Growth Kinetics of Gold Nanoparticle Formation from Glycated Hemoglobin

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ABSTRACT: Gold nanostructures have always been a subject of interest to physicists, chemists, and material scientists. Despite the extensive research associated with gold nanoparticles, their actual formation mechanism is still debatable. The nanoscale rearrangements leading to the formation of gold nanostructures of definite size and shape are contradictory. The study presented in here details out a mechanism for gold nanoparticle formation in the presence of a biological template. The kinetics of gold nanostructure formation was studied using glycated hemoglobin as a biological template as well as the reducing agent. Particle formation was studied in a time- and temperature-dependent manner using different biophysical techniques. Here, we report for the first time spontaneous formation of gold nanostructures which gradually dissociates to form smaller spherical particles. In addition, our experiments conclusively substantiate the existing postulations on gold nanoparticle formation from relatively larger precursor structures of gold and contradict with the popular nucleation growth mechanism.

INTRODUCTION

Gold nanostructures find application in a range of fields of biological, physical, chemical, and medical sciences. The in situ synthesis of gold nanoparticles (GNPs) involves two major reactions, reduction of gold ions to atomic gold and the stabilization of the resultant structures. A number of chemical as well as biological agents are reported to be capable of synthesizing GNPs when used as a template or reducing agent. Among them, trisodium citrate is well studied and frequently employed owing to its use as both reducing agent and stabilizer for the fabrication of a range of gold nanostructures. Recently, biological agents have gained popularity toward their use in GNP synthesis, principally for applications in the field of medical sciences because of its superior biocompatibility and biocompatibility compared to other chemical reducing agents. In the bottom-up chemical synthesis of metallic nanomaterials, the post-reduction growth kinetics of nanostructures has drawn particular attention from scientists all over the world, ever since Turkevich studied the nucleation and growth in gold colloids. Studying the growth kinetics of gold nanostructures is important owing to its versatile application potentials.

Biological and chemical sensing is an emerging application of GNPs considering its unique physical and optical properties. Taking into account the use of GNPs for sensing applications, the mechanism of sensing differs either by synthesis, aggregation, or interaction. Here, aggregation and interaction studies are based on the already prepared GNPs, and sensing enabled through synthesis depends on the growth of GNPs from a template which is the target molecule. Different proteins are reported to carry out the formation of gold nanoclusters based on their activity towards the reduction of gold salt. Extracts from different plant species known to be rich in proteins, sugars, amino acids, and secondary metabolites including flavonoids and alkaloids can direct the growth of GNPs of different sizes and shapes. Leng et al. in 2016 suggested that synthesis of GNPs using different proteins such as hemoglobin (Hb) and myoglobin can be used as a means for sensing of proteins on the basis of colorimetric profile of the formed GNPs. GNPs which are found to be able to differentiate among structural and conformational alterations in proteins are also reported to be capable of sensing the protein conformational changes associated with glycation.

Nonenzymatic glycation is an important physiological phenomena having clinical significances in diabetes and associated complications. The products of nonenzymatic glycation are known to possess high reducing potentials.

In a previous study, it was demonstrated that the color of colloidal GNPs when synthesized using the glycated Hb
(HBF) template varied among differentially glycated samples.27 The present study discusses the biological synthesis of GNPs using a glycated Hb template. Glycated proteins are capable of synthesizing stable gold nanostructures in combination with a chemical reducing agent28 owing to their higher reactivity post glycation. Also, in a previous study, we have found that advanced glycation end products (AGEs) produced as a result of glycation provide a good reducing environment required for the synthesis of GNPs.29 Here, we have performed in vitro glycation of human hemoglobin A0 (Hb) at 37°C by using fructose as a reducing sugar and synthesized GNPs from glycated Hb (HBF) without the use of any additional reducing agents. Unlike the conventional methods reported for chemical or biological syntheses of GNPs, our method was performed at room temperature (RT) (25°C).30 Here, the formation of GNPs was postulated to be mediated by the AGEs produced as a result of the nonenzymatic glycation reaction. The HBF_GNPs thus formed were characterized in detail, and the kinetics of formation of the particles were studied using absorption spectroscopy and electron microscopy. Our kinetically controlled approach for the synthesis of GNPs enabled greater understanding of the growth kinetics of GNPs from a biological template along with identification of predominant intermediates in HBF-mediated GNP synthesis.

