Antimicrobial activity of silver-coated hollow poly(methylmethacrylate) microspheres for water decontamination

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Abstract
Background: Growing microbial resistance towards the existing antimicrobial materials appears as the greatest challenge for the scientific community and development of new antimicrobial materials has become an important research objective.

Results: In this work, antimicrobial activity of silver-coated hollow poly(methylmethacrylate) microspheres (PMB) having a diameter of 20–80 µm was evaluated against two bacterial strains, Gram-positive Bacillus subtilis (MTCC 1305) and Gram-negative Escherichia coli (MTCC 443). The polymeric PMMA microspheres were synthesized by solvent evaporation technique and were further coated with silver (Ag) under microwave irradiation on their outer surface using an electroless plating technique. It was observed that Ag was uniformly coated on the surface of microspheres. Characterization of the coated microspheres was performed using optical microscope (OMS), scanning electron microscope (SEM), energy dispersive X-ray spectroscopy (EDX), UV–Vis spectroscopy, FTIR spectroscopy and thermo-gravimetric analysis (TGA) techniques. We have shown that the silver-coated microspheres were potent bactericidal material for water as they are highly active against the tested microorganisms. The results of the antibacterial tests indicated that APMB particles showed enhanced inhibition rate for both Gram-positive and Gram-negative bacteria and also exhibited dose-dependent antibacterial ability. The diameters of zone of inhibition were 14.3 ± 0.2 mm against B. subtilis and 15.2 ± 0.9 mm against E. coli at a concentration of 8 mg. At this concentration, total removal of both Bacillus subtilis and Escherichia coli was observed. The results of shake flask technique for a concentration of 8 mg showed no bacterial presence after 24 h in both the cases. In other words, the material acted efficiently in bringing down the bacterial count to zero level for the tested strains. During the experiments, we have also confirmed that use of this material for water disinfection does not cause leaching of silver ion in to the water solution. The material can be successfully regenerated by backwashing with water.

Conclusions: Considering the cost-effective synthesis, ability to regenerate and very low level of leaching of the material, it can be projected as an advanced material for water disinfection and antimicrobial application.

Keywords: Antimicrobial, Poly(methylmethacrylate), Silver-coated hollow microsphere, Water disinfection, Gram-positive bacteria, Gram-negative bacteria, Zone of inhibition

Background
The purity of drinking water is one of the primary requisites for maintaining public health and well-being. But many countries in the world are still suffering due to unavailability of safe drinking water, which has put millions of people at vulnerable risk from waterborne diseases [1–3]. Even waterborne diseases still remain the prominent...
cause of death for many countries. Many of these diseases are related to pathogenic microorganisms living in the drinking water, which find their way into human bodies by ingestion of contaminated water [4]. Disinfection is the process by which these microorganisms are deactivated prior to human consumption. Conventional disinfection methods include chlorination, ultraviolet light, reverse osmosis, and use of silver catalyst. But some of these methods lead to harmful disinfection byproducts [5]. Further studies reveal that these pathogens develop resistance for conventional chemicals used for disinfection resulting in the use of large dosages [6, 7]. Other methods like UV purification and reverse osmosis require special systems and power for their functioning and are thus not cost-effective [8, 9]. Hence the exploration for an ideal decontamination process has impelled researchers throughout the globe to investigate into the use of nanotechnology-based approaches for water disinfection using diverse metallic or non-metallic nanoparticles (NPs) to deactivate the microorganisms [10, 11]. Many different studies were carried out involving the synthesis, characterization, and assessment of various NPs for their application in water disinfection [12–19].

The antimicrobial properties of copper, silver and other metal ions, have been well known for centuries [2, 20, 21]. Among the metal ions, silver exhibits the highest toxicity for microorganisms and minimum toxicity towards animal cells. The excellent biocidal property combined with nontoxicity towards human cells [22, 23] makes silver one of the highest priority materials for water purification. The antimicrobial spectrum of silver is extensive, including Gram-negative enterobacteria and Gram-positive cocci [24–26]. Available literature indicates that silver possess virucidal properties against bovine rotavirus, adenovirus, poliovirus, herpesas well as vaccina virus. It also possesses anti-algal properties [27–29].

