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Effect of stabilizers on physical stability and protein adsorptive capacity of aluminum hydroxide nanoparticles suspension

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ABSTRACT
Despite the greater adjuvant activity of aluminum hydroxide at smaller particle size, nanoparticles suspension of aluminum hydroxide has low stability due to ease particle aggregation. In an attempt to find approaches to produce aluminum hydroxide nanoparticle suspension with high stability, a study was undertaken of the effect of stabilizers on the physical stability and protein adsorptive capacity. Three types of stabilizers, i.e., trehalose, polyvinyl alcohol, and sodium polyphosphate at various concentrations were investigated. Aluminum hydroxide nanoparticles were synthesized by precipitation-homogenization method using aluminum chloride and sodium hydroxide solution. The physical stability of suspension was evaluated by measuring the decantation degree. The protein adsorptive capacity of aluminum suspension was evaluated using BSA as a model antigen. The results indicated that the physical stability and protein adsorptive capacity of aluminum suspension was highly depended on stabilizer type and concentration. Sodium polyphosphate seems to be most suitable stabilizer of aluminum hydroxide nanoparticles suspension.

Keywords: Aluminum hydroxide, Stabilizer, Protein adsorptive capacity, Vaccine adjuvant

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I. INTRODUCTION

Aluminum hydroxide (AH) has been widely used as vaccine adjuvant for decades due to its favorable safety profile. AH adsorb antigens of vaccine on the surface and inside of particles, submit the reposited antigens to the immune cells and promote interactions between antigens and immune cells for long duration to induce immune responses [1]. AH is chemically crystalline aluminum oxyhydroxide, AlOOH. It is prepared by mixing an aluminum solution, usually $AlCl_3$ or $AlK(SO_4)_2$, with sodium hydroxide. AH is composed of nanoscale primary particles. When dispersed in aqueous solution, the primary nanoparticles tend to aggregate and form larger microparticles (1 to 20 µm) [2-3].

In recent year, several studies have demonstrated that the smaller nanoparticles showed more potent adjuvant activity than larger particles [4-6]. Our previous study also revealed that AH nanoparticles of 243 nm has better adjuvant activity compared to that of the traditional AH of around 5.67 µm [7]. Therefore, simply reducing the particle size of AH adjuvant in suspension from micrometers into nanometers represents a novel and effective approach to improve the potency of AH particles as vaccine adjuvant.

A simple technique to disrupt the particle aggregates is by homogenization [8]. The disrupted particle aggregates could be possible to re-aggregate due to strong hydrophobic interactions. Therefore, addition of stabilizer to prevent re-aggregation is required. In this study, we are focusing on finding suitable stabilizer to improve the stability of AH nanoparticles suspension. The effect of type and concentration of stabilizer on the sedimentation degree of AH nanoparticles was investigated experimentally. Furthermore, since the presence of stabilizer may influence the adsorption of protein onto AH particles, the effect of stabilizer addition on the protein adsorptive capacity was also studied.

II. MATERIALS AND METHODS

2.1 Materials

Aluminium chloride hexahydrate (Merck), sodium hydroxide (Merck), polyvinyl alcohol (Merck), sodium polyphosphate (Merck), trehalose (Sigma), bovine serum albumin (Sigma), and Bradford protein assays (Thermo Scientific) were obtained commercially. Commercial aluminum hydroxide gel Alhydrogel® (Brentagg) was used as AH standard product.

2.2 Methods

2.2.1 Preparation of aluminum hydroxide nanoparticles suspension

Aluminum hydroxide was synthesized by precipitation method as described in previous study [9]. An equal volume of a 3.6 mg/ml $AlCl•6H_2O$ 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 15 min of agitation at room temperature, particle suspension was decanted and washed with distilled water two times to remove soluble salt. Three types of stabilizers, i.e., trehalose, polyvinyl alcohol and sodium polyphosphate were added to the AH suspension at various concentrations, and homogenized at 10,000 rpm for 1 hour to produce nanoparticles suspension.

2.2.2 Stability test of AH nanoparticle suspension

The stability of AH nanoparticle suspension at room temperature was evaluated by measuring the sedimentation degree of suspension. 10 ml of each sample was transferred to a test tube and kept at room temperature for 15 days. The sedimentation degree of AH nanoparticle in suspension was measured at days 3 and 15. A blank sedimentation degree of AH commercial product, AH microparticle, and AH nanoparticle without any stabilizer were also investigated.

2.2.3 Determination of protein adsorptive capacity of AH nanoparticle suspension

The adsorption of protein (use BSA as a model protein) on AH particles was carried out by mixing the particles in suspension with the protein in solution. Briefly, 0.2 ml of 10.000 ppm BSA solution was added into a microtube, followed by the addition of 1.8 ml of AH nanoparticles suspension. After 20 min of gentle stirring at room temperature, the protein-AH mixtures were centrifuged at 9.000 rpm for 15 min at 4ºC. The supernatant were collected and assessed for protein concentration by Bradford assay method at the absorbance of 601 nm. The amount of protein adsorbed to AH particles was calculated by subtracting the amount of protein remaining in solution from the amount of protein added initially [10].

