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TEM Analysis of Gold Nanoparticles Synthesisin Glycerin: Novel Safety Materials in Cosmeticsto Recovery Mercury Damage.

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ABSTRACT

Nanogold or gold nanoparticles (GNPs) have been widelystudied in the past 10 years because of their unique properties, such as catalysis, quantum size effect, and optical property.In the recent years responsibilities for the control and regulatory mechanisms increased to guard the utilization of GNPs into cosmetic products. On March 24, 2009, the European Parliament (EP) approved an update of EU legislation on cosmetics. The emergent applications gold nanoparticles in cosmetics related the current issue that mercury steel in cosmetics.Mercury is a common ingredient found in skin lightening soaps and creams. It is also found in other cosmetics, such as skin lightening soaps and creams, eye makeup cleansing products and mascara. The presence of mercury is bound to the thiol group on the cysteine residue is causing the function of cysteine and enzyme are not properly.This research is synthesis of gold nanoparticles use glycerin as matrix and sodium citric as reduction agent. TEM characterization result of synthesis used in this research to analyze form and size of the clusters. It was estimated that optimal size of interacting with the skin nanoparticles would be in the range 10-50 nm. Smaller tend to penetrate the skin more easily than large particles.Glycerin used in this synthesis because glycerin always present in cosmetics formulation than the result prepare for novel safety material in cosmetics. GNPs can synthesized with glycerin as matrix with diameter of clusters size 10-40 nm in various concentrate. This size of clusters can be used in cosmetic application because these clusters can penetration in pore of skin.The size of clusters bigger in higher concentration of GNPs. This phenomenon is caused aggregation process intra two or more clusters.Gold and Mercury (Hg) availably form binding with sulfur from Cysteine in human enzyme. Gold or gold NPs bioavailable in human but Mercury is not bioavailable, so that gold is allowed in cosmetics but Mercury is very dangerous in cosmetics.

Key word: Nanogold, cosmetics, mercury, clusters, novel safety material

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INTRODUCTION

Nanoparticles (NPs) form a new class of materials possessing unique properties that are characteristic of neither the molecular nor the bulk solid-state limits. They havebecome the focus of considerable fundamental and applied research leading toimportant technological applications in areas such as heterogeneous catalysis, optical communications, gas sensing, nano-electronics, and medicine. NPs come in a widerange of sizes and shapes, with varied electronic, optical, and chemical properties. However, throughout this diversity a universal concept is applicable: the properties of NPs are intimately connected to their nano-scale size and atomic-scale structure [1].

The perception of gold as a highly inert material has been transformed by the discovery that nano-sized gold particles are chemically active for numerous reactions. They also possess unique electronic and optical properties which can be tuned for specific applications by controlling their size and atomic structure. Surface science studies and theoretical models that have helped to understand these complex structure-property relationships suggest that confinement of electronic states, under coordination of atoms, charging, and NP-support interactions all play important roles. These effects are not only relevant to Au but to other metallic NP systems as well, such as transition metals. However, Au is a particularly interesting example because its bulk surfaces are inert whereas bulk transition metal surfaces are active catalysts. Many of the applications of Au NPs, for example, in biology, gas sensing, and catalysis, involve interactions with molecules that can influence NP structure. The dynamical interplay between molecules and NPs is, in general, both complex and non-equilibrium in nature[1].

Recent advances in techniques such as TEM, STM, and atomic forcemicroscopy mean that atomic-scale resolution of NP structure is now becoming possible. This is complemented by increasingly powerful computers that allow for quantum mechanical calculations on larger and more complex NP systems (e.g.including the substrate or interfaces between NPs). One of the key remaining challenges (for experiment and theory) concerns the atomic-scale dynamics of NPs.This is important for understanding NP growth, an important step towards controllingstructure, and for understanding the role of temperature in real applications. A relatedissue concerns the interaction of NPs with their environment, for example, molecules in a surrounding liquid or gas, which can influence their structure and properties [2].

