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Original Article

Design and Fabrication of Gold Nanoparticles for Anti-Asthma Drug Delivery

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Abstract

Background and Aim: Nanoparticle drug delivery has recently found a special place in medicine and treatment. Different nanoparticles have different capabilities and functions. Gold nanoparticles are one of the most widely used nanoparticles and have many uses in pharmaceuticals and medical purposes, including diagnostic, therapeutic, and imaging methods, and due to their unique characteristics, such as high contact surface area compared to volume. Gold nanoparticles have many advantages over other nanoparticles such as their neutral nature, stability, high diffusion property, non-toxicity, environmental compatibility, optical adjustment. Our goal is to synthesize and characterization gold nanoparticles with specific applications to produce the best delivery system of drugs to the asthmatic lung.

Methods: Turkevich method has been used for the synthesis of gold nanoparticles and approving studies have been done.

Results: The produced GNP has the average diameter 100-200 nm and the Z-average was 137.9 d.nm and in positive charge area. PDI for GNP was 0.358.

Conclusion: In this study, we were able to produce the applicable gold nanoparticles for carrying drugs to asthmatic bronchi. Gold nanoparticles easily reach target cells due to their high dispersion power. Drug side effects are reduced when gold nanoparticles are used in conjunction with the drug for drug delivery purposes.

Keywords: Asthma; Nanoparticles; Drug Delivery; Treatment.

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Introduction

Asthma is a complex disease that is classified by differences in severity, the natural history of the disease, response to treatment, and its clinical signs include airways contraction and narrowing bronchial epithelium swelling and thickening, mucus secretion, coughing, wheezing, and breathlessness. For this reason, asthma is considered a chronic airway disorder (1). For some people, asthma is a major problem and interferes with their daily activities and lives, leading to severe and dangerous attacks. According to the World Health Organization, asthma is a rapidly progressive disease its economic and social pressures are greater than both TB and HIV. The high rates of death and disability caused by asthma have become serious public and social health concerns (2, 3).

Symptoms and clinical manifestations of asthma include; chest pain, difficulty breathing, sleep disturbances, wheezing during exhaustion, frequent coughing. Exacerbation of asthma with respiratory triggers such as respiratory viral infections, allergens, airborne substances, such as dust mites, pollen, pet dander, mold spores, cold air, and smoking are usual asthma triggers. Having other allergic diseases such as atopic dermatitis, allergic rhinitis, urticaria, eczema, and also, overweight, being a smoker increase chance of asthma developing (1-3). Currently, the use of anti-asthma

drugs has significantly reduced asthma mortality. When drugs are used traditionally and routinely, larger amounts are often needed because the drug may not be fully and properly delivered to the target tissue and significant amounts may be metabolized before reaching the destination, and that is itself Excess amounts can cause side effects in the body. Recent advances in nanotechnology and the use of nanoparticles have led to the production of more effective drugs for asthma control. The side effects of drugs are minimized by the use of gold nanoparticles, and this substance also reduces the duration of the disease. Gold nanoparticles reach the target cells easily due to their high dispersion power (4).

Nano drugs can be divided into two categories, traditional drugs that are improved by nanotechnology and new nano-drugs that use the nano-materials themselves as drugs (3). Nanoparticles are the carrier of genes, DNA, and RNA molecules that are either adsorbed on the surface of the nanoparticles or wrapped in the nanoparticles. Molecules such as specific ligands and monoclonal antibodies bind to the surface of the nanoparticles. After attaching target molecules to specific receptors on the cell surface, the nanoparticles enter it through cellular uptake thus safely and effectively deliver targeted therapeutic genes and drugs. For this reason, the selection and fabrication of nanocarriers are one of the most important issues in the treatment of diseases such as asthma at present (1, 3).