The development of silver in the form of nanoparticles (AgNPs) has resulted in more effective and useful application of silver for bacterial decontamination [30, 31]. AgNPs are thought to disinfect [34] via: (1) release of silver ion (Ag); (2) damage of cell membrane via direct contact and/or; (3) generation of reactive oxygen species (ROS). Some of the present applications that exploit the antibacterial property of AgNPs include medical devices, clothing, portable water filters and coating for food containers and washing machines [30, 34].

The only drawback associated with nanoparticles is that they tend to agglomerate due to the high surface energy. Hence the immovability of silver nanoparticles towards aggregation is considered as the most important feature for their efficient antibacterial action, as bacterial interaction with AgNPs is more rigorous when the NPs are in well-dispersed state [35]. To achieve this objective, AgNPs need to be immobilized to control their contact and release kinetics (of either ionic or particulate form) so that they can be applied in a cost-effective manner [30]. Hence, AgNPs have been either adsorbed on or embedded in various organic or inorganic substrates which include filter materials made of silica, zeolite, natural macroporous materials, carbon materials, fiberglass and polymers or paper of different types [36–41]. For instance, nanoparticles of silver were embedded in materials like, montmorillonites [23], polysulfone [42] cellulose acetate [37], fiberglass [43], polyurethane foams [44], ceramic filters [45–48] and activated carbon [49] to enhance their applicability, especially for water purification system. However, many times these materials associate with the drawbacks like relatively poor disinfection of water and poor structural stability in water purification system. Therefore, primary focus of the work presented here is to develop a silver-coated hollow PMMA microsphere for water purification system with enhanced disinfection capacity and which is also highly mechanically durable. The lightweight polymethyl methacrylate is also an economical alternative to many substrate/mediums referred above. Apart from great mechanical properties, PMMA does not contain potential harmful units and, therefore, it is truly an incomparable choice as substrate for water purification system.

Numerous studies have been performed on the usage of silver nanoparticles embedded polymer composites for water disinfection [23, 27, 38]. However, for many polymeric materials, polymer degradation is very common which causes leaching of monomer and if the polymer contains potentially harmful units like bisphenol-A, the material becomes harmful for health. Polymers embedded with silver nanoparticles deliver antibacterial property by means of sustained release of silver [37, 45, 50]. In near future, nonleaching silver nanoparticles embedded copolymer beads will be the pioneer material for safe water purification technology.

Present study, therefore, aims to evaluate the water filter-related antibacterial efficacy of the silver-coated PMB (APMB) against Gram-positive Bacillus subtilis and Gram-negative Escherichia coli using different microbiological methods, so that a novel material with enhanced disinfection capacity can be developed for water purification systems.
Materials and methods

Materials
Polymethylmethacrylate (PMMA) [Sigma-Aldrich, MW (avr.): 1,200,000, 98%, viscosity 0.20 dL/g (lit.)], dichloromethane [Merck, 99.5%, M = 84.93 g/mol], polyvinyl alcohol [Central Drug House, Delhi, MW (avr.): 1,250,000, 99.25%, viscosity 35-50cP at 4% cold aqueous solution], Stannous chloride (SnCl₂ 98%, Acros Organics), concentrated hydrochloric acid (Conc. HCl 98%, Lancaster), silver nitrate (AgNO₃ 99%, Lancaster) and 2-amino-2-methyl-1-propanol (AMP 98%, Lancaster), ethylene glycol (Anhydrous, 99.8% Merck), ethyl alcohol (AR 99.9% Jiangsu Huaxi International China), and ammonium hydroxide solution (28% NH₃ in H₂O, ≥ 99.99% trace metals basis Sigma-Aldrich) were used as such.

Synthesis of PMMA microspheres
Hollow PMMA microspheres were prepared using modified solvent evaporation technique as shown in Fig. 1 [51]. Initially a solution was prepared by dissolving PMMA (5–6% W/V) in dichloromethane by magnetic stirring. The solution was added dropwise to a stirring aqueous medium with occasional stirring. The aqueous medium comprises of (0.5%, w/v) of poly (vinyl alcohol) which acts as stabilizer. The stirring was maintained at 550 rpm with propeller-type mechanical stirrer. PMMA microspheres (PMB) were formed by slow evaporation of dichloromethane at room temperature (RT). At the end of the reaction obtained hollow PMMA microspheres were washed with water and dried at 70 °C. The bulk density of the PMB was calculated as 0.69 gm/cc.