The near mono dispersity of the nanoparticles of the last two decades (indeed, the reason they have become so interesting) is the analogue to compoundstoichiometry. For example, a 5.0-nm diameter gold particle ligated withdodecanethiol can be written Au3870 (C12SH)365where the standard deviation on the numbers is about 10%.Gold nanoparticles are the most common, largely because gold is relatively inert, yet it is the most electrophillic of metallic elements and this makes ligation propitious. Figure 3.2 shows a cartoon of what one might call a canonical gold nanoparticle molecule and Table 3.1 gives some useful statistics. There is a great variety in sizes and ligands. Sizes can range from approximately 1 to 20 nm, although 4 to 7 nm, is most common. The most common ligands are the alkyl thiols; the electrophilic gold binds the lone

pairs of the sulfur readily. Alkane chain lengths from C6 to C16 are typical and aromatics have been used as well. Other ligands such as amines and phosphines have been used. Narrow sizes dispersions have been obtained through fractionation or digestive ripening. The surface capping ligands keep the nanoparticles from irreversibly aggregating and largely determine their solution and super-latticeproperties. With regard to gold and thiols, the properties of self-assembled monolayers, SAMs, of organic thiolsadsorbed on bulk gold surfaces have been studied for some time, and this has beenused to understand the ligation of thiols at the curved surfaces of the nanoparticles.Control of the ligand capping can also allow formation of amphiphilic nanoparticle molecules by capping with both hydrophilic and hydrophobic ligands. Such mixedcoatings have been reported in the literature (38–40) and phase equilibrium of such nanoparticle molecules have been studied with simulations to yield a potentiallyrich phase diagram [1].

The novel synthesis of goldnanoparticles of different sizes and hence colors in a wool fiber matrix, simultaneously utilising the chemical affinity of gold for sulfur to bind the nanogold to the disulfide linkages in cysteine amino acids in the keratin protein. For this, the wool fibers act as a solid matrix to control the particle size and prevent agglomeration of the gold nanoparticles and hence facilitate a range of attractive colors in the wool due to the surface plasmon resonance effects of such gold nanoparticles. Because the nanogold is chemically bound to the cysteine, it does not wash or rub out and is also stable to UV light, unlike organic colorants. Theresearch innovatively links the high value and prestige of gold through nano-science for high value textiles and fashion apparel, wherein the nanogold wool composite fibers contain only pure wool and pure gold and are environmentally desirable [3].

Gold has long been known to form stable colloids of nano-size particles. Such nanoparticles exhibit different colors due to surface plasmon resonance effects which result from the resonance interaction of incoming electromagnetic radiation in the visible region with the collective plasmon oscillations at the metal surface [4]. This gives rise to intense absorptions in the visible spectrum. The color is dependent on the particle size and shape and the dielectric constant of the surrounding medium. Colloids of spherical nanogold particles of about 10–20 nm in size are red. As the particle size increases,the color changes through darker shades of red to purple,then to blue-grey for particles or agglomerates up to about80–100 nm. The colors relate to a shift and broadening of the transverse plasmonresonance absorption band, originally about 520 nm. Non-spherical gold nanoparticles also exhibit different colors depending upon their shape and aspect ratio, as they show a longitudinal plasmon resonance band at about 720 nm along with the transverse band [5]. Short gold nano-rods are typically blue in color. The change in shape from rods to dumbbell or phi-shaped particles leads to a shift in the longitudinal plasmon band to the blue and red directions respectively [6]. All the colors are stable in sunlight and UV light and do not change providing there is no change in particle size through the growth of discrete nanoparticles or agglomeration [3].

The synthesis ofpoly(MMA-co-MSMA) and MPS–Au nanoparticles were used to prepare acrylic polymer–nanogoldnanocomposites. The covalent bounds between GNPs and acrylic polymers were formed through the condensation of Si–OMethyl groups in MPS and MSMA. The GNPs were thus well dispersed and maintained nano-scale in the acrylic

polymer. TGA results indicate that functionalized GNPsincrease the crosslink density and the thermal stability. The earlier Td of high Au-content nanocomposites might be dueto the residual –OH groups, reactants, or by-products. Light scatterings are observed and increase with the particle sizesand volumes. The nanocomposites of high Au-content present more dense structures. The hardness increases withdecreasing the size and increasing the Au content [7].

