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THE PATENT OFFICE

पेटेंट प्रमाणपत्र
PATENT CERTIFICATE
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पेटेंट सं. / Patent No. : 286423
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फाइल करने की तारीख / Date of Filing : 25/03/2011
पेटेंटी / Patentee : INDIAN INSTITUTE OF TECHNOLOGY

प्रमाणित किया जाता है कि पेटेंटी को उपरोक्त आवेदन में यथाप्रकरित A SUSTAINED SILVER RELEASE COMPOSITION FOR WATER PURIFICATION नामक आविष्कार के लिए, पेटेंट अधिनियम, १९७० के उपबंधों के अनुसार आज तारीख 25th day of March 2011 से बीस वर्ष की अवधि के लिए पेटेंट अनुदत्त किया गया है।

It is hereby certified that a patent has been granted to the patentee for an invention entitled A SUSTAINED SILVER RELEASE COMPOSITION FOR WATER PURIFICATION as disclosed in the above mentioned application for the term of 20 years from the 25th day of March 2011 in accordance with the provisions of the Patents Act, 1970.



अनुदान की तारीख : 18/08/2017
Date of Grant :

पेटेंट नियंत्रक
Controller of Patent

टिप्पणी - इस पेटेंट के नवीकरण के लिए फीस, यदि इसे बनाए रखा जाना है, 25th day of March 2013 को और उसके पश्चात प्रत्येक वर्ष में उसी दिन देय होगी।

Note. - The fees for renewal of this patent, if it is to be maintained will fall / has fallen due on 25th day of March 2013 and on the same day in every year thereafter.

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 SUSTAINED SILVER RELEASE COMPOSITION FOR WATER PURIFICATION

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3. Preamble to the Description

COMPLETE SPECIFICATION

The following specification particularly describes the invention and the manner in which is to be performed.

COMPLETE SPECIFICATION

5 TITLE OF THE INVENTION

A SUSTAINED SILVER RELEASE COMPOSITION FOR WATER PURIFICATION

FIELD OF THE INVENTION

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This invention relates to the constant release of silver ion from silver nanoparticles embedded in the organic-templated-boehmite nanoarchitecture (OTBN). Silver nanoparticles impregnated OTBN is capable of removing bacteria and virus from water. The antibacterial activity of the composition is due to the embedded silver nanoparticles and silver ions released from the same. The advantage with this composition is the constant concentration release of silver ions in water over extended periods, which is novel and important from the perspective of maintaining silver ion concentration below permissible limits while maintaining antimicrobial activity. The composition utilizes the virus removal capability of OTBN, as reported in previous patent application (1529/CHE/2010). Therefore, a simultaneous removal of both bacteria and virus from drinking water is achieved using an adsorbent composition.

BACKGROUND OF THE INVENTION

Drinking water contamination is a major health concern across the world, especially in the developing and under-developed countries. A number of contaminants affect the water quality. They include biological (eg. bacteria and virus), inorganic (eg. fluoride, arsenic, iron) and organic (eg. pesticides, volatile organics) species. Government of India has been extensively supporting the endeavor to bring quality drinking water to people of the country. The eleventh schedule in the Constitution of India along with article 47 requires the State to take care of the drinking water and nutrition needs of its citizens. Government of India has made a number of proactive efforts for ensuring good quality drinking water supply throughout the nation. An investment of more than Rs. 100,000 crore has already been made by the Central and State Governments in rural water supply sector and nearly Rs. 90,000 crore is envisaged to be spent during the XIth five year plan. India is also committed to the

Millennium Development Goal 2015 of reducing the population without access to drinking water by half. A number of governmental agencies are working together to address the drinking water contamination problem. These include, Ministry of Health and Family Welfare, Ministry of Rural Development, Department of Drinking Water Supply, Housing and Urban Development Corporation, Central Pollution Control Board, State Public Health Engineering Departments, etc. However, a significant cost burden still remains on the shoulders of the poor, with an estimated expenditure of Rs. 6,700 crore being made annually on treatment of waterborne diseases (<http://articles.timesofindia.indiatimes.com/2008-04-13/special-report/277807901-potable-water-drinking-water-fluoride> accessed on January 18, 2011).

Despite all the efforts made so far, contradicting reports continue to emerge on the availability of protected drinking water in Indian rural households. Some reports suggest that nearly 50% of villages still do not have any source of protected drinking water; however, as per the 2001 census, 68.2% households have access to safe drinking water. A report by WaterAid suggests that out of the 1.42 million villages in India, 1,95,813 are affected by chemical contamination of water. More than 400,000 children die under an age of five due to diarrhoea; more than 35 million people are afflicted by water borne diseases every year. It is estimated that 1.5 million children die of diarrhoea alone and 73 million working days are lost due to waterborne diseases each year. The resulting economic burden is estimated at \$600 million a year. While the statistics collected by various sources vary, the conclusion is the same: water is the source of a number of diseases for a large population of the country. In contrast, water has always been regarded as the elixir of life.

In our earlier work, we removed various contaminants from drinking water: organic contaminants such as pesticides (T. Pradeep, A. Sreekumar Nair, Indian Patent 200767 and PCT Application PCT/IN05/0002), biological contaminants such as E. coli (T. Pradeep, Prashant Jain, Indian Patent 20070608), fluoride (T. Pradeep, S. M. Maliyekkal, Anshup, Indian Patent Application 2089/CHE/2009; T. Pradeep, S. M. Maliyekkal, Anshup, M. Udhaya Sankar, Indian Patent Application 1529/CHE/2010) and heavy metals (T. Pradeep, Lisha K. P., Anshup, Indian Patent Application 169/CHE/2009; T. Pradeep, Amrita Chaudhary, T. Saraladevi, M. Udhaya Sankar, S. M. Maliyekkal, Anshup, Indian patent application, 2433/CHE/2010; T. Pradeep, S. M. Maliyekkal, T. S. Sreeprasad, Indian patent application, 2563/CHE/2010) in addition to developing a water purification device (T.

Pradeep, M. Udhaya Sankar, Amrita Chaudhary, Anshup, Indian patent application, 2892/CHE/2010). As a continuation of our research activities on nanomaterials for environmental remediation and applications, we have impregnated silver nanoparticles on the organic templated boehmite nanoarchitecture. This composition works actively for the removal of microorganisms from drinking water.

An important aspect of the utilization of such composition for the removal of microorganisms at household level in India is the affordability. A low cost of ownership for household-based water purifier is likely to play a major role in improving the health of citizens of this country (<http://www.nextbillion.net/news/hindustan-lever-to-take-water-purifiers-to-villages>, accessed on January 18, 2011). Percentage of population in India below international poverty line of US\$1.25 per day is 42% (UNICEF estimate, 2005) and 25% (CIA estimate, 2007). While the Government of India continues to uplift the economic well-being of people, it is also imperative to produce state-of-the-art products for people at the bottom of the pyramid. This invention aims to contribute to the initiative of improving health of people by safeguarding them against waterborne diseases at extremely affordable cost.

PRIOR ART

Silver is widely known for its antibacterial property. Silver has been employed as inorganic silver salt, as organic silver salt and as colloids of its salt, oxide and in metallic states. Although it is well known that silver is a good antibacterial agent, the nature of silver present in the water determines its antibacterial efficiency. For examples, various forms of silver such as those from inorganic salts and organic compounds, electrochemical dissolution process, nanoparticles, etc. show different antibacterial performance. In recent decades, silver has been extensively used as metallic nanoparticles. Without getting into the theoretical concepts of silver nanoparticles, the antibacterial property of silver nanoparticles emerges either from nanoparticle-bacteria surface interaction or from released silver ion from nanoparticles or both.

Antibacterial property of silver nanoparticles has been covered in a number of patent applications, wherein method of synthesis has been improved (Pal et. al. in Appl Environ Microbiol., 2007, 73(6), 1712; De Windt et. al. in United States Patent Application 20100272770; Sastry et. al. in 936/MUM/2008), made in media other than water (Chen et. al.

in United States Patent 7329301), and loaded various substrates (Rautaray et. al. in Indian patent application 1571/MUM/2008). The enhanced antibacterial property of silver nanoparticles is exclusively due to size confinement of silver metal. Although a number of methods have been developed for the synthesis of silver nanoparticles, keeping reactive particles in nanometer size for long time in real water composed of various species is very difficult. This is due to ion induced aggregation, surface modification and salt deposition, etc. Hence, an important requirement while employing reactive silver nanoparticles in water purification is size stabilization and prevention of surface modification for extended periods.

The importance of stability of silver nanoparticles in real water has been insisted in the literature. For example, Hoek et al. J Nanopart Res. 2010, 12, 1531, Hoek et al. Environ. Sci. Technol. 2010, 44, 7321, Bonzongo et al. Environ. Sci. Technol. 2009, 43, 3322 and Lead et al. Environ. Sci. Technol. 2009, 43, 7285 demonstrated that the antibacterial property of silver nanoparticles depends on intrinsic features (such as particle size, stability and shape of the nanoparticle) as well as extrinsic features (such as hardness of the water, soluble ligands in the water, ionic composition, organic matter and pH).

Another important aspect of use of silver nanoparticles for anti-bacterial performance is the fraction of silver ion released (quantity of silver ion released/quantity of silver nanoparticle used). It is known that though significant quantity of silver nanoparticles is used, very less silver ions are released into the contaminated water from silver nanoparticles. For example, Hoek et al. Environ. Sci. Technol. 2010, 44, 7321 reported that in reproduced real water having total dissolved solids (TDS) around 340 ppm, the fraction of dissolved silver is less than 0.1% of the total mass of silver added, regardless of the initial source, i.e., AgNO₃ or silver nanoparticles. This is attributed to the presence of various anions in real water such as chloride (many silver salts have very low solubility product). Hence, more than required quantity of silver nanoparticles is used in water filters. This results in increase in the filter size and cost.

While the fraction of silver ion released from silver nanoparticles is important, the rate of release is the most important step in employing the nanoparticles for long time usage. Constant release of silver ions from silver nanoparticles for longer time is most important for their use in water filters. This is important from the perspective of ensuring consistent anti-bacterial performance and release of silver ions below permissible limit as prescribed by

WHO. The rate of silver ion release has been discussed in the literature. For example, Epple et al. Chem. Mater. 2010, 22, 4548 and Hurt et al. Environ. Sci. Technol. 2010, 44, 2169 demonstrated that release of silver ion from silver nanoparticles in distilled water depends on temperature, incubation days, and species present in water such as dissolved oxygen level, salt and organic matters. They showed that the rate of dissolution is not constant with time but attains saturation in short period.

Hence, stability of reactive nanoparticles for long time in real water is very important for controlled release of silver ions. Metal oxides have been widely considered as good substrates (matrices). Silver nanoparticles have been ex-situ and in-situ loaded in/on metal oxides. In-situ loading in metal oxide has showed more promising stability even at high loading percentage. For example, In-situ syntheses of silver nanoparticles in metal oxide matrices have been reported earlier. Chen et al. Environ. Sci. Technol. 2009, 43, 2905 demonstrated the sol-gel synthesis of silver nanoparticles (<5 nm) loaded TiO₂ nanocomposite where TiO₂ particle acts as anti-aggregation supports. He showed that 7.4 wt% Ag loading in TiO₂ showed highly potent antibacterial properties against E.coli. Similar examples are available with rice husk ash (Rautaray et. al. in Indian patent application 1571/MUM/2008) and Fe₂O₃-fiber glass (Nangmenyi et. al. in Mater. Lett., 2011, 65, 1191).