Synthesis of silver-coated PMMA microspheres
Figure 2 shows the synthesis of silver coated PMMA microspheres. The surface of synthesized PMMA microspheres was first sensitized by treating with tin chloride solution. 5 g of synthesized PMMA microspheres were suspended in 50 ml deionized water. To this suspension, 10 ml of 3% SnCl₂ solution in 1 M HCl was added. The mixture was shaken and left undisturbed for 10–15 min. The resulting mixture was filtered and washed using several aliquots of water. The microspheres were then dried at 80 °C. A tube was loaded with the following starting materials: sensitized PMB (0.4 g), Deionized water (3 mL), ethylene glycol (EG) (1 mL), ethanol (1 mL), NH₄OH (0.1 mL), and AgNO₃ (0.085 g). The reaction mixture was purged with argon for 30 min to expel dissolved oxygen/air. After the bubbling of argon, the reaction solution was irradiated for 5 min in a modified [17] microwave digestive system (Milestone Ethos Easy Advanced microwave Digestion System 3500VA). Finally, the silver-coated microspheres were collected, washed with water and ethanol, and dried overnight in vacuum. Their change in color to grey was an immediate indication of the successful silver coating of the PMMA. Bright shiny grey coloured silver-coated microspheres were obtained. Titration of the prepared product with potassium thiocyanate (KSCN) shows that ∼ 23 wt % Ag is found on the PMMA.

1. \[ \text{Ag}^{+} + 2 \text{NH}_3 \rightarrow [\text{Ag(NH}_3)_2]^+ \]
2. \[ \text{HOCH}_2\text{CH}_2\text{OH} \rightarrow \text{CH}_3\text{CHO} + \text{H}_2\text{O} \]
3. \[ 2\text{CH}_3\text{CHO} + 2[\text{Ag(NH}_3)_2] \rightarrow 2\text{Ag} + 4\text{NH}_4^+ + \text{CH}_3\text{COCOCH}_3 \]
Bacterial strains
The synthesized silver-coated hollow PMMA microspheres (APMB) were tested for their antimicrobial activity against a Gram-positive bacterial strain *Bacillus subtilis* (MTCC 1305) and a Gram-negative bacterial strain *Escherichia coli* (MTCC 443). The strains were obtained from microbial type culture and collection (MTCC), Chandigarh, India. The lyophilized culture was pre-cultured in nutrient broth medium overnight in a rotary shaker at 37°C. After that, each strain was adjusted to a concentration of $10^8$ cells/ml using 0.5 McFarland standards [52].

Agar well diffusion assay
Preliminary antibacterial activity of APMB microspheres and uncoated PMMA microspheres (PMB) for comparison was evaluated using the agar well diffusion assay. The bacterial test organisms were cultured in the nutrient broth overnight to attain the colony-forming unit (CFU) of $\sim 10^8$ cells/ml. One-hundred microlitres (µL) of each bacterial culture was spread on the Luria–Bertani (LB) agar plates. Upon solidification, wells (6 mm in diameter) were punched using a sterile cork borer and loaded with 10 mg of PMB and different amounts of APMB (2, 4, 6, 8 mg), respectively. The plates were then incubated for 18 h at 37°C and diameters of zone of inhibition were recorded in millimeter (mm).

Antimicrobial test by dynamic contact
3 ml of the stock culture with CFU $\sim 10^8$ cells/ml was first pelleted down through centrifugation at 1000 rpm for 5 min. After discarding the supernatant layer, the pellet was washed twice with distilled water to remove the remaining broth. It was then suspended with 3 ml of distilled water to make up the stock volume. Further, 10-ml test tubes were taken each filled with 2 ml of distilled water and mixed with different concentrations of the test sample along with 10 µl of the bacterial stock culture. One test tube was considered as blank which contained only 2-ml distilled water along with 10 µl of the bacterial culture. These test tubes were then put inside the shaker incubator at 150 rpm and 37°C for 24 h. After 24 h of incubation, the samples are tested for microbial presence by plate count [53] and OD measurement in UV–Vis spectrophotometer leading to bacterial count [54].

Effect of concentration of APMB on removal efficiency of bacteria
Percent reduction calculation
Percent reduction = \( (A - B) \times 100 / A \).

where: A represents the number of viable microorganisms before treatment; B represents the number of viable microorganisms after treatment.