GNPs are one of the most widely used nanoparticles and have many applications in pharmaceuticals and medical purposes, including diagnostic, therapeutic, and imaging modalities. Au NPs have unique properties, such as a high Specific surface area to particle volume, neutral nature, stability, high dispersibility, non-toxic, environmentally friendly, optical adjustable, in comparison with other nanoparticles. Gold nanoparticles are readily functionalized for binding to proteins, amino acids, DNA, carboxylic acids, thiols, disulfides, and aptamers are available. The side effects of drugs using gold nanoparticles are minimized and this substance significantly reduces the duration of the disease (1, 5, 6). In fact, the Au NPs can easily reach target cells because of their high dispersion power (5). In this study, we designed and produced gold nanoparticles to bind to a biomolecule.

Methods

Synthesis

Gold (III) chloride trihydrate (HAuCl4) and tri-sodium citrate were purchased (Merck, Germany). Synthesis of AuNPs was accomplished by the Turkevich method (7) which involves the reduction of tetra chloroaurate by tri-sodium citrate. Citrate anions play a dual role in this reaction, both as a reducing agent by transferring electrons to gold ions and then adsorbing citrate anions to the surface of the gold nanoparticle through the central carboxylate group and acting as a nanoparticle stabilizing agent. The repulsive effect between the citrate layers leads to the stability of the gold nanoparticle and the not formation of particle aggregation. In this work, we modified the Terkovich method to obtain AuNPs with diameters ranging from 100 to 200 nm, by controlling the ratio of reducing agent/stabilizing agent (tri-sodium citrate/gold). In brief, 7.5 mL of 0.001 molar HauCl4 solutions were placed on a hot plate while constantly stirring to the boiling point at 100°C. Then, 0.75 mL sodium citrate solution 1% (obtained by dissolving 0.5 gr in 50 mL ionized distilled water) is rapidly added into the boiling solution under vigorous stirring. Stirring and warming were continued until the color of the solution changes from light yellow to wine red. Then the heating was stopped, but the stirring continues for a few more minutes. Due to the low volume of the solution, the reaction is rapid. After cooling the residual solution containing clearly defined gold nanoparticles, distilled water was added and placed the container in an oven at 100°C to dry the nanoparticles.

Characterization

SEM analysis was used to determine the properties of nanoparticles. The device used is Hitachi S-4700 FE-SEM which is a cold field emission high resolution scanning electron microscope. This SEM permits ultra-high-resolution imaging of thin films and semi-conductor materials on exceptionally

clean specimens. The S-4700 is configured to detect secondary and backscattered electrons as well as characteristic X-rays. The system is fully automated and operated via menu-driven software.

The subsequent analysis is X-ray diffraction. Philips PW 1390 Diffractometer using CUK radiation and Ni-filter was used for crystallographic analysis. The X-ray diffractograms were performed in the interval 2θ between 10˚ and 80˚, at 2˚/min. The X-ray tube was operated at 40 KV and 20 mA. We use XRD analysis to determine the characteristics of the crystals. The principles of this work are based on the radiation of the X-beam to the sample at different angles (θ) and the analysis of its diffraction or reflection pattern. The maximum peak angle indicates the distance between the crystal plates and the intensity of each peak contains information about how the atoms are arranged. The average size, distribution, and amount of electric charge of the nanoparticles were determined by Zeta Plus, Zeta Particle Size Analyzer (Malvern Zetasizer Nano range, UK).

Results

After the synthesis process and production, determining the properties of the nanoparticles was done by SEM study and the results show that the distribution of particles is uniform and homogeneous. The particles have an average diameter of 100-200 nm. The particle structure is observed in cubic orbitals which were shown in Figure 1.

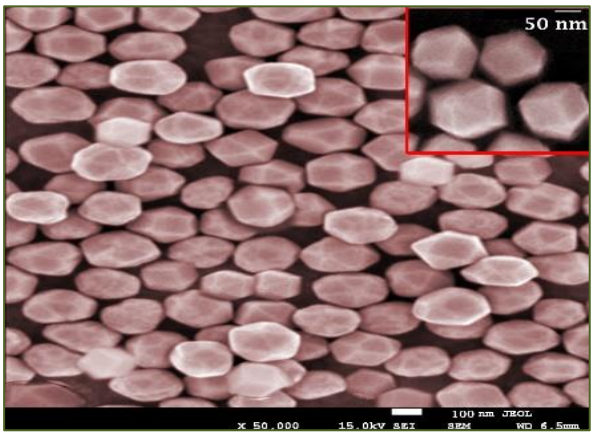

Figure 1. SEM study shows that the distribution of particles is uniform and the particles have an average diameter of 100-200 nm and cubic orbitals form.