Various attempts have been done to synthesize silver nanoparticles on low-cost substrates. For example, Shankar et al. J Chem Technol Biotechnol. 2008, 83, 1177 loaded silver on cheap activated carbon at high silver loading percentage. They reported that an optimum of 9-10.5 wt% of Ag loaded in activated carbon (5 g) is necessary for the complete killing of E.coli (concentration: 10³ CFU/ml) in the contact-mode for up to 350 L of flowing water (flow rate: 50 mUmin). This implies the use of ~0.5 g of silver for 350 L of bacterial free water (cost of water per liter is above 10 paisa per liter).

Therefore, the problem with the current systems is making reactive silver nanoparticles well stabilized by a supporting matrix without altering the surface chemistry for controlled silver ion release for extended period using less quantity of material.

Although synthesizing reactive silver nanoparticles and subsequently stabilizing its size by a supporting matrix is achievable easily, maintaining its reactivity over a long period by preventing surface modification and salt deposition for continuous controlled silver ion

release is very difficult. Controlled constant silver release is very important for long term use, effectiveness, life of the device and cost.

Therefore, the main object of the present invention is to provide optimum dissolution
5 of silver ions in real water from silver nanoparticles for long term use.

The another object of the present invention is increase of the volume of water treated
and time independent constant release of silver ions from silver nanoparticles loaded on
organic polymer-metal oxide/hydroxide composite such as chitosan stabilized boehmite.

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The another object of the present invention is the art of using organic polymer-metal
oxide/hydroxide composite as a dual stabilizing agent for the synthesis of highly dispersed
and stable silver nanoparticles having potent antibacterial activity at high flow rate at very
less loading wt%.

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SUMMARY OF THE INVENTION

Generally, more than required quantity of silver nanoparticles is used in water filters
due to very poor silver ion release in real water. This is in agreement with the literature
20 mentioned above (Shenker et al. J Chem Technol Biotechnol. 2008, 83, 1177). This results in
increase in the filter size and the cost. In the view of the need, the present invention
demonstrates that more than 10 % of the silver present in nanoparticles can be released as
silver ion into the tap water having moderately high TDS from silver nanoparticles loaded
OTBN over an extended period. The embodiment of the present invention includes the
25 volume of water treated and time independent constant release of silver ion from Ag-OTBN
matrix.

The present invention describes the art of making antibacterial filtration medium
made up of less than 10 nm sized silver nanoparticles, in-situ synthesized in organic-
30 templated-boehmite-nanoarchitecture (Ag-OTBN).

In the present invention, the potent antibacterial material for long term use is obtained
when silver nanoparticles are synthesized in organic templated metal oxide/hydroxide
nanoarchitecture. Stability of silver nanoparticles in real water for longer time is very

important for its constant antibacterial property. In the view of the importance of the stability of silver nanoparticles, those are in-situ synthesized in the OTBN matrix. We disclose that the OTBN matrix enhances the antibacterial property of silver nanoparticles in real water as the matrix controls the size, stabilizes the particles from aggregation and prevents the adsorption/deposition of soluble ligands, organic matters and dissolved solids on the silver nanoparticles.

The surface reactivity of silver nanoparticles is maintained by both chitosan and metal oxide/hydroxide. Silver nanoparticles are capped by chitosan - an organic polymer and also dispersed in metal oxide support and vice-versa. The dual stabilization prevents the surface modification and also salt deposition over a period of time.

According to the method of present invention, we demonstrate that even 0.5 wt% Ag loaded in OTBN is capable for the complete inhibition of 10^5 CFU/mL E.coli in the contact-mode for several hundred litres of flowing water at very high flow rate. This is achieved due to controlled constant release of silver ion for long time.

The present invention also demonstrate that the dissolved silver from silver nanoparticles in the tap water itself is capable of killing 10^5 CFU/mL E.coli without being contacted with the antibacterial material.

DESCRIPTION

DETAILED DESCRIPTION OF THE INVENTION

In this patent application, we describe the synthesis, characterization and application of silver nanoparticles impregnated organic-templated-boehmite-nanoarchitecture (Ag-OTBN). Impregnation of silver nanoparticles in OTBN is demonstrated using a number of procedures. The as-synthesized Ag-OTBN composition is characterized by a number of spectroscopic and microscopic techniques. The capability of Ag-OTBN to remove microorganisms from drinking water is demonstrated through the use of E.Coli and MS2 bacteriophage as model organisms for bacteria and virus, respectively. However, the result presented here should not be construed as limiting the scope of the invention.

Experimental methods

Material characterization

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The identification of the phase(s) of the as-prepared sample was carried out by X-ray powder diffraction (Bruker AXS, D8 Discover, USA) using Cu-K α radiation at $\lambda = 1.5418 \text{ \AA}$. Surface morphology, elemental analysis and elemental mapping studies were carried out using a Scanning Electron Microscope (SEM) equipped with Energy Dispersive Analysis of X-rays (EDAX) (FEI Quanta 200). For this, sample was re-suspended in water by sonication for 10 min and drop casted on an indium tin oxide (ITO) conducting glass and dried. High resolution Transmission Electron Microscopy (HRTEM) images of the sample were obtained with JEM 3010 (JEOL, Japan). The samples prepared as above were spotted on amorphous carbon films supported on copper grids and dried at room temperature. X-ray Photoelectron Spectroscopic (XPS) analysis was done using ESCA Probe TPD of Omicron Nanotechnology. Polychromatic Mg K α was used as the X-ray source ($h\nu = 1253.6 \text{ eV}$). Spectra in the required binding energy range were collected and an average was taken. Beam induced damage of the sample was reduced by adjusting the X-ray flux. Binding energy was calibrated with respect to C 1s at 284.5 eV. Silver ion concentration in the water was detected using inductively coupled plasma optical emission spectrometry (ICP-OES).

Some examples are provided to illustrate the present invention. The examples should not be construed as limiting the scope of the invention.

25 Example 1

This example describes the in-situ impregnation of silver nanoparticles on the OTBN. OTBN is prepared as reported in the previous patent application (1529/CHE/2010). The OTBN gel obtained after washing the salt content, is used for the formation of silver nanoparticles. The OTBN gel is again re-dispersed in water, to which 1 mM silver precursor (silver nitrate, silver fluoride, silver acetate, silver permanganate, silver sulfate, silver nitrite, silver bromate, silver salicylate or any combination of the above) is added drop-wise. The weight ratio of Ag to OTBN can be varied anywhere between 0.1-1.5%. After stirring the solution overnight, 10 mM sodium borohydride is added to the solution drop wise (in ice-cold condition,

temperature $< 5^{\circ}\text{C}$). Then, the solution was allowed to stir for half an hour, filtered and washed with copious amount of water. The obtained gel is then dried at room temperature for further studies.

5 **Example 2**

This example describes the in-situ impregnation of silver nanoparticles on the OTBN powder. The dried OTBN powder was crushed to a particle size of 100-150 micron. The powder is stirred in water, using a shaker. 1 mM silver precursor solution is then slowly added. The weight ratio of Ag to OTBN can be varied anywhere between 0.1-1.5%. After stirring the mixture overnight, 10 mM sodium borohydride is added to the mixture drop wise (in ice-cold condition, temperature $< 5^{\circ}\text{C}$). Then, the mixture was allowed to stir for half an hour, filtered and washed with copious amount of water. The obtained powder is then dried at room temperature for further studies.

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Example 3

This example describes the ex-situ impregnation of silver nanoparticles on the OTBN. The OTBN gel obtained after washing the salt content, is used for the impregnation of silver nanoparticles. The OTBN gel is again re-dispersed in water, to which 1 mM silver nanoparticles solution (prepared by any route reported in the literature) is added drop-wise. The weight ratio of Ag to OTBN can be varied anywhere between 0.1-1.5%. After stirring the solution overnight, it was filtered and washed with copious amount of water. The obtained gel is then dried at room temperature for further studies.

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Example 4

This example describes the ex-situ impregnation of silver nanoparticles on the OTBN powder. The dried OTBN powder was crushed to a particle size of 100-150 μm . The powder is stirred in water, using a shaker. 1 mM silver nanoparticles solution (prepared by any route reported in the literature) is added drop-wise. The weight ratio of Ag to OTBN can be varied anywhere between 0.1-1.5%. After stirring the solution overnight, it was filtered and washed with copious amount of water. The obtained powder is then dried at room temperature for further studies.

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Example 5

This example describes the silver nanoparticles impregnation in transition and rare-earth metal doped OTBN composition (as claimed in previous patent application 1529/CHE/2010). Transition and rare-earth metals are chosen from the following list: zinc, manganese, iron, titanium, zirconium, lanthanum, cerium, silicon. The synthesis procedure for transition and rare earth metal doping in OTBN composition is as follows: the chosen metal salt is mixed with the aluminum salt solution in an appropriate ratio, preferably 1:9 (wt/wt). The salt solution was added slowly into the chitosan solution (dissolved in 1 - 5 % glacial acetic acid or HCl or combination thereof) with vigorous stirring for 60 minutes and kept overnight. Aqueous ammonia or NaOH solution was slowly added into the metal-chitosan solution with vigorous stirring to facilitate the precipitation of the metal-chitosan composites. All these steps were carried out at room temperature. Stirring was continued for two hours. The precipitates were filtered, washed to remove any unwanted impurities, converted to bead shape and dried.

The OTBN gel is again re-dispersed in water, to which 1 mM silver precursor is added drop-wise. The weight ratio of Ag to OTBN can be varied anywhere between 0.1-1.5%. After stirring the solution overnight, 10 mM sodium borohydride was added to the solution drop-wise (in ice-cold condition). Then, the solution was allowed to stir for half an hour, filtered and washed with copious amount of water. The obtained gel is then dried at room temperature for further studies.

Example 6

This example describes the testing protocol in batch for antibacterial activity of silver nanoparticles impregnated OTBN composition. 100 mL of water was shaken with the material and 1×10^5 CFU/mL of bacterial load was added to the water. Tap water having the TDS between 300-500 ppm was used in the study. After one hour of shaking, 1 mL of the sample along with nutrient agar was plated on sterile petridish using the pour plate method. After 48 hrs of incubation at 37 °C, the colonies were counted and recorded. This procedure was repeated 25 to 30 times.

Example 7

This example describes the testing protocol for antibacterial activity of silver nanoparticles impregnated OTBN powder packed in a column. The column in which a known quantity of the material is packed has a diameter anywhere between 35 mm to 55 mm. The feed water was passed at a flow rate in the range of 10 mL/min to 2000 mL/min. The tap water was periodically -subjected to a E.coli load of 1×10^5 CFU/mL. The output water collected from the column was screened for bacterial presence by pour plate method. The bacterial colonies were counted and recorded after 48 hours of incubation at 37°C.

Example 8

This example describes the testing protocol in batch for antiviral activity of silver nanoparticles impregnated OTBN composition. 100 mL of water was shaken with the material and 1×10^3 PFU/mL of MS2 coliphage load was added to the water. The tap water having the TDS between 300-500 ppm was used in the study. After one hour of shaking, virus count was done by plaque assay method. After 24 hrs of incubation at 37 °C, the plaques were counted and recorded. This procedure was repeated for 35 to 40 times.

The OTBN matrix defined in the present invention can be made up of chitosan stabilized any metal oxide/hydroxide nanoarchitectures. The metal precursor can be Fe(II), Fe(III), Al(III), Si(IV), Ti(IV), Ce(IV), Zn(II), La(III), Mn(II), Mn(III), Mn(IV) or a combination thereof. And the metal oxide/hydroxide nanoparticle may serve as an inert filler material or an active filtration medium.

It should be noted that the silver nanoparticles can be impregnated in transition and rare-earth metal doped OTBN compositions. It should also be noted that it can be of mixed metal oxide/hydroxide nanoarchitecture. The mixture can be binary or a mixture of all the above mentioned metal oxide/hydroxide.