Recently, the functionalized GNPs weresynthesized through different stabilizing agents and showed the potential in several applications. For example, nanocomposites synthesized from silica and 3-mercapto-propyltrimethoxysilane (MPS) encapsulated GNPs have excellent catalytic application. Although many block copolymers and dendrimers have been used as the stabi-lizing agents to synthesize GNPs in the literatures, reports of the preparation and characterization of polymer nanogoldnanocomposites are only occasional. In the literatures, poly(methylphenylphosphazene) (PMPP),polyacrylamide, acrylic polymers, polyurethane (PU), polystyrene (PS), poly(ethylene glycol) (PEG), poly(vinylphenol) (PVP), poly(vinyl alcohol) (PVA), poly(o-phenylenediamine), poly(amic acid) (PAA), polyimide (PI), and polyfluorene (PF) were used to prepared the polymer–GNPs nanocomposite.

Nanogold or gold nanoparticles (GNPs) have been widelystudied in the past 10 years because of their unique properties, such as catalysis, quantum size effect, and optical property.In the recent years responsibilities for the control and regulatory mechanisms increased to guard the utilization of nano-particles into cosmetic products. On March 24, 2009, the European Parliament (EP) approved an update of EU legislation on cosmetics. As requested by the EP, the new regulation introduces a definition, safety assessment procedure and labeling requirement for all nanomaterials that are used in cosmetics [8].

The current applications of gold nanoparticles span a wide range of sectors. Current niche for such applications is in the areas where there is an overlap between medicines and cosmetics sectors. Many products are marketed as a means to enhance performance for different lifestyles and age groups, as an aid to health, beauty and wellbeing. Recently there is widespread use of nanoparticle gold containing cosmetics like skin creams that are used on the whole body surface for appearance of shining glow, lipsticks, anti-aging face creams and many other products. Overview of state-of-the-art exploration of nanoparticle gold compounds will handle us with special knowledge about differences in physical and chemical properties of this material, dependent on size, shape, charge, even solvent used in processing of this metal. Although such applications are relatively new and emergent, they appear to have started to make a global impact. Number one question is if quality of life will improve thanks to synthesis of new materials (gold nanoparticles) with new properties[8] The emergent applications gold nanoparticles in cosmetics related the current issue that mercury steel in cosmetics.

Mercury is a common ingredient found in skin lightening soaps and creams. It is also found in other cosmetics, such as skin lightening soaps and creams, eye makeup cleansing products and mascara. These productsare commonly used in certain African, Asian nations, among dark-skinned populations in Europe and North America. Mercury salts inhibit the formation of melanin, resulting in a lighter skin tone. Mercury in cosmetics exists in two

forms: inorganic and organic. Inorganic mercury (e.g. ammoniated mercury) is used in skin lightening soaps and creams. Organic mercury compounds (thiomersal [ethyl mercury] and phenyl mercuric salts) are used as cosmetic preservatives in eye makeup cleansing products and mascara [9].

Long-term use of mercury-based skin lighteners often produces a characteristic slate grayl skin color. Over-pigmented skin is a common problem among African women, caused by mercury and/or hydroquinone in skin-lightening creams, among other factors. Ammoniated mercury, used in some skin-lightening creams, can cause rashes and allergic reactions. Other ingredients in these products, in particular steroids, also damage the skin, sometimes severely. Kidney damage caused by long-term use of mercury-containing skin-lightening creams has been reported by investigators in China, Hong Kong2 and the UK. In those cases, once the source of mercury exposure was identified and the women stopped using the harmful products, their kidney function gradually returned to normal. Unfortunately, most women who use skin-lightening products are unlikely to be seen by a medical professional who could detect such kidney damage at an early stage and eliminate their mercury exposure before more serious disease develops [10].

