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Nanogold – IgY antibodies. An immunoconjugated for the detection of house dust mite (Dermatophagoides) allergens

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ABSTRACT

Conjugation of avian IgY antibodies to nanosensors has been extensively explored for the diagnostics of virus and parasite infection, as well as for the detection of pharmaceutically and toxicologically relevant molecules. However, to date this strategy has only been minimally applied the detection of allergens. In this study, gold nanoparticles (GNPs) were conjugated to a polyvalent IgY antibodies raised against Dermatophagoides group I allergens. GNPs were synthesized by $HAuCL_4$ reduction using 1% trisodium citrate, and characterized them by absorption spectroscopy and transmission electron microscopy (TEM). The most stable immunoconjugates were obtained with 18-nm monodisperse GNPs and a minimal concentration of 12.5 μg/mL of IgY at pH 7.5. The immunoconjugate was capable of detecting up to 1.5 μg of a total *Dermatophagoides farinae* protein extract in an immuno-dot blot assay. This immunoreactant conjugate represents a new tool for the detection and control of indoor dust mite allergens.

1. Introduction

In Colombia, there is an increasing prevalence of respiratory allergies especially among paediatric patients (Dennis et al., 2004; Dennis et al., 2012). Dust mites allergens, which can be found in indoor dust, are the main triggering factors of both asthma and allergic rhinitis. In fact, these allergy diseases are so widespread and regarded public health issue (Pawankar, 2014). Consequently, there is a high demand for novel methods capable of assessing and controlling contamination in indoor spaces. Early intervention techniques require the development of innovative analytical reagents able to be sensitive, specific, rapid and simple for detecting these allergens (Chapman, 1998).

Gold nanoparticles (GNPs) have been widely used for the identification of both biological and chemical contaminants (Dykman and Khlebtsov, 2011). When it has been combined with recognition proteins such as antibodies or receptors, this nanomaterial can act as a biosensor molecule (Zeng et al., 2011). In particular, the binding of GNPs to antibodies, whether polyclonal or monoclonal, produces immunoconjugates that are able to rapidly and reliably detect toxins, pathogens and other substances harmful to health (Lin et al., 2013; Koedrith et al., 2015). With respect to environmental control, GNP immunoconjugates designed against organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) (Lisa et al., 2009), methamidophos, and haptens from carbaryl, carburofan and triazophos herbicides, have been used in diagnostic testing (Kaur et al., 2007; Guo et al., 2009). Furthermore, colorimetric immunoassays with gold nanoparticles, for the detection of allergens in food samples have been conducted in both direct solid phase and sandwich- type formats (Sánchez-Martínez et al., 2009; Wang, 2015; Alves et al., 2015). Most immunoconjugates intended for the detection of mite allergens within indoor dust samples, consist of monoclonal IgG antibodies (Abm) (Chapman et al., 1987; Lau et al., 2001; Härfast et al., 1996). These antibodies are mainly used in laborious immunoassays (for example, enzyme-linked immunosorbent assays, ELISA) with long execution times that must be performed by well-trained, specialized staff.

On the other hand, Wongkamchai et al., 2000 developed a sandwich-type immunoblot assay for the detection of the major allergens of the mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Der p 1 and Der f 1) in dust samples using two IgG monoclonal antibodies; this assay had an execution time of only 30 min. Tsay et al.,

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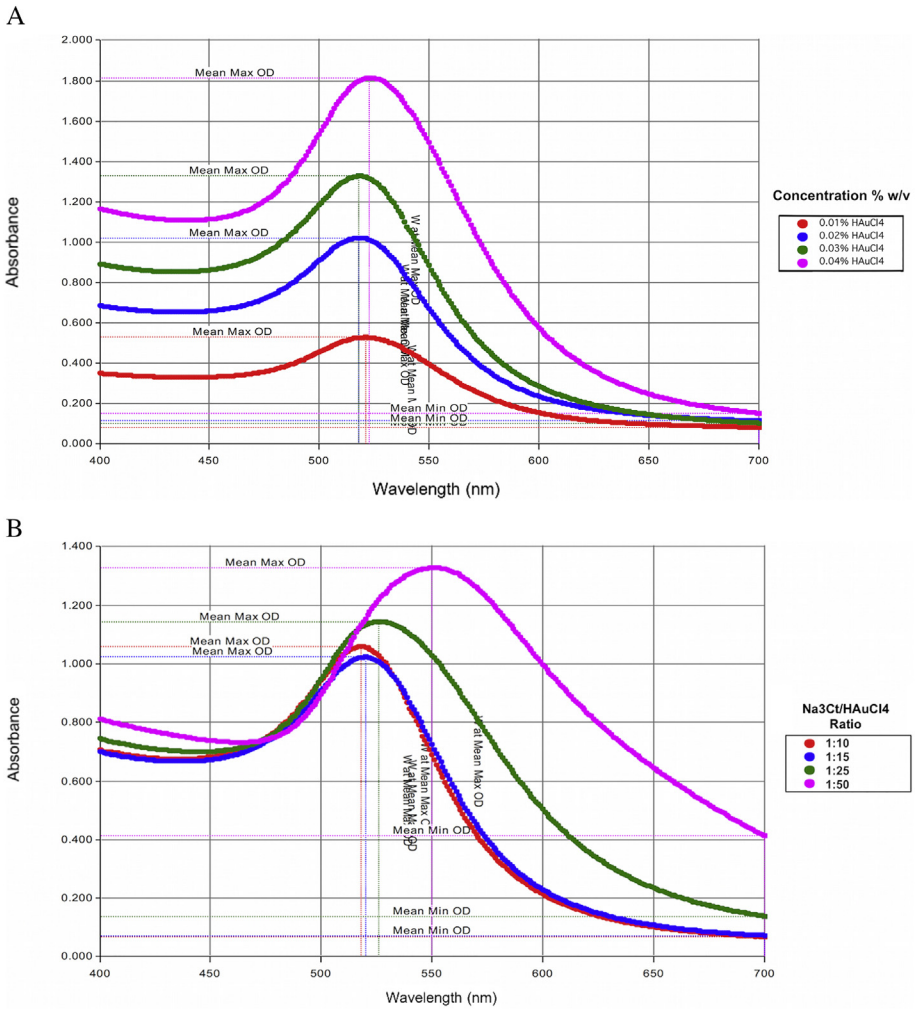