The Ag-OTBN defined in the present invention can have chitosan polymer to metal oxide/hydroxide weight ratio in between 5 to 50%. And Ag to OTBN weight ratio can vary from 0.1 to 10 %.

It should be noted that the silver nanoparticles can also be synthesized in OTBN using any other reducing agent at any temperature for any application. The reducing agent can be ascorbic acid, tri sodium citrate, dextrose, hydrazine, etc., and at any temperature in between 4° to 95° C.

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DESCRIPTION WITH REFERENCE TO DRAWINGS AND TABULATED DATA

Figure 1. X-ray diffraction pattern of (a) OTBN (b) Ag nanoparticles loaded OTBN. Peaks marked by * correspond to the organic template — chitosan.

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The as-synthesized OTBN showed peaks corresponding to (120), (013), (051), (151), (200), (231) and (251) planes (Figure 1 — curve (a)). All these peaks can be indexed as orthorhombic-AIOOH (JCPDS 21-1307). The broadened XRD peaks imply that the crystallite size of OTBN particles is very small. The mean crystallite size calculated from the Scherrer formula shows that nanocrystals are of an average size of 3.5 nm. The presence of organic template (chitosan) is also clear from the XRD data. The peaks marked by * in Figure 1 corresponding to 2θ (in degrees) = 18.7°, 20.6°, 41.2° are attributed to the presence of the organic template. It is to be observed that there is a definite difference in the full-width at half maxima (FWHM) for the peaks corresponding to AIOOH and organic template.

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Upon impregnation of silver nanoparticles in the OTBN, there are no new peaks observed in the diffraction pattern (Figure 1 — curve b). This is attributed to the low loading percentage of silver nanoparticles and homogeneous distribution of silver nanoparticles in OTBN. Comparing the diffraction peaks of OTBN and silver nanoparticles impregnated OTBN, a negative shift in the 2θ value is observed. However, the interplanar distance of OTBN increases after loading of silver nanoparticles loading.

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Figure 2. (a to c) HRTEM micrographs of Ag nanoparticles loaded OTBN system (d) EDAX spectrum of Ag nanoparticles loaded OTBN.

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In order to understand the interaction between OTBN and silver nanoparticles, silver nanoparticles impregnated OTBN matrix was analyzed under transmission electron microscope (Figure 2). The TEM image shows that all the three components such as silver nanoparticle, organic polymer and metal oxide/hydroxide nanoparticles in the Ag-OTBN. The

OTBN matrix stabilizes the silver nanoparticles from aggregation which results in the homogenous distribution of silver nanoparticles in the matrix (Figure 2a). It is clear from the TEM images that homogeneously sized silver nanoparticles are anchored in the organic polymer-metal oxide/hydroxide nanoparticle matrix (Figure 2b, 2c) and the silver nanoparticles are of 5-10 nm in size (Figure 2c). The sheet-like organic polymer chitosan is seen clearly (Figure 2a, 2b, 2c).

Figure 2d is the EDAX spectrum measured from the area shown in Figure 2b. And the presence of silver is confirmed.

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Figure 3. EDAX elemental imaging of Ag nanoparticles loaded OTBN matrix. Top left extreme is the TEM image and others are elemental maps from the region.

EDAX coupled with TEM was used to image the elemental mapping of Ag loaded OTBN (Figure 3). Elements present in the Ag-OTBN such as C, N, O, Al and Ag were mapped. The presence of three components such as chitosan (C, N and O), boehmite (Al and O) and silver nanoparticles (Ag) was confirmed.

Figure 4. ICP-OES data for silver ion leaching in E.coli contaminated water at 30° C. (a) Allowed silver ion concentration in drinking water (WHO norms). (b) Released silver ion concentration in output water.

The Ag-OTBN material as explained in example 1 was used for column study. As explained in the example 7, the antibacterial activity was tested for Ag-OTBN column. The tap water having TDS between 300-500 ppm was used in the study. E.coli concentration of 1×10^5 CFU/mL was periodically spiked in tap water at the passage of 0, 250, 500, 750, 1000, 1250 and 1500 L. Contaminated water was filtered at 10-1000 mL/min flow rate. At regular intervals, the microbial de-contaminated output water was collected. Quantitative detection of concentration of silver ion released from the Ag-OTBN material was done using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Figure 4 shows the relation between the concentration of silver ion released into the contaminated tap water and the volume of water passed. Curve (a) in figure 4 shows the allowed silver ion concentration in drinking water and curve (b) shows the released silver ion concentration from Ag-OTBN. It is evident from figure 4 that silver ions are continuously released into the contaminated tap

water at a constant rate and the concentration found was significantly below the permitted level of silver ion in drinking water. The present invention demonstrates that the low concentration of 18-25 ppb silver ion released into the tap water having TDS between 300–500 ppm is enough for destroying all E.coli present in the water. From the ICP-OES, it was found that more than 10 % of silver content was released from silver nanoparticles loaded in OTBN into the tap water.

Figure 5. Antibacterial activity of Ag nanoparticles loaded OTBN tested in batch mode. (a) Input E. coli concentration (b) output E. coli concentration.

The Ag-OTBN material as explained in example 1 was used for batch study. As explained in the example 6, the antibacterial activity was tested for batch mode. Figure 5 shows the antibacterial efficiency of Ag-OTBN with number of trials. Curve (a) in Figure 5 shows the input concentration of E. coli and curve (b) shows the number of E.coli colonies after 1 hr of shaking. It is confirmed from curve (b) that the Ag-OTBN completely kills the E.coli present in the water. For up to 30 trials, complete killing of E.coli was seen. It should be noted that the number of trials or the output E.coli counts does not indicate the saturation point of the Ag-OTBN material but shows the continuous release of silver ions at constant rate. It should also be noted that the concentration of released silver ions from silver nanoparticles is higher under vigorous shaking for an hour (150 rpm). And hence one cannot expect it run for many trials under such unrealistic tough conditions. However, the antibacterial activity of Ag-OTBN in batch method indirectly demonstrates the promising long-time antibacterial activity of Ag-OTBN column in real water.

The material was also tested for antibacterial study without contact mode. The 100 mL of the shaken water was filtered and 1×10^5 cfu/mL of bacterial load was added to the water. It was plated as mentioned earlier. The performance of the material tested without contact mode is similar to the material tested with contact mode (data not shown). It showed that the anti-bacterial property is due to the released silver ions from silver nanoparticles.

Figure 6. Antibacterial activity of Ag nanoparticles loaded OTBN tested in column mode. (a) Input E. coli concentration (b) output E.coli concentration.

As explained in the example 7, the antibacterial activity was tested for a column filled with Ag-OTBN. Figure 6 shows the antibacterial efficiency of Ag-OTBN with volume of contaminated water passed. Curve (a) in figure 6 shows the input concentration of 10^5 CFU/mL E.coli and curve (b) shows the number of surviving E. coli colonies after filtration.

It is evident from curve (b) that the Ag-OTBN material shows the complete inhibition of E. coli for 1500 L at 100-1000 mL/min flow rate. It should be noted that complete killing was observed both at 100 mL/min and 1000 mL/min. Hence, the present invention demonstrates that the complete inhibition of E.coli at the concentration of $\sim 10^5$ CFU/mL can be achieved using Ag-OTBN material even at very high flow rate such as ~ 1000 mL/min.

Figure 7. Antiviral activity of Ag nanoparticles loaded OTBN tested in batch. (a) Input MS2 coliphage concentration (b) output MS2 phage concentration.

The Ag-OTBN material as explained in example 1 was used for batch study. Figure 7 shows the antiviral efficiency of Ag-OTBN with number of trials. Curve (a) in figure 7 shows the input concentration of MS2 coliphage and curve (b) shows the number of MS2 coliphage plaques after 1 hr of shaking. It is confirmed from curve (b) that the MS2 coliphage is completely removed from the water. For up to 35 trials, complete removal of MS2 coliphage was seen. It should be noted that the number of trials or the output counts does not indicate the saturation point of the Ag-OTBN material but shows the continuous performance of its antiviral property. Antiviral activity of Ag-OTBN in batch method indirectly demonstrates the promising long time performance of Ag-OTBN column in real water.

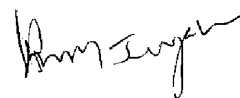
We Claim:

1. A method of preparing adsorbent composition having silver nanoparticles impregnated on organic-templated-boehmite-nanoarchitecture (OTBN), with particle size of silver nanoparticles less than 50 nm, for sustained and continuous release of silver into water below the permissible limit of 100 ppb wherein the adsorbent composition is used for destroying microorganisms in water.
2. A method as claimed in claim 1 wherein silver nanoparticles are supported on OTBN through impregnation of silver ions with OTBN in gel state and reducing the silver ions to zerovalent state by the use of a reducing agent.
3. A method as claimed in claim 1 wherein silver nanoparticles are supported on OTBN through impregnation of externally prepared silver nanoparticles with OTBN in the gel state.
4. A method as claimed in claim 1 wherein silver nanoparticles are supported on OTBN through impregnation of externally prepared silver nanoparticles with OTBN in the solid state.
5. A method as claimed in claim 1 wherein impregnation procedure involves drop-wise addition of silver ion or silver nanoparticles to the OTBN and consequently soaking for a duration of 30 minutes to 12 hours and preferably for a period of 1 hour.
6. A method as claimed in claim 1 wherein the organic template is chitosan.
7. A method as claimed in claim 1 wherein templates such as chitosan, banana silk and cellulose is used individually or in combination, with varying ratios.
8. A method as claimed in claim 2 wherein the reducing agent is chosen from sodium borohydride, ascorbic acid, tri-sodium citrate or hydrazine hydrate or the support itself or their mixtures.

9. A method as claimed in claim 1 wherein the silver precursor used for the preparation of silver nanoparticles is chosen from silver nitrate, silver fluoride, silver acetate, silver sulfate or silver nitrite or their mixtures.
- 5 10. A method as claimed in claim 1 wherein the concentration of silver is in the range of 0.001 M to 1 M, preferably in the range of 0.001 M to 0.1 M.
11. A method as claimed in claim 2 wherein the concentration of reducing agent is in the range of 0.005 M to 1M.
- 10 12. A method as claimed in claim 1 wherein the metal particles impregnated in adsorbent composition is of size in between 3 nm to 10 μ m.
13. A method as claimed in claim 10 wherein metal nanoparticles is based on silver, gold, copper, iron, nickel or a combination thereof.
- 15 14. A method as claimed in claim 1 wherein the BN in OTBN is boehmite nanoparticles or any other individual or mixed or doped metal oxide/hydroxide nanoparticles.
- 20 15. A method as claimed in claim 1 wherein the silver nanoparticles impregnated adsorbent composition is used as a filter in a filtering device in a variety of formats including but not limited to candle, molded porous block, filter bed, column, packets and bags.
- 25 16. A method as claimed in claim 15 wherein the adsorbent composition is used in the form of granules having a particle size between 0.3 mm to 5 mm and preferably between 0.3 mm to 1 mm.

Dated at Chennai this July 25, 2017

Signature:



D. Moses Jeyakaran
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IN/PA — 369

COMPLETE SPECIFICATION

5 TITLE OF THE INVENTION

A SUSTAINED SILVER RELEASE COMPOSITION FOR WATER PURIFICATION

FIELD OF THE INVENTION

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This invention relates to the constant release of silver ion from silver nanoparticles embedded in the organic-templated-boehmite nanoarchitecture (OTBN). Silver nanoparticles impregnated OTBN is capable of removing bacteria and virus from water. The antibacterial activity of the composition is due to the embedded silver nanoparticles and silver ions released from the same. The advantage with this composition is the constant concentration release of silver ions in water over extended periods, which is novel and important from the perspective of maintaining silver ion concentration below permissible limits while maintaining antimicrobial activity. The composition utilizes the virus removal capability of OTBN, as reported in previous patent application (1529/CHE/2010). Therefore, a simultaneous removal of both bacteria and virus from drinking water is achieved using an adsorbent composition.