**RESULTS**

Characterization of Hemoglobin Glycation and AGE Formation. At first, HBF was characterized for the formation of AGEs and the development of reducing properties, prior to GNP synthesis (Figure 1A). HBF showed significantly higher fluorescence emission at 450 nm (p = 0.0000338) compared to its nonglycated counterpart (HBC), confirming the formation of AGEs as a result of nonenzymatic glycation.32 HBC showed a minimal reducing property as measured by the ferric ion reduction test (Materials and Methods), whereas HBF showed significantly higher reducing activity (p = 0.00000793). Once AGE formation was confirmed, this was used as a template to synthesize GNPs.

**Formation of GNPs from Glycated Hb (HBF).**

Consistent with the reducing properties and presence of AGEs, only HBF produced stable GNPs in colloidal solution (Figure 1B inset). GNPs produced from HBF showed surface plasmon resonance (SPR) peak centered around 525 nm (marked red) (Figure 1B). The HBF_GNPs were found to be stable with a negative zeta potential of −33.8 mV. The size and morphology of the particles were analyzed using transmission electron microscopy (TEM). The population of particles was nearly spherical in shape except for a few elongated structures (Figure 1D). The elongated structures marked in Figure 1D are expected to be the intermediates formed during the synthesis. A single gold particle with the 111 plane marked is shown in the Figure 1D as inset. The mean particle size was calculated to be 16.824 ± 2.998 nm (Figure 1E) which was also confirmed by dynamic light scattering (DLS) size measurements (Figure S1A). The polycrystalline nature of the synthesized particles was confirmed by X-ray diffraction (XRD) (Figure 1C) and selective area electron diffraction (SAED) (Figure 1C inset) and confirmed the growth of particles in 111, 200, 220, and 311 planes of the fcc lattice. The elemental composition of the synthesized particles was analyzed using energy-dispersive X-ray spectroscopy. The presence of gold was confirmed in HBF_GNPs and traces of carbon, oxygen, and nitrogen substantiated the capping of synthesized GNPs with the products of glycation of Hb (Figure S1B). Thus, the use of HBF for the synthesis of GNPs was established, and it was found to direct the synthesis of particles of less than 20 nm diameters with a negative zeta potential.

**Mechanism of Formation of HBF_GNPs.** The kinetics of the reaction involving the synthesis of HBF_GNPs was studied in detail till stable GNPs were formed. After the addition of gold salt and HBF at 25°C, the reaction was monitored up to 303 hrs. Initially, the color of the gold colloid changed from colorless to black and later to purple and finally stabilized as

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**Figure 1.** GNP Formation from HBF. (A) Fluorescence and reducing properties of HBF and HBC, (B) absorption profiles of HBF_GNPs and HBC_GNPs, (C) XRD analysis and SAED of HBF_GNPs (inset), (D) TEM image of HBF_GNPs and a single particle (inset), and (E) size distribution of HBF_GNPs.
as evident from Figure 2C, the intensity of the gold colloid which is a measure of the number of particles present at a distinct time point increased slowly at the start of the reaction followed by a steady enhancement till 50 h followed by its stabilization at around 80 h. Similarly, the SPR peak centered around 545 nm at the start of the reaction first shifted slightly to higher wavelengths and then steadily dropped to 525 nm post 50 h, showing a reduction in particle size with time. The observed nature is in agreement with the color transitions of colloidal solutions over time. The spectroscopic study of the kinetics of GNP synthesis from HBF indicated a mechanism that involves the generation of larger structures of gold which gradually dissociates to smaller nanostructures which is in contrary to the LaMer’s model of nucleation and growth.33

In order to support this notion, TEM analysis was carried out for the gold colloids at different time points during the course of the reaction. Aliquots were taken out at regular intervals and were drop-casted onto carbon-coated copper grids for performing TEM. Figure 3 outlines the maturation of GNP nanostructures from HBF as observed under the electron microscope. TEM images of gold nanostructures with 0.3 mg/mL (Figure 4A–C) concentration of HBF. Figure 3A–G represents the morphology of the nanostructures as viewed under TEM at 1, 12, 18, 36, 44, 65, and 180 h from the start of the synthesis, respectively.