Log reduction calculation
Log reduction = \( \log_{10} (A / B) \),

or
Log reduction = \( \log_{10} (A) - \log_{10} (B) \),

where A and B denotes the same as mentioned above formula to convert log reduction to percent reduction.

\[ P = (1 - 10^{-L}) \times 100, \]

where P is the percent reduction; L is the log reduction.

\[ P = (1 - 10^{-L}) \times 100, \]
\[ P/100 - 1 = 10^{-L}, \]
\[ -P/100 + 1 = 10 - L. \]

Logarithm rule
Filtration under gravity using a pen shell
To test the antibacterial efficiency of APMB in real-life situation a small filter was fabricated using a pen shell and the synthesized material was put between two presterilized glass wool layers at top and bottom. Each slot of the filtration tube contained 2 mg of the sample separated by thin layer of glass wool so that there is no physical contact between each slot/layers of APMB. For a low dose of 2 mg only one layer was used while for 4, 6 and 8 mg the layers increased from 2, 3 to 4 each separated by glass wool as shown in Fig. 3. 100 ml bacterial contaminated solution was passed through it. The filtrate was tested for microbial presence using standard plate count method [53] and optical density (OD) measurement using UV–Vis spectrophotometer leading to bacterial count [54]. The mean average value for both the bacterial testing is compiled into the results.

\[ \log_{10} X = N \quad aN = X \]

In this case
\[ a = 10, \]
\[ N = -L, \]
\[ X = -\frac{P}{100} + 1. \]

Therefore,
\[ \log_{10}(-\frac{P}{100} + 1) = -L, \]
\[ \text{or} \]
\[ L = -(\log 10(-\frac{P}{100} + 1)). \]

The leaching test was performed by already reported method [55].

Characterization
The materials synthesized were characterized using an optical microscope with Leica DMLM/P, Leica Microsystems AG Switzerland at 50× magnification, scanning electron microscope attached with energy dispersive X-ray analysis (Carl ZEISS, EVO50), Double beam UV–Vis Spectrophotometer (Analytica JENA Model Specord 205), FTIR Spectrophotometer (Bruler Alpha model with KBr) and Thermogravimetric analysis (TGA) (TA Instrument USA, Model 2950 and 2910).

Results and discussion
Visual inspection of sample
Visual observation of uncoated PMMA microspheres Fig. 4a displayed white coloured texture which darkened to greyish after silver coating. The colour change after the coating is very clearly visible to the naked eye as shown in the Fig. 4b.

Characterization of PMB and APMB
Figure 5a, b show the images of the uncoated PMB and APMB respectively, recorded under Optical microscope. Optical micrographs show clearly the coating by shining appearance of coated microspheres. Such shining feature was not noticed in the uncoated microspheres. Scanning electron microscope (SEM) images of the
uncoated PMB and APMB are shown in Fig. 6a and b, respectively.

It is observed that the microspheres show a perfectly spherical morphology with diameter in the range of 20–100 µm. The SEM micrograph 6(a) of uncoated microspheres shows smooth surfaces whereas the same for silver-coated PMMA microspheres. Figure 6b shows rough surfaces due to the attachment of silver on the microspheres surface. This shows that the silver salt gets reduced to elemental silver which adheres to the surface of microspheres due to sensitization by tin chloride. The elemental composition of the silver-coated surface of PMMA microspheres was analyzed using the energy dispersive X-ray spectrometer attached to the SEM. Figure 7a and b show the EDX spectra of PMB and APMB, respectively. The EDX spectrum of uncoated PMMA microspheres (Fig. 7a) shows only the carbon peak, which comes from the PMMA microsphere. The spectrum of coated PMMA microspheres shows an additional peak of silver which confirms the coating by silver. Thus, the presence of Ag on surface of coated microspheres is confirmed by EDX.

Figure 8 shows the UV–Vis absorption spectra of PMMA and silver-coated PMMA microspheres recorded in the wavelength range 200 to 800 nm. The spectra show a characteristic absorption band [56] for the pure PMB in the range 250–370 nm which is due to 250 nm π–π* (carbonyl group) electronic transition and 335 nm n–π*(aldehyde carbonyl group) electronic transition. The spectra clearly show that both the microspheres do not exhibit any significant absorption peaks beyond the 400 nm range to 800 nm. This is very interesting to observe that no peak is present around 400 nm which signifies that silver is deposited over the surface of
PMMA microsphere in the form of smooth coating instead of nanoparticles as reported in literature [57]. But another observation is seen that the intensity of n–π* electronic transition is significantly enhanced upon the coating. This is due to extended metal polymer interaction [36] causing full width of half maximum (FWHM) peak intensity.