Fig. 1. UV-Visible absorption spectra of GNPs. A GNPs synthesized with different concentration of $HAuCl_4$ (from 0.01% to 0.04%). B GNPs synthesized with different ratios (*v*/v) of $Na_3Ct/HAuCl_4$ (from 1:10 to 1:50).

2002 developed a GNP-conjugated monoclonal IgG antibody directed against group 2 allergens of the Dermatophagoides spp. (Der p 2), this conjugate was used as capture antibody in a one-step immune-chromatographic test. Currently, at least two rapid tests are available for detecting house dust mites allergens "In-house". One is in a dipstick format (Aclotest; Lofarma, Milan, Italy) and the other is an immune-chromatographic format kit (Ventia Rapid Allergen Test; Indoor Biotechnologies, Charlottesville, Virginia) (Portnoy et al., 2013). Both tests use IgG for allergens detection. To our knowledge, IgY immunoconjugates for indoor mite detection are not available.

Recently, we have developed a polyclonal avian antibody (named PO4-IgY), which was directed against a consensus sequence of Der f1 and Der p1. PO4-IgY showed a high reactivity and sensitivity toward allergens of Dermatophagoides spp. and, a low and nonspecific recognition against proteins in an allergenic extract of the cockroach *Periplaneta americana* (another common source of indoor allergens) (Egea et al., 2018). In the present study PO4-IgY was conjugated to colloidal GNPs, and their ability to detect dust mite allergens was evaluated using an immuno-dot blot assay. This immunoreactant conjugate could play a key role in the development of a high-sensitivity analytical test that allow dust mites allergens to be monitored in real time without complex sample preparation.

2. Materials and methods

2.1. Reagents

All chemicals and solvents were obtained from Merck (Darmstadt, Germany) unless otherwise stated. All reagents used were molecular biology standard.

2.2. Colloidal gold nanoparticles (GNPs)

Uniform gold sols were synthetized following the protocol described by Frens, 1973. Briefly GNPs were prepared by chemical reduction of $HAuCl_4$ (Aldrich, 254,169) using 1% (p/v) trisodium citrate (Na_3Ct) (Sigma-Aldrich, S1804), heating at 100 °C under reflux, and constant stirring at 600 rpm. The size, shape and distribution of synthesized GNPs in the sols were studied by varying the $HAuCl_4$ concentration (from 0.01% to 0.04% *w*/*v*) and $Na_3Ct/HAuCl_4$ ratio (from 1:10 to 1:50 *v*/v).

UV–Visible absorption spectra of GNPs were obtained using the Synergy™ HT Multi- Mode Microplate Reader. Subsequently, the acquired data were analyzed with the Gen5™ data analysis software (BioTek® Instruments, Inc.) to identify the wavelength of maximum absorption (Ab_{max}).

The average size and distribution of the GNPs was determined by transmission electron microscopy (TEM) using a TECNAI F20 microscope operated at 120 kV. Samples for TEM were prepared by placing