In a study with XRD, The powder XRD pattern of the GNPs was shown in Figure 2. The major diffraction peaks can be indexed as the gold FCC phase based on the data of the JCPDS file (JCPDS no.04-0784).

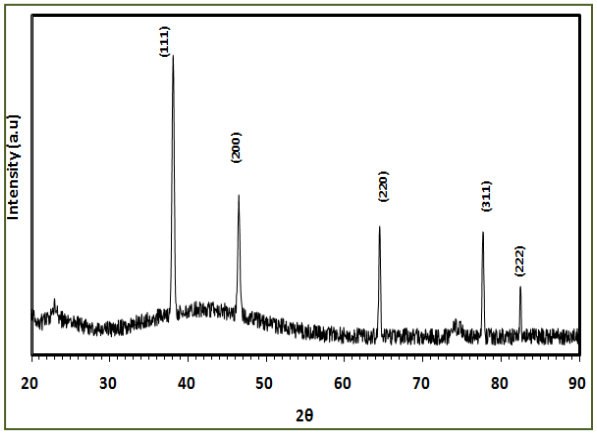

Figure 2. The XRD pattern of the GNPs.

The diffraction peaks of GNPs appeared in 38.1°, 46.2°, 64.6°, 77.8°, and 82.4°, which can be assigned to (111), (200), (220), (311), and (222) crystalline plane diffraction peaks of gold, respectively. On the nanometer scale metals (most of them are FCC) tend to nucleate and grow into twinned and multiply twinned particles with their surfaces bounded by the lowest-energy (111) facets. Other morphologies with less stable facets have only been kinetically achieved by adding chemical capping reagents to the synthetic systems. Zeta potent curves indicated that Z-average was 137.9 d.nm and in positive charge area. PDI for gold-nanoparticle was 0.358 (Figure. 3).

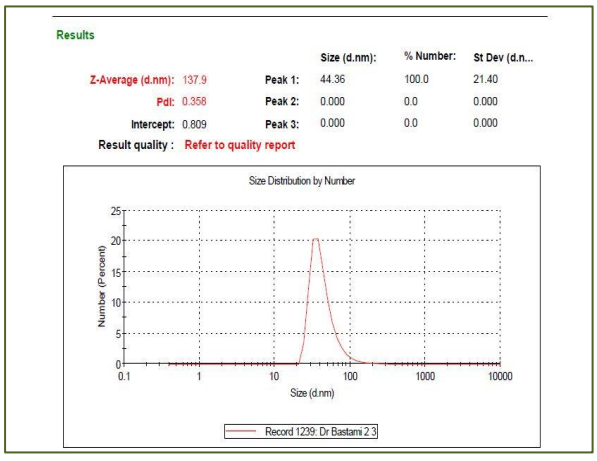

Figure 3. Zeta potent curves with Pdi of the synthesized gold nanoparticles.

Discussion

In medical Nano-drug cases, there are two important factors, drug release, and drug delivery. These two factors can be modified on the nanoparticles by reaction non-covalent or covalent loading. This manipulation is performed in the preparation of drugs to deliver to the cells, with high potential. Meanwhile, nanoparticles have high flexibility and system performance in this regard (1, 3, 5). Modification of traditional drugs, including research and development of carrier nanoparticles for delivery of existing drugs through targeted drug delivery systems, nanoparticles are used to reduce toxicity, side effects, dosage and improve the solubility of insoluble drugs which are applicable with the design of suitable nanoparticles (3). The second category is new nanoparticles, high efficacy drugs, lower toxicity, higher absorption, and better targeting, as well as diagnostic drugs based on nanoparticles (1, 6).

