**FORM 2**

**THE PATENTS ACT, 1970**

**(39 OF 1970)**

**&**

**The Patents Rules, 2003**

**COMPLETE SPECIFICATION**

**(Refer section 10 and rule 13)**

**TITLE OF THE INVENTION:**

**A METHOD FOR PREPARING MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

**2. APPLICANT:**

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(B) NATIONALITY: Indian

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Chennai - 600 036

**3. Preamble to the Description**

**COMPLETE SPECIFICATION**

The following specification describes the invention

## COMPLETE SPECIFICATION

## TITLE OF THE INVENTION

# A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS

## FIELD OF THE INVENTION

The present invention relates to a method for preparing monolayer protected silver clusters as antibacterial agents.

## BACKGROUND OF THE INVENTION

Few-atom noble metal clusters or quantum clusters (QCs) with discrete electronic structure, exhibiting distinct HOMO-LUMO transitions in optical absorption, intrinsic magnetism, enhanced photoluminescence, and modified redox properties are the materials of interest. These properties are substantially different from larger metallic nanoparticles which exhibit surface plasmon resonance arising from the coherent oscillations of free electrons in the conduction band. QCs are good candidates for applications in areas such as catalysis, nanoelectronics, sensing, etc. Several such clusters of noble metals have been synthesized using various templates such as peptides, thiols, dendrimers, and proteins. Among them, the thiol protected ones are widely studied with diverse techniques as well-defined compositions can be obtained. QCs are also called by other names in the scientific literature such as atomically precise clusters, artificial atoms, monolayer protected clusters or simply, clusters.

Varity of chemical and physical methods have been developed to produce them and most of the synthetic routes are limited to gold QCs. Although gold and silver belong to the same group, because of differences in their chemical reactivity, the area of silver QCs has not been exploited significantly. Silver may be a better system for sensor applications due to the high extinction coefficient. *Angew. Chem. Int. Ed.* 2010, 49, 3925-3929 and *J. Am. Chem. Soc.* 2010, 132, 16304-16307 demonstrate the possibility to synthesize thiol protected silver clusters using interfacial and solid state routes. Glutathione protected clusters may be prepared through high

temperature nucleation route as well as reported in *Chem. Commun.* 2012, 48, 6788-6790. *J. Am. Chem. Soc.* 2009, 131, 16672-16674 describes dimercaptosuccinic acid protected $Ag_7$ quantum clusters. *J. Phys. Chem. C* 2010, 114, 16010-16017 describes glutathione (SG) protected silver clusters in a single step. In all of the cases, synthesis involves the use of chemical reducing agents such as $NaBH_4$, HCOOH, etc. However, it is a challenge to synthesize the desired materials using natural resources. The preparation of plasmonic nanoparticles using natural sources such as light irradiation and by using materials of plant origin is described in *Chem. Commun.* 2002, 792-793, *Photochem. Photobiol. Sci.* 2005, 4, 154-159 and *Journal of Colloid and Interface Science* 2004, 275, 496-502. There are also other methods such as microwave irradiation.

PCT Publication No. WO 2012/090034 describes the preparation of gold and silver quantum clusters using glutathione to form a composition. Related descriptions may be found in US Patent Application No. 2011/111002, US Patent No. 5,514,501, US Patent Application No. 2010/215766, PCT Publication No. WO 2011/124187, PCT Publication No. WO 2012/033097, *Langmuir*, 2006, 22(26), 11376-11383, *Nano Reviews*, 2012, *Langmuir*, 2012, 28(24), 8915-8919, *Journal of American Chemical Society*, 2010, 132(46), 16304-16307, *ACS Applied Materials & Interfaces*, 2010, 2(4), 1206-1210, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2011, 79, 594-598, *Materials Letters*, 2011, 65, 999-1002, and *Nanomedicine: Nanotechnology, Biology and Medicine*, 2012, 8, 37-45.

A scalable, cheaper and environmentally friendly method involving natural sources is a need of the art for preparing monolayer protected silver clusters.

## SUMMARY OF THE INVENTION

The present invention provides a method for preparing monolayer protected silver as well as other metals such as Au, Pt, etc. quantum clusters (AgQCs), wherein the method comprises,

a) sonicating a mixture of silver nitrate and glutathione (GSH)/mercaptosuccinic acid (MSA)/cysteine in an aqueous base to obtain a solution containing oligomeric silver thiolate or other metal thiolates,

b) mixing the solution containing oligomeric silver thiolate or other thiolates with a gel solution to obtain a uniform solution,

c) treating the solution obtained in step b) with ammonium persulfate (APS) and N,N,N',N'-tetramethylethylene diamine (TEMED) to initiate polymerization and formation of a gel, other organic gels such as polyethylene glycol,..... can also be used.

d) subjecting the mixture obtained in step c) to sunlight or other light sources for a sufficient time to obtain monolayer protected silver or other quantum clusters, and

e) isolating monolayer protected silver quantum clusters or other clusters from the mixture thereof.

The silver quantum clusters of the present invention exhibit enhanced antibacterial activity. The present method is scalable, cheaper and environment-friendly involving the use of natural resources.

**BRIEF DESCRIPTION OF THE DRAWINGS:**

**Figure 1** depicts I) photograph of the polymerized form of acrylamide gel along with oligomeric Ag(I)SG, taken in a petri dish. II), III), IV), V) and VI) are photographs corresponding to the exposure to sunlight at different time intervals such as 5 minutes, 30 minutes, 1 h, 3 h, and 6 h, respectively. Petri dish was placed on a white paper with a label, 'Ag' printed on it. The change in transparency may be seen from I) to VI). The gel shrinks upon irradiation as water evaporates and the dry gel gets detached from the petri dish.

**Figure 2** depicts UV/Vis spectrum of the prepared AgQC. Dominant spectral peaks at 480 nm and 650 nm are marked on the enlarged optical spectrum. Insets: I) Photograph of the gel templates containing silver cluster after exposure to sunlight for 6 h. II) Extracted clusters which are readily soluble in water whereas the gel was insoluble and the solution appears dark brown in color. III) Upon dilution to 1000 times, the solution appears reddish brown whose spectrum is shown as the main figure.

**Figure 3** depicts I) luminescence spectra of the as-synthesized cluster, which shows three excitation wavelengths and all of these give emission at the same wavelength. II) X-ray

photoelectron spectrum of the cluster in the Ag 3d region. The corresponding peaks are assigned. The peaks are fitted with spin-orbit split components.

**Figure 4** depicts I) UV/Vis spectrum of the AgQCs prepared using various filters such as without filter (CW), blue (CB), red (CR), yellow (CY) and green (CG). II) Photographs of AgQC clusters under visible light (b, a1) and UV light (a, $b^1$), before (a, b) and after ($a^1$, $b^1$) phase transfer.

**Figure 5** depicts I) Inset (1 to 6) are photographs of AgQC synthesized without gel precursors (APS and TEMED) at different time intervals of exposure to sunlight, starting form initial to 6 h. UV/Vis spectrum of AgQC cluster extracted from VI. II) UV/Vis spectra of AgQC synthesized in different media. Among them, acetonitrile shows enhanced intensity.

**Figure 6** depicts the antibacterial activity of I) monolayer protected AgQCs compared with II) glutathione, III) Ag (I)@SG, IV) Au25@SG and V) Ag@SG nanoparticle. In all the cases, petri dish of 2.5 cm diameter and escherichia coli bacteria (ATCC 8739) were used.

**Figure 7** depicts I) polymerized acrylamide gel along with Ag (I) MSA. Photographs are of different periods of exposure of sample to sunlight. II) UV/Vis spectrum of Ag@MSA clusters extracted after 6h of exposure. Inset of II) shows a photograph of the sample collected under UV lamp showing red luminescence.

**Figure 8** depicts polymerized acrylamide gel along with Ag(I)cysteine. I) to VI) are different periods of exposure of sample I) to sunlight. Inset is the UV/Vis spectrum of Ag@cysteine clusters extracted from sample VI).

**Figure 9** depicts the photographs of gold cluster made using the substantially same synthetic method. I) Under visible light, and II) under UV light. Intense red fluorescence confirms the formation of clusters.

**Figure 10** depicts polyacrylamide solution along with Ag (I) SG was spread on a glass plate and kept under sunlight for six hours to complete the reaction. I) to VI) show the progress of the reaction with time.

**Figure 11** depicts photograph of the gel template containing Ag@SG clusters. II) These templates were soaked in water for 30 min. III) The clusters were dispersed and the gel remained insoluble.

**Figure 12** depicts time dependent UV/Vis spectra during Ag@SG cluster evaluation. Corresponding photographs are shown in inset (I). HRTEM image of the cluster after 6 h irradiation is given as inset (II).

**Figure 13** depicts XPS survey spectrum of the as-synthesized cluster. Individual peaks are labeled.

**Figure 14** depicts XPS spectra for individual regions. Spectra were fitted using Casa XPS software.

**Figure 15** depicts comaprative IR spectra of AgQCs and GSH. The absence of band at 2552 cm-1 confirms the attachement of glutathione to the cluster core.

**Figure 16** depicts ESI mass spectrum of as-synthesized cluster in negetive mode. Inset shows some fragments with good isotope distribution.

**Figure 17** depicts Exciation and emission spectra of $C_R$ (I), $C_Y$ (II), $C_G$ (III) and $C_B$ (IV).

**Figure 18** depicts I) photograph of the polymeric gel + Ag(I)SG taken in a petri dish kept in dark. Note that gel is transparent. II) After six hours, the same petri dish shows no change in color indicating the absence of reaction under dark conditions.