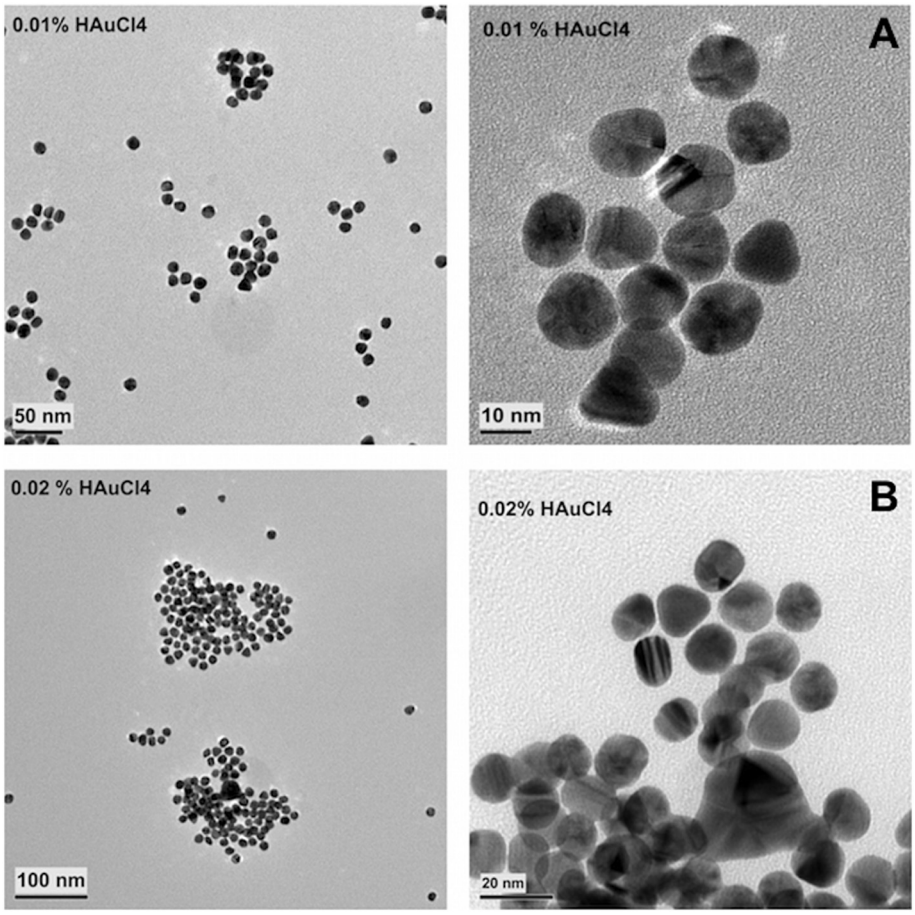

Fig. 2. Transmission electron microscopy (TEM) images of GNPs. (A) Gold nanoparticles obtained with 0.01% of $HAuCl_4$ and (B) 0.02% of $HAuCl_4$.

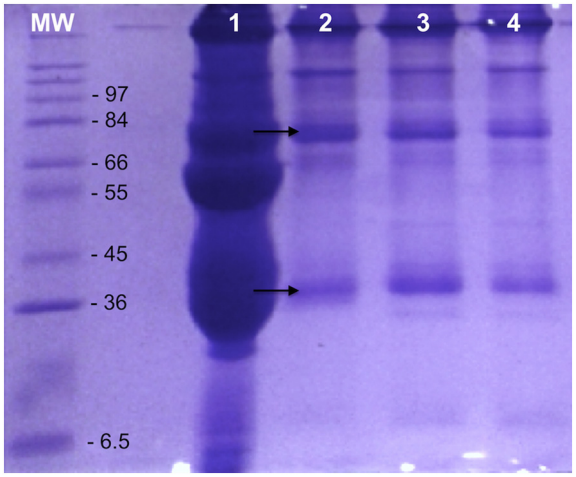

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of PO4-IgY antibody. MW: Molecular weight (kDa). Lane 1: IgY before purification; Lane 2–4: IgY after purification by thiophilic chromatography. Arrows shows the heavy and light chains of IgY.

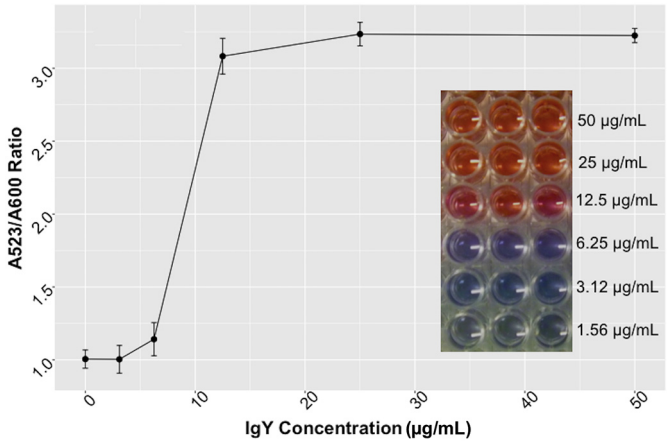

Fig. 4. Result of flocculation assay. Different concentrations of PO4-IgY antibody (from 50 to 1.56 µg/mL) were conjugated to 0.02% GNPs at pH 6.5. The minimum IgY concentration required for a stable conjugation was 12.5 µg/mL. Lower concentrations of IgY produced GNP flocculation and a change of red to blue colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5 µL of the gold sols on a 300-mesh copper grid covered with charcoal. The solution was incubated for 5 min and afterwards any excess was cautiously removed. The size and the distribution of the GNPs were determined using the STEM digital imaging software.

2.3. IgY antibodies

Non-glycosylated synthetic oligopeptides were designed *in silico* based on a consensus sequence of dust mite group 1 allergens (Der f 1 and Der p 1) and then were used for producing polyclonal IgY antibodies in chickens as described by Egea et al., 2018. The purification of these antibodies from the chickens´ eggs was done by thiophilic chromatography as follow the method described by Sosa et al., 2011. Antibody purification was carried out by the Inborn Errors of Metabolism Laboratory at the Universidad Pontificia Javeriana de Colombia using

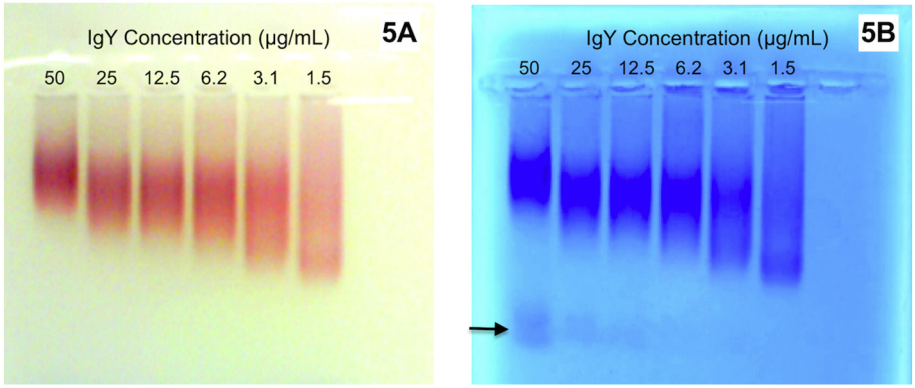

Fig. 5. Electrophoretic mobility shift assay (EMSA) of IgY conjugated with GNPs. A and B show the agarose gel before and after of staining with Coomassie Brilliant Blue R-250. Each lane corresponds to GNPs coated to different amount of PO4-IgY. Increasing PO4-IgY concentration decrease the shift extent of conjugated antibody in the gel. Arrow shows the PO4-IgY non-conjugated to GNPs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