Mercury is also toxic to the nervous system. Users of mercury-containing soaps in Kenya had symptoms of nervous system toxicity including tremors, lassitude, vertigo, loss of memory, and generalized aches and pains, all classic signs of inorganic mercury poisoning.
Two German women who used skin lighteners for up to 20 years each suffered from headaches, abdominal cramps and shortness of breath; both were repeatedly hospitalized.
On the other hand, a large study in Hongshortness of breath; both were repeatedly hospitalized. On the other hand, a large study in Hong Kong found that 78 percent of women using mercury-containing skin lighteners reported no symptoms, although two-thirds of them had significantly elevated blood and/or urine mercury levels. However, the fact that even a majority of users may suffer no evident adverse effects does not make this category of products less dangerous to consumers [11].

The dose-dependent effects of heavy metals including mercury on the cell proliferation, collagen synthesis, and non-collagen protein synthesis were studied in early passage cultures of human synovial cells exposed to 1-100 µM concentration of mercury for 5 days. (Goldberg, Kaplan, & Fuller, 2002) Mercury can form a bond with the thiol group which formed very strong bonds and stable that caused by the high constant stability of mercury-thiol. In the formation of mercury complexes with thiolgroups(from glutathione, albumin, cysteine and others) mercury binds to the free thiol groups available. The presence of mercury is bound to the thiol group on the cysteine residue is causing the function of cysteine is not properly. It is caused that thiol groups play an important role in the metabolism of the body, such as the active center of the enzyme. The presence of mercury atoms causes the enzyme does not work because the enzyme has done at specifically site. The incorporation of [3H]thymidine into trichloro acetic acid insoluble material was inhibited 50% by each of the heavy metals at concentrations between 1 and 10 µM. Mercury 10 µM decrease the DNA content of the cultures by less than 15%, which was

attributed to cytotoxicity. A dose-dependent inhibition of [3H]proline incorporation into bacterial collagenase resistant (non-collagen) protein was observed after incubation with 10µM mercury [12].

Gold nanoparticles are also recognized in their ability to bind to DNA, which may be exploited within the treatment of disease, such as anticancer agents or within gene therapy, but may also contribute to genotoxicity, or block transcription [13]. HammadShifferli et al. have demonstrated that transmitted radio signals influence integrity of DNA strand, while is bound to nanoparticles gold molecules. This discovery opens up the possibility to control more complex biological processes of living cells, such as enzymatic activity, protein folding and bio-molecular assembly [14]. Furthermore, the ability of gold nanoparticles to bind to DNA is of concern, due to their potential cytotoxic or gene-toxic consequences, which may be exploited within anticancer drugs, or gene therapy, which warrants further investigation. In addition, the ability of goldnanoparticles to interrupt transcription is of concern [15].

This research is synthesis of gold nanoparticles use glycerin as matrix and sodium citric as reduction agent. Glycerin molecule have 3 –OH groups that binding and stabilizing colloidal gold or gold nanoparticles(NPs) clusters and α-keto (oxidation form of citric) together. The stability of gold NPs cluster can minimalize aggregation of clusters. TEM characterizationresult of synthesis used in this research to analyze form and size of the clusters. It was estimated that optimal size of interacting with the skin nanoparticles would be in the range 10-50 nm. Smaller tend to penetrate the skin more easily than large particles.Glycerin used in this synthesis because glycerin always present in cosmetics formulation than the result prepare for novel safety material in cosmetics.

MATERIAL AND METHOD

Material

Gold chloride (HAuCl4), glycerin and sodium citrate were obtained from Sigma (www.sigmaaldrich.com,USA). 1,000ml of water. All chemicals and solvents used were of analyticalgrade and were used as received. All solutions were madeup with twice-distilled water. Gold colloids were prepared according to the literature by adding 2gsodium citrate solution to 100 ml boiling solution of HAuCl4. The size of the prepared gold colloids was about 10-40 nm, which was estimated from transmissionelectron microscopy(TEM) JEOL/E0 version 1,0 JEM-1400.