In the present study, we have synthesized gold nanoparticles with a size of about 100-200 nanometers using the Turkevich method, which is suitable for our intended purposes. In the current study, the aim was the production of gold nanoparticles as carriers for respiratory system drug delivery and especially as an anti-asthma drug carrier. After synthesis, produced nanoparticles will be used to carry drugs to the bronchi. The smaller size of the gold nanoparticles than 50-100 nm cannot be effective for our purpose (1, 5) such as, protect and carrying drugs (for example peptide form drugs), to the lung tissue. Therefore, by making slight changes in the details of the work and mixing ratios, we were able to synthesize gold nanoparticles with a size of about 100-200 nm. The size and distribution size has been investigated by the Zeta sizer method. Particles smaller than 50-100 nm are exhaled out of the lungs and are not used to treat asthma, and drugs without any useful effect, can be returned out by expiration. Particles larger than 500 nm are also trapped in the upper airways and oropharynx, therefore, cannot enter the airway of the lungs, and have no therapeutic effects.

Three methods have been used to synthesize gold nanoparticles; thermal heating (with chemical or organic reagents), UV irradiation, and the use of ascorbic acid as a reducing agent. In the thermal method, a double-walled reactor located in the thermostat bath is used, and the necessary heat of the reaction is provided. In the UV irradiation method, a 5 mm PMMAUV coat is filled with gold chloride solution and by adding citrate and stirring, irradiation is carried out with a UV at 366nm. In the third method, the reduction of gold salt is done by ascorbic acid at room temperature. In the recent method, by decreasing the temperature, the size and quality of the final synthesized particles will proceed to the particles with larger diameters and irregular shapes. The final study of gold nanoparticle synthesis using two methods of thermal and UV irradiation yielded similar results. These two methods are based on the Turkevich protocol, and the only difference is the method of heat supply required for the reaction. In this study, the Turkevich method has been used, but to reach a useful and fit product, the current method has been manipulated and modified (3-6).

In a study by J. Kimling et al, 2006, the synthesis of gold nanoparticles from the reduction of gold chloride by citrate and ascorbic acid was investigated (8). In another study by Harihar Nath Verma et al, 2013 the synthesis and characterization of gold nanoparticles for therapeutic approaches and diagnostic tools for various diseases were performed (9). The reduction of gold salt by a reducing agent, such as tri-sodium citrate, was performed in an aqueous solution. Changes in the initial concentration of gold and trisodium citrate and temperature have been performed to investigate their effect on particle size and distribution. Zeta Sizer and SEM have been used to investigate the properties of synthesized nanoparticles under different conditions. The results show that the optimal concentration of gold salt is 20mM and the optimal concentration of trisodium citrate is 1.5% and the optimum temperature is 97C. The gold nanoparticles obtained with optimal concentrations and temperatures were 15-20 nm in size. Therefore, we have to change these parameters to achieve ideal parameters for design and anti-asthma drugs carrier.

In a study conducted by Amir Tabrizi et al, 2009 the method of reducing gold salt by trisodium

citrate was used to synthesize gold nanoparticles (10). Changes in the initial concentration of gold chloride and trisodium citrate and the rate of mixing have been followed to investigate their effect on nanoparticle size. In the first step, by keeping the initial concentration of tri-sodium citrate at 0.17 mM and the mixing rate constant at 450 rpm, and changing the initial concentration of gold chloride from 0.03 mM-0.3 mM, they have reached the optimal concentration of gold chloride at 0.06 mM, which the nanoparticle size 23-120 nm has been obtained. A high initial concentration of gold chloride leads to the formation of particle accumulation and turbidity of the solution. Micron-sized nanoparticles were also found at low initial concentrations of gold chloride. In the second part, the changing sodium citrate concentration was investigated. In the initial low concentrations of this reducing agent, no nanoparticles were formed, which indicates the extraordinary importance of sodium citrate concentration in the synthesis of gold nanoparticles and their sizes. The third part of this study included the study of the effect of the mixing rate of the synthesis of gold nanoparticles (10). Nanoparticle formation was observed at all velocities. Higher mixing speeds led to the production of very small particles, but the size distribution was larger. The optimal speed was obtained at 450 rpm.