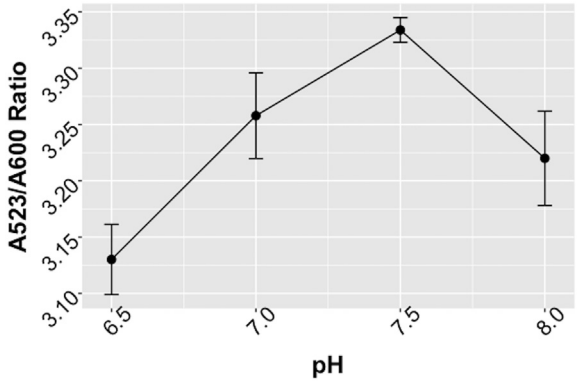

Fig. 6. Optimal pH for conjugation. IgY antibody at concentration of 12.5 µg/mL was conjugated to GNPs at different pH values. The most stable conjugate was at pH 7.5.

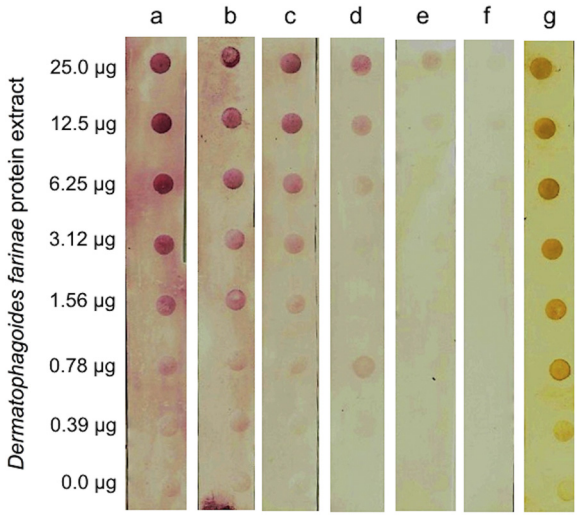

Fig. 7. Immuno-dot blot with *Dermatophagoides farinae* protein extract. Strips were incubated with (a) 1/5; (b) 1/10; (c) 1/20; (d) 1/50; (e) 1/100 dilutions of the PO4-IgY- GNP. (f) Strip incubated with pre-immune antibody (dilution 1/5). (g) Strip incubated with biotin-IgY (dilution 1/200).

the BioLogic™ LP chromatography system (Bio- Rad). Chromatography experiments were monitored with the LP Data View software. The Sepharose ® CL-4B (Sigma-Aldrich, CL4B200) stationary phase was activated with divinyl sulfone (DVS) and β-mercaptoethanol. The activated matrix was equilibrated using a 50 mM phosphate buffer pH 7.4, 0.5 M Na_2SO_4. Afterwards, the sample in dilution 1:2 in equilibration buffer was added to the activated Sepharose. The bound IgY was eluted with.

50 mM phosphate buffer (pH 7.4) and was monitored by measuring the absorbance at 280 nm. The protein concentration of each elution fractions were measured using the QuickStart™ Bradford protein assay kit (Bio-Rad). The purified PO4-IgY was stored at −20 °C in 1.5 mL aliquots.

2.4. Conjugation of GNPs to IgY antibodies

2.4.1. Flocculation assay

This assay was performed as described by Wangoo et al., 2008 for determining the minimal quantity of PO4-IgY necessary to stabilize the GNPs. The conjugate solution was prepared as follow: 1 mL of each gold sol was mixed with 100 µL of PO4-IgY (from 50 µg/mL to 3.1 µg/mL) in 2 mL polystyrene vials, followed by incubation for 10 min at room temperature and gently mixing by inversion in a rotary mixer. Afterwards, 100 µL of 2 M NaCl was added to conjugate solution and mixed during 1 h at room temperature. A volume of 300 µL of each reaction mixture was transferred to polystyrene 96 well plate (NUNC, Roskilde, Denmark) and the maximum absorbance (Ab_{max}) and absorbance at 600 nm (Ab_{600}) was determined. The amount of protein necessary to prevent flocculation was deduced graphically from the concentration at which the ratio Ab_{max}/Ab_{600} becomes nearly constant.

2.4.2. Optimal pH

To establish the optimum pH of absorption, an assay was conducted in which the PO4-IgY concentration was maintained constant and the gold sol pH was modified with 1% (*w/v*) carbonate buffer (pH range between 6.5 and 8.0).