Method

Glycerin (5ml) dissolved in95 ml of water was prepared in double distilled water. The solution boiled at temperature of 100^{o}C. Sodium citrate about 2 g is added in boiling solution and then exposed to a solution of $HAuCl_4$ about specific volume (ml) 1000ppm. The specific Volume of $HAuCl_4$are 5, 10, 15, 20, 25 and 30ml. The synthesis was carried out for 10 minutes. Characterization synthesis result with transmission electron microscopy (TEM).

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RESULTS

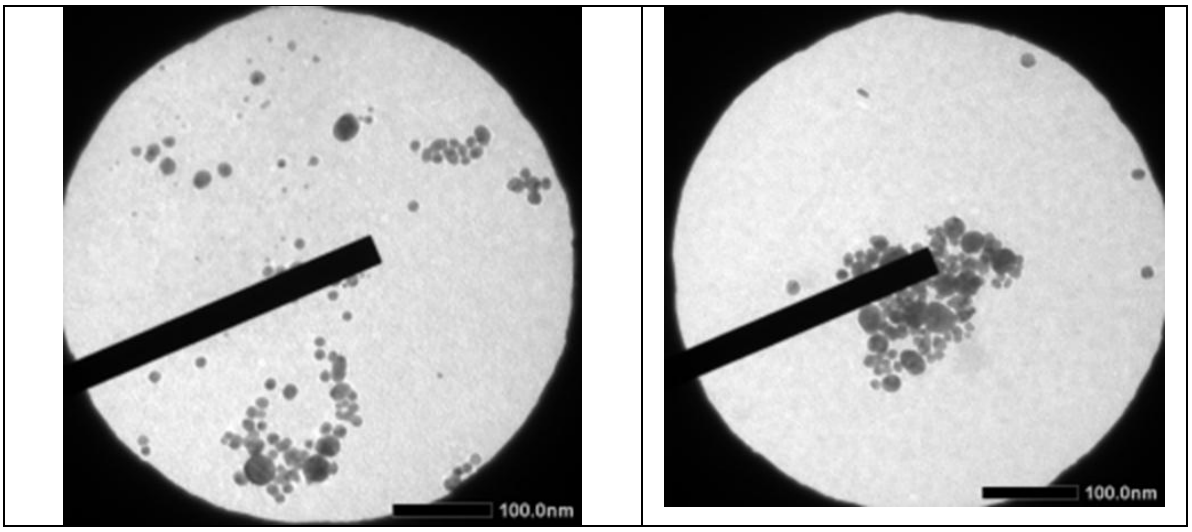

Figure 1. The clusters of*gold NPs* concentration 5 ppm(left) and 10 ppm(right).