Chao Lin et al, 2013 conducted a study using nanoparticles synthesized in catalysts to reduce P-Nitrophenol and examined the effect of nanoparticle size on catalytic activity. The gold nanoparticles were obtained by $NaBH_4$ gold resuscitation. In this work, it has been shown that the size of the gold nanoparticles can be changed by adjusting the amount of $NaBH_4$ consumed in the synthesis. The colloidal solution uses an optimal pH of 5.9 to prevent the growth of gold nanoparticles. And also, it has been found that the catalytic activity of the catalyst depends on the size of the gold nanoparticle, and the highest catalytic activity was observed at 3.4 nm. Larger size Particles have been also synthesized as the initial concentration of $NaBH_4$ increased (11). In a study by Zhao Jingyue, 2015 the synthesis of spherical gold nanoparticles in an aqueous solution has been investigated using the reduction method. The aim was to obtain efficient synthesis methods for the industrial production of high-quality gold nanoparticles in high quantities. In this work, two chemical reduction methods have been used; citrate reducing agent and $NaBH_4$ (12).

In the citrate reduction method, the pH was changed and the ionic strength of the environment and the presence of other organic matter were controlled, so the size and distribution of the particles can be controlled. Also, in this method, different amounts of gold salt and trisodium citrate have been used to achieve different size distribution. The results show that in the process of reduction of citrate at 100˚C, the morphological particles have a more uniform and smaller size distribution, although the effect of temperature is affected by the initial concentration of citrate. In addition, the high concentration of gold salt leads to a wider range size distribution, larger and more irregular size (1, 3, 8). At concentrations above 40 mgAu/100 mL gold nanoparticles accumulated strongly. After the nanoparticle synthesis process, the dialysis membranes were used to reduce impurities, including unreacted sodium ions, an excess citrate salt, and by-products. When the $NaBH_4$ reducing agent was used, synthesis was performed using the classical Brust process, which is a two-phase method. In this method, a phase transfer factor is required. This study shows that the two-phase method leads to the synthesis of high-quality materials and the distribution of narrow size. In the reduction process with $NaBH_4$ reducing agent, $NaBH_4$ is a reducing agent and citrate only acts as a stabilizer. While in the citrate regeneration method, trisodium citrate itself plays both in reducing and stabilizing role (7, 10, 12). In the process of regenerating with $NaBH_4$, the faster the reaction produces the more irregular the gold nanoparticles.

In a study by Muhammad Iqbal et al. 2015 $NaBH_4$ was used to reduce gold salt. After obtaining the gold nanoparticles, the particle size was calculated by both experimental and theoretical methods (13). $NaBH_4$ as a reducing agent in different concentrations has been added to $HAuCl_4$ 0.06 mM to investigate the effect of reducing agents on particle size and distribution and morphology. The

physicochemical aspect of the colloidal system, such as particle size and Zeta potential, were studied to investigate the effect of colloidal stability and the rate of release and interaction of particles with cells and the biological environment. The Characterizing of particles with TEM indicates that the produced nanoparticles were spherical and have an average size of less than 30 nm. TEM images show that low concentrations of the reducing agent, and the particles, are relatively separate and homogeneous, but in high concentrations and excess of the reducing agent. A small aggregation between the particles is observed and the particle size has also increased, due to the aggregation of particles and excessive reduction of gold salt in the presence of excess amounts of $NaBH_4$. The gold nanoparticles were synthesized in this study have been used for biomedical applications, in vivo and in vitro with modified shape and size according to our purpose (5, 9, 13).

In the method of calculating the particle size, theoretically, the Mie theory has been used, and the optical absorption spectra of the samples have been fitted using the Lorentz equation, and the particle size has been obtained in good agreement with the findings of the standard UV curve and SEM analysis. At least, the gold nanoparticles were synthesized in this study have been used for biomedical application in vivo and in vitro as a drug delivery system for treatment and control of asthma . Depending on the type of work studied, different sizes of gold nanoparticles are required, which can be obtained by changing the reaction conditions such as temperature and, changing the initial concentration of the reactants and the type of reducing agent, and changing the mixing speed.

Gold nanoparticle synthesis is a simple, fast, and very efficient method with good adaptability and can be used in biomedical studies.