2.4.3. Electrophoretic mobility shift assay (EMSA)

The binding of GNPs to PO4-IgY was verified by EMSA using 1% (w/v) agarose (A9539, Sigma-Aldrich) gels in 1× TBE running buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA). For each of the different antibody concentration assays, 15 µL of IgY-GNP conjugate was loaded onto the gel. EMSA was performed at 80 v for 3 h in a Bio-Rad Mini-Sub® Cell GT Cell (Bio-Rad, 1664288EDU) following the protocol previously described (Wangoo et al., 2008). The gel was stained with Coomassie Brilliant Blue R-250 solution (Bio-Rad, 1,610,436).

2.4.4. Stabilization of the PO4-IgY-GNPs

Bovine serum albumin (BSA) was used to stabilize any free GNPs. The conjugate solution.

(prepared with 10 mL of the gold sol and 1 mL of PO4-IgY) was mixed with 1.1 mL of 20% (*w/v*) BSA. The mixture was softly stirred for 2 h at room temperature, and subsequently centrifuged at 12,000 × g for 30 min at 4 °C. The precipitate (immunoconjugate) was washed with Mili-Q water, centrifuged once again, and stored at 4 °C in 20 mM Tris (pH 7.5), 1% (w/v) BSA and 0.02% (w/v) NaN_3.

2.5. PO4-IgY-Biotin

PO4-IgY antibody was also conjugated to biotin and was used as a reference control in the immuno-dot blot assay. The EZ-Link™ Sulfo-NHS-LC-Biotin kit (Thermo Fisher Scientific, 21,435) was used to tag the antibody with biotin according to the manufacturer's instructions.

2.6. Immuno-dot blot assay

The ability of the conjugates to detect dust mite allergens was determined by an immune-dot blot assay. The allergens source was a native extract of the *D. farinae* mite, which was obtained in our laboratory. We did not quantify Der f 1 in this extract for those experiments; however, previously we have demonstrated that this extract has allergenic activity (Mendoza et al., 2011) and our PO4-IgY polyclonal antibody recognizes a 25.6 kDa electrophoretic band in the SDS-PAGE (Egea et al., 2018), corresponding to molecular weight that identify the allergen Der f 1 (Chruszcz et al., 2009). Serial dilutions of mite extract (0–500 μg/mL) in TBS buffer (50 mM Tris-HCl pH 7.2 and 100 mM NaCl) were prepared. The negative control for the conjugate consisted of pre-immune IgY conjugated to GNPs (IgYp-GNP), and the negative control for the allergen source was TBS buffer.

Mite allergen proteins were spotted onto a Zeta-Probe® Blotting Membrane (Bio-Rad, 162-.

0153). A volume of 50 μL of mite extract were absorbed onto membranes for 2 h, using the Bio Dot® SF Microfiltration Apparatus (Bio-Rad, 170–6542) microfiltration system. Then, these were washed with TBS buffer and blocked with TBST-milk solution [0.05% (*v*/v) Tween-20 and 5% (*w*/*v*) skimmed milk in TBS] for 30 min at room temperature.

For allergen detection, the membranes were incubated with the PO4-IgY-GNPs conjugate diluted in TBST-milk buffer containing 0.1% (w/v) gelatin. Conjugate dilutions of 1/50, 1/20, 1/10, 1/50 and 1/100 were tested. After dots became visible (red coloration), membranes were washed with the TBST buffer containing 0.01% (v/v) Tween-20.

For allergen detection with biotinylated antibodies, one membrane previously absorbed with serial dilutions of mite extract, was incubated for 45 min with PO4-IgY-biotin (dilution 1/200 in TBS-milk buffer). Then the membrane was washed three times with TBST buffer and incubated for 30 min with Streptavidin-Peroxidase from *Streptomyces avidinii* (Sigma-Aldrich, S5512) diluted 1/2000 in TBS-milk buffer. The membrane was developed in 30 mL of developing solution [0.1% (*w*/*v*) imidazole, 0.01% (w/v) HPLC- grade 3,3′-Diaminobenzidine (Sigma-Aldrich, D8001), and 0.015% (*v*/v) hydrogen peroxide in 50 mM phosphate buffer (pH 7.4)], and after signals became visible (yellow coloration), the reaction was stopped using TBS buffer.

2.7. Data analyses

Experiments with IgY-antibody conjugates were conducted in triplicate. Data obtained were subjected to univariate statistical analyses by means of frequency, central, and dispersion tendency tests, using the statistical package SPSS Statistics 19 for Windows by IBM (SPSS Inc., an IBM Company, Chicago, h, IL, USA). Charts were created using statistical package R, version 3.2.1 (2015).

3. Results

3.1. Gold nanoparticles (GNPs)

In all experiments, optimum nucleation time for GNP synthesis was 15 min. UV–Visible absorption spectra of GNPs showed a maximum absorption peak (Ab_{max}) around 523 nm (Fig. 1). $HAuCl_4$ concentration had an effect in the absorption intensity of GNPs. Besides, when the ratio $Na_3Ct/HAuCl_4$ was increased to 1:25 and 1:50, the maximum absorbance peaks showed a red shift, away from the characteristic absorption zone of GNPs (Fig. 1B). For this reason, the GNPs were synthetized using 1:10 (*v*/v) $Na_3Ct/HAuCl_4$ ratio. TEM analyses showed that 0.02% GNPs had triangular and spherical shapes, a monodisperse distribution, and sizes between 16 and 18 nm (Fig. 2), these characteristics are desirable for the antibodies conjugation (Frens, 1973). In contrast, 0.03% and 0.04% GNPs were mostly aggregated in the form of bars, with only a few particles as monodisperse spheres, for this reason they were not used in the conjugation reaction. Although 0.01% GNPs also exhibited the required size, shape and distribution, the intensity of their colour was very low (A520 $<$ 0.52), and as such, they were not used in the IgY conjugation.