III. RESULTS AND DISCUSSION

In order to select suitable stabilizer for improving AH nanoparticle suspension stability, we examined the sedimentation degree of AH nanoparticle which treated with several type of stabilizers. The physical observation on suspension stability at days 3 and days 15 is shown in Figure 1.

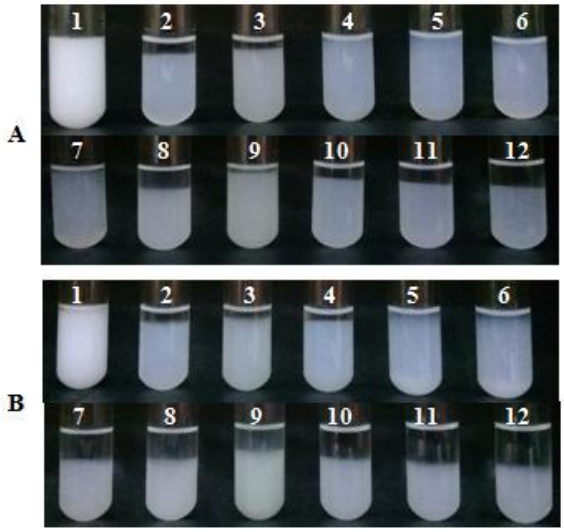

Figure 1: Observation on suspension stability of AH nanoparticle without and with stabilizers. Observation at (A) days 3; (B) days 15. Samples: (1) AH commercial; (2) AH microparticle; (3) AH nanoparticle; (4) AH nanoparticle + sodium polyphosphate 0.2%; (5) AH nanoparticle + sodium polyphosphate 1.0%; (6) AH nanoparticle + sodium polyphosphate 4.0%; (7) AH nanoparticle + trehalose 0.2%; (8) AH nanoparticle + trehalose 1.0%; (9) AH nanoparticle + trehalose 4.0%; (10) AH nanoparticle + PVA 0.2%; (11) AH nanoparticle + PVA 1.0%; (12) AH nanoparticle + PVA 4.0%.

It can be seen from Fig. 1 that the commercial product of aluminum hydroxide has excellent suspension stability (sample no 1). In contrast of that, AH microparticle (sample no 2) and nanoparticle (sample no 3) we prepared tend to settle down even after 1 day. The addition of 3 types of stabilizer gave different results. The sedimentation was higher when trehalose and PVA were used. The stability of AH nanoparticle suspension was improved when sodium polyphosphate was used as stabilizer.

Further evaluation on suspension stability was carried out by quantitatively measuring the volume fraction of suspension that settled down. The results are shown in Table 1.

Table 1: Sedimentation degree of AH nanoparticle suspension treated with various stabilizers

Samples	Sedimentation (%)	
	Days 3	Days 15
1. AH commercial	0	0
2. AH microparticle	5.18	10.37
3. AH nanoparticle	7.76	12.98
4. AH nanoparticle + sodium polyphosphate 0.2%;	0	5.18
5. AH nanoparticle + sodium polyphosphate 1.0%	0	5.18

6. AH nanoparticle + sodium polyphosphate 4.0%	0	12.98
7. AH nanoparticle + trehalose 0.2%	5.18	33.68
8. AH nanoparticle + trehalose 1.0%	31.10	36.29
9. AH nanoparticle + trehalose 4.0%	15.55	28.49
10. AH nanoparticle + PVA 0.2%	16.55	33.68
11. AH nanoparticle + PVA 1.0%	25.92	41.47
12. AH nanoparticle + PVA 4.0%	25.92	41.47

Sedimentation degree of AH microparticle and nanoparticle at days 3 was 5.18 and 7.76 %, respectively. The sedimentation degree increased, around twice higher at days 15. No sedimentation was observed at days 3 when AH nanoparticle suspension treated with sodium polyphosphate, indicating that the suspension was stable. However, the stability of suspension decreased slowly, in which at days 15 the sedimentation degree of suspension was 5.18 % at sodium polyphosphate concentration of 0.2 and 1.0 %. The sedimentation degree of suspension treated with trehalose and PVA was higher compared to the non-treated one, indicating that these type of stabilizers incapable to stabilize the AH nanoparticle suspension.

One of main factor that influence the adjuvant activity of AH particles is protein adsorptive capacity. They may be most effective as adjuvants when vaccine antigens are optimally adsorbed to the surface of the AH particles [11]. The presence of stabilizer in the particle suspension may affect the interaction of AH particle and protein, the effect of stabilizers on the protein adsorptive capacity was evaluated. In this study, BSA was used as protein model. The result is shown in Table 2.