**Figure 19** depicts I) polymerized acrylamide gel along with Ag(I)SG. II) Sample covered with aluminium foil and exposed to sunlight in outdoor air. III) No visible color change after 6 h of exposure to sunlight. The ambience was at 35°C.

**Figure 20** depicts UV/Vis spectrum of the material formed when acetonitrile was taken in place of gel. Inset shows photographs at different time intervals. This shows the formation of plasmonic nanoparticles.

**Figure 21** depicts effect of various solvents: (I) toluene, II) methanol, III) acetonitrile, IV) tetrahydrofuran, V) dimethyl formamide and VI) water during cluster growth. Solvent volume was kept constant. In water, the clusters were extracted.

Referring to the drawings, the embodiments of the present invention are further described. The figures are not necessarily represented to scale, and in some instances the drawings have been exaggerated or simplified for illustrative purposes only. One of ordinary skill in the art may appreciate the many possible applications and variations of the present invention based on the following examples of possible embodiments of the present invention.

**DETAILED DESCRIPTION OF THE INVENTION**

One aspect of the present invention provides a method for preparing monolayer protected silver quantum clusters (AgQCs), wherein the method comprises,

a) sonicating a mixture of silver nitrate and glutathione (GSH) in an aqueous base to obtain a solution containing oligomeric silver thiolate,

b) mixing the solution containing oligomeric silver thiolate with a gel solution to obtain a uniform solution,

c) treating the solution obtained in step b) with ammonium persulfate (APS) and N,N,N',N'-tetramethylethylene diamine (TEMED) to initiate polymerization and formation of a gel,

d) subjecting the mixture obtained in step c) to sunlight for a sufficient time to obtain monolayer protected silver quantum clusters, and

e) isolating monolayer protected silver quantum clusters from the mixture thereof.

The gel solution is used in step b) to control the growth of AgQCs. The gel may be, for example, acrylamide:bisacrylamide. Gel solutions are synthesized by following the method reported in *J. Hazard. Mater.* 2012, 211-212, 396-403 with appropriate modifications. The gel solution may be prepared by dissolving acrylamide and bisacrylamide in 50 ml of water followed by sonication to get a clear solution. The gel solution may be stored at about 10°C.

A mixture of silver nitrate and glutathione (GSH) in an aqueous base is sonicated to obtain a solution containing oligomeric silver thiolate. The aqueous base may be 1 M sodium hydroxide. the sonication is carried out until the solution color changes from turbid to clear pale yellow which is due to the formation of oligomeric silver thiolate. The solution containing oligomeric silver thiolate is mixed with the gel solution to obtain a uniform solution. The solution obtained in above step is treated with ammonium persulfate (APS) and N,N,N',N'-tetramethylethylene diamine (TEMED) to initiate polymerization and formation of a gel. It may be achieved by keeping the sample without disturbance. The polymerization and formation of the gel may be visible after about 15 to about 20 minutes.

The mixture obtained above is subjected to sunlight for a sufficient time to obtain monolayer protected silver quantum clusters. The mixture may be kept under sunlight for about 5 to about 10 hours, for example, about 6 hours to yield AgQCs. Formation of AgQCs may be visible by a change of color, for example, from colorless to black-brown.

The monolayer protected silver quantum clusters are isolated from the mixture thereof. For example, addition of water to the gel results in the extraction of the cluster selectively, leaving gel pieces at the bottom. They may be separated from the cluster solution through filtration, followed by centrifugation. The final cluster solution may be subjected to freeze drying to yield a powder sample. Cluster may be phase transferred to the organic medium to observe its enhanced luminescence properties.

Initially, the silver thiolate containing polymerized gel appears transparent. Upon exposure to sunlight, there is a gradual color change form colorless to light yellow and finally to brown-black

(Figure 1). At the same time, the softness of the gel decreases and it becomes harder, due to evaporation of water. The conversion to brown-black color indicates the completion of the reaction and formation of the clusters. In conventional synthesis, the initially turbid metal thiolates become dark brown or black upon addition of an external reducing agent such as sodium borohydride, due to the formation of clusters or nanoparticles. Figure 1 shows the evolution of cluster formation with the duration of light exposure as manifested by the change in color of the gel (All the images are collected from the same petri dish).

During the progress of the reaction, the transparency of the gel is reduced and finally the gel becomes opaque due to cluster formation (Figure 1). The method of the present invention is scalable, for example, to make hundreds of milligram quantities of AgQCs in a single step, by increasing the amount of reagents used in the reaction.

The present method is capable to get clusters protected with other ligands including mercaptosuccinic acid (Figure 7) and cysteine (Figure 8). The gold clusters preparation is also possible by following substantially the same method described in the present invention (Figure 9).

Another aspect of the present invention provides a method of using monolayer protected silver quantum clusters (AgQCs) as an antibacterial agent. The monolayer protected silver quantum clusters (AgQCs) of the present invention is useful treat bacterial infections, for example, the infections caused by Gram negative organism. The monolayer protected silver quantum clusters (AgQCs) may be administered through appropriate dosage forms, for example, solid orals, injections, liquid orals, topical applications, and the like.

UV/Vis spectra were measured with a Perkin Elmer Lambda 25 instrument in the range of 200-1100 nm. Luminescence measurements were carried out on a Jobin Yvon NanoLog instrument. The band pass for excitation and emission was set as 2 nm. X-ray photoelectron spectroscopy (XPS) measurements were conducted using an Omicron ESCA Probe spectrometer with polychromatic MgKα X-rays (hυ=1253.6 eV). The samples were spotted as drop-cast films on a sample stub. Constant analyzer energy of 20 eV was used for the measurements. High

resolution transmission electron microscopy of clusters was carried out with a JEOL 3010 instrument. The samples were drop casted on carbon-coated copper grids and allowed to dry under ambient conditions. FT-IR spectra were measured with a Perkin Elmer Spectrum One instrument. KBr crystals were used as the matrix for preparing samples.

*E. Coli* (ATCC 8739) bacterial cells (1*10-8 CFU /ml CFU- Colony Forming Unit.) were grown at the growth phase on agar-agar gel.

It may be appreciated by those skilled in the art that the drawings, examples and detailed description herein are to be regarded in an illustrative rather than a restrictive manner.

## EXAMPLE

### METHOD FOR PREPARING MONOLAYER PROTECTED SILVER QUANTUM CLUSTERS AND ANALYSIS

10 g of acrylamide and 0.75 g of bisacrylamide were dissolved in 50 ml of water and sonicated to get a clear solution (which was stored at 10°C and used as stock solution). 47 mg of silver nitrate and 110 mg of GSH were added to 1 mL of 1M NaOH; the resultant mixture was sonicated until the solution color changes from turbid to clear pale yellow which is due to the formation of oligomeric silver thiolate. 3 mL of the former solution was poured into a petridish which contains 1 mL of silver thioalate and the two were mixed well to form a uniform solution. Then 30 uL of APS (0.1%) and 20 µL TEMED were added to intiate the poymerization. Upon keeping the sample without disturbance, polymerization and formation of the gel was visible after 15-20 min. Sample was kept under sunlight for 6 hours (starting at 9 am, in open air) to yield AgQCs. Formation of AgQCs was visible by a change of color form colorless to black-brown. Addition of 10 mL water to the gel results in the extraction of the cluster selectively, leaving gel pieces at the bottom. These were separated from the cluster solution through filtration, followed by centrifugation. The final cluster solution was subjected to freeze drying to yield a powder sample. Cluster was phase transferred to the organic medium to observe it's enhanced luminescence properties.

Ag@SG nanoparticles were made with $AgNO_3$ (47 mg) and GSH (110 mg in 50 mL water) followed by rapid addition of $NaBH_4$ (90 mg in 12.5 mL water). It was further purified by ethanol washing and dried in rotavapor.

During the progress of the reaction, the transparency of the gel was reduced and finally the gel became opaque due to cluster formation (Figure 1). In a typical synthesis, about 70 mg of the cluster powder was obtained following the present method. For larger scale synthesis, 50 ml of the acrylamide gel solution was used and the reagents necessary for polymerization along with Ag(I)SG were taken in a 3'x2' glass tray and the polymerization was started. The tray was irradiated to make the clusters. Photographs of the process are given in Figure 10.

When the cluster formation was complete, the gel appeared like flakes as shown in Figure 2 (inset I). For extraction of the clusters, water was added to the final hard gel and the solution was kept at about 20°C for about 1 h (Figure 11). Clusters were extracted to water while a colorless gel residue settled at the bottom. A peak at 480 nm (2.6 eV) and a shoulder at 640 nm (1.9 eV) were observed in the UV/vis spectrum of the cluster (Figure 2). The cluster in water showed a dominant step-like behavior, typical of this size regime, indicating that the material is composed of a few atoms.

The cluster in water was stable for several months without change in its absorption peaks. Cluster in high concentrations appeared to have brown-black color (Figure 2II) but upon 1000 times dilution, the color became reddish brown (Figure 2II). Time dependent profiles showed that the process of reduction was slow and it took nearly about 6 h to get AgQCs. In the initial stages of the reaction, a broad peak at 480 nm appeared (1 h), which became narrow with time and increased in intensity. No observable changes in absorption profiles and peak intensities were seen even after about 6 h of irradiation time. The evolution of peaks within 6 h indicated that it was a slow reaction. Time dependent UV/Vis for the evolution of cluster is provided in Figure 12. Clusters appear as tiny dots in TEM (Figure 12). Nanoparticles of larger dimension were not observed.

The cluster exhibits luminescence at 650 nm at all (395, 440 and 518 nm) excitation wavelengths (Figure 3I). The emission and excitation are comparable with the cluster reported in *J. Hazard. Mater.* 2012, 211-212, 396-403. In view of the specific optical absorption and emission features and due to the fact no nanoparticles are seen in the TEM image, it may be concluded that atomically precise clusters are formed in the method of the present invention.