3.2. PO4-IgY antibodies

Fig. 3 shows the IgY antibodies before and after the purification by thiophilic chromatography. IgY antibody purification yielded a concentration of 1.23 ± 0.31 mg/mL.

3.3. Conjugation of GNPs to IgY antibodies

Fig. 4 show the result of flocculation test with GNPs coated to PO4-IgY at pH 6.5. The minimal concentration of antibody that produced stable PO4-IgY-GNP conjugates was 12.5 μg/mL. Lower concentrations of IgY resulted in unstable conjugates, producing a quickly changed from red to blue colour in the gold sol after addition of 2 M NaCl. Such a colour change is indicative of aggregate formation, and coincides with optical properties that are different from those presented by antibody-saturated GNPs (Haiss et al., 2007). On the other hand, IgY concentrations equal to or $>$ 12.5 μg/mL decreased the shift extent of conjugated antibody in the EMSA assay and produced unconjugates IgY antibodies that were visible on the agarose gel (Fig. 5).

The optimum pH for IgY absorption to GNPs was established in 7.5 (Fig. 6). From this results, the PO4-IgY-GNP conjugates were prepared at pH 7.5 with 12.5 μg/mL of IgY and a ratio 1:10 *v*/v of IgY:GNP.

3.4. Immuno-dot blot assay

PO4-IgY-GNP was able to recognize allergenic proteins from *D. farinae* mite, displaying a detection limit of 0.78 μg of total mite protein when used at a dilution of 1/5 and 1/10 in TBS-milk buffer with a detection time of approximately 15 min. A PO4-IgY-GNP dilution of 1/20 and 1/50 resulted in a weaker signal (Fig. 7). In comparison, the reference antibody, PO4-IgY-Biotin (dilution 1/200), required only 7 min for signal development, and had a detection limit of 0.39 μg of total mite protein.

4. Discussion

Use of IgY technology in the production of polyclonal antibodies directed against dust mites is an innovative biotechnological development. This method provides an economical alternative for the generation of immunoreactants that are capable of detecting and monitoring medically important environmental allergens. However, in order for IgY antibodies to be effective as biosensors in analytical assays, they need to be marked with some kind of readout tag that produces an easy-to-measure chemical or physical signal (Tansil and Gao, 2006). Here, we conjugated IgY antibodies to GNPs to create a simple, rapid, sensitive, reproducible and economic biosensor molecule.

Various parameters were critical in the conjugation of IgY to GNPs. Minimum PO4-IgY concentration require for producing stable immunoconjugated was 12.5 μg/mL. Lowest quantities of PO4-IgY produced unstable conjugates according to flocculation assay. Rudolf (2009) described that low quantities of antibody produce unstable GNPs because many regions are left uncovered. On the other hand, when the quantities of antibody are too high, both leaching and poor binding of the antibody from/to the GNPs can occur (Polson et al.,

1980). Although we did not directly assess leaching effects in this study, we can conclude that antibody concentrations > 12.5 ± 0.2 μg do not produce significant variations in conjugate stability. Other important and determinant step of conjugate preparation is the purification because it establishes the most quantity of antibody that can be used in the GNP coupling reactions (Rudolf, 2009).

The pH is another critical parameters for antibody absorption to GNPs. The optimum pH was established in 7.5, this value is greater than the average isoelectric point (pI) of the IgY (pI between 5.7 and 7.6) (Chalghoumi et al., 2009; Polson et al., 1980). It is known that absorption of antibodies at colloid surfaces occurs through both hydrophobic and electrostatic interactions (Dávalos-Pantoja et al., 2000). As the GNPs are negatively charged, electrostatic interactions would be increased by the presence of positive charges within the hydrophilic zones of the antibody (pH < pI). However, as the antigen binding sites on antibodies are usually hydrophilic zones, the binding of GNPs to these regions should be limited. In order to direct the binding of GNPs toward the most hydrophobic regions of the antibody – the Fc portion - the absorption of the GNPs should thus be carried out with pH > pI (Dávalos-Pantoja et al., 2000).

The immuno-dot blot assay showed that PO4-IgY-GNPs conjugates are capable to recognized allergens from *D. fariane*, in a few steps, achieving a detection limit of 0.78 μg of total allergenic protein, under the experimental conditions applied. PO4-IgY-Biotin conjugates also recognized allergens from *D. farinae*, but with a greater reactivity and sensitivity compared with PO4-IgY-GNPs (detection limit: 0.39 μg of total allergenic protein). However, the detection reaction with biotin requires additional two steps that negatively impact the simplicity and speed of the immune-test with this conjugated. More specifically, when using the biotin conjugates, dots must not only be incubated with a streptavidin-peroxidase conjugate but also be developed using a peroxidase-based colorimetric reaction. An enzyme-dependent detection reaction brings into play other factors, as pH and temperature, that could be affect the results of the test (Rai and Venkateswaran, 2013).