BACKGROUND OF THE INVENTION

Drinking water contamination is a major health concern across the world, especially in the developing and under-developed countries. A number of contaminants affect the water quality. They include biological (eg. bacteria and virus), inorganic (eg. fluoride, arsenic, iron) and organic (eg. pesticides, volatile organics) species. Government of India has been extensively supporting the endeavor to bring quality drinking water to people of the country. The eleventh schedule in the Constitution of India along with article 47 requires the State to take care of the drinking water and nutrition needs of its citizens. Government of India has made a number of proactive efforts for ensuring good quality drinking water supply throughout the nation. An investment of more than Rs. 100,000 crore has already been made by the Central and State Governments in rural water supply sector and nearly Rs. 90,000 crore is envisaged to be spent during the XIth five year plan. India is also committed to the

Millennium Development Goal 2015 of reducing the population without access to drinking water by half. A number of governmental agencies are working together to address the drinking water contamination problem. These include, Ministry of Health and Family Welfare, Ministry of Rural Development, Department of Drinking Water Supply, Housing and Urban Development Corporation, Central Pollution Control Board, State Public Health Engineering Departments, etc. However, a significant cost burden still remains on the shoulders of the poor, with an estimated expenditure of Rs. 6,700 crore being made annually on treatment of waterborne diseases (<http://articles.timesofindia.indiatimes.com/2008-04-13/special-report/277807901-potable-water-drinking-water-fluoride> accessed on January 18, 2011).

Despite all the efforts made so far, contradicting reports continue to emerge on the availability of protected drinking water in Indian rural households. Some reports suggest that nearly 50% of villages still do not have any source of protected drinking water; however, as per the 2001 census, 68.2% households have access to safe drinking water. A report by WaterAid suggests that out of the 1.42 million villages in India, 1,95,813 are affected by chemical contamination of water. More than 400,000 children die under an age of five due to diarrhoea; more than 35 million people are afflicted by water borne diseases every year. It is estimated that 1.5 million children die of diarrhoea alone and 73 million working days are lost due to waterborne diseases each year. The resulting economic burden is estimated at \$600 million a year. While the statistics collected by various sources vary, the conclusion is the same: water is the source of a number of diseases for a large population of the country. In contrast, water has always been regarded as the elixir of life.

In our earlier work, we removed various contaminants from drinking water: organic contaminants such as pesticides (T. Pradeep, A. Sreekumar Nair, Indian Patent 200767 and PCT Application PCT/IN05/0002), biological contaminants such as E. coli (T. Pradeep, Prashant Jain, Indian Patent 20070608), fluoride (T. Pradeep, S. M. Maliyekkal, Anshup, Indian Patent Application 2089/CHE/2009; T. Pradeep, S. M. Maliyekkal, Anshup, M. Udhaya Sankar, Indian Patent Application 1529/CHE/2010) and heavy metals (T. Pradeep, Lisha K. P., Anshup, Indian Patent Application 169/CHE/2009; T. Pradeep, Amrita Chaudhary, T. Saraladevi, M. Udhaya Sankar, S. M. Maliyekkal, Anshup, Indian patent application, 2433/CHE/2010; T. Pradeep, S. M. Maliyekkal, T. S. Sreeprasad, Indian patent application, 2563/CHE/2010) in addition to developing a water purification device (T.

Pradeep, M. Udhaya Sankar, Amrita Chaudhary, Anshup, Indian patent application, 2892/CHE/2010). As a continuation of our research activities on nanomaterials for environmental remediation and applications, we have impregnated silver nanoparticles on the organic templated boehmite nanoarchitecture. This composition works actively for the removal of microorganisms from drinking water.

An important aspect of the utilization of such composition for the removal of microorganisms at household level in India is the affordability. A low cost of ownership for household-based water purifier is likely to play a major role in improving the health of citizens of this country (<http://www.nextbillion.net/news/hindustan-lever-to-take-water-purifiers-to-villages>, accessed on January 18, 2011). Percentage of population in India below international poverty line of US\$1.25 per day is 42% (UNICEF estimate, 2005) and 25% (CIA estimate, 2007). While the Government of India continues to uplift the economic well-being of people, it is also imperative to produce state-of-the-art products for people at the bottom of the pyramid. This invention aims to contribute to the initiative of improving health of people by safeguarding them against waterborne diseases at extremely affordable cost.

PRIOR ART

Silver is widely known for its antibacterial property. Silver has been employed as inorganic silver salt, as organic silver salt and as colloids of its salt, oxide and in metallic states. Although it is well known that silver is a good antibacterial agent, the nature of silver present in the water determines its antibacterial efficiency. For examples, various forms of silver such as those from inorganic salts and organic compounds, electrochemical dissolution process, nanoparticles, etc. show different antibacterial performance. In recent decades, silver has been extensively used as metallic nanoparticles. Without getting into the theoretical concepts of silver nanoparticles, the antibacterial property of silver nanoparticles emerges either from nanoparticle-bacteria surface interaction or from released silver ion from nanoparticles or both.

Antibacterial property of silver nanoparticles has been covered in a number of patent applications, wherein method of synthesis has been improved (Pal et. al. in Appl Environ Microbiol., 2007, 73(6), 1712; De Windt et. al. in United States Patent Application 20100272770; Sastry et. al. in 936/MUM/2008), made in media other than water (Chen et. al.

in United States Patent 7329301), and loaded various substrates (Rautaray et. al. in Indian patent application 1571/MUM/2008). The enhanced antibacterial property of silver nanoparticles is exclusively due to size confinement of silver metal. Although a number of methods have been developed for the synthesis of silver nanoparticles, keeping reactive particles in nanometer size for long time in real water composed of various species is very difficult. This is due to ion induced aggregation, surface modification and salt deposition, etc. Hence, an important requirement while employing reactive silver nanoparticles in water purification is size stabilization and prevention of surface modification for extended periods.

The importance of stability of silver nanoparticles in real water has been insisted in the literature. For example, Hoek et al. *J Nanopart Res.* 2010, 12, 1531, Hoek et al. *Environ. Sci. Technol.* 2010, 44, 7321, Bonzongo et al. *Environ. Sci. Technol.* 2009, 43, 3322 and Lead et al. *Environ. Sci. Technol.* 2009, 43, 7285 demonstrated that the antibacterial property of silver nanoparticles depends on intrinsic features (such as particle size, stability and shape of the nanoparticle) as well as extrinsic features (such as hardness of the water, soluble ligands in the water, ionic composition, organic matter and pH).

Another important aspect of use of silver nanoparticles for anti-bacterial performance is the fraction of silver ion released (quantity of silver ion released/quantity of silver nanoparticle used). It is known that though significant quantity of silver nanoparticles is used, very less silver ions are released into the contaminated water from silver nanoparticles. For example, Hoek et al. *Environ. Sci. Technol.* 2010, 44, 7321 reported that in reproduced real water having total dissolved solids (TDS) around 340 ppm, the fraction of dissolved silver is less than 0.1% of the total mass of silver added, regardless of the initial source, i.e., AgNO₃ or silver nanoparticles. This is attributed to the presence of various anions in real water such as chloride (many silver salts have very low solubility product). Hence, more than required quantity of silver nanoparticles is used in water filters. This results in increase in the filter size and cost.

While the fraction of silver ion released from silver nanoparticles is important, the rate of release is the most important step in employing the nanoparticles for long time usage. Constant release of silver ions from silver nanoparticles for longer time is most important for their use in water filters. This is important from the perspective of ensuring consistent anti-bacterial performance and release of silver ions below permissible limit as prescribed by

WHO. The rate of silver ion release has been discussed in the literature. For example, Epple et al. Chem. Mater. 2010, 22, 4548 and Hurt et al. Environ. Sci. Technol. 2010, 44, 2169 demonstrated that release of silver ion from silver nanoparticles in distilled water depends on temperature, incubation days, and species present in water such as dissolved oxygen level, salt and organic matters. They showed that the rate of dissolution is not constant with time but attains saturation in short period.

Hence, stability of reactive nanoparticles for long time in real water is very important for controlled release of silver ions. Metal oxides have been widely considered as good substrates (matrices). Silver nanoparticles have been ex-situ and in-situ loaded in/on metal oxides. In-situ loading in metal oxide has showed more promising stability even at high loading percentage. For example, In-situ syntheses of silver nanoparticles in metal oxide matrices have been reported earlier. Chen et al. Environ. Sci. Technol. 2009, 43, 2905 demonstrated the sol-gel synthesis of silver nanoparticles (<5 nm) loaded TiO₂ nanocomposite where TiO₂ particle acts as anti-aggregation supports. He showed that 7.4 wt% Ag loading in TiO₂ showed highly potent antibacterial properties against E.coli. Similar examples are available with rice husk ash (Rautaray et. al. in Indian patent application 1571/MUM/2008) and Fe₂O₃-fiber glass (Nangmenyi et. al. in Mater. Lett., 2011, 65, 1191).

Various attempts have been done to synthesize silver nanoparticles on low-cost substrates. For example, Shankar et al. J Chem Technol Biotechnol. 2008, 83, 1177 loaded silver on cheap activated carbon at high silver loading percentage. They reported that an optimum of 9-10.5 wt% of Ag loaded in activated carbon (5 g) is necessary for the complete killing of E.coli (concentration: 10³ CFU/ml) in the contact-mode for up to 350 L of flowing water (flow rate: 50 mUmin). This implies the use of ~0.5 g of silver for 350 L of bacterial free water (cost of water per liter is above 10 paisa per liter).

Therefore, the problem with the current systems is making reactive silver nanoparticles well stabilized by a supporting matrix without altering the surface chemistry for controlled silver ion release for extended period using less quantity of material.

Although synthesizing reactive silver nanoparticles and subsequently stabilizing its size by a supporting matrix is achievable easily, maintaining its reactivity over a long period by preventing surface modification and salt deposition for continuous controlled silver ion

release is very difficult. Controlled constant silver release is very important for long term use, effectiveness, life of the device and cost.

Therefore, the main object of the present invention is to provide optimum dissolution
5 of silver ions in real water from silver nanoparticles for long term use.

The another object of the present invention is increase of the volume of water treated
and time independent constant release of silver ions from silver nanoparticles loaded on
organic polymer-metal oxide/hydroxide composite such as chitosan stabilized boehmite.

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The another object of the present invention is the art of using organic polymer-metal
oxide/hydroxide composite as a dual stabilizing agent for the synthesis of highly dispersed
and stable silver nanoparticles having potent antibacterial activity at high flow rate at very
less loading wt%.

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SUMMARY OF THE INVENTION

Generally, more than required quantity of silver nanoparticles is used in water filters
due to very poor silver ion release in real water. This is in agreement with the literature
20 mentioned above (Shenker et al. J Chem Technol Biotechnol. 2008, 83, 1177). This results in
increase in the filter size and the cost. In the view of the need, the present invention
demonstrates that more than 10 % of the silver present in nanoparticles can be released as
silver ion into the tap water having moderately high TDS from silver nanoparticles loaded
OTBN over an extended period. The embodiment of the present invention includes the
25 volume of water treated and time independent constant release of silver ion from Ag-OTBN
matrix.