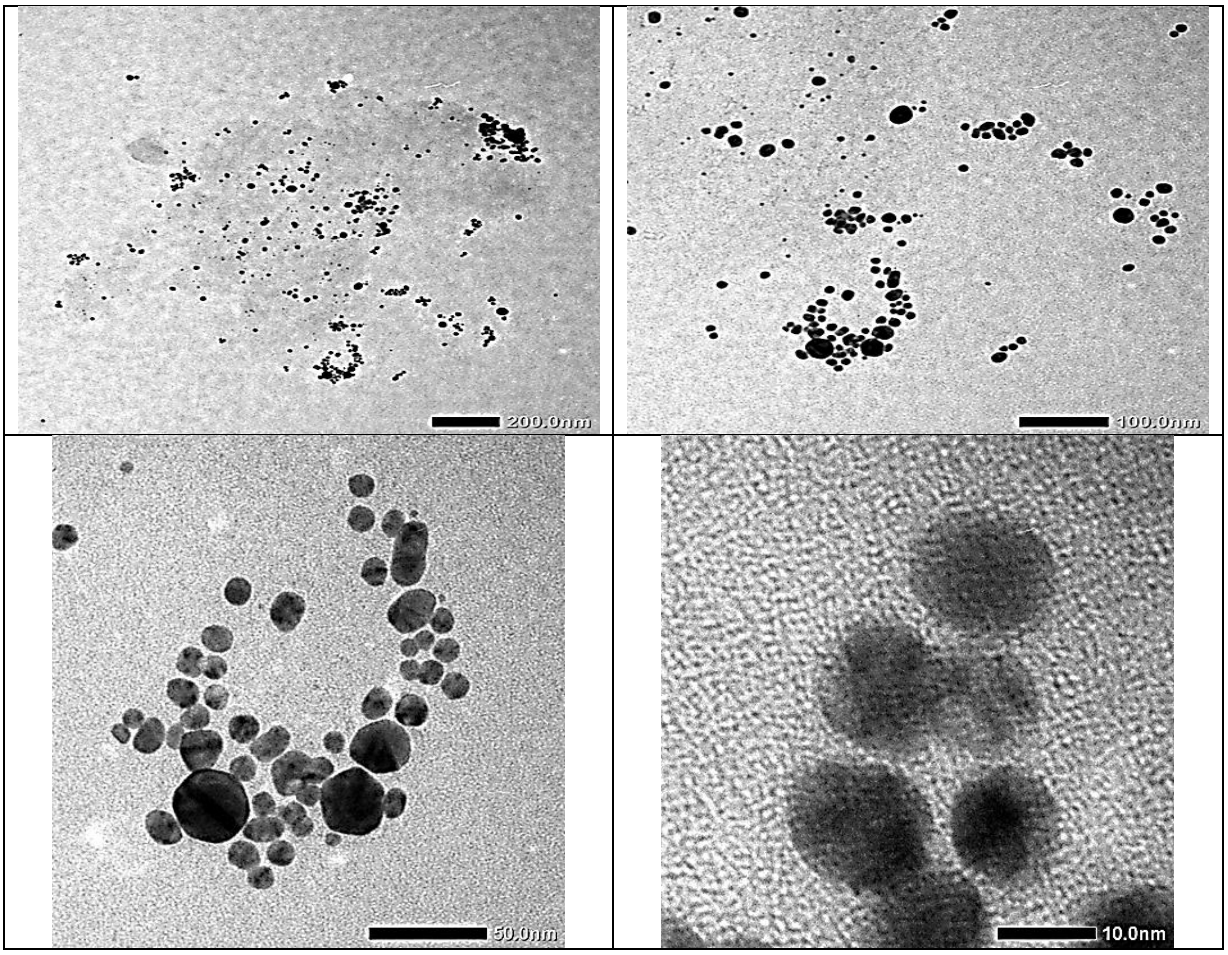

Figure 2.TEM of nanogold with several magnifications to measure diameters of clusters.

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The clusters sizes are hetero but they have dominant size in various concentration. The size in 5 ppm concentration is 10nm, 10 ppm is 12nm, 15 ppm is 14nm, and closed in Table 1. These size are the rate of diameter that accounted from magnifications of TEM picture with scale-bar prediction. The clusters with diameters 10nm interaction one by one form the big one. If the clusters diameters have big size or more 50nm they are suspended and go out from colloidal system. These phenomena caused gravitation effect. The clusters with big size have more weight than the little one. Aggregation process is started by clusters magnification. At the higher concentration clusters magnification easily occurred, than predicted that the concentrate is correlation with clusters sizes. The correlation can see in Figure 3.

Table 1. Diameter clusters of gold NPs at various concentrate.

No	*Nanogold concentration*	Diameter (nm)
1	5 ppm	10
2	10 ppm	12
3	15 ppm	14
4	20 ppm	16
5	25 ppm	20
6	30 ppm	27
7	35 ppm	30
8	40 ppm	40

DISCUSSION

The relation concentration and diameter clusters of nanogold in Table 1 can explain with following diagram or Figure 3.