Figure 9 shows the FTIR analysis results of PMMA microspheres and silver-coated PMMA microspheres. FTIR analysis was performed to confirm the presence of functional groups and polymeric backbone linkages of microspheres. The FTIR spectrum of PMMA shows characteristic peaks at 1,152 cm\(^{-1}\) to 1,257 cm\(^{-1}\), for C–O–C stretching vibration, two bands at 753 cm\(^{-1}\)
and 1390 cm\(^{-1}\) for \(\alpha\)-methyl group vibrations. The band at 987 cm\(^{-1}\) is the characteristic absorption vibration of PMMA, along with the bands at 1062 cm\(^{-1}\) and 845 cm\(^{-1}\). The band at 1760 cm\(^{-1}\) confirms the presence of the acrylate carboxyl group and the band at 1443 cm\(^{-1}\) can be attributed to the bending vibration of the C–H bonds of the \(-\text{CH}_3\) group. The two bands at 2981 cm\(^{-1}\) and 2940 cm\(^{-1}\) can be assigned to the C–H bond stretching vibrations of the \(-\text{CH}_3\) and \(-\text{CH}_2\) groups, respectively. Furthermore, there are two weak absorption bands at 3413 cm\(^{-1}\) and 1640 cm\(^{-1}\) corresponding to the stretching and bending vibrations of \(-\text{OH}\) group of physisorbed moisture respectively.

The FTIR spectrum of silver-coated PMMA microsphere also reveals all the characteristic peaks of PMMA microspheres but with slightly reduced intensities. This shows that the synthesis procedure had not affected the main structure of PMMA. However, silver coating does not give rise to any additional peaks [58] as silver does not show any characteristic absorption in FTIR.

Thermogravimetric analysis (TGA) was also carried out for the synthesized materials. Figure 10 shows the thermogravimetric analysis (TGA) spectra of uncoated (PMB) and Ag-coated PMMA microspheres (APMB). TGA analysis revealed single-step weight loss for both the materials. For the PMMA microspheres the spectrum shows total 100% weight loss from 300 to 440 °C. The silver-coated microspheres show nearly 77% weight loss from 300 to 430 °C. Thus, polymer microsphere and Ag had a relative weight percentage of approximately 80 and 20. No weight change was observed above 440 °C, revealing the complete removal of polymeric cores.

**Antibacterial test**

**Plate assay method**

The results of inhibitory effect of Ag-coated PMB against *E. coli* and *B. subtilis* along with the zones of inhibition (mm) around each well containing APMB and PMB
microspheres are shown in Figs. 11 and 12. Here the diameter of the zone of inhibition strongly reveals the efficiency of APMB material as potential antibacterial agent as shown in Tables 1 and 2.

The diameter of the zone of inhibition against *B. subtilis* was 10.1 ± 0.3, 10.4 ± 0.4, 12.2 ± 0.3 and 14.3 ± 0.2 mm for the concentrations 2, 4, 6 and 8 mg of APMB, respectively. At same concentrations of APMB, the diameter of the zone of inhibition against *E. coli* was 11.2 ± 0.4, 11.4 ± 0.3, 13.3 ± 0.3 and 15.2 ± 0.9 mm.

However the uncoated PMMA (without Ag) showed no zone of inhibition against *E. coli* and *B. subtilis* (Fig. 12). These results indicated that the antibacterial properties exhibited by APMB can be attributed to the presence of silver on their large surface area that provides more surface contact with microorganisms.

Figures 13 and 14 show the percentage reduction of *B. subtilis* and *E. coli* respectively. The results of antibacterial effect for different concentrations of APMB against *E. coli* and *B. subtilis* under dynamic conditions are shown in Table 3. The results show that after contacting for 24 h, all bacterial cells were removed by APMB, which also proves the bactericidal effect of APMB against both pathogenic *E. coli* as well as *B. subtilis*.