Table 2: Protein adsorptive capacity of AH nanoparticle suspension treated with various stabilizers

Samples	Protein adsorptive capacity (%)
1. AH commercial	100.06
2. AH microparticle	87.67
3. AH nanoparticle	87.87
4. AH nanoparticle + sodium polyphosphate 0.2%;	96.88
5. AH nanoparticle + sodium polyphosphate 1.0%	98.88
6. AH nanoparticle + sodium polyphosphate 4.0%	98.27
7. AH nanoparticle + trehalose 0.2%	88.15
8. AH nanoparticle + trehalose 1.0%	88.55
9. AH nanoparticle + trehalose 4.0%	87.85
10. AH nanoparticle + PVA 0.2%	87.83
11. AH nanoparticle + PVA 1.0%	88.52
12. AH nanoparticle + PVA 4.0%	89.43

Protein adsorptive capacity of AH commercial product was 100%, whereas the AH micro- and nanoparticle were around 88%. The addition of sodium tripolyphosphate significantly improved the protein adsorptive capacity, close to the value of protein absorptive capacity of AH commercial. The concentrations of sodium tripolyphosphate used, however, were not linearly related to the protein adsorptive capacity. Meanwhile, the addition of trehalose and PVA as stabilizers did not affected the protein adsorptive capacity of AH particle, which was indicated by their same value of protein adsorptive capacity.

IV. CONCLUSION

The stability of aluminum hydroxide adjuvant can be improved by addition of stabilizer. Sodium polyphosphate seems to be most appropriate stabilizer, since it not only improve the suspension stability but also increase the protein adsorptive property. Further study on the effect of stabilizer addition on immune response activity of AH nanoparticle is required.

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REFERENCES

[1]. P. He, Y. Zou, and Z. Hu. Advances in aluminum hydroxide-based adjuvant research and its mechanism. *Human Vaccines & Immunotherapeutics, 11(2),* 2015, 477-488.

[2]. S.L. Hem and H. HogenEsch. Relationshio between physical and chemical properties of aluminum-containing adjuvants and immunopotentiation. *Expert Review of Vaccines, 6(5),* 2007, 685-698.

[3]. H. HogenEsch, D.T. O'Hagan and C. B. Fox. Optimizing the utilization of aluminum adjuvants in vaccines: you might just get what you want. *npj Vaccines, 3(51),* 2018, 1-11.

[4]. X. Li, A.M. Aldayel, Z. Cui. Aluminum hydroxide nanoparticles show a stronger vaccine adjuvant activity than traditional aluminum hydroxide microparticles. *Journal of Controlled Release, 173*, 2014, 148–157.

[5]. R.R. Shah, D.T. O'Hagan, M.M. Amiji, L.A. Brito. The impact of size on particulate vaccine adjuvants. *Nanomedicine (Lond.), 9(17)*, 2014, 2671–2681.

[6]. M.T. Orr, A.P. Khandhar, E. Seydoux, H. Liang, E. Gage, T. Mikasa, E.L. Beebe, N.D. Rintala, K.H. Persson, A. Ahniyaz, D. Carter, S.G. Reed, C.B. Fox. Reprogramming the adjuvant properties of aluminum oxyhydroxide with nanoparticle technology. *npj Vaccines, 4(1)*, 2019, 1-10.

[7]. S. Pambudi, E. Mardliyati, S. Rahmani, D.R. Setyawati, T. Widayanti, A. Gill, A. Sulfianti, W. Lestari. The Potency of Aluminum Hydroxide Nanoparticles for Dengue Subunit Vaccine Adjuvant. *Microbiology Indonesia, 12(3)*, 2018, 99-105.

[8]. C.D. Doan and S. Ghosh. Formation and Stability of Pea Proteins Nanoparticles Using Ethanol-Induced Desolvation. *Nanomaterials, 9(949)*, 2019, 1-15.

[9]. E. Mardliyati, D.R. Setyawati, S. Pambudi, Suryandaru, R. Kusumaningrum, M.. Amal. Preparation of Aluminum Hydroxide by Precipitation Method for Vaccine Adjuvant Application. *International Journal of Engineering Research and Application 7(11)*, 2017, 21-25.

[10] J.R Harris, A.Soliakov, R.J. Lewis, F. Depoix, A. Watkinson, J.H. Lakey. Alhydrogel® adjuvant, ultrasonic dispersion and protein binding: A TEM and analytical study. *Micron, 43*, 2012, 192–200.

[11] S.L. Hem, H. HogenEsch, Aluminum-containing adjuvants: properties, formulation, and use, in M. Singh (Ed.), *Vaccine Adjuvants and Delivery Systems*(New Jersey: John Wiley & Sons, 2007) 81-114.

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