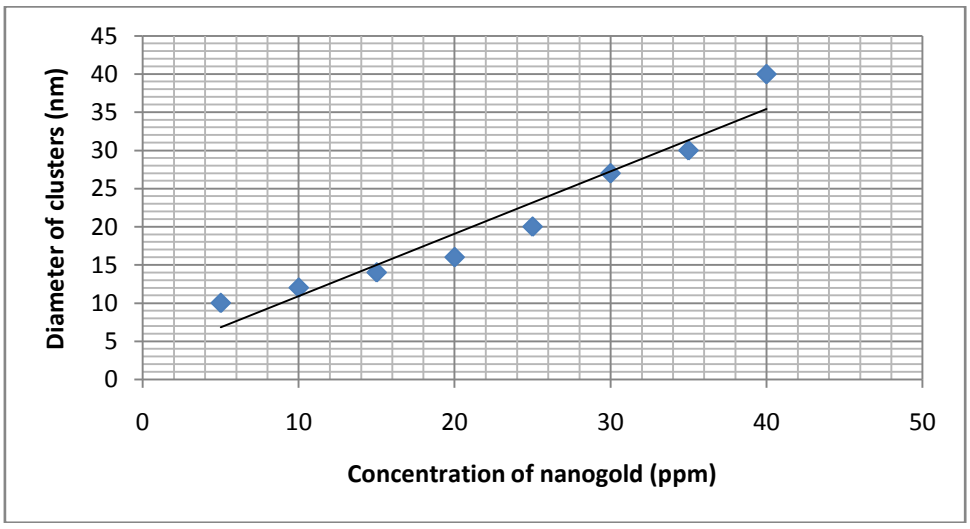

Figure 3. The relation concentration and diameter clusters

The clusters diameteris bigger by increasing concentration. This phenomena caused by aggregation processoccurredintraclusters. This process can see at the TEM characteristic in Figure 2. Two or more clusters move and attract each other to form new clusters in big size.

The glycerin matrix protects the clusters gold NPs so that aggregation process not occur. In high concentration the number of clusters increasing, glycerin could not retain aggregation inter clusters. The precipitate of cluster occur and the colloidal of gold NPs change to be suspension.

Au-S bond is the covalent conjugation, this bond form then gold nanoparticles were adsorbed onto the surface of $CoFe_2O_4$-MPS, one of applied of gold nano particles as anti-AFP antibodies in human serum [16].Gold NPs particles were covalently linked to agenetically modified Sup35p-NM variant. After placing the gold-labeled protein fibers across gold electrodes, highly specific chemical enhancement of the gold NPs particles yielded 50–100 nm wide gold wires. These bio-templated metal wires demonstrated the conductive properties of a solid metal wire, such as low resistance and ohmic behavior [17] Conducting nanowires based on self-assembling protein fibers. (a) A genetically modified yeast prion protein that presents reactive, surface accessible cysteine residues was employed to covalently couple gold NPs after fiber assembly [18]

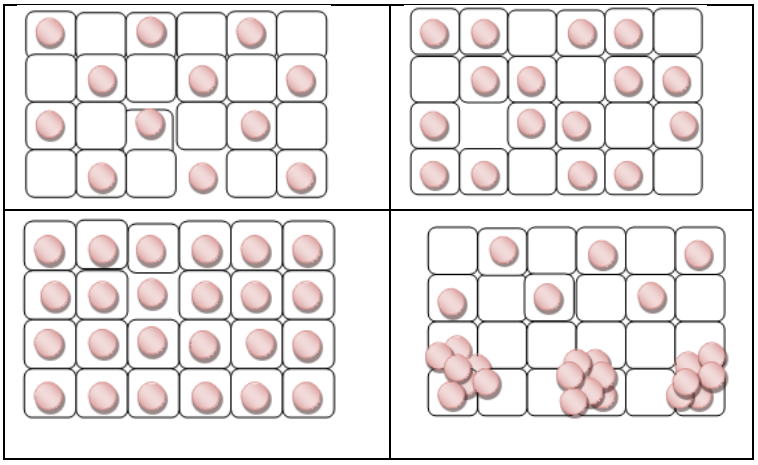

Figure 4.The illustrate of clusters aggregation process in glycerin matrix.