Over the last decade, reports on IgY production have begun to flourish. Currently, IgYs have been generated against a diverse array of molecules including pharmaceuticals (Gandhi et al., 2009), viral antigens (Reschová et al., 2000; Zhang, 2015; Bentes et al., 2015) parasites (Cai et al., 2012), pollens and food allergens (Alessandro et al., 2009; Trashin et al., 2011). With respect to dust mite allergens, there is still a need for the development of new, alternative technologies that are capable of rapidly detecting indoor dust mite allergens. Importantly, for these methods to be successful, they also need to be both economical and accessible for the potential users. The results described in this work, related to the use of the polyclonal antibody PO4-IgY, point to a future development of a dust mite allergen monitoring method, which will require experimental data of accuracy and precision experiments in order to compared with those currently used. The limitations of this study correspond to the fact that standardized allergen extracts of Dermatophagoides spp. were not included.

In conclusion, a polyvalent avian IgY antibody against Dermatophagoides group 1 allergen conjugated to colloidal GNPs was obtained. This conjugate is capable of recognizing *D. farinae* allergenic proteins in a solid phase detection test, thus representing a novel immunoreactant that could be used for the rapid and sensitive detection of dust mite allergens. In the future, rapid detection of *Dermatophagoides spp* using these IgY methodology could be high beneficial for monitoring indoor allergens affecting atopic patients. However, precision and accuracy studies should be performed with this methodology to compare our results with existing commercial products.

Competing interests

The authors declare no competing interests.

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References

Alessandro, R., et al., 2009. Production of an egg yolk antibody against Parietaria judaica 2 allergen. Poult. Sci. 88, 1773–1778. https://doi.org/10.3382/ps.2009-00054.
Alves, R.C., et al., 2015. Detection of Ara h 1 (a major peanut allergen) in food using an electrochemical gold nanoparticle-coated screen-printed immunosensor. Biosens. Bioelectron. 64, 19–24. https://doi.org/10.1016/j.bios.2014.08.026.
Bentes, G.A., et al., 2015. Using immunoglobulin Y as an alternative antibody for the detection of hepatitis A virus in frozen liver sections. Mem. Inst. Oswaldo Cruz 110, 577–579. https://doi.org/10.1590/0074-02760140457.
Cai, Y.C., et al., 2012. Chicken egg yolk antibodies (IgY) for detecting circulating antigens of Schistosoma japonicum. Parasitol. Int. 61, 385–390. https://doi.org/10.1016/j.parint.2012.01.008.
Chalghoumi, R., Beckers, Y., Portetelle, D., 2009. Hen egg yolk antibodies (IgY), production and use for passive immunization against bacterial enteric infections in chicken: a review. Biotechnol. Agron. Soc. Environ. 13, 295–308.
Chapman, M.D., 1998. Environmental allergen monitoring and control. Allergy 53, 48–53. https://doi.org/10.1111/j.1398-9995.1998.tb04939.x.
Chapman, M.D., Heymann, P.W., Wilkins, S.R., Brown, M.J., Platts-Mills, T.A., 1987. Monoclonal immunoassays for major dust mite (Dermatophagoides) allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. J. Allergy Clin. Immunol. 80, 184–194.
Chruszcz, M., et al., 2009. Crystal structures of mite allergens Der f 1 and Der p 1 reveal differences in surface exposed residues that may influence antibody binding. J. Mol. Biol. 386, 520–530. https://doi.org/10.1016/j.jmb.2008.12.049.
Dávalos-Pantoja, L., Ortega-Vinuesa, J.L., Bastos-González, D., Hidalgo-Alvarez, R., 2000. A comparative study between the adsorption of IgY and IgG on latex particles. J. Biomater. Sci. Polym. Ed. 11, 657–673.
Dennis, R., et al., 2004. Asthma and other allergic conditions in Colombia: a study in 6 cities. Ann. Allergy Asthma Immunol. 93, 568–574.
Dennis, R., et al., 2012. Prevalence of asthma and other allergic conditions in Colombia 2009–2010: a cross-sectional study. BMC Pulm. Med. 13, 12–17. https://doi.org/10.1186/1471-2466-12-17.
Dykman, L.A., Khlebtsov, N.G., 2011. Gold nanoparticles in biology and medicine: recent advances and prospects. Acta Nat. 3, 34–55.
Egea, E., et al., 2018. IgYespecíficos anti- alérgenos del grupo 1 de ácaros del polvo doméstico inducidos por oligopéptidos sintéticos no glicosilados. Vol. 38. pp. 232–243. https://doi.org/10.7705/biomedica.v38i0.3689.
Frens, G., 1973. Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. Nat. Phys. Sci. 241, 20–22.
Gandhi, S., Caplash, N., Sharma, P., Raman Suri, C., 2009. Strip-based immunochromatographic assay using specific egg yolk antibodies for rapid detection of morphine in urine samples. Biosens. Bioelectron. 25, 502–505. https://doi.org/10.1016/j.bios.2009.07.018.
Guo, Y.R., Liu, S.Y., Gui, W.J., Zhu, G.N., 2009. Gold immunochromatographic assay for simultaneous detection of carbofuran and triazophos in water samples. Anal. Biochem. 389, 32–39. https://doi.org/10.1016/j.ab.2009.03.020.
Haiss, W., Thanh, N.T., Aveyard, J., Fernig, D.G., 2007. Determination of size and concentration of gold nanoparticles from UV-vis spectra. Anal. Chem. 79, 4215–4221.
Härfast, B., Johansson, E., Johansson, S.G., van Hage-Hamsten, M., 1996. ELISA method for detection of mite allergens in barn dust: comparison with mite counts. Allergy 51, 257–261.
Kaur, J., et al., 2007. Immunochromatographic dipstick assay format using gold nanoparticles labeled protein-hapten conjugate for the detection of atrazine. Environ. Sci. Technol. 41, 5028–5036.
Koedrith, P., et al., 2015. Recent trends in rapid environmental monitoring of pathogens and toxicants: potential of nanoparticle-based biosensor and applications. Sci. World J. 2015, 510982. https://doi.org/10.1155/2015/510982.
Lau, S., Schulz, G., Sommerfeld, C., Wahn, U., 2001. Comparison of quantitative ELISA and semiquantitative Dustscreen for determination of Der p 1, Der f 1, and Fel d 1 in domestic dust samples. Allergy 56, 993–995.
Lin, M., Pei, H., Yang, F., Fan, C., Zuo, X., 2013. Applications of gold nanoparticles in the detection and identification of infectious diseases and biothreats. Adv. Mater. 25, 3490–3496.
Lisa, M., Chouhan, R.S., Vinayaka, A.C., Manonmani, H.K., Thakur, M.S., 2009. Gold nanoparticles based dipstick immunoassay for the rapid detection of dichlorodiphenyltrichloroethane: an organochlorine pesticide. Biosens. Bioelectron. 25, 224–227. https://doi.org/10.1016/j.bios.2009.05.006.
Mendoza, D., Ruiz, T., Lagares, A., Garavito, G., Egea, E., 2011. Caracterización de la actividad alergénica y enzimática de extractos somáticos producidos a partir de cultivos in vitro del ácaro Dermatophagoides farinae. Salud Uninorte 27, 11–21.
Pawankar, R., 2014. Allergic diseases and asthma: a global public health concern and a call to action. World Allergy Organ. J. 7, 12. https://doi.org/10.1186/1939-4551-7-12.
Polson, A., Von Wechmar, M.B., Regenmortel, M.H.V.V., 1980. Isolation of viral IgY