To study the effect of initial concentration of APMB, its concentration was varied from 2 to 8 mg against both the bacterial strains. It was further observed that uncoated PMB does not have any effect on the removal of studied bacteria. At the same time, a little amount of 2 mg showed substantial effect on the removal of *E. coli* and *B. subtilis* with reduction of 95 and 94 percent, respectively. The Log reduction values for *B. subtilis* and *E. coli* is shown in Figs. 15 and 16 respectively. On increasing the amount to 4 mg, removal increases to 97 and 96 percent, respectively. On further increase to 6 mg, removal enhancement to 99 and 98 percent was observed. It was observed that 8 mg of the material effectively removed both *E. coli* and *B. subtilis* with 100% reduction in
bacterial count after 24 h of incubation with 100 ml of contaminated solution. These results are in sequence of the minimum inhibitory concentration (mic) value which was calculated at 80 mg/L. It is to be noted that the material was found to be slightly more effective against \textit{E. coli} than \textit{B. subtilis}.

The probable mechanism of action can be reviewed from the literature. Li et al. [59] demonstrated that silver particles display antimicrobial activity comparable to silver nanoparticles while having more grounded antibacterial movement.

The antibacterial activity of silver particles (Ag\(^+\)) is legitimately relative to the natural grouping of silver particles. Due to the oligodynamic impact, silver shows high antibacterial adequacy even in low doses [25]. Silver particles created in an electrolytic manner are preferable antibacterial operators over those acquired by dissolving the silver mixes.

The mechanism of antibacterial activity of silver particles is associated with: (I) connection with the bacterial cell envelope (destabilization of the film—loss of K\(^+\) particles and diminishing of ATP level, reinforced with phospholipids), (ii) collaboration with atoms inside the cell (e.g., nucleic acids and proteins), (iii) the creation of responsive oxygen species (ROS) [7]. The association of silver particles with bacterial inner membrane is one of the most significant components of Ag\(^+\) harmfulness [6]. Woo et al. [25] demonstrated that the accumulation of Ag\(^+\) in the bacterial cell envelope is trailed by the partition of the cytoplasmic membrane (CM) from the cell divider in both Gram-positive and Gram-negative microbes. Süterlin et al.

| Table 1 Zone of inhibition (mean + standard error) for \textit{E. coli} |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Low dose | Low dose | Medium dose | High dose |
| 2 mg | 4 mg | 6 mg | 8 mg |
| 11.2±0.4 | 11.4±0.3 | 13.3±0.3 | 15.2±0.9 |

| Table 2 Zone of inhibition (mean + standard error) for \textit{B. subtilis} |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Low dose | Low dose | Medium dose | High dose |
| 2 mg | 4 mg | 6 mg | 8 mg |
| 10.1±0.3 | 10.4±0.4 | 12.2±0.3 | 14.3±0.2 |

![Fig. 12](image) Results of PMB zone of inhibition test against \textit{E. coli} and \textit{B. subtilis}

![Fig. 13](image) 3D graph for percentage reduction of \textit{B. subtilis}

![Fig. 14](image) 3D graph for percentage reduction of \textit{E. coli}

| Table 3 Antibacterial test results under shaking conditions |
|-----------------------------|-----------------------------|-----------------------------|
| SI No | APMB concentration (mg) | Percentage of removal % \textit{(B. subtilis)} | Percentage of removal % \textit{(E. coli)} |
| 1 | 0 (control) | Trace | Trace |
| 2 | 2 | 94 | 95 |
| 3 | 4 | 96 | 97 |
| 4 | 6 | 98 | 99 |
| 5 | 8 | 99.99 | 99.99 |

* Shaking time: 24 hr, concentration of \textit{E. coli} and \textit{B. subtilis}: 10\(^8\) CFU/ml, quantity of contaminated solution: 100 ml
Fig. 15 Log reduction for *B. subtilis*

Fig. 16 Log reduction for *E. coli*
[7] demonstrated that an insignificant minimum bactericidal concentration (MBC) of Ag\(^+\) for Gram-positive microscopic organisms was in excess of multiple times higher than the MBC estimates for the Gram-negative bacterial cells. As indicated by reference [25], carboxyl groupings (–COOH) in glutamic acid and phosphate branches in teichoic acid are for the most part answerable for authoritative silver particles. Then again, Randall et al. [60] recommended that the harm brought about by Ag\(^+\) in the internal membrane (IM) is one of the most significant systems in staphylococci. It has been demonstrated that silver particles enter microscopic organism cells within 30 min of interaction and tie to cytoplasm segments, proteins and nucleic acids [24]. It was proved by the TEM (transmission electron microscopy) that the two kinds of bacterial cells (Gram-positive and Gram-negative) treated with Ag\(^+\) were lysed resulting in the spillage of cytoplasm. It was also recommended that silver particles prompt an “active but non-culturable” state (ABNC) in microscopic organisms. Stress induced by Ag\(^+\) caused that microorganisms to maintain its metabolism yet halted the development, hence the quantity of viable cells diminished in the performed in vitro tests. The percentage reduction for B. subtilis and E. coli are shown in Figs. 17 and 18.