The present invention describes the art of making antibacterial filtration medium
made up of less than 10 nm sized silver nanoparticles, in-situ synthesized in organic-
30 templated-boehmite-nanoarchitecture (Ag-OTBN).

In the present invention, the potent antibacterial material for long term use is obtained
when silver nanoparticles are synthesized in organic templated metal oxide/hydroxide
nanoarchitecture. Stability of silver nanoparticles in real water for longer time is very

important for its constant antibacterial property. In the view of the importance of the stability of silver nanoparticles, those are in-situ synthesized in the OTBN matrix. We disclose that the OTBN matrix enhances the antibacterial property of silver nanoparticles in real water as the matrix controls the size, stabilizes the particles from aggregation and prevents the adsorption/deposition of soluble ligands, organic matters and dissolved solids on the silver nanoparticles.

The surface reactivity of silver nanoparticles is maintained by both chitosan and metal oxide/hydroxide. Silver nanoparticles are capped by chitosan - an organic polymer and also dispersed in metal oxide support and vice-versa. The dual stabilization prevents the surface modification and also salt deposition over a period of time.

According to the method of present invention, we demonstrate that even 0.5 wt% Ag loaded in OTBN is capable for the complete inhibition of 10^5 CFU/mL E.coli in the contact-mode for several hundred litres of flowing water at very high flow rate. This is achieved due to controlled constant release of silver ion for long time.

The present invention also demonstrate that the dissolved silver from silver nanoparticles in the tap water itself is capable of killing 10^5 CFU/mL E.coli without being contacted with the antibacterial material.

DESCRIPTION

DETAILED DESCRIPTION OF THE INVENTION

In this patent application, we describe the synthesis, characterization and application of silver nanoparticles impregnated organic-templated-boehmite-nanoarchitecture (Ag-OTBN). Impregnation of silver nanoparticles in OTBN is demonstrated using a number of procedures. The as-synthesized Ag-OTBN composition is characterized by a number of spectroscopic and microscopic techniques. The capability of Ag-OTBN to remove microorganisms from drinking water is demonstrated through the use of E.Coli and MS2 bacteriophage as model organisms for bacteria and virus, respectively. However, the result presented here should not be construed as limiting the scope of the invention.

Experimental methods

Material characterization

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The identification of the phase(s) of the as-prepared sample was carried out by X-ray powder diffraction (Bruker AXS, D8 Discover, USA) using Cu-K α radiation at $\lambda = 1.5418 \text{ \AA}$. Surface morphology, elemental analysis and elemental mapping studies were carried out using a Scanning Electron Microscope (SEM) equipped with Energy Dispersive Analysis of X-rays (EDAX) (FEI Quanta 200). For this, sample was re-suspended in water by sonication for 10 min and drop casted on an indium tin oxide (ITO) conducting glass and dried. High resolution Transmission Electron Microscopy (HRTEM) images of the sample were obtained with JEM 3010 (JEOL, Japan). The samples prepared as above were spotted on amorphous carbon films supported on copper grids and dried at room temperature. X-ray Photoelectron Spectroscopic (XPS) analysis was done using ESCA Probe TPD of Omicron Nanotechnology. Polychromatic Mg K α was used as the X-ray source ($h\nu = 1253.6 \text{ eV}$). Spectra in the required binding energy range were collected and an average was taken. Beam induced damage of the sample was reduced by adjusting the X-ray flux. Binding energy was calibrated with respect to C 1s at 284.5 eV. Silver ion concentration in the water was detected using inductively coupled plasma optical emission spectrometry (ICP-OES).

Some examples are provided to illustrate the present invention. The examples should not be construed as limiting the scope of the invention.

25 Example 1

This example describes the in-situ impregnation of silver nanoparticles on the OTBN. OTBN is prepared as reported in the previous patent application (1529/CHE/2010). The OTBN gel obtained after washing the salt content, is used for the formation of silver nanoparticles. The OTBN gel is again re-dispersed in water, to which 1 mM silver precursor (silver nitrate, silver fluoride, silver acetate, silver permanganate, silver sulfate, silver nitrite, silver bromate, silver salicylate or any combination of the above) is added drop-wise. The weight ratio of Ag to OTBN can be varied anywhere between 0.1-1.5%. After stirring the solution overnight, 10 mM sodium borohydride is added to the solution drop wise (in ice-cold condition,

temperature $< 5^{\circ}\text{C}$). Then, the solution was allowed to stir for half an hour, filtered and washed with copious amount of water. The obtained gel is then dried at room temperature for further studies.

5 **Example 2**

This example describes the in-situ impregnation of silver nanoparticles on the OTBN powder. The dried OTBN powder was crushed to a particle size of 100-150 micron. The powder is stirred in water, using a shaker. 1 mM silver precursor solution is then slowly added. The weight ratio of Ag to OTBN can be varied anywhere between 0.1-1.5%. After stirring the mixture overnight, 10 mM sodium borohydride is added to the mixture drop wise (in ice-cold condition, temperature $< 5^{\circ}\text{C}$). Then, the mixture was allowed to stir for half an hour, filtered and washed with copious amount of water. The obtained powder is then dried at room temperature for further studies.

15

Example 3

This example describes the ex-situ impregnation of silver nanoparticles on the OTBN. The OTBN gel obtained after washing the salt content, is used for the impregnation of silver nanoparticles. The OTBN gel is again re-dispersed in water, to which 1 mM silver nanoparticles solution (prepared by any route reported in the literature) is added drop-wise. The weight ratio of Ag to OTBN can be varied anywhere between 0.1-1.5%. After stirring the solution overnight, it was filtered and washed with copious amount of water. The obtained gel is then dried at room temperature for further studies.

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Example 4

This example describes the ex-situ impregnation of silver nanoparticles on the OTBN powder. The dried OTBN powder was crushed to a particle size of 100-150 μm . The powder is stirred in water, using a shaker. 1 mM silver nanoparticles solution (prepared by any route reported in the literature) is added drop-wise. The weight ratio of Ag to OTBN can be varied anywhere between 0.1-1.5%. After stirring the solution overnight, it was filtered and washed with copious amount of water. The obtained powder is then dried at room temperature for further studies.

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Example 5

This example describes the silver nanoparticles impregnation in transition and rare-earth metal doped OTBN composition (as claimed in previous patent application 1529/CHE/2010). Transition and rare-earth metals are chosen from the following list: zinc, manganese, iron, titanium, zirconium, lanthanum, cerium, silicon. The synthesis procedure for transition and rare earth metal doping in OTBN composition is as follows: the chosen metal salt is mixed with the aluminum salt solution in an appropriate ratio, preferably 1:9 (wt/wt). The salt solution was added slowly into the chitosan solution (dissolved in 1 - 5 % glacial acetic acid or HCl or combination thereof) with vigorous stirring for 60 minutes and kept overnight. Aqueous ammonia or NaOH solution was slowly added into the metal-chitosan solution with vigorous stirring to facilitate the precipitation of the metal-chitosan composites. All these steps were carried out at room temperature. Stirring was continued for two hours. The precipitates were filtered, washed to remove any unwanted impurities, converted to bead shape and dried.

The OTBN gel is again re-dispersed in water, to which 1 mM silver precursor is added drop-wise. The weight ratio of Ag to OTBN can be varied anywhere between 0.1-1.5%. After stirring the solution overnight, 10 mM sodium borohydride was added to the solution drop-wise (in ice-cold condition). Then, the solution was allowed to stir for half an hour, filtered and washed with copious amount of water. The obtained gel is then dried at room temperature for further studies.

Example 6

This example describes the testing protocol in batch for antibacterial activity of silver nanoparticles impregnated OTBN composition. 100 mL of water was shaken with the material and 1×10^5 CFU/mL of bacterial load was added to the water. Tap water having the TDS between 300-500 ppm was used in the study. After one hour of shaking, 1 mL of the sample along with nutrient agar was plated on sterile petridish using the pour plate method. After 48 hrs of incubation at 37 °C, the colonies were counted and recorded. This procedure was repeated 25 to 30 times.

Example 7

This example describes the testing protocol for antibacterial activity of silver nanoparticles impregnated OTBN powder packed in a column. The column in which a known quantity of the material is packed has a diameter anywhere between 35 mm to 55 mm. The feed water was passed at a flow rate in the range of 10 mL/min to 2000 mL/min. The tap water was periodically -subjected to a E.coli load of 1×10^5 CFU/mL. The output water collected from the column was screened for bacterial presence by pour plate method. The bacterial colonies were counted and recorded after 48 hours of incubation at 37°C.

Example 8

This example describes the testing protocol in batch for antiviral activity of silver nanoparticles impregnated OTBN composition. 100 mL of water was shaken with the material and 1×10^3 PFU/mL of MS2 coliphage load was added to the water. The tap water having the TDS between 300-500 ppm was used in the study. After one hour of shaking, virus count was done by plaque assay method. After 24 hrs of incubation at 37 °C, the plaques were counted and recorded. This procedure was repeated for 35 to 40 times.

The OTBN matrix defined in the present invention can be made up of chitosan stabilized any metal oxide/hydroxide nanoarchitectures. The metal precursor can be Fe(II), Fe(III), Al(III), Si(IV), Ti(IV), Ce(IV), Zn(II), La(III), Mn(II), Mn(III), Mn(IV) or a combination thereof. And the metal oxide/hydroxide nanoparticle may serve as an inert filler material or an active filtration medium.

It should be noted that the silver nanoparticles can be impregnated in transition and rare-earth metal doped OTBN compositions. It should also be noted that it can be of mixed metal oxide/hydroxide nanoarchitecture. The mixture can be binary or a mixture of all the above mentioned metal oxide/hydroxide.

The Ag-OTBN defined in the present invention can have chitosan polymer to metal oxide/hydroxide weight ratio in between 5 to 50%. And Ag to OTBN weight ratio can vary from 0.1 to 10 %.

It should be noted that the silver nanoparticles can also be synthesized in OTBN using any other reducing agent at any temperature for any application. The reducing agent can be ascorbic acid, tri sodium citrate, dextrose, hydrazine, etc., and at any temperature in between 4° to 95° C.

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DESCRIPTION WITH REFERENCE TO DRAWINGS AND TABULATED DATA

Figure 1. X-ray diffraction pattern of (a) OTBN (b) Ag nanoparticles loaded OTBN. Peaks marked by * correspond to the organic template — chitosan.

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The as-synthesized OTBN showed peaks corresponding to (120), (013), (051), (151), (200), (231) and (251) planes (Figure 1 — curve (a)). All these peaks can be indexed as orthorhombic-AIOOH (JCPDS 21-1307). The broadened XRD peaks imply that the crystallite size of OTBN particles is very small. The mean crystallite size calculated from the Scherrer formula shows that nanocrystals are of an average size of 3.5 nm. The presence of organic template (chitosan) is also clear from the XRD data. The peaks marked by * in Figure 1 corresponding to 2θ (in degrees) = 18.7°, 20.6°, 41.2° are attributed to the presence of the organic template. It is to be observed that there is a definite difference in the full-width at half maxima (FWHM) for the peaks corresponding to AIOOH and organic template.

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Upon impregnation of silver nanoparticles in the OTBN, there are no new peaks observed in the diffraction pattern (Figure 1 — curve b). This is attributed to the low loading percentage of silver nanoparticles and homogeneous distribution of silver nanoparticles in OTBN. Comparing the diffraction peaks of OTBN and silver nanoparticles impregnated OTBN, a negative shift in the 2θ value is observed. However, the interplanar distance of OTBN increases after loading of silver nanoparticles loading.

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Figure 2. (a to c) HRTEM micrographs of Ag nanoparticles loaded OTBN system (d) EDAX spectrum of Ag nanoparticles loaded OTBN.