antibodies from yolks of immunized hens. Immunol. Commun. 9, 475–493.
Portnoy, J., et al., 2013. Environmental assessment and exposure control of dust mites: a practice parameter. Ann Allergy Asthma Immunol 111, 465–507. https://doi.org/10.1016/j.anai.2013.09.018.
Rai, G.O., Venkateswaran, K.S., 2013. Limitations and practical problems in enzyme linked immunosorbent assays. Def. Sci. J. 42, 71–84.
Reschová, S., Franz, J., Stepánek, J., Rozhasá, A., 2000. Immunochromatographic detection of bovine rotavirus using egg yolk antibodies. Vet. Med. Czech. 45, 33–37.
Rudolf, J., 2009. Differences in usability of rabbit IgG and chicken IgY after clean-up and impact on gold labelling properties. J. Immunol. Methods 350, 79–88. https://doi.org/10.1016/j.jim.2009.08.006.
Sánchez-Martínez, M.L., Aguilar-Caballos, M.P., Gómez-Hens, A., 2009. Homogeneous immunoassay for soy protein determination in food samples using gold nanoparticles as labels and light scattering detection. Anal. Chim. Acta 636, 58–62. https://doi.org/10.1016/j.aca.2009.01.043.
Sosa, A.C., et al., 2011. Development of a sandwich enzyme linked immunosorbent assay (ELISA) for the quantification of iduronate-2-sulfate sulfatase. J. Immunol. Methods 368, 64–70. https://doi.org/10.1016/j.jim.2011.03.004.
Tansil, N.C., Gao, Z., 2006. Nanoparticles in biomolecular detection. NanoToday 1, 28–37. https://doi.org/10.1016/S1748-0132(06)70020-2.
Trashin, S.A., Cucu, T., Devreese, B., Adriaens, A., De Meulenaer, B., 2011. Development of a highly sensitive and robust Cor a 9 specific enzyme-linked immunosorbent assay for the detection of hazelnut traces. Anal. Chim. Acta 708, 116–122. https://doi.org/10.1016/j.aca.2011.09.036.
Tsay, A., Williams, L., Mitchell, E.B., Chapman, M.D., 2002. A rapid test for detection of mite allergens in homes. Clin. Exp. Allergy 32, 1596–15601.
Wang, Y., 2015. Rapid and sensitive detection of the food allergen glycinin in powdered milk using a lateral flow colloidal gold immunoassay strip test. J. Agric. Food Chem. 63, 2172–2178. https://doi.org/10.1021/jf5052128.
Wangoo, N., Bhasin, K.K., Mehta, S.K., Suri, C.R., 2008. Synthesis and capping of water-dispersed gold nanoparticles by an amino acid: bioconjugation and binding studies. J. Colloid Interface Sci. 323, 247–254. https://doi.org/10.1016/j.jcis.2008.04.043.
Wongkamchai, S., Mahakittikun, V., Vichyanond, P., Malainual, N., Wititsuwannaku, D., 2000. Dot-blot assay for the semiquantitative detection of major dust mite allergens Derp1 and Derf1 in house dust. Allergology Int. 49, 151–155. https://doi.org/10.1046/j.1440-1592.2000.00173.x.
Zeng, S., et al., 2011. A review on functionalized gold nanoparticles for biosensing applications. Plasmonics 6, 491–506.
Zhang, L., 2015. Development of a colloidal gold immunochromatographic strip for the rapid detection of soft-shelled turtle systemic septicemia spherical virus. J. Virol. Methods 221, 39–45. https://doi.org/10.1016/j.jviromet.2015.04.016.