Table 1 summarizes the morphological features of these nanostructures along with the histogram plots of mean diameters. As the reaction progresses, the irregularly shaped slightly elongated nanostructures reshapes into regular particles which are predominantly spherical, whereas the crystallinity of particles at all the time points remained the same, corresponding to a polycrystalline particle with lattice points of an fcc crystal (Figure S2). As observed in here, the diameter of the particles remained more or less the same even though the color of the colloidal solutions was distinct at these time points (Figure 2A). The morphological differences in the gold nanostructures from HBF as observed under the electron microscope hints at a transformation of structures during the course of the reaction rather than growth and maturation of a preformed nuclei.

Structural Evolution of HBF_GNPs. In order to investigate the insights into the structural transformations associated with GNP synthesis from HBF, we lowered the rate of the reaction by reducing the HBF concentration. This allowed us to carefully analyze the structures formed at the start of the reaction. Figure 4 represents the transmission electron micrographs of gold nanostructures with 0.3 mg/mL (Figure 4A–C) concentration of HBF. At this significantly lower concentration of HBF, we observed concurrent occurrence of large networks of gold nanochains with diameters less than 10 nm and gold nanoflowers of 100 nm diameter with each petals having a diameter less than 10 nm (Figure 4D). It is to be noted that at this concentration where larger structures of gold were observed, the colloidal solution of gold remained colorless or grayish in appearance. The inset of Figure 4D shows the electron micrograph of a single flower. With the increase in concentration of HBF, the morphology of particles changed from large networks of gold/gold nanoflowers to irregularly shaped particles to regular spheres (Figure 5A–C) and the size of the particles reduced (Figure 5D,E).

Because HBF_GNPs with the lowest concentration of HBF (0.3 mg/mL) had both chains and flowers, it was difficult to elucidate if the chain evolved from the flowers or chains transformed into flowers. Either way, it was confirmed that the particles formed at the end of the reaction with HBF were generated as breakdown products of the larger structures as the reaction proceeded. Here, as the larger structures broke down, initially, the particles formed were around 12 nm in size (Figure 5D,E), which later rearranged to form spherical particles (Figure 5F). Throughout the concentrations used, the polycrystalline nature of the gold nanostructures remained consistent (Figure S3). Thus, here, we established that in HBF-

Figure 2. Absorption kinetics of HBF_GNP formation. (A) Color profile of HBF_GNP formation, (B) absorption profile of HBF_GNPs, and (C) absorption maxima profile for HBF_GNPs from the start of the reaction (0H) to 303H.

Figure 3. TEM analysis of formation of HBF_GNPs. TEM images of HBF_GNPs at (A) 1H, (B) 12H, (C) 18H, (D) 36H, (E) 44H, (F) 65H, and (G) 180H, respectively.
mediated GNP synthesis, the reaction starts with the formation of large networks of gold which periodically disintegrates to form stable spherical nanoparticles. Although the intermediates during the synthesis ultimately rearranges to form spherical particles, these intermediates were found to be stable for longer periods when stored at 4°C.

### Resolving the Structural Transformations in HBF_GNPs

The results discussed previously confirm the formation of large networks of gold from the HBF template which gradually dissociates to form small spherical particles. In order to differentiate between the stages of formation of gold nanochains and gold nanoflowers during the synthesis, we performed the GNP synthesis at lower temperatures while keeping the concentration of HBF the same and compared the particle formation at two different temperatures. As demonstrated in Figure 6, reaction performed at 25°C generated only flowers (Figure 6A) and the one performed at 37°C formed both nanoflowers and nanochains (Figure 6B). Flowers formed at 37°C in which the reaction was kinetically faster had a denser core compared to the one formed at 25°C. These results suggested that the formation of nanoflowers must have taken place at the start of the reaction which then unfolds to form networks of gold nanochains that further breaks down to form smaller spherical particles.

To further confirm this notion, we took three different samples of HBF generated by decreasing the concentration of protein (2, 1, and 0.5 mg/mL) and used them for the synthesis of GNPs. Although the samples differed in the concentration of the protein (Figure 7A), the amount of AGE was kept the same as indicated in Figure 7B. The reducing property of the three samples also remained more or less the same with 0.5 mg/mL being slightly less reducing in nature (Figure 7C). Figure 7D−F represents the TEM image of nanostructures synthesized from the three different HBF samples. The sample with a higher protein concentration (2 mg/mL) generated both nanoflowers and nanochains (Figure 7D), whereas the samples with lower protein concentrations (1 and 0.5 mg/mL) generated only nanoflowers (Figure 7E,F). Here, the density of the core of the nanoflower and the diameter decreased as the protein concentration decreased. Here, although reducing power was a constant, the amount of protein in each samples determined the kinetics of the reaction, wherein availability of higher number of templates (2 mg/mL protein) enhanced the rate of reaction considerably.