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In order to understand the interaction between OTBN and silver nanoparticles, silver nanoparticles impregnated OTBN matrix was analyzed under transmission electron microscope (Figure 2). The TEM image shows that all the three components such as silver nanoparticle, organic polymer and metal oxide/hydroxide nanoparticles in the Ag-OTBN. The

OTBN matrix stabilizes the silver nanoparticles from aggregation which results in the homogenous distribution of silver nanoparticles in the matrix (Figure 2a). It is clear from the TEM images that homogeneously sized silver nanoparticles are anchored in the organic polymer-metal oxide/hydroxide nanoparticle matrix (Figure 2b, 2c) and the silver nanoparticles are of 5-10 nm in size (Figure 2c). The sheet-like organic polymer chitosan is seen clearly (Figure 2a, 2b, 2c).

Figure 2d is the EDAX spectrum measured from the area shown in Figure 2b. And the presence of silver is confirmed.

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Figure 3. EDAX elemental imaging of Ag nanoparticles loaded OTBN matrix. Top left extreme is the TEM image and others are elemental maps from the region.

EDAX coupled with TEM was used to image the elemental mapping of Ag loaded OTBN (Figure 3). Elements present in the Ag-OTBN such as C, N, O, Al and Ag were mapped. The presence of three components such as chitosan (C, N and O), boehmite (Al and O) and silver nanoparticles (Ag) was confirmed.

Figure 4. ICP-OES data for silver ion leaching in E.coli contaminated water at 30° C. (a) Allowed silver ion concentration in drinking water (WHO norms). (b) Released silver ion concentration in output water.

The Ag-OTBN material as explained in example 1 was used for column study. As explained in the example 7, the antibacterial activity was tested for Ag-OTBN column. The tap water having TDS between 300-500 ppm was used in the study. E.coli concentration of 1×10^5 CFU/mL was periodically spiked in tap water at the passage of 0, 250, 500, 750, 1000, 1250 and 1500 L. Contaminated water was filtered at 10-1000 mL/min flow rate. At regular intervals, the microbial de-contaminated output water was collected. Quantitative detection of concentration of silver ion released from the Ag-OTBN material was done using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Figure 4 shows the relation between the concentration of silver ion released into the contaminated tap water and the volume of water passed. Curve (a) in figure 4 shows the allowed silver ion concentration in drinking water and curve (b) shows the released silver ion concentration from Ag-OTBN. It is evident from figure 4 that silver ions are continuously released into the contaminated tap

water at a constant rate and the concentration found was significantly below the permitted level of silver ion in drinking water. The present invention demonstrates that the low concentration of 18-25 ppb silver ion released into the tap water having TDS between 300–500 ppm is enough for destroying all E.coli present in the water. From the ICP-OES, it was found that more than 10 % of silver content was released from silver nanoparticles loaded in OTBN into the tap water.

Figure 5. Antibacterial activity of Ag nanoparticles loaded OTBN tested in batch mode. (a) Input E. coli concentration (b) output E. coli concentration.

The Ag-OTBN material as explained in example 1 was used for batch study. As explained in the example 6, the antibacterial activity was tested for batch mode. Figure 5 shows the antibacterial efficiency of Ag-OTBN with number of trials. Curve (a) in Figure 5 shows the input concentration of E. coli and curve (b) shows the number of E.coli colonies after 1 hr of shaking. It is confirmed from curve (b) that the Ag-OTBN completely kills the E.coli present in the water. For up to 30 trials, complete killing of E.coli was seen. It should be noted that the number of trials or the output E.coli counts does not indicate the saturation point of the Ag-OTBN material but shows the continuous release of silver ions at constant rate. It should also be noted that the concentration of released silver ions from silver nanoparticles is higher under vigorous shaking for an hour (150 rpm). And hence one cannot expect it run for many trials under such unrealistic tough conditions. However, the antibacterial activity of Ag-OTBN in batch method indirectly demonstrates the promising long-time antibacterial activity of Ag-OTBN column in real water.

The material was also tested for antibacterial study without contact mode. The 100 mL of the shaken water was filtered and 1×10^5 cfu/mL of bacterial load was added to the water. It was plated as mentioned earlier. The performance of the material tested without contact mode is similar to the material tested with contact mode (data not shown). It showed that the anti-bacterial property is due to the released silver ions from silver nanoparticles.

Figure 6. Antibacterial activity of Ag nanoparticles loaded OTBN tested in column mode. (a) Input E. coli concentration (b) output E.coli concentration.

As explained in the example 7, the antibacterial activity was tested for a column filled with Ag-OTBN. Figure 6 shows the antibacterial efficiency of Ag-OTBN with volume of contaminated water passed. Curve (a) in figure 6 shows the input concentration of 10^5 CFU/mL E.coli and curve (b) shows the number of surviving E. coli colonies after filtration.

It is evident from curve (b) that the Ag-OTBN material shows the complete inhibition of E. coli for 1500 L at 100-1000 mL/min flow rate. It should be noted that complete killing was observed both at 100 mL/min and 1000 mL/min. Hence, the present invention demonstrates that the complete inhibition of E.coli at the concentration of $\sim 10^5$ CFU/mL can be achieved using Ag-OTBN material even at very high flow rate such as ~ 1000 mL/min.

Figure 7. Antiviral activity of Ag nanoparticles loaded OTBN tested in batch. (a) Input MS2 coliphage concentration (b) output MS2 phage concentration.

The Ag-OTBN material as explained in example 1 was used for batch study. Figure 7 shows the antiviral efficiency of Ag-OTBN with number of trials. Curve (a) in figure 7 shows the input concentration of MS2 coliphage and curve (b) shows the number of MS2 coliphage plaques after 1 hr of shaking. It is confirmed from curve (b) that the MS2 coliphage is completely removed from the water. For up to 35 trials, complete removal of MS2 coliphage was seen. It should be noted that the number of trials or the output counts does not indicate the saturation point of the Ag-OTBN material but shows the continuous performance of its antiviral property. Antiviral activity of Ag-OTBN in batch method indirectly demonstrates the promising long time performance of Ag-OTBN column in real water.

1. A method of preparing adsorbent composition having silver nanoparticles impregnated on organic-templated-boehmite-nanoarchitecture (OTBN), with particle size of silver nanoparticles less than 50 nm, for sustained and continuous release of silver into water below the permissible limit of 100 ppb wherein the adsorbent composition is used for destroying microorganisms in water.
2. A method as claimed in claim 1 wherein silver nanoparticles are supported on OTBN through impregnation of silver ions with OTBN in gel state and reducing the silver ions to zerovalent state by the use of a reducing agent.
3. A method as claimed in claim 1 wherein silver nanoparticles are supported on OTBN through impregnation of externally prepared silver nanoparticles with OTBN in the gel state.
4. A method as claimed in claim 1 wherein silver nanoparticles are supported on OTBN through impregnation of externally prepared silver nanoparticles with OTBN in the solid state.
5. A method as claimed in claim 1 wherein impregnation procedure involves drop-wise addition of silver ion or silver nanoparticles to the OTBN and consequently soaking for a duration of 30 minutes to 12 hours and preferably for a period of 1 hour.
6. A method as claimed in claim 1 wherein the organic template is chitosan.
7. A method as claimed in claim 1 wherein templates such as chitosan, banana silk and cellulose is used individually or in combination, with varying ratios.
8. A method as claimed in claim 2 wherein the reducing agent is chosen from sodium borohydride, ascorbic acid, tri-sodium citrate or hydrazine hydrate or the support itself or their mixtures.

9. A method as claimed in claim 1 wherein the silver precursor used for the preparation of silver nanoparticles is chosen from silver nitrate, silver fluoride, silver acetate, silver sulfate or silver nitrite or their mixtures.

5 10. A method as claimed in claim 1 wherein the concentration of silver is in the range of 0.001 M to 1 M, preferably in the range of 0.001 M to 0.1 M.

11. A method as claimed in claim 2 wherein the concentration of reducing agent is in the range of 0.005 M to 1M.

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12. A method as claimed in claim 1 wherein the metal particles impregnated in adsorbent composition is of size in between 3 nm to 10 μ m.

13. A method as claimed in claim 10 wherein metal nanoparticles is based on silver, gold, copper, iron, nickel or a combination thereof.

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14. A method as claimed in claim 1 wherein the BN in OTBN is boehmite nanoparticles or any other individual or mixed or doped metal oxide/hydroxide nanoparticles.

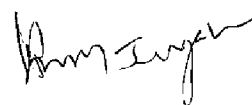
20 15. A method as claimed in claim 1 wherein the silver nanoparticles impregnated adsorbent composition is used as a filter in a filtering device in a variety of formats including but not limited to candle, molded porous block, filter bed, column, packets and bags.

25 16. A method as claimed in claim 15 wherein the adsorbent composition is used in the form of granules having a particle size between 0.3 mm to 5 mm and preferably between 0.3 mm to 1 mm.

Dated at Chennai this July 25, 2017

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Signature:



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A SUSTAINED SILVER RELEASE COMPOSITION FOR WATER PURIFICATION

ABSTRACT

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A single adsorbent composition having simultaneous antibacterial and antiviral property for use in water purification is described. The antibacterial-cum-antiviral system consists of organic-templated-boehmite nanoarchitecture (OTBN) impregnated with silver nanoparticles, wherein the size of silver nanoparticles is below 50 nm. A sustained release of silver ions at
10 nearly 25 ppb, well below the permissible limit of 100 ppb as recommended by WHO, from silver nanoparticle-loaded OTBN for extended periods is described. The organic template-chitosan and metal oxyhydroxide-boehmite serve as dual stabilizing agents for silver nanoparticles for constant silver ion release allowing long term use of the composition for water purification. The antibacterial property arises from the silver nanoparticles and released
15 silver ions from the same and, the composition utilizes the virus removal capability of OTBN, reported in our Indian Patent application 1529/CHE/2010. A water purification device based on granular Ag-OTBN adsorbent composition is also demonstrated.