The thiolate form of the glutathione being attached to cluster core is supported by XPS (Figure 3II). XPS survey spectrum of the clusters shows all the expected elements (Figure 13). Ag $3d_{5/2}$ (BE of 368.1 eV) supports the Ag(0) state (Figure 3II). It may be noted that there is not much difference in BE between Ag(0) and Ag(I) states. The S $2p_{3/2}$ BE is thiolate-like and a value of 162.0 eV is observed (Figure 14). An additional S $2p_{3/2}$ peak at 164.1 eV observed upon peak fitting may be due to other ligand binding sites or X-ray induced damage of the monolayer. IR spectrum of the cluster shows that the cluster is connected to glutathione through the thiolate link (Figure 15). The S-H stretching at 2552 $cm^{-1}$ of glutathione is absent in the case of the cluster. ESI MS analysis (in negative mode) was performed to know the composition of the cluster but only some fragmented ions with good isotopic distribution (Figure 16) was seen. The peaks corresponding to m/z 933 and 1443 were assigned to $Ag_3(SG)_2^{2+}$ and $Ag_4(SG)_3^{+}$. Upon a closer view, another peak with a difference of m/z 22 was seen for both the cases which could be attributed to replacement of Na in place of H. Efforts are going on to find the composition of the cluster. The tiny quantities of the polymeric gel remaining with the cluster seem to prevent it from creating intact ions in the gas phase. ESI MS analysis of silver cluster has been difficult in a number of cases.

Several experiments were performed by exposing the sample to sunlight under different filters. Red, blue, green and yellow filters were used to allow specific light to be exposed to the sample. Depending on the filter used in the synthesis, these clusters are labeled $C_W$, $C_R$, $C_B$, $C_G$ and $C_Y$ (W, R, B, G and Y refer to without filter, with red, blue, green and yellow filters, respectively). The clusters formed were extracted into water. Variation in the absorption profiles were observed by varying the filter as shown in Figure 4. At the same time, few similarities were also there, like the 480 nm peaks appeared for both the CW and CR cluster, but with different width. All these clusters show intense luminescence in the red region. Their QY is of the order of

10-3. Clusters exhibit significant differences in their excitation and emission profiles. Excitation and emission spectra for CW, CR, CB, CY, CG clusters are given in Figure 17. As these clusters are protected with glutathione which is a dicarboxylic acid, it is possible to connect polar end of quaternary ammonium salts so that the resultant cluster is soluble in organic solvents. This kind of phase transfer helps in the exploration of properties of the clusters in both organic and aqueous phases, in detail. The procedure of phase transfer is given in the supporting information. Absorption profiles of phase transferred clusters are not changed. Luminescence still remained as in the case of parent clusters indicating that the cluster core is intact even after phase transfer. Photographs of the $C_W$, $C_R$, $C_B$, $C_Y$ and $C_G$ clusters in water and toluene before and after phase transfer in both ambient and UV light are given in Figure 4II (a, $a^1$, b and $b^1$). Several control experiments were done to understand the formation and to improve the yield of the cluster. The same reaction in the absence of sunlight did not produce clusters showing the importance of light exposure for the synthesis (Figure 18). Petri dish containing polymerized acrylamide gel with Ag(I)SG was kept under the sun but covered with aluminum foil so that there was no light penetration while there was heat input. After 6 h of exposure, there was no observable color change during the reaction (Figure 19). This ruled out the possibility of any thermal effect in cluster synthesis. Upon sunlight exposure of aqueous oligomeric Ag (I) SG, silver clusters were not formed. It shows featureless UV/Vis spectra which resembled the spectrum of oligomeric Ag(I)SG (figure not shown). In the absence of gel polymerizing agent (APS and TEMED), clusters were formed but optical features were not prominent. The photographs and UV/Vis spectra of cluster, synthesized without gel precursors are given in Figure 5I.

In another experiment, Ag(I)SG was taken in acetonitrile and exposed to sunlight for 8 h (Figure 20). A color change from blackish powder to reddish brown powder occurred indicating the reduction of silver. The final powder was insoluble in acetonitrile but dispersible in water. UV/Vis spectrum of the sample in aqueous medium showed a surface plasmon resonance at 400 nm indicating the formation of silver nanoparticles (Figure 20). In the case of acetonitrile, the growth step was not controlled so it produces nanoparticles whereas in the gel system, growth of clusters was controlled within the gel templates.

It is known that the quantum efficiency of absorption (number of photons absorbed/total number of photons) plays an important factor for the conversion of Ag(I) to Ag(0). This can be improved by choosing a given solvent as photochemical reaction in the liquid phase proceeds under the influence of the solvent cage. Not wishing to be bound by any theory, the primary reaction of photo-reduction is assumed to be electron transfer from the solvent molecule to the silver ion: $(Ag^{+}, ROH/NH_2/CN)_{cage} + hv \rightarrow (Ag, ROH+/NH_2+/CN+)_{cage}$. Cluster formation is initiated by the photons of sunlight, through the influence of the solvent cage and then through autocatalytic nucleation process further growth happens. Growth is controlled by the concentrations, ligand as well as the cavity available in the gel. Several other aspects such as temperature may also have influence on cluster formation.

Various solvents such as toluene, acetonitrile, tetrahydrofuran and dimethyl formamide were taken on top of the reaction medium (polymerized gel + Ag(I)SG) and exposed to sunlight (Figure 21). This was to check the effect of solvent properties on cluster synthesis. Variations in their absorption profile were observed as the extent of cluster formation was different for each solvent (Figure 5II). Product obtained in acetonitrile shows better intensity compared to all other solvents.

## TESTING OF ANTIBACTERIAL ACTIVITY

Antibacterial activity of monolayer protected AgQCs was evaluated against the Gram negative organism, *Escherichia coli* (Figure 6). The average diameter of the zone of inhibition for the microorganism was about 12, 14, 16, 19 mm, respectively for 10, 20, 30 and 40 µL of synthesized AgQCs. Experiments using glutathione, Ag(I)SG complex and $Au_{25}(SG)_{18}$ clusters did not show any zone of inhibition which confirms that the antibacterial activity is due to the AgQCs alone. Not wishing to be bound by any theory, the fast internalization of silver nanoclusters, the enhanced release of silver ions from the clusters and consequent antibacterial action of silver ions may be the reasons for the activity. The consequent structural changes end up in cell death. It is also known that Ag nanoclusters interact with sulfur containing proteins which in turn affects the cell viability. Glutathione protected nanoparticles did not show any antibacterial effect.

WE CLAIM: CLEAN COPY

1. A method for preparing monolayer protected silver quantum clusters (AgQCs), wherein the method comprises,
   a) sonicating a mixture of silver nitrate and glutathione (GSH)/mercaptosuccinic acid (MSA)/cysteine in an aqueous base to obtain a solution containing oligomeric silver thiolate
   b) mixing the solution containing oligomeric silver thiolate with a gel solution to obtain a uniform solution,
   c) treating the solution obtained in step b) with ammonium persulfate (APS) and N,N,N',N'-tetramethylethylene diamine (TEMED) to initiate polymerization and formation of a gel,
   d) subjecting the mixture obtained in step c) to sunlight for a sufficient time to obtain monolayer protected silver quantum clusters, and
   e) isolating monolayer protected silver quantum clusters from the mixture thereof.

2. The method as claimed in claim 1, wherein the gel is used in step b) to control the growth of AgQCs.

3. The method as claimed in claim 2, wherein the gel is acrylamide:bisacrylamide.

4. The method as claimed in claim 1, wherein the aqueous base is 1 M sodium hydroxide.

5. The method as claimed in claim 1, wherein polymerization and formation of a gel in step c) is achieved by keeping the sample without disturbance.

6. The method as claimed in claim 1, wherein the polymerization and formation of the gel is visible after about 15 to about 20 minutes.

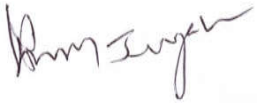
7. The method as claimed in claim 1, wherein the mixture is kept under sunlight for about 5 to about 10 hours.

8. The method as claimed in claim 1, wherein the formation of AgQCs is visible by a change of color from colorless to black-brown.

9. The method as claim in claim 1, wherein the monolayer protected silver quantum clusters used as an antibacterial agents.

10. The method of making metal clusters as claimed in claim 1 where the metal salt is Au, Pt, Cu, Pd and Ni separately or as a mixture of two or more, with or without Ag.

Dated at Chennai this Jun 13, 2018

Signature: 

D. Moses Jeyakaran
Advocate & Patent Agent
IN/PA — 369

# A METHOD FOR PREPARING MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS

## ABSTRACT

The present invention relates to a method for preparing monolayer protected, atomically precise silver clusters as antibacterial agents.