One of the contrasts between the method of Ag\(^+\) activity against Gram-positive and Gram-negative bacteria respects the method for silver uptake into the cell. Silver particles enter Gram-negative cells by means of major outer membrane proteins (OMPs), particularly OmpF (and its homolog OmpC) [61, 62], which is a 39 kDa transmembrane protein with trimeric β-barrel structure. Every monomer of OmpF is worked by sixteen transmembrane, antiparallel β-strands amassed with one another by means of hydrogen bonds. Those strands structure a stable β-sheet which subsequently overlays into a round and hollow cylinder with a channel work. Other than porin and particle transporter movement, OmpF is engaged with the vehicle of other little atoms (e.g., drugs) over the bacterial outer membrane (OM) [63–65]. The significance of the OmpF/OmpC in the component of protection from silver has been talked about more than once in a couple of distributed papers [57, 66, 67]. Here and there the consequences of the directed trials were very unique. Radzig et al. [57] guaranteed that E. coli lacking OmpF (or OmpC) in the outer membrane was 4–8 times more resistant to Ag\(^+\) or AgNPs than E. coli which had those proteins. In another study, Randall et al. [62] demonstrated that longer exposure to silver particles caused missense transformations in the cusS and ompR quality. The later led to the loss of capacity of OmpR protein (which is an interpretation factor of OmpF and OmpC) and, at last, the absence of OmpF/C proteins in the outer membrane. E. coli (BW25113) without the referenced OMPs is portrayed by a low penetrability of the OM and a significant level of protection from Ag\(^+\). Those highlights were observed distinctly when both the proteins were absent in the OM. Yen et al. [68] remain contrary to the outcomes appeared previously. In their research, with or without the presence of OmpF/OmpC in the bacterial OM, they watched no progressions in bacterial sensibility to silver particles.

Another molecular mechanism of silver particles is associated with their connection with structural and functional proteins, particularly those with thiol groups (–SH) [6, 24, 69]. Hindrance of the primary respiratory chain proteins (e.g., cytochrome b) causes an expansion of ROS inside the cell, which adds to the death of the microorganisms. Reaction to silver outcomes in the expansion of the degree of intracellular receptive oxygen species, what prompts oxidative pressure, protein damage, DNA strand breakage, and, therefore, cell death [24]. One of the significant targets inside the cell is the S2 protein. The binding of silver particles to ribosomal proteins results in the denaturation of ribosomal native structure as well as inhibition of protein biosynthesis [24]. It has been shown that silver particles interact with nucleic acids resulting in bond formation with pyrimidine bases. In the outcome, DNA condenses and replication is stopped [70]. The most probable mechanism for the mode of action by silver ion is shown in Fig. 19.

**Antibacterial efficiency of APMB via gravity filtration using a pen shell as filter column**

Additionally, in an attempt to simulate real environmental conditions, water contaminated with E. coli and B. subtilis having concentration of \(10^8\) CFU/ml was prepared and passed through pen shell filter column having dimension (87.3 × 1.2 mm). The concentration of material loading varied from 2 to 8 mg. As a result of purification through the pen shell filter column loaded with APMB, 100% bacteria-free water was obtained when 100 ml bacterial contaminated solution was passed through it.

Since exposure to silver can have negative effects on human health, its effluent levels need to be below the allowable limit [55, 71]. The Environmental Protection Agency (EPA) of United States (US) regulates the maximum contaminant level of silver ions in drinking water to be less than 0.1 mg/L [72]. Hence to check the possible health effect, silver leaching from APMB loaded pen shell filter was investigated using standard protocol. The
**Fig. 17** Percentage reduction for *B. subtilis*

**Fig. 18** Percentage reduction for *E. coli*
filtrate did show presence of silver even in trace quantities. Leaching test is carried out using standard \[55\], showing a presence of 0.03 mg/L of silver. This finding suggests that the adsorption of Ag on the PMB surface is a stable process which otherwise indicates the general stability of the APMB loaded filter for its application in water disinfection system.

