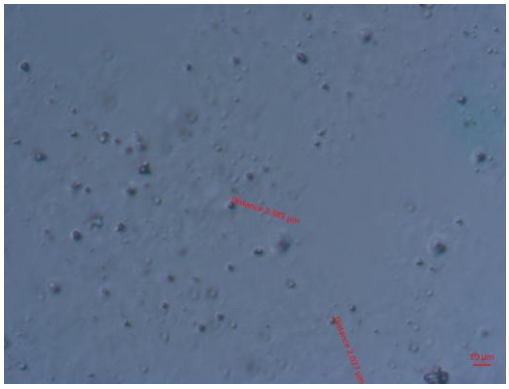

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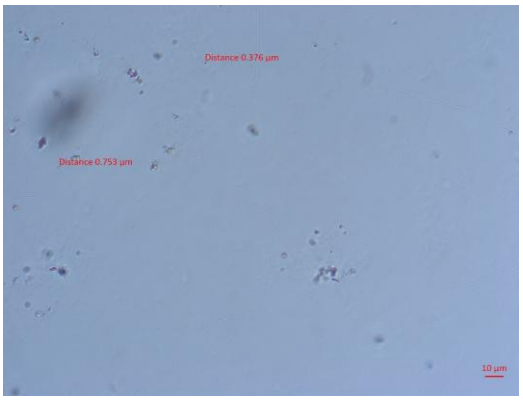

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Figure 3. Optical Micrograph of Aluminum Hydroxide Prepared

The adsorption capacity of aluminum hydroxide using BSA as protein model and show that adsorption capacity increase with the decreasing the particle size (Table 2). However, protein adsorption of the aluminum hydroxide particles prepared at 10000 rpm was lower than the other. We assumed that it could be caused by the existence of sodium chloride in the sample due to the highly agitation rate, since the sample is difficult to be decanted and washed.

Table 2. Protein adsorption capacity of aluminum hydroxide

Agitation Rate (rpm)	Protein Adsorption Capacity (%)
200	90.05
5000	90.80
10000	89.90

IV. CONCLUSION

Aluminum hydroxide could be prepared by precipitation method and the particles size depend to agitation rate. The highly agitation rate produced smaller particles size. Agitation rate on 200 rpm given 3422 nm, 305 nm for 5000 rpm and 279 nm for 10000 rpm. The protein adsorption capacity of aluminum hydroxide also associated with the particle size of aluminum hydroxide, in which the adsorption of BSA to the aluminum hydroxide on 3422 nm was 90.05 % and increase with the decrease of particle size that 305 nm given 90.80% protein adsorption. However, the smallest particle which on 279 nm given 89.90% it may be due to the highly agitation rate that replaced the equilibrium of aluminum hydroxide formation.

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