# A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS



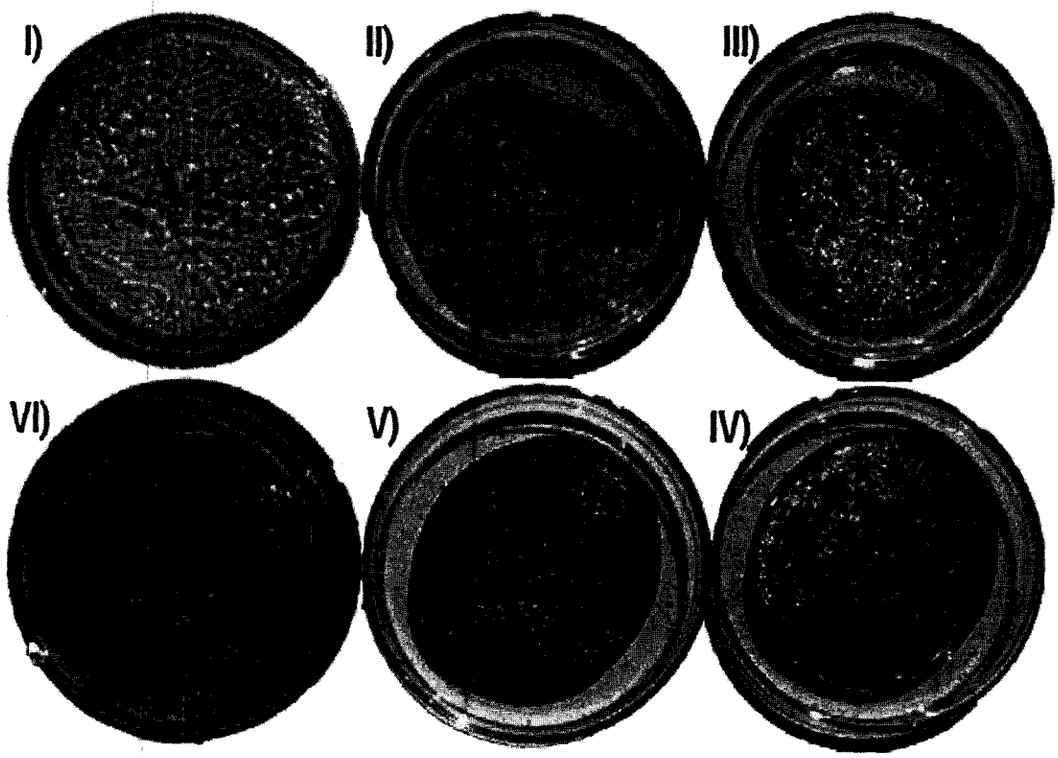



**FIGURE 1**

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A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS

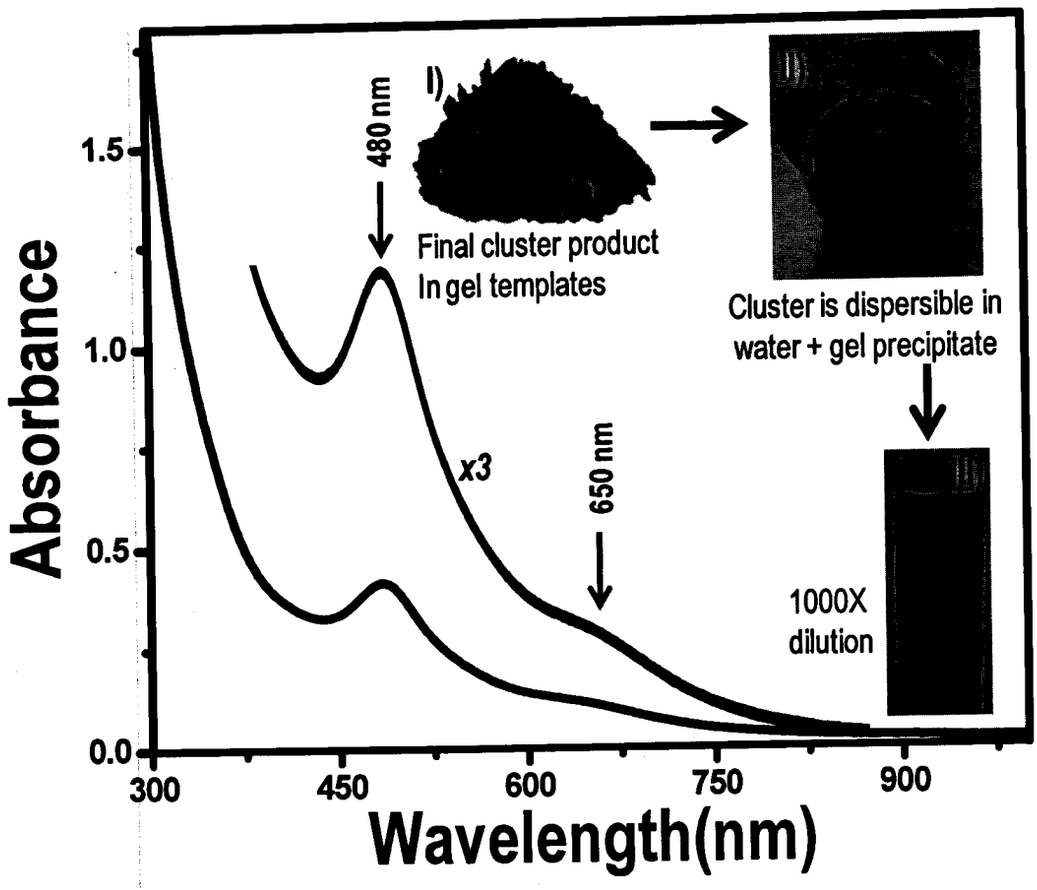


FIGURE 2

Signature:

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

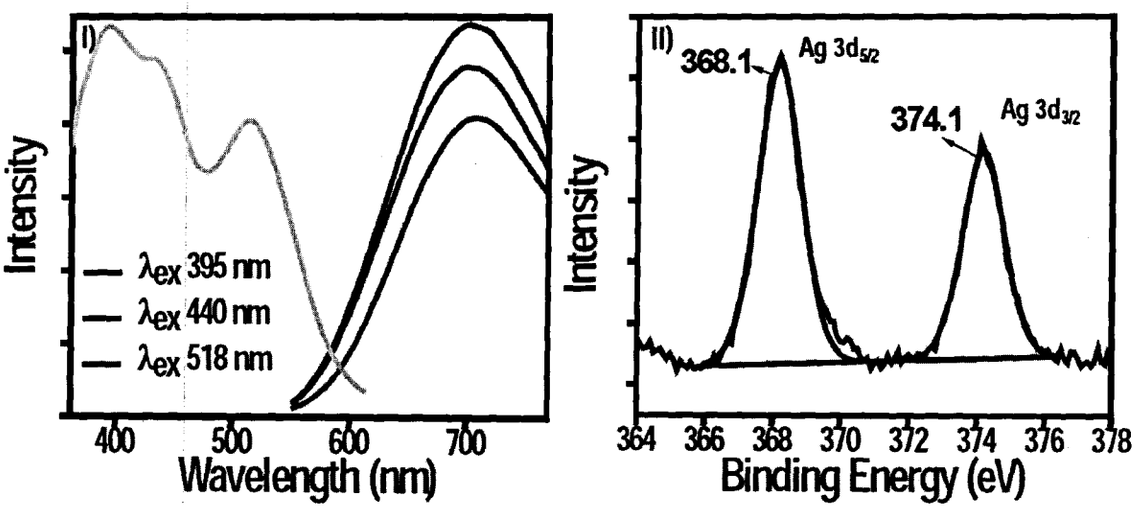


**FIGURE 3**

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

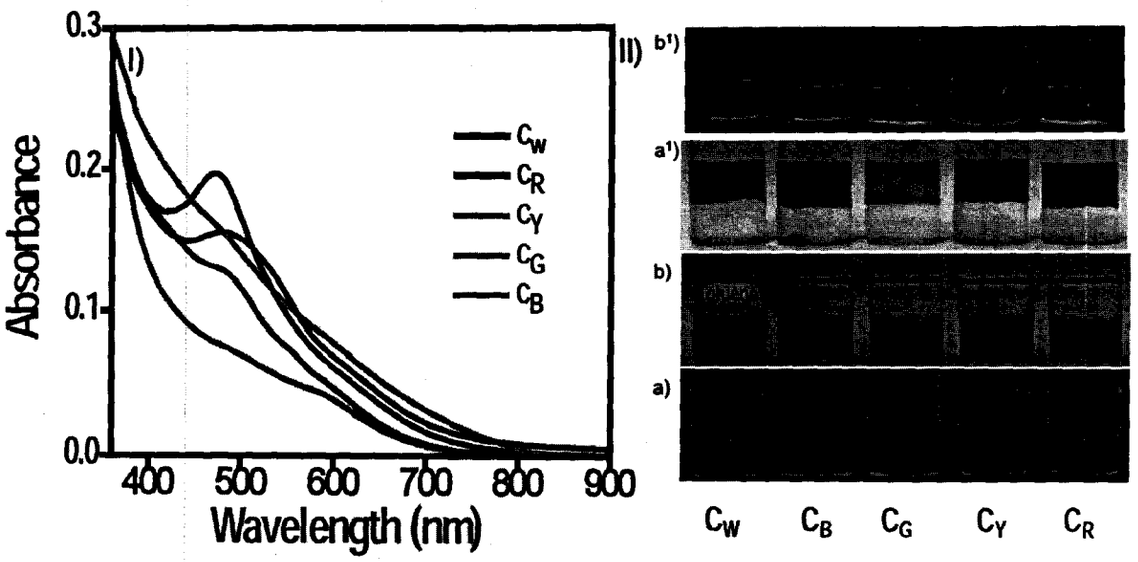


**FIGURE 4**

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IN/PA — 369

**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

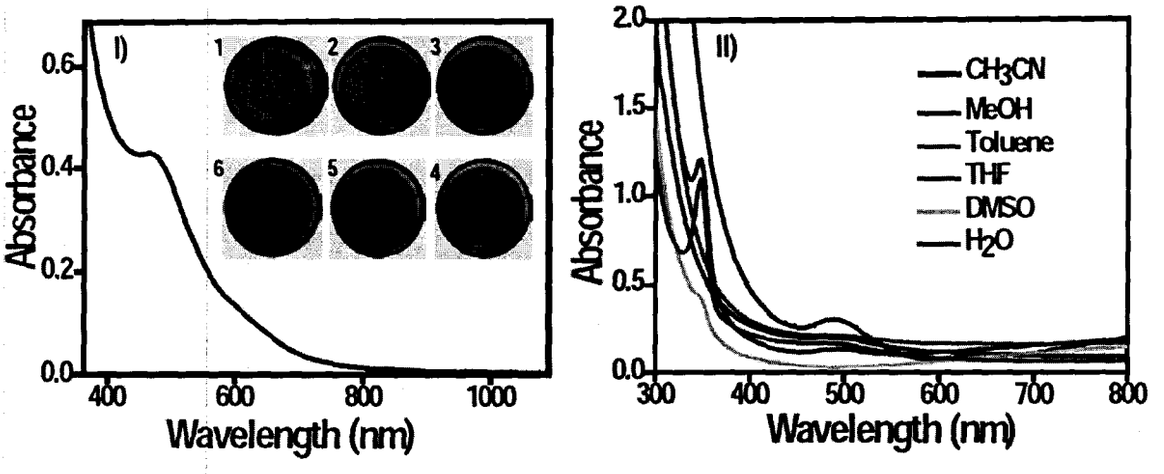


**FIGURE 5**

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

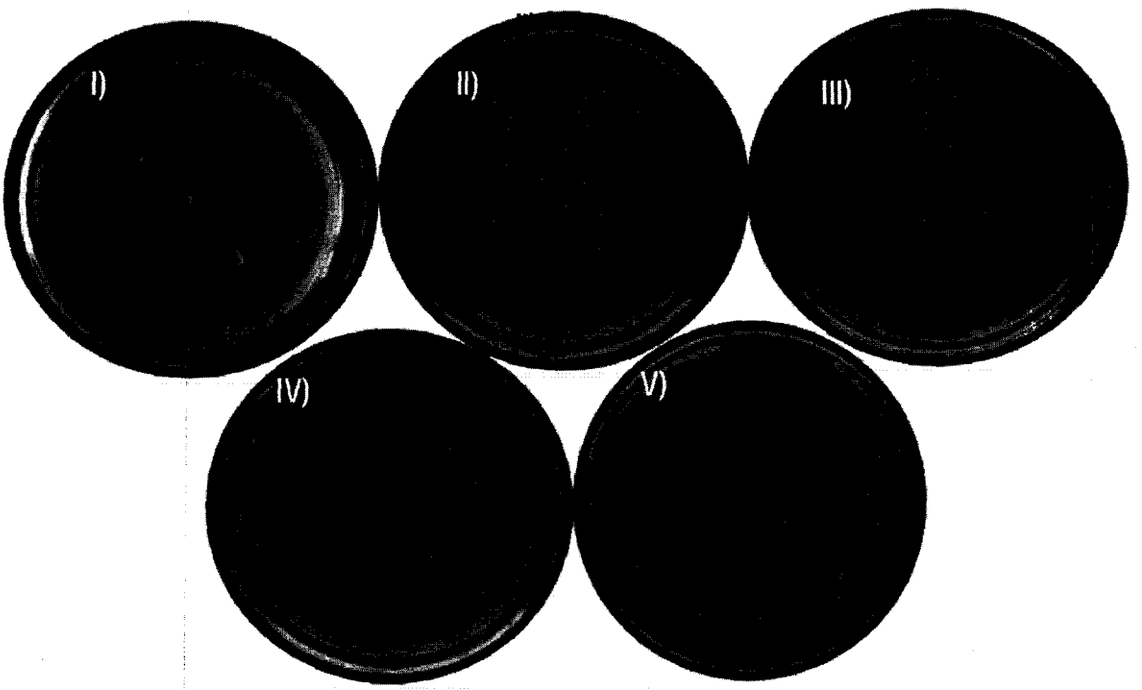


**FIGURE 6**

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

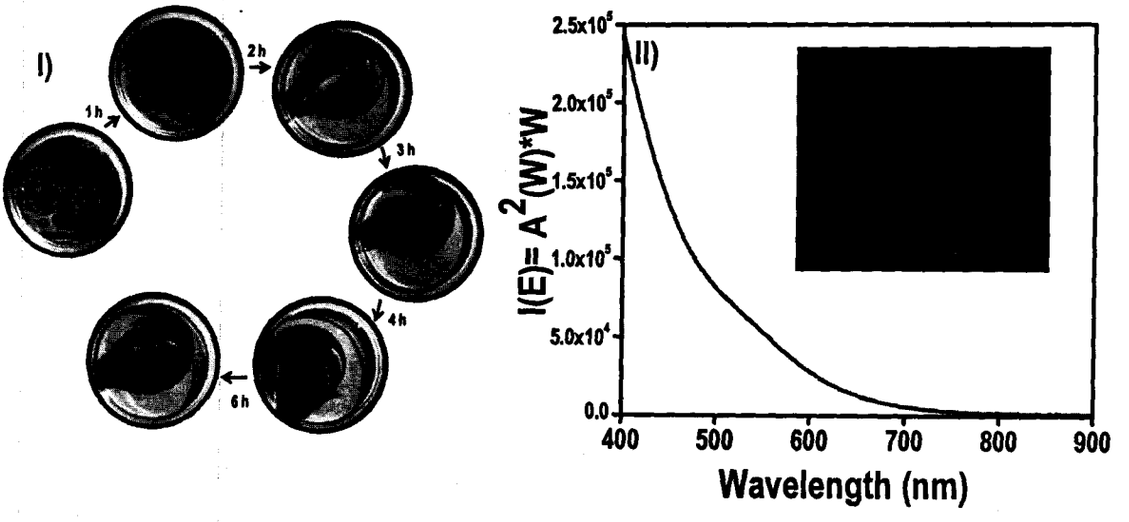


**FIGURE 7**

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# A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS

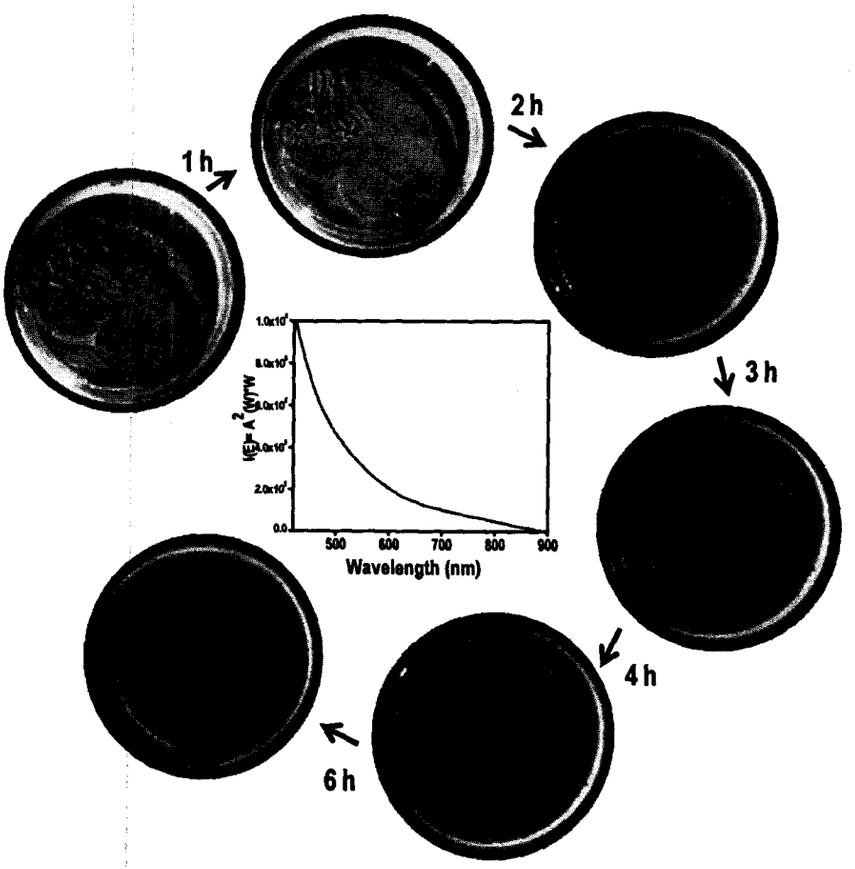


**FIGURE 8**

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

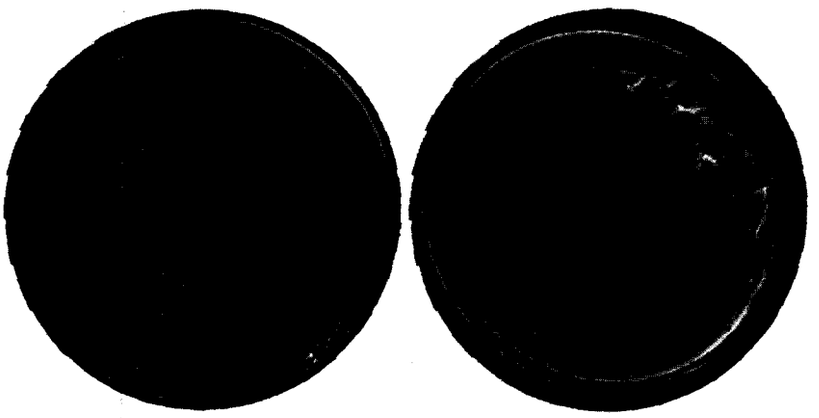

**FIGURE 9**

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

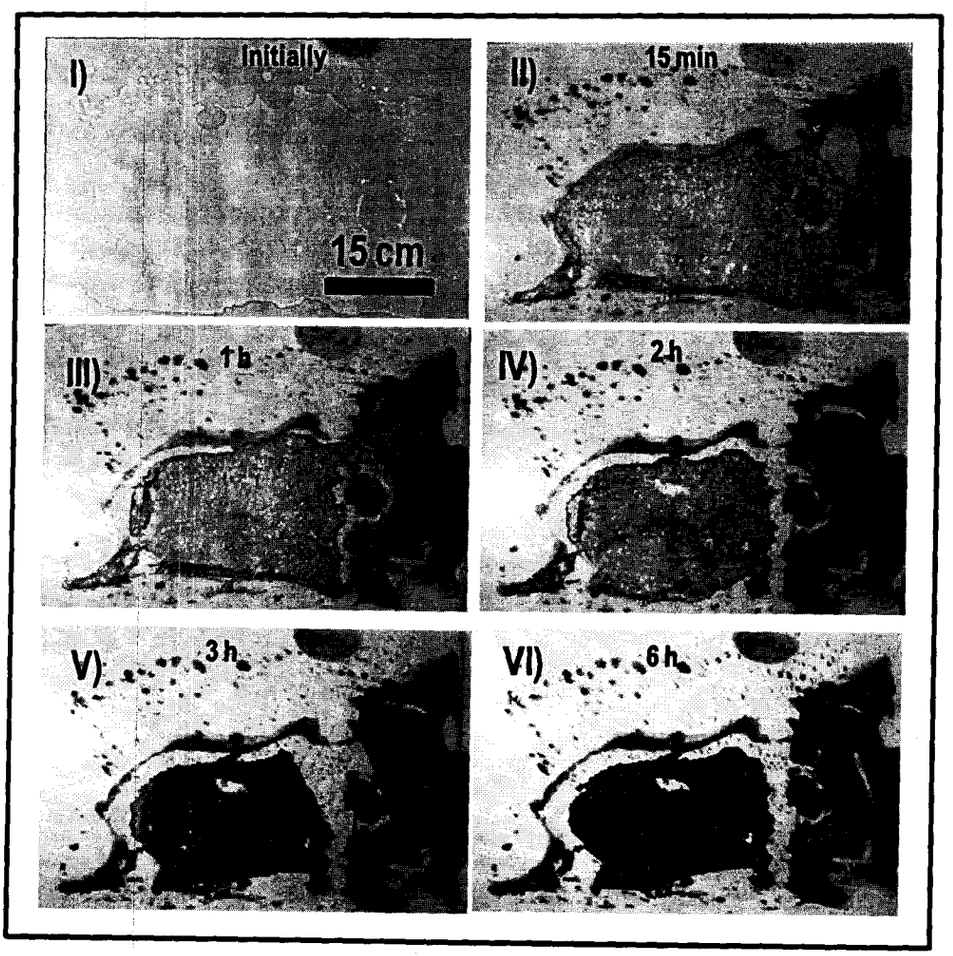


**FIGURE 10**

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

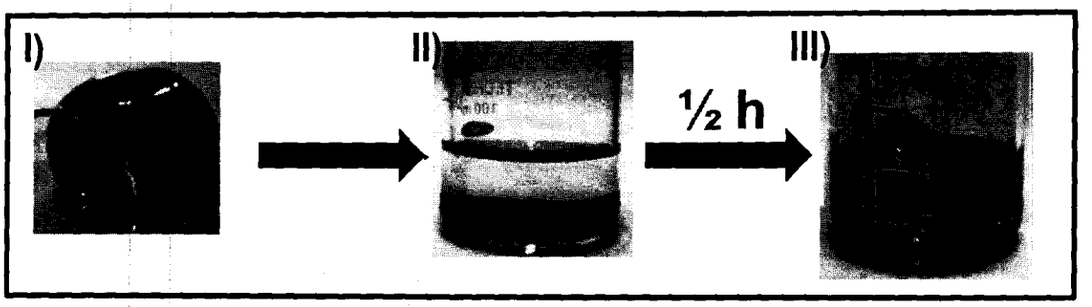


**FIGURE 11**

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A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS

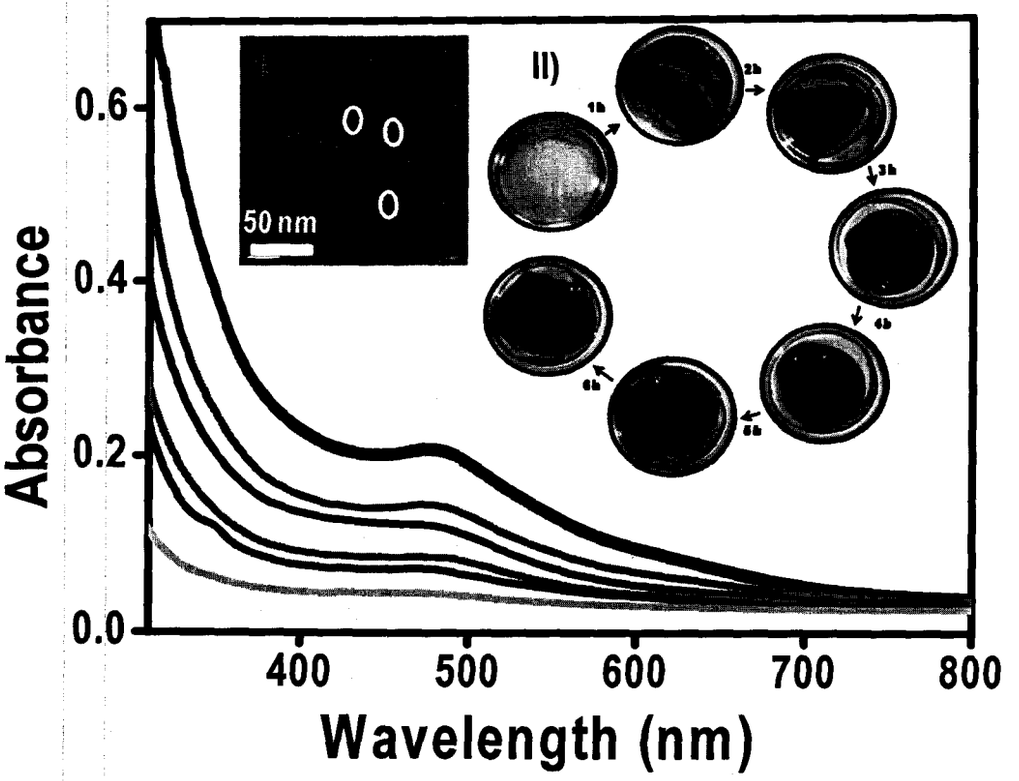


FIGURE 12

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

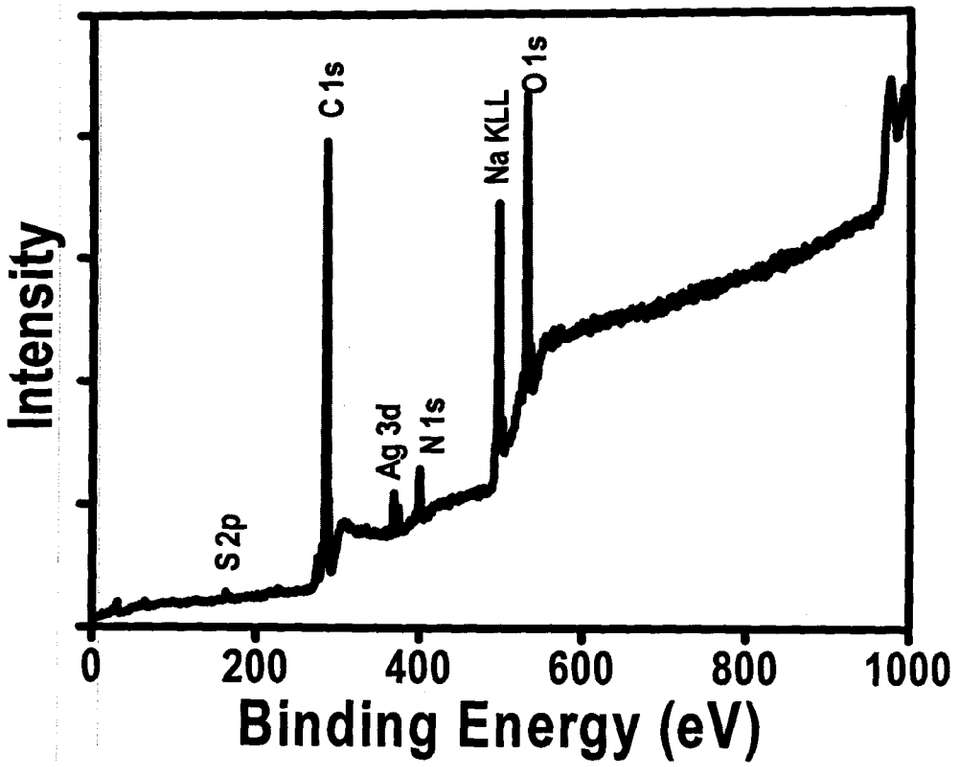


**FIGURE 13**

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A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS

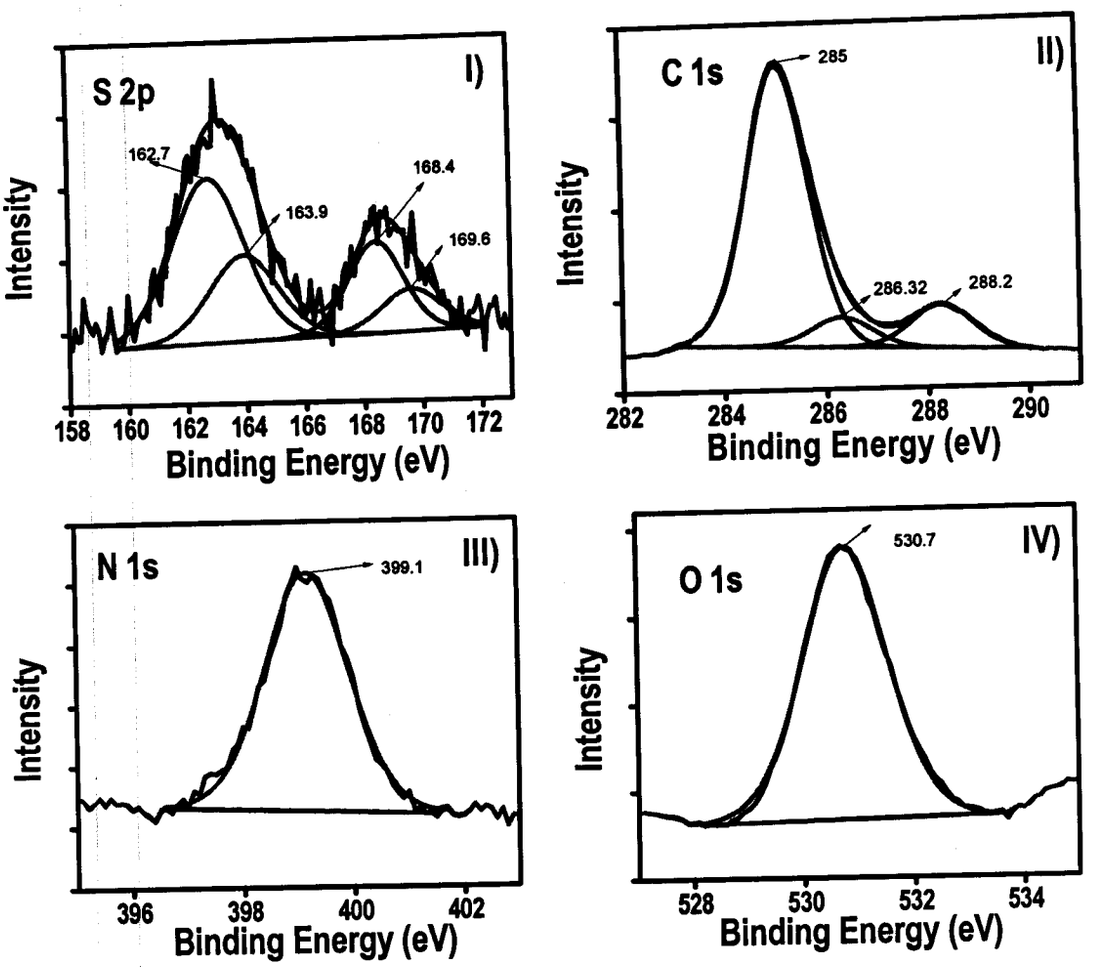


FIGURE 14

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A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS

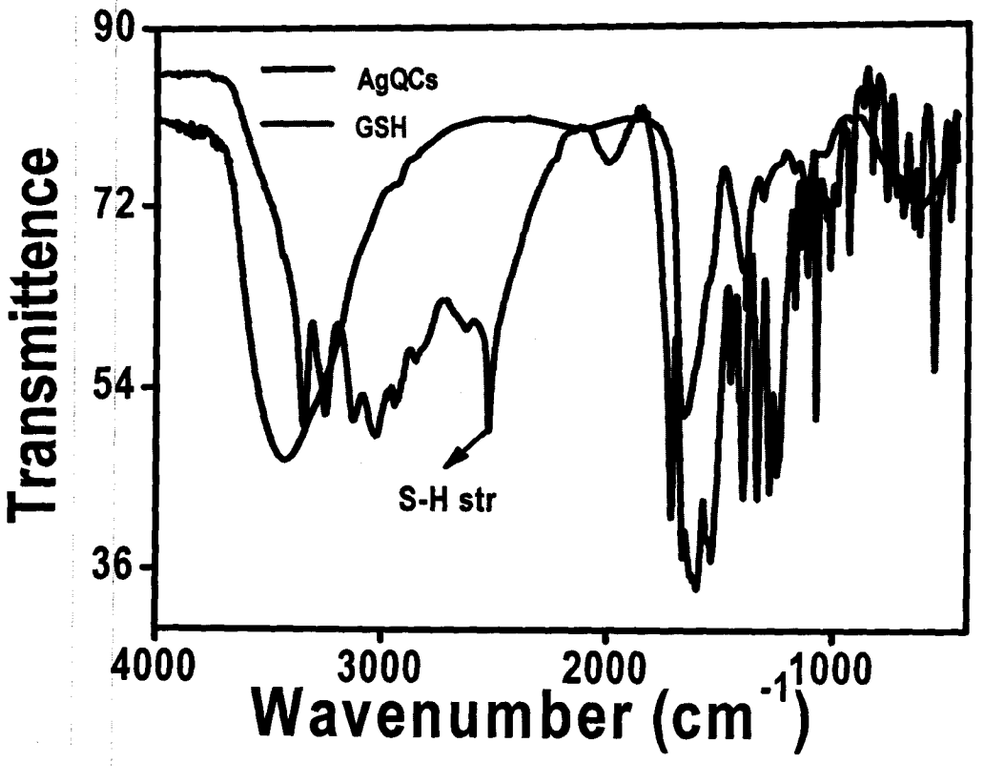


FIGURE 15

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A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS

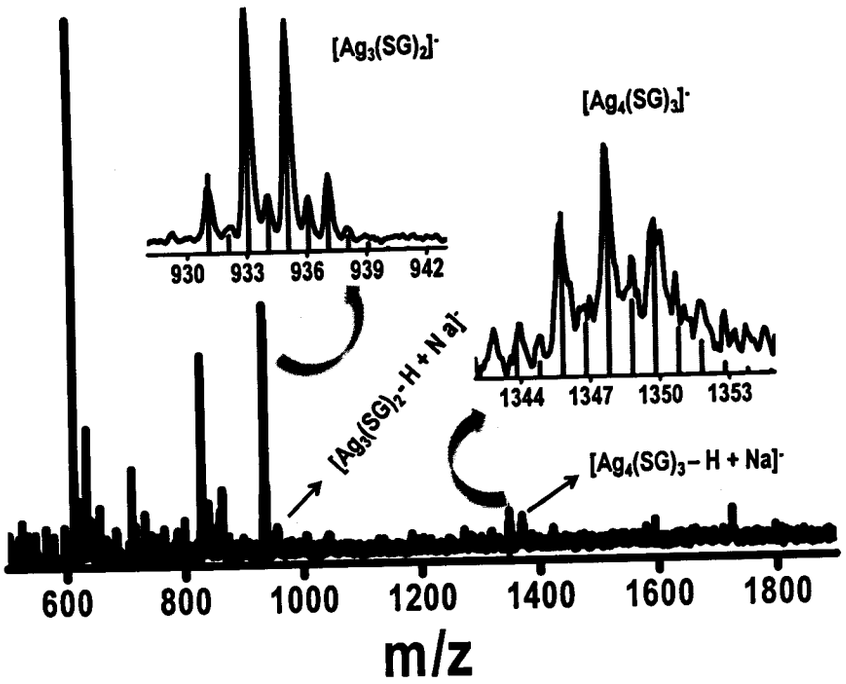


FIGURE 16

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A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS

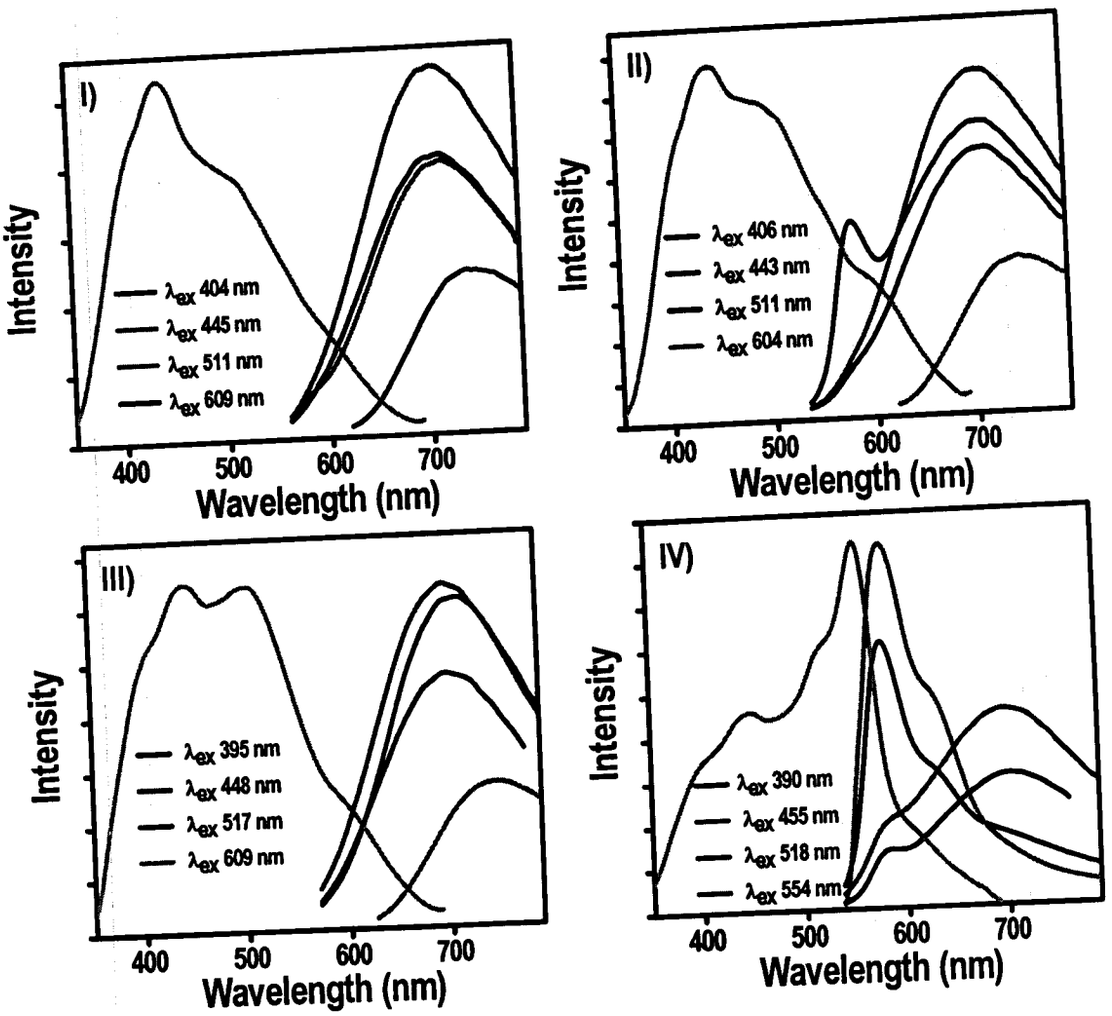


FIGURE 17

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

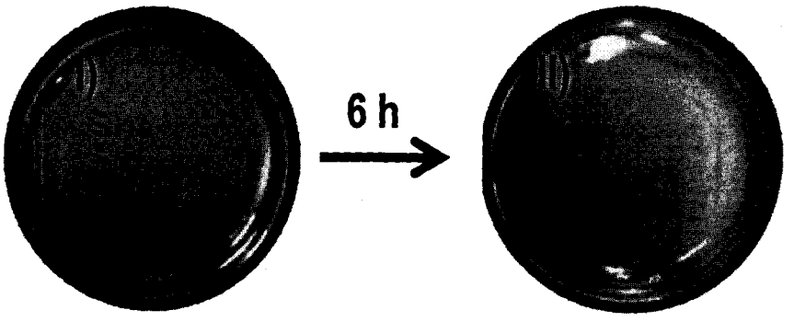


**FIGURE 18**

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

**FIGURE 19**

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**A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS**

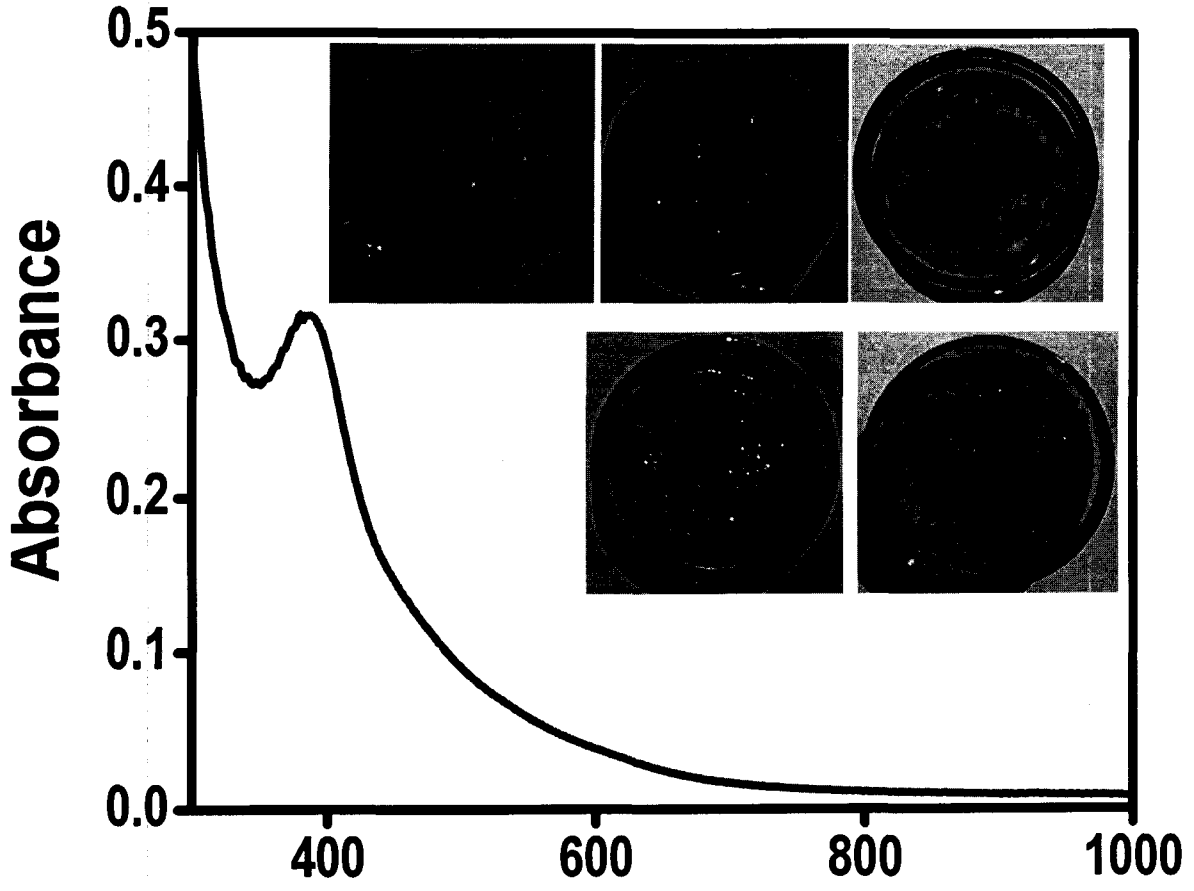


**FIGURE 20**

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# A METHOD FOR PREPARING OF MONOLAYER PROTECTED SILVER CLUSTERS AS ANTIBACTERIAL AGENTS

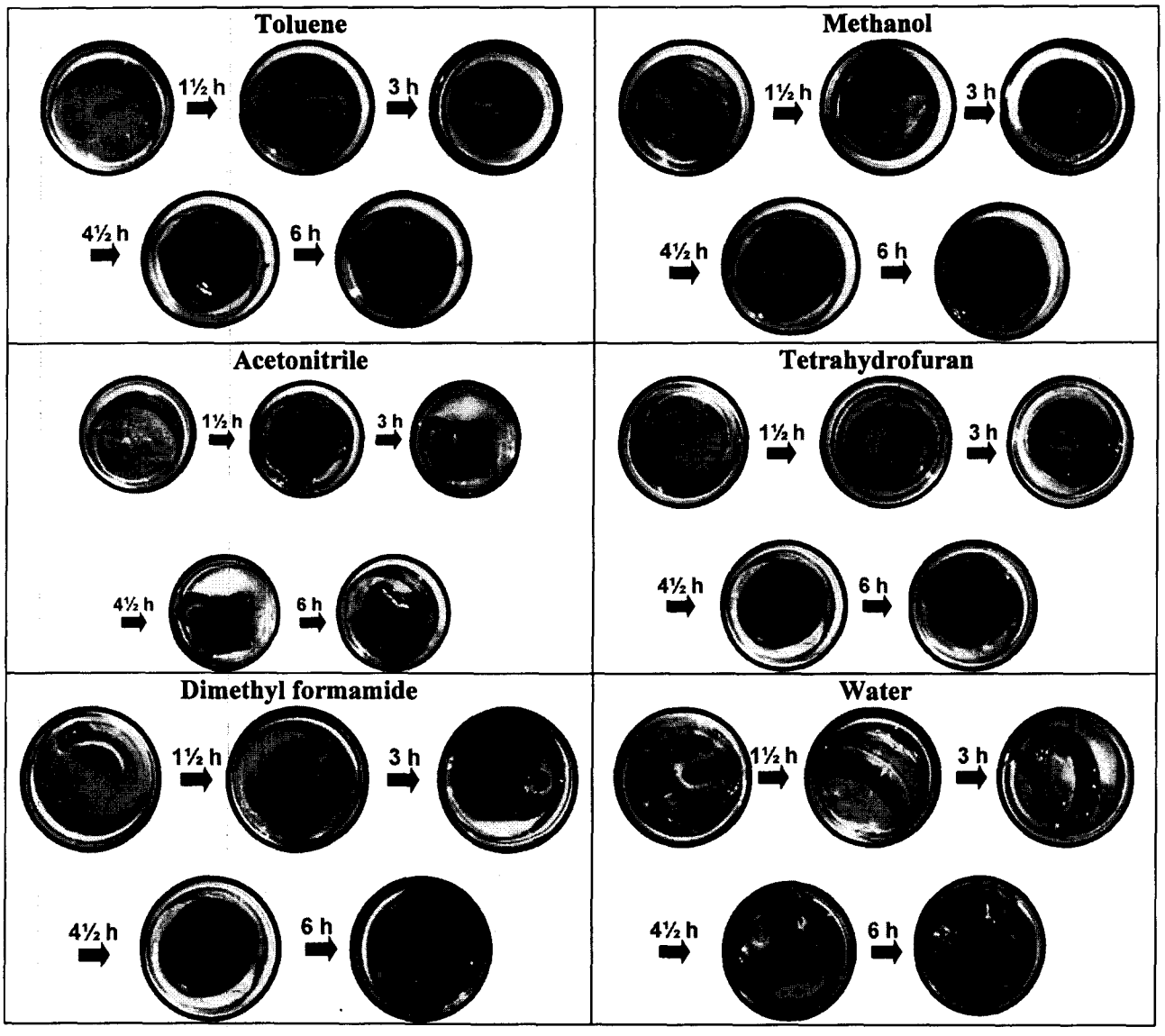


**FIGURE 21**

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