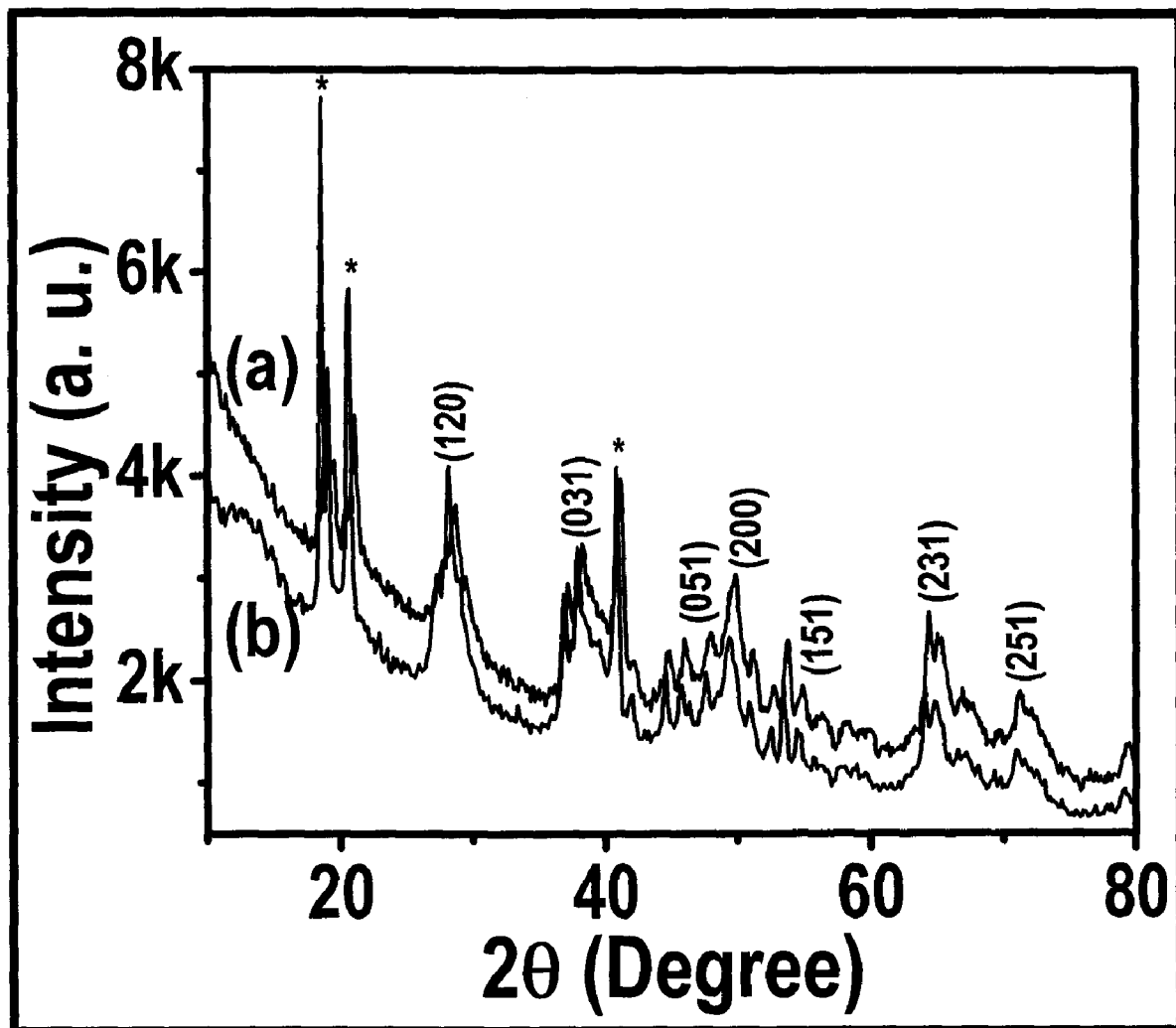


Figure 1

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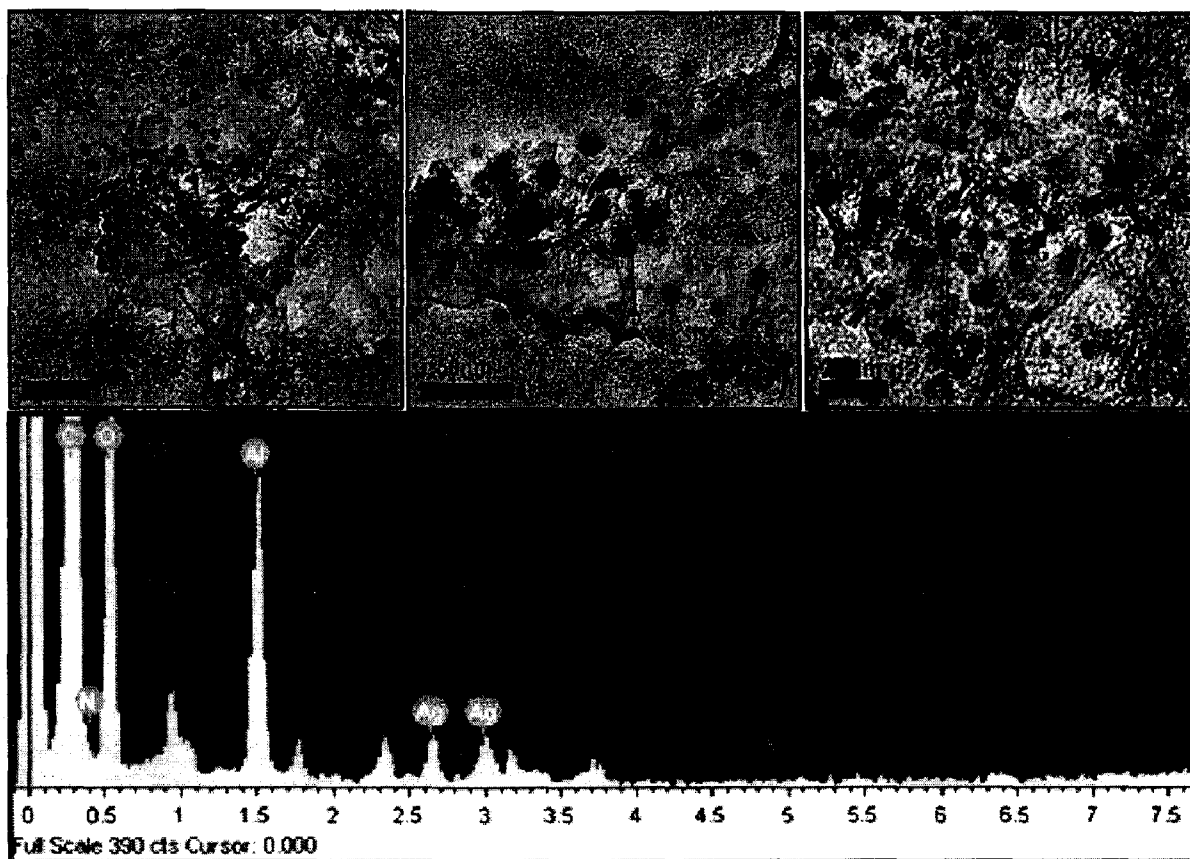
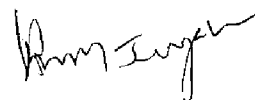


Figure 2

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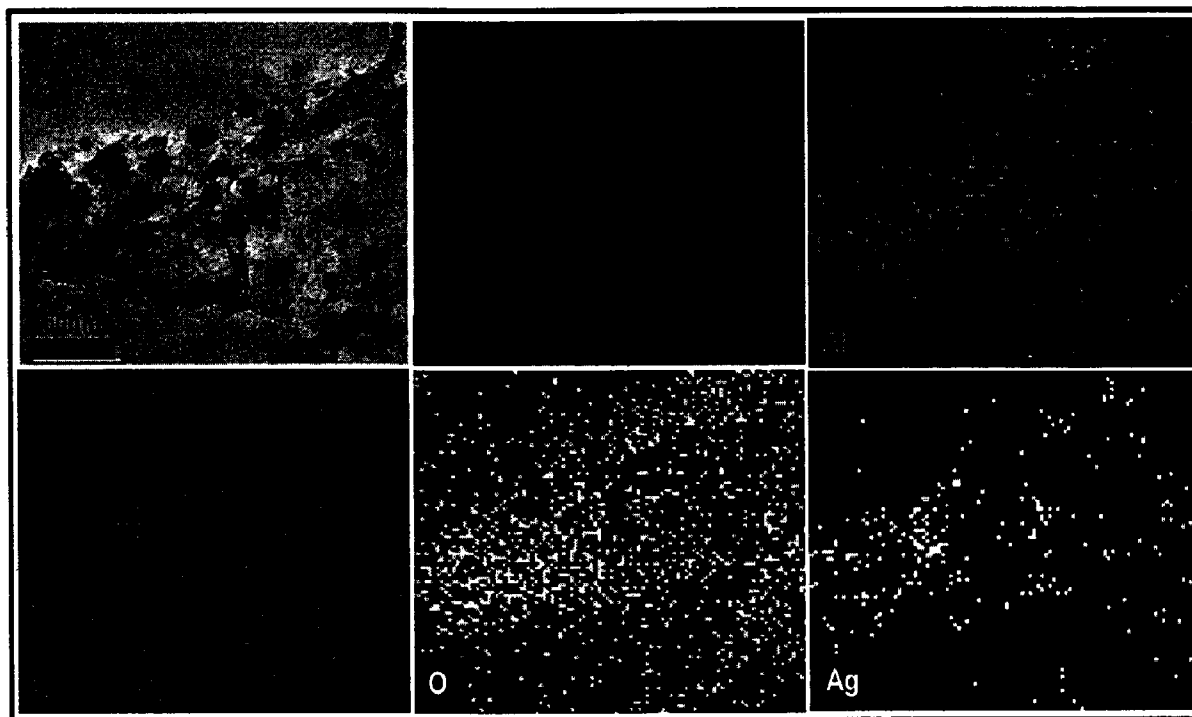


Figure 3

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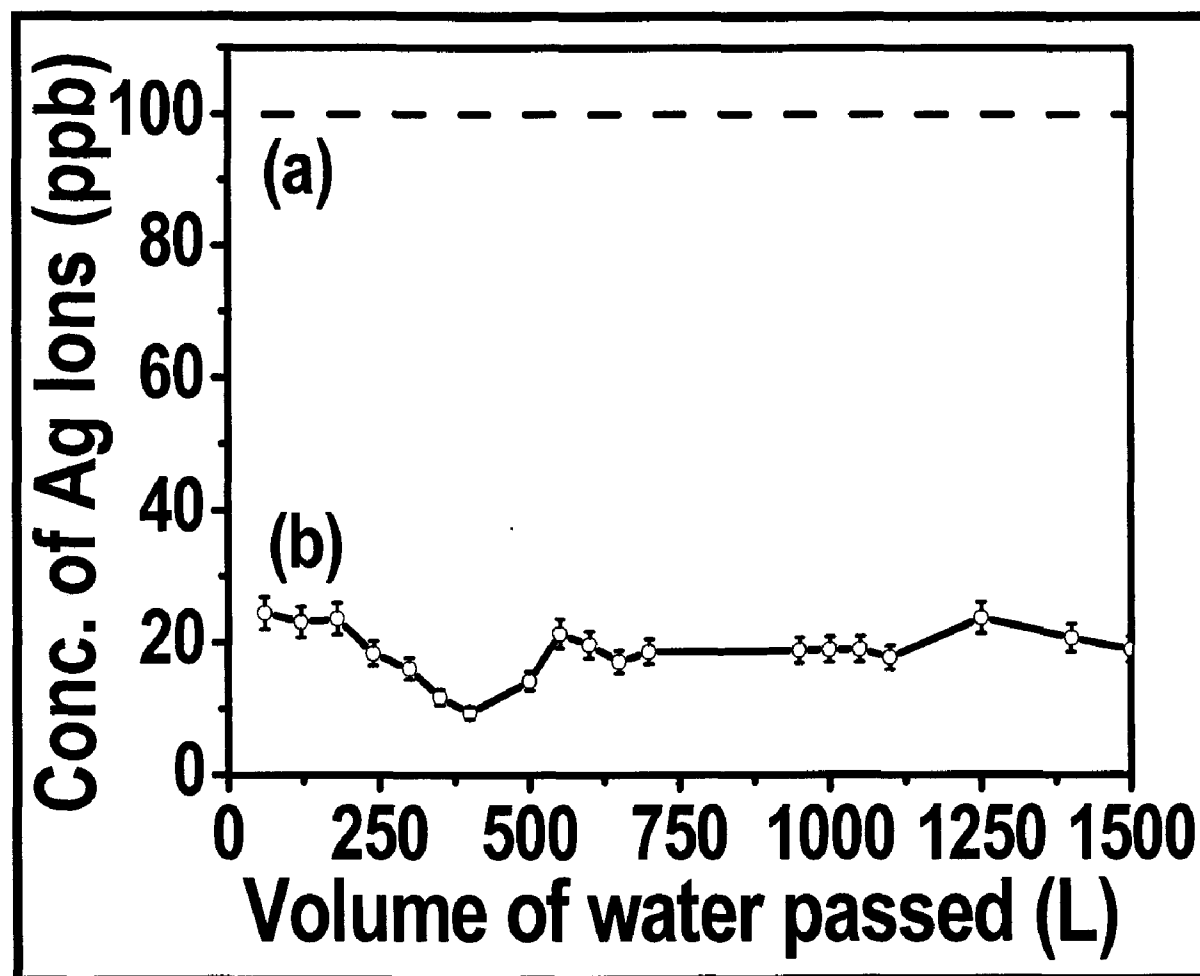
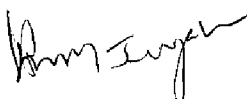