The Au-S (gold nanoparticles-Sulfur) arechemically bound to the sulfur in the cysteine amino acids in the keratin protein. The comparable deconvoluted high resolution XPS spectrum of the S 2p electrons for sulfur in the gold NPs wool fibers shows the typical 3/2 and 1/2 peaks for sulfur consistent with the –S–S–disulfide entities in cysteine. Because of the strong chemical affinity of gold for sulfur, it is likely that clusters gold NPs form in the vicinity of the –S–S– units in the cysteine, amino acids of the keratin. Here, the Au3+ is reduced to $Au^{o}$and the resulting gold nanoparticles then chemically bind to the sulfur in the remaining –S–S– cysteine linkages to form a stable gold NPs wool fibers [17].

Effect of mercury on the disulfide bonds can cause two problems. The first is that methyl mercury causes breaking disulfide bonds. Disulfide bond is forming the tertiary structure of a protein. This disulfide bond rupture resulting protein loses its biological properties (protein denaturation). The second problem is that mercury is forming a disulfide bond replaces previous bridge. Although it seems no effect on the structure initially, the body will naturally detect that there are foreign proteins in the body. Adverse reactions may occur due to the influence of elemental mercury in the protein. Furthermore, this complex can cause damage to proteins that have been formed. The mechanism of its formation can

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be observed in Figure 5 and Figure 6 [19] Mercury has electron-sharing affinities that can result in formation of covalent attachments [20] These attachments are mainly formed between mercury and sulfhydryl groups of protein, gold NPs substitute mercury to normalization tissue health [21].

Figure 5.Sulfur-gold NPs interaction to recovery human tissue damage.

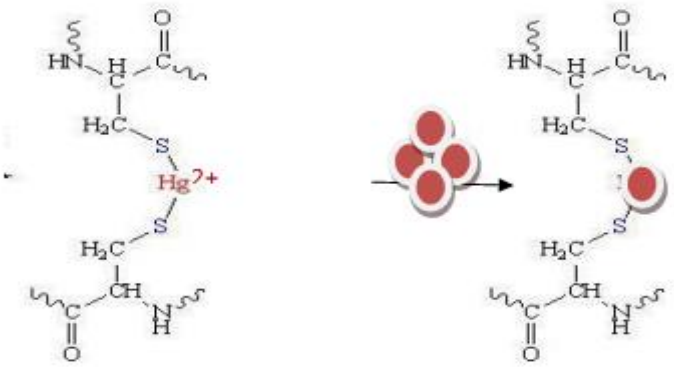

Figure 6.Disulfide-gold NPs interaction to recovery human tissue damage.

Effect of gold NPs (red circle on Figure 5 and Figure 6) on the disulfide bonds can cause two problems. The first is recovery methyl mercury binding that causes breaking disulfide bonds. The disulfide bonds formed again. Disulfide bond is forming the tertiary structure of a protein. This disulfide bond rupture resulting protein loses its biological properties (protein denaturation). After the process recovery protein denaturation can be stopped. The second resolving problem is: if that mercury is forming a disulfide bond replaces previous bridge, gold NPs replaces the bridge and normalizing this condition. Although it seems no effect on the structure initially, the body will naturally detect that there are foreign proteins in the body. Gold NPs effect the body detect that foreign protein have being body's protein. Furthermore, this complex gold NPs-body's protein can stop damage and other proteins that have been formed.

CONCLUSION

- Nanogold can synthesized with glycerin as matrix with diameter of clusters size 10-50 nm in various concentrate. This size of clusters can be used in cosmetic application because these clusters can penetration in pore of skin.
- The size of clusters bigger in higher concentration of nanogold. This phenomenon is caused aggregation process intra two or more clusters.

- Gold and Mercury (Hg) availably form binding with sulfur from Cysteine in human enzyme.
- Gold or gold NPs bioavailable in human but Mercury is not bioavailable, so that gold is allowed in cosmetics but Mercury is very dangerous in cosmetics.

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