Conclusion

To achieve the optimal size of gold nanoparticles (100-500 nm) as anti-asthma drugs and peptides carriers, this modified and manipulated method can be useful at low cost and will be done in minimum time. The particles small than 100 nm can return with exhalation and more than 500 nm will be trapped in the upper airways. Therefore, to use GNPs for therapeutic purposes in asthma by changing the ratio of sodium citrate to gold salt, we succeeded in syntheses gold nanoparticles in the range of 100-200, which was the ideal size for our pharmaceutical and therapeutic purposes.

Conflict of Interest

The authors declared that they have no conflict of interest.

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References

1. Seyyed Shamsadin Athari, Zahra Pourpak, Gert Folkerts, Johan Garssen, Mostafa Moin, Ian M Adcock, Masoud Movassaghi, Mehdi Shafiee Ardestani, Seyed Mohammad Moazzeni, Esmaeil Mortaz. Conjugated Alpha-Alumina nanoparticle with vasoactive intestinal peptide as a Nano-drug in treatment of allergic asthma in mice. Eur J Pharmacol 2016; 791:811-20.

2. Jan Lotvall, Cezmi A. Akdis, Leonard B. Bacharier, Leif Bjermer, Thomas B. Casale, Adnan Custovic, Robert F. Lemanske, Jr, Andrew J. Wardlaw, Sally E. Wenzel, and Paul A. Greenberger. Asthma endotypes: A new approach to classification of disease entities within the asthma syndrome. Journal of the Allergy Clin Immunol. 2011; 127(2): 355-60.

3. Lingwei Wang, Mengjie Feng, Qiuwen Li, Chen Qiu and Rongchang Chen, Advances in nanotechnology and asthma. journal of the Annals of Translational Medicine. 2019; 7(8):180.

4. Brussino L, Heffler E, Bucca C, Nicola S, Rolla G. Eosinophils Target Therapy for Severe Asthma: Critical Points. Biomed Res Int. 2018; 2018:7582057.

5. Wang L, Feng M, Li Q, Qiu C, Chen R.Wang L, et al. Advances in nanotechnology and asthma. Ann Transl Med. 2019; 7(8):180.

6. Yan X, Zhang A, Yu L, Chen C, Cui H.Yan X, et al. Differential Expression of Serum Proteins in Rats with Allergic Asthma: A Study Based on the Nanoliter Two-Dimensional Liquid Chromatography Technique. Can Respir J. 2017; 2017:8015703.

7. J. Turkevitch, P.C. Stevenson and J. Hillier, Nucleation and growth process in the synthesis of colloidal gold, Discuss. Faraday Soc. 1951; 11:55–5.

8. J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot, and A. Plech, Turkevich Method for Gold Nanoparticle Synthesis Revisited, journal of the Phys. Chem. 2006; 110(32):15700-7.

9. Harihar Nath Verma1, Praveen Singh and R. M. Chavan, Gold nanoparticle: synthesis and characterization, journal of the Veterinary world. 2014; 7:72-7.

10. Amir Tabrizi, Fatma Ayhan and Hakan Ayhan, Gold Nanoparticle Synthesis and Characterisation, journal of the Hacettepe Journal of Biology and Chemistry. 2009; 37:217-26.

11. Chao Lin, Kai Tao, Dayin Hua, Zhen Ma and Shenghu Zhou, Size Effect of Gold Nanoparticles in Catalytic Reduction of p-Nitrophenol with $NaBH_4$, Molecules. 2013; 18:12609-20.

12. Zhao Jingyue and Friedrich Bernd, Synthesis of Gold nanoparticles via Chemical Reduction Methods, NanoCon, 2015, Brno, Czech Republic, EU.

13. Muhammad Iqbal, Gisele Usanase, Kafia Oulmi, Fairouz Aberkane, Tahar Bendaikha, Hatem Fessi, Nadia Zine, Géraldine Agusti, Errachid El-Salhi and Abdelhamid Elaissari, Preparation of gold nanoparticles and determination of their particles size via different Methods. Materials Research Bulletin. 2016; 79:97-104.