**Fig. 19** Comparison of mode of action by silver ion against a Gram-positive bacteria and b Gram-negative bacteria
Time of backwashing requirement study
Backwashing is another important parameter in fabrication of any online water filtration system because the contaminant adsorbent gets saturated after decontamination of a specific volume of contaminated water or a certain amount of time. When the decontamination medium gets saturated, the overall performance or efficiency of filtration decreases. Then the backwashing has to be done to regenerate the adsorbent and regain the filtration efficiency of the system. In this particular setup, seven simultaneous experiments were carried out to observe any decrease in performance. It was found that till 5th experiment, there was little or negligible drop of performance. But from 6th experiment there was slight drop of effectiveness, which could be recovered by simple backwashing with normal water. Backwashing was carried out using the same distilled water which was used for making the stock solution.

Conclusion
In summary, silver-coated hollow PMMA microspheres were successfully demonstrated as an efficient antibacterial material for water purification system. The silver coating of PMMA microspheres involved simple procedure using microwave method. Structural characterization performed using conventional spectroscopic and imaging techniques have revealed the formation of silver-coated microspheres with a size distribution of 20 to 100 µm and efficient coating of silver particles over the hollow PMMA surface. The silver-coated hollow PMMA microspheres possess enhanced disinfection power and remarkably minimum leaching tendency, in addition to other intriguing features like low density and mechanically robust nature. Thermogravimetric analysis showed that the material does not exhibit any weight loss till 300°C. The zone of inhibition test and batch experiments have established enhanced antimicrobial efficacy of the material, APMB, against the two bacteria (Gram-positive Bacillus subtilis and Gram-negative Escherichia coli) and the rate for both bacteria exhibited dose-dependent antibacterial ability. Depending on the bacterial concentration, complete removal of both the bacterial strain is possible as demonstrated for the case of bacterial concentration of 10^8 CFU/ml, where 100 ml of contaminated water was completely disinfected with 8 mg of APMB, after 24 h. Therefore, the developed hollow PMMA microsphere, which possesses significant biocompatibility, is truly an efficient choice as substrate for silver coating to obtain economically viable and mechanically durable low-density antimicrobial material for water purification system.

The above findings suggest that the material can be easily incorporated in any system as simple as a common pen shell and convert it into effective filtration system. Additionally this study also signifies that the synthesized material may possibly provide a cost-effective and versatile technology for a wide variety of water disinfection applications. This disinfection technology may find its suitability for point-of-use water treatment devices, particularly at sites affected by natural disasters as earthquakes, tsunamis and flooding.

Abbreviations
AgNPs: Silver nanoparticles; APMB: Silver-coated PMB microspheres; Ag: Silver; Ag+: Silver ion; AMP: 2-Amino-2-methyl-1-propanol; ABNC: Active but non-culturable; AgNO₃: Silver nitrate; B. subtilis: Bacillus subtilis; Conc. HCl: Concentrated hydrochloric acid; CFU: Colony-forming unit; CM: Cytoplasmic membrane; EG: Ethylene glycol; E. coli: Escherichia coli; EDX: Energy dispersive X-ray spectroscopy; H₂O: Water; IM: Internal membrane; KSCN: Potassium thiocyanate; LB: Luria–Bertani; MTCC: Microbial type culture and collection; MBC: Minimum bactericidal concentration; MIC: Minimum inhibitory concentration; NPs: Nanoparticles; NH₄OH: Ammonium hydroxide; OMPs: Outer membrane proteins; OD: Optical density; OMS: Optical microscope; PMMA: Polymethylmethacrylate; PMB: Poly(methylmethacrylate); ROS: Reactive oxygen species; RT: Room temperature; SEM: Scanning electron microscope; SnCl₂: Stannous chloride; TEM: Transmission electron microscopy; TGA: Thermogravimetric analysis; US EPA: United States Environmental Protection Agency; µL: Microlitres.

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DO performed material synthesis and wrote the manuscript, SG performed microbial experiments, RD conceptualized experiments, SKD facilitated the infrastructure and provided necessary guidance for the article, AP final editing.

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It is to be noted that the authors have no competing financial or personal interests.

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