In all, these results confirmed the formation of spherical nanoparticles from larger structures of gold when HBF was used as a template as well as the reducing agent. Evident from

### Table 1. Particle Size Distribution of HBF_GNPs with Time

| Time point | Particle morphology | Size Distribution | Particle Size (nm) |
|------------|---------------------|-------------------|--------------------|
| 1 Hour     | Polydispersed, Flower like morphology | ![Size Distribution](image) | 61.93 ± 13.512nm |
| 12 Hours   | Polydispersed, elongated, nearly spherical morphology | ![Size Distribution](image) | 22.359 ± 6.321nm |
| 18 Hours   | Polydispersed, elongated, nearly spherical morphology | ![Size Distribution](image) | 19.330 ± 6.582nm |
| 36 Hours   | Polydispersed, nearly spherical morphology | ![Size Distribution](image) | 18.031 ± 4.630nm |
| 44 Hours   | Polydispersed, nearly spherical morphology | ![Size Distribution](image) | 20.980 ± 4.850nm |
| 65 Hours   | Polydispersed, nearly spherical morphology | ![Size Distribution](image) | 21.930 ± 2.423nm |
| 180 Hours  | Polydispersed, nearly spherical morphology | ![Size Distribution](image) | 20.662 ± 2.992nm |

Figure 4. Structural evolution of HBF_GNPs. TEM images of HBF_GNPs synthesized by using (A−C) 0.3 mg/mL concentration of HBF and (D) the size distribution of flower-like particles (inset: a single flower of gold, scale bar = 20 nm).
the TEM studies, HBF seeds the gold atoms and are self-assembled to form bulky flowers of gold which gradually unfolds into long networks of nanochains which further dissociates to form regular spherical GNPs.

**DISCUSSION**

According to the LaMer nucleation growth model, in a typical citrate reduction approach, nuclei are formed as a result of random collision of gold atoms, and the nucleus grows progressively till the size of the particles is stabilized. Polte et al. in 2010 studied the mechanism of GNP formation in the classical citrate reduction method using coupled in situ X-ray absorption near-edge spectroscopy and small-angle X-ray spectroscopy (SAXS) and proposed a model in which coalescence of nuclei and further attachment of monomers result in particles of desired size. But the development of colours during the reaction of GNP synthesis cannot be explained by this.

Our results substantiate the presence of larger intermediates during the reaction of GNP synthesis (Figures 2−4). Even at different concentrations of the HBF used, network-like structures of gold were observed which entitles the same as an intermediate during the reaction rather than being produced at a particular concentration of the HBF used. For the first time, we here report the spontaneous formation of gold nanoflowers with the use of HBF as a template as well as the reducing agent. These structures were stable for longer time periods and do not undergo any structural alterations when stored at 4 °C. Although the flower is formed as an intermediate during the reaction, the seeds formed at the start

![Figure 5](image-url) **Figure 5.** Structural evolution of HBF_GNPs. TEM images of HBF_GNPs synthesized by using (A) 0.6, (B) 0.8, and (C) 1 mg/mL of HBF, and (D−F) the respective particle size distributions.

![Figure 6](image-url) **Figure 6.** HBF_GNP synthesis at two different temperatures. TEM image of HBF_GNP synthesized at (A) 25 and (B) 37 °C. Insets showing the TEM image of a single gold nanoflower (scale bar = 20 nm).

![Figure 7](image-url) **Figure 7.** HBF_GNP synthesis with different protein−protein concentrations. (A) Intrinsic protein fluorescence (excitation at 280 nm), (B) AGE fluorescence (excitation at 350 nm), and (C) reducing properties of HBF samples with Hb concentration 2, 1, and 0.5 mg/mL, respectively. TEM images of HBF_GNPs synthesized from HBF with Hb concentrations of (D) 2, (E) 1, and (F) 0.5 mg/mL.
were anisotropic in nature which is highly unlikely in a typical GNP synthesis approach.