Figure 4

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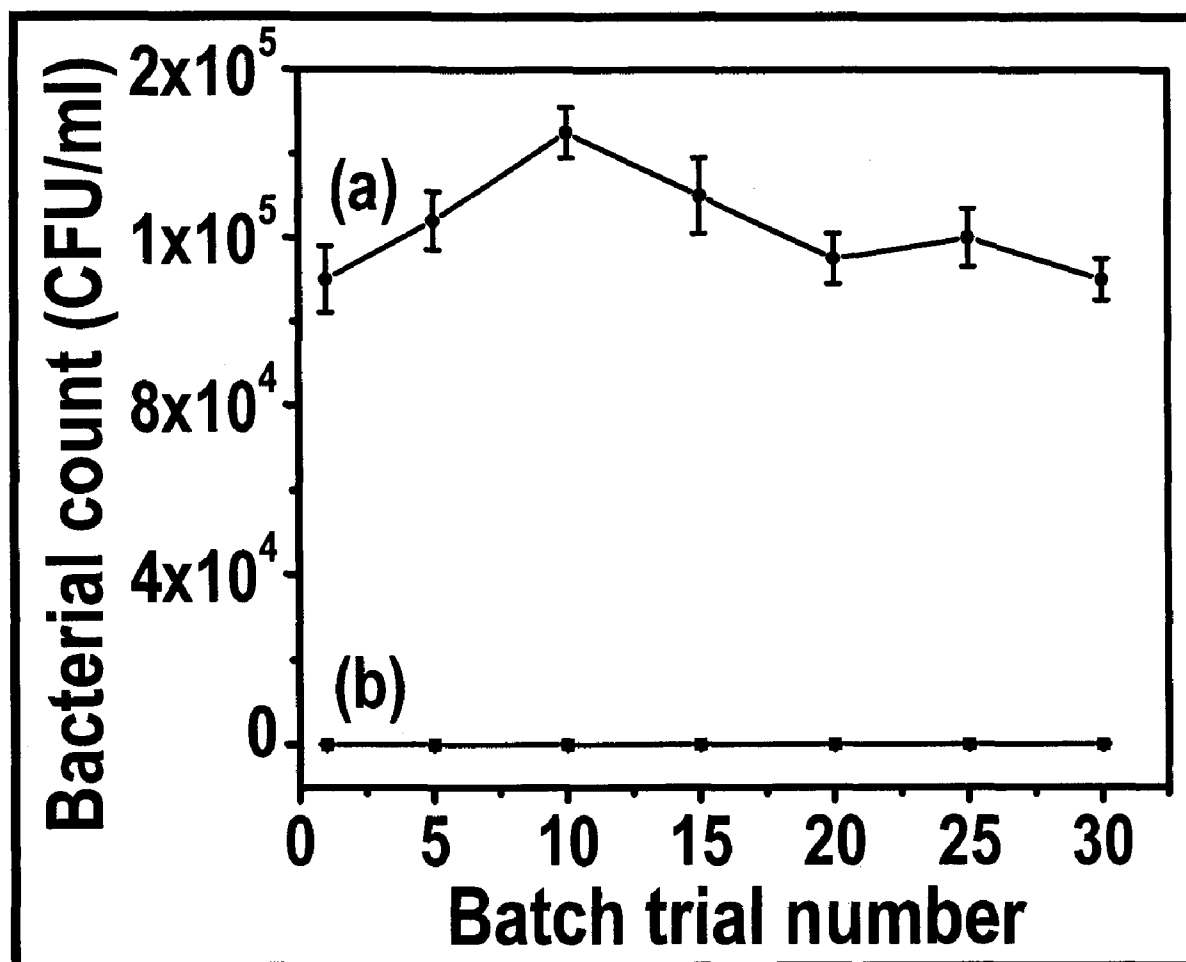


Figure 5

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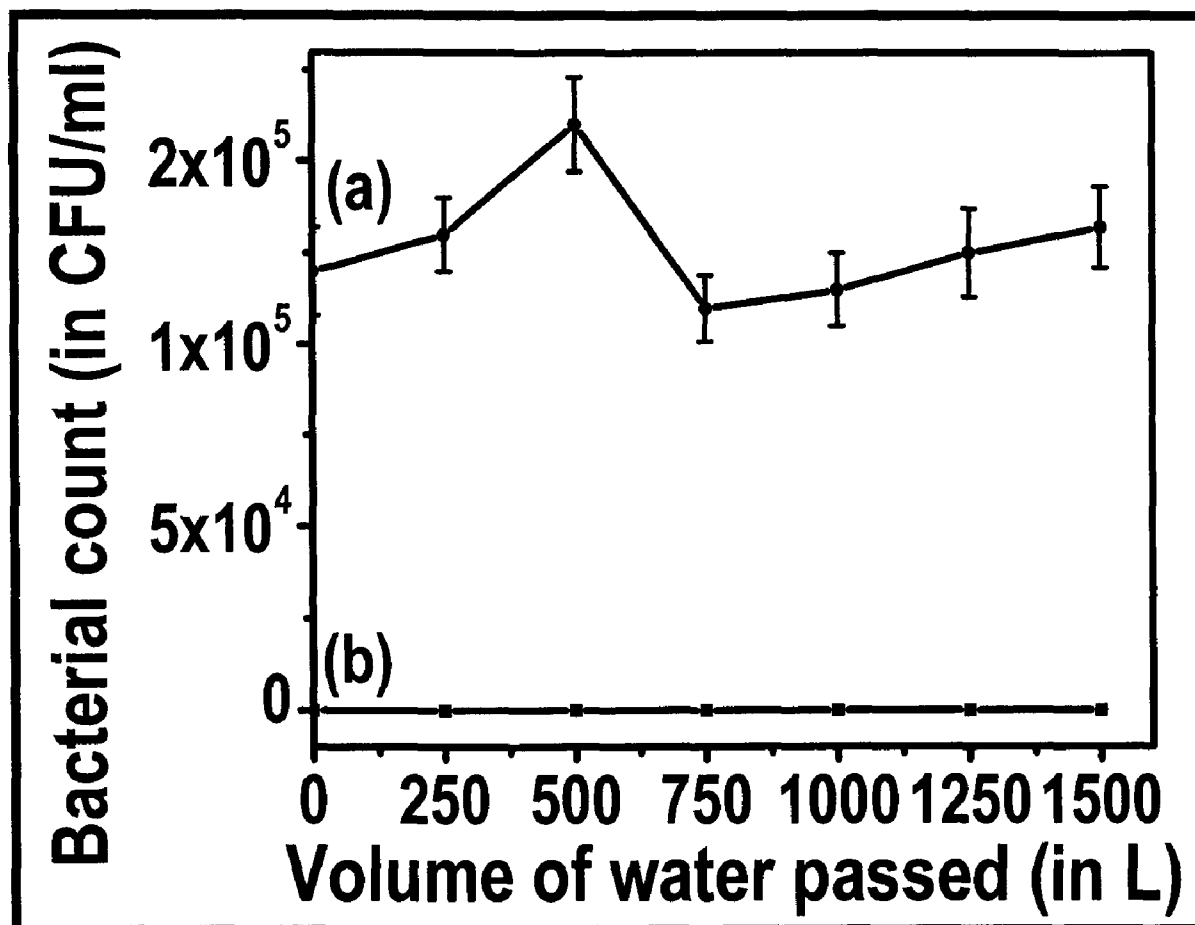


Figure 6

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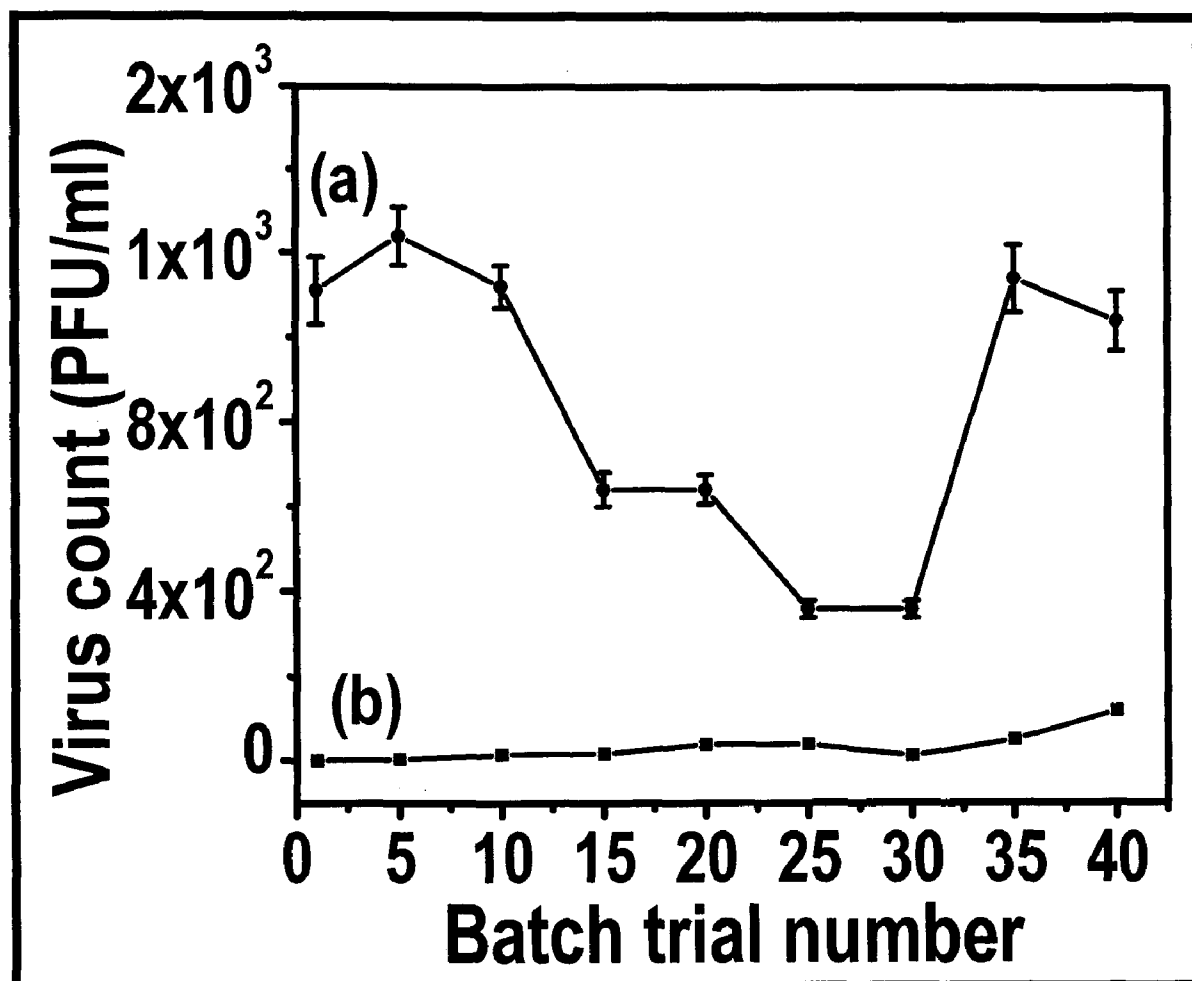


Figure 7

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