In 2004, in an attempt to synthesize 2D networks of gold, Pei et al. showed that once the nucleus was formed, gold atoms were found to be attached to the nucleus in a strong manner, forming elongated chain-like structures of gold and as the reaction proceeded, particles were formed from the chains.\(^{35}\) Although subsequent studies substantiated this observation, the phenomenon could not be explained in a unified manner\(^ {36–38}\) and it was only in 2017 that Jakhmola et al. confirmed the formation of networks of gold as an intermediate during GNP synthesis involving lower reaction rates.\(^{39}\) In 2018, Lee et al. reported the spontaneous formation of gold nanostructures in aqueous microdroplets. In this kinetically controlled reaction, linear nanowires and aggregated structures of gold were observed. However, rather than referring to it as an intermediate of GNP formation, this study reported the spontaneous formation of different nanostructures.\(^ {40}\) Also, recently, Yan et al. reported the controlled synthesis of branched structures of gold with the use of engineered peptoids, which are structurally similar to what we obtained as an intermediate in our work.\(^ {41}\) A number of perspectives still remain unanswered regarding the kinetics associated to GNP formation. Taking into account the enormous reports published in this area, scientists were able to confirm the presence of large intermediates during the formation of GNPs using TEM, atomic force microscopy, and DLS, whereas in situ SAXS and XPS failed to catch the intermediates or these techniques captured the progressive growth of nanoparticles in size during the reaction. Our study clearly established the presence of mesoscale structures of gold during the synthesis, proving the formation of smaller spherical nanoparticles from larger intermediates. The parameters to control the growth of nanoparticles were kept the minimum as in the synthesis was performed at RT, and no external mixing was provided. The entire synthesis was dependent on the concentration of the reactants used.

From what we observed in our study and what is already available in the literature, the evolution of mesoscale structures of gold can be regarded as an intermediate during GNP synthesis when carried out at extremely low reaction rates. In this study, the low reaction kinetics was obtained by using HBF, which acts as both reducing agent and template. Earlier reports suggest that the mesoscale gold nanostructures can be obtained with the commonly used reducing agent trisodium citrate as well; hence, the evolution of such structures must be dependent on the rate of reduction of gold ions to gold atoms which in turn relies on the concentration of the reactants and temperature. In a report published in 2008 by Ji et al., pH of the reaction mixture was found to be a determining factor for the particle formation through nucleation growth or nucleation aggregation.\(^ {42}\) Though conclusive reports are not available stating whether particle formation occurs through nucleation growth or breakdown of mesoscale structures or it is a combination of both, using HBF as a template, we could prove important aspects involved in the mechanism of GNP formation that could add to the long debated postulations on GNP synthesis.

**CONCLUSIONS**

Studying the mechanism of GNP formation from different templates is an important aspect owing to the ever growing applications of gold nanostructures in biological, physical, and chemical sciences. Our study aimed at studying the general route of GNP formation using a biological reducing agent. In order to draw any similarities or differences, we compared the synthesis with that from the commonly used reducing agent TSC as well. Our results substantiated the existence of mesoscale structures of gold during the synthetic route for particle formation, which was clearly established using electron micrographs and spectroscopic studies. This study contradicts the well-known LaMer model of nucleation and growth\(^ {33}\) which states that gold atoms fuse to form nuclei and combines to form particles until a stable size is approached. Several studies later were able to prove that a typical synthetic protocol for the synthesis of GNPs involves the formation of larger intermediates of gold which gradually breaks down to form stable GNPs.\(^ {36,38,39,42}\) Although our results clearly explains the presence of mesoscale structures during the formation of GNPs, we observed the formation of anisotropic nuclei at first which grows into a marigold-like gold nanoflower. These larger structures of gold had diameters of more than 100 nm, which further unfold into linear networks and finally break down to particles of around 20 nm in diameter. These mesoscale structures of gold formed during the synthesis of GNPs can find applications in sensing, catalysis, and drug delivery owing to their distinct structural aspects. Also, results from this study is assumed to contribute to the many of the quests that still remains unclear in the colloidal synthesis of GNPs.

**MATERIALS AND METHODS**

**Materials.** Hemoglobin A0 (Hb) was purchased from Sigma-Aldrich India Pvt. Ltd. Hydrogen tetrachloroaurate-(III) trihydrate (HAuCl₄·3H₂O) was purchased from Loba Chemie. Fructose, potassium dihydrogen orthophosphate (KH₂PO₄), dipotassium hydrogen orthophosphate (K₂HPO₄), sodium dihydrogen orthophosphate (NaH₂PO₄), disodium hydrogen orthophosphate (Na₂HPO₄), potassium ferricyanide (K₃[Fe(CN)₆]), trichloroacetic acid (TCA), ferric chloride (FeCl₃), and hydrochloric acid (HCl) were of analytical grade purchased from Thermo Fisher Scientific India Pvt. Ltd and used without further purification. Milli-Q ultrapure water (>18 MΩ) was used for all of the experiments.

**Methods.** Glycation of Hemoglobin (Hb). Glycation of Hb was performed under sterile conditions, and all of the glassware and plastic were autoclaved before use. Stock solutions of Hb and fructose were prepared in 100 mM potassium phosphate buffer (pH 7.4). For glycation, 1 mg/mL Hb was incubated with 100 mM fructose in an incubator set at 37 °C. The concentration of reactants for the preparation of glycated Hb (HBF) was kept a constant unless mentioned. Samples were taken after 0 and 10 days of incubation and stored at −20 °C until used. The formation of AGEs in the HBF samples was confirmed using UV-visible absorption spectroscopy and fluorescence emission spectroscopy. The controls for the glycation experiment consisted of the physical mixture of fructose and Hb without incubation (HBC) and fructose alone (F).

**Evaluation of Reducing Properties of HBF and HBC.** The reducing property of HBF was determined by the method described by Gu et al. with slight modifications.\(^ {43}\) HBF was mixed with 1 mL of 200 mM sodium phosphate buffer of pH 7 and 1 mL of 1% potassium ferricyanide following which it was incubated in a water bath set at 50 °C for 20 min. The mixture was brought back to RT and mixed with 1 mL of 10% TCA. To 1 mL of this, 1 mL of distilled water and 200 μL of 0.1%
ferric chloride were added. The light absorbance of the resultant mixture was measured at 700 nm in a 1 mL quartz cuvette of 1 cm path length using PerkinElmer, LAMBDA 25 UV–visible Spectrometer. For comparison, the reducing property of HBC was measured and 100 mM potassium phosphate buffer of pH 7.4 served as the negative control. A higher absorbance at 700 nm indicates a higher reducing property.

Synthesis of GNPs from HBF (HBF_GNPs). All of the glassware used for GNP synthesis were washed with 1 N HCl and rinsed with ethanol and ultrapure water. Briefly, 32 μL of aqueous solution of HAuCl₄ (1% w/v) was added to 3.868 mL of Milli-Q water. HBF (100 μL) (1 mg/mL) was then added to the gold salt solution and mixed for 1 min. The reaction mixture was left at 25 °C and the color change of the gold colloid from gray to purple to pink was observed till it stabilized. As a negative control, synthesis was also attempted with HBC.

Physical Characterization of GNPs. The extinction profile for the synthesized HBF_GNPs was obtained from the UV–visible absorption spectra performed using the LAMBDA 25 UV–visible spectrometer, PerkinElmer. Spectra were obtained by performing a scan from 200 to 700 nm in a 1 mL quartz cuvette of 1 cm path length. The gold colloids were diluted with 100 mM potassium phosphate buffer of pH 7.4 before performing the scans.

The size and morphological features of the HBF_GNPs and SAED pattern were obtained by performing electron microscopy using the transmission electron microscope, JEOL 2100F, with an incident energy of 200 keV. Prior to viewing, the GNPs were concentrated by centrifugation of the colloidal gold solutions, and the concentrated samples were drop-casted onto copper grids of 300 mesh size.

The crystallinity of the HBF_GNPs was investigated using X-ray diffraction (PANanalytical Xpert PRO) with Cu Kα radiation (λ = 1.54 Å). For this, thin films of HBF_GNPs were prepared on clean glass slides by repeated drop-casting. The elemental analysis of GNPs was performed using the scanning electron microscope coupled with energy-dispersive X-ray spectroscopy (Zeiss EVO40). Zeta potential and particle size analysis using DLS were carried out with Zetasizer Nano Z.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b02200.

Physical and chemical characterization of HBF_GNPs, SAED for HBF GNP at different time points of formation, SAED for HBF_GNPs with different concentrations of HBF (PDF)

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J.B. and R.G.M. have conceptualized the work. A.A.M. has performed all of the experiments. A.A.M., J.B., R.G.M., and S.J. have taken part in the writing the manuscript.

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Abbreviations
HBF, glycated hemoglobin; GNPs, gold nanoparticles; AGEs, advanced glycation end products

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