# Iodinated P(MMA-NVP): An Efficient Matrix for Disinfection of Water

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**ABSTRACT:** Polymethyl methacrylate-N-vinyl-2-pyrrolidone P(MMA-NVP) copolymer was synthesized in an inert atmosphere by bulk polymerization using azobis-isobutyronitrile (AIBN) as initiator. The copolymer was crushed, and ground to a defined particle size and iodinated. The iodinated copolymer matrix was washed thoroughly in a suitable solvent so as to remove any residual iodine and characterized using solubility, swelling, viscosity, FT-IR, and $^1$H-NMR studies. The iodinated copolymer was then packed in a refill cartridge specially designed for the purpose. The release of iodide ions from the iodinated matrix was measured in a continuous flow system by directly attaching the cartridge to the water tap and adjusting the flow rate. The water samples were collected at regular intervals and estimated for the concentration of iodide ions using an iodide ion selective electrode. The antimicrobial activity of the copolymer was estimated against bacterial (both Gram-positive and -negative) and fungal species using the zone of inhibition technique. In addition, efficacy of the iodinated copolymer against a variety of microbes was established by inoculating a microbial culture in the water reservoir and measuring the rate of survival of microbes after passing through the copolymer-filled column. The iodinated copolymer was found to be effective against a variety of microbes and remained so completely until 5000 L of water had passed.
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**Key words:** water disinfection; Water purification; Antimicrobial polymers; Ion-exchange resin; iodine containing polymers; MMA/NVP

## INTRODUCTION

In the developing world, and more so in India, which is densely populated, water scarcity is a major problem. Owing to the acute shortage of drinking water in both urban as well as rural areas, various alternatives are being tried to meet the demand. Recycling of water and its distribution is commonly in use today. The water distributed in urban areas is often improperly treated or, even if it is well treated, then gets contaminated during storage or distribution. Several such cases, where there has been a drainage leakage into the pipelines for domestic water, have been reported from urban areas in recent years. This contamination in water leads to various waterborne diseases among the population. As a result, the popularity of several in-house water treatment devices is rapidly increasing. Various techniques/methods employed for treatment of water include reverse osmosis,[1] ultrasonic cleaning,[2] polymeric aluminium salts,[3] antibacterial-activated carbon fibers,[4] ion-exchange resins,[5] ultaviolet (UV) irradiation,[6] electrochemical methods,[7,8] and magnetic separators.[9]

Several workers in the past few decades have attempted to produce insouble polymeric disinfectants by incorporating antibacterial agents into

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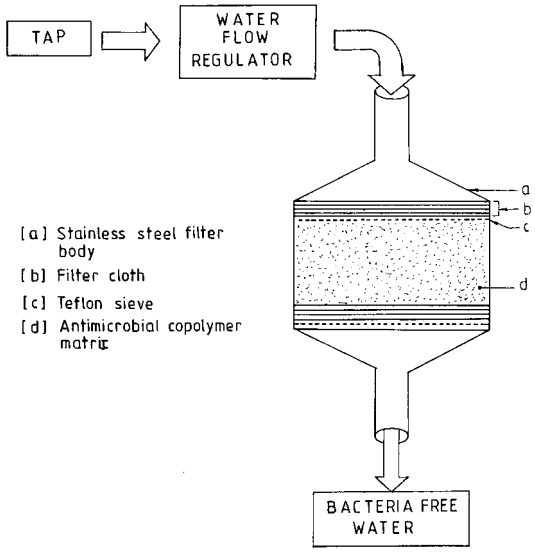


**Figure 1** Schematic diagram of the refill cartridge.

the ion exchange resins to achieve a slow release of active agent into the medium. Some of these products, especially the polyhalide-anion exchange resins, have shown promise as effective water disinfection systems.[10–12] The present paper deals with the synthesis of a hydrophilic but water-insoluble copolymer matrix to which a suitable antimicrobial agent could be immobilized so as to achieve a slow release of antimicrobial agent into the medium for prolonged duration of time. Iodine was chosen as an antibacterial agent for the present study owing to its wide spectrum activity against bacteria, fungi, virus, and some protozoa.[13–15] Moreover, it has been proved by Chang and Morris[16,17] that iodine can be used for disinfection of water successfully. In addition, it is easily available and quite cheap as compared with any other antibacterial agent. Iodine also has a low toxicity and, unlike antibiotics, does not lead to generation of resistance in the microbial species.[18]

## EXPERIMENTAL

### Materials

Reagent grade methyl methacrylate (MMA) and N-vinyl-2-pyrrolidone (NVP) were obtained from Qualigen (India) and Fluka (Germany), respectively. These were purified by passing through a packed column of activated alumina (neutral). The eluting monomers free of inhibitor were then used as such. Resublimed iodine, azobis-isobutyronitrile (AIBN), reagent grade sodium iodide and sodium nitrate for release studies, and various solvents were obtained from Qualigens and were used as such. The nutrient agar, Saboraud dextrose agar (SDA), and peptone water required for microbiological assay were obtained from Sigma (USA) and were used for antimicrobial studies. The various microbes *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive) and *Candida* (fungi) for antimicrobial assessment of the iodinated copolymer were obtained from the Department of Microbiology, AIIMS, India. The refill cartridge made of steel was designed and fabricated in the central workshop of IIT Delhi and the filter cloth was obtained from market. An overview of the entire assembly is shown in Figure 1.

### Synthesis

MMA and NVP were taken in a 1:1 (w/w) ratio and 0.5% AIBN (w/w) was added to it. The contents were sealed into glass ampoules after passing nitrogen through them for 5 min. The ampoules were heated in a water bath at $55\pm1$°C for 3 h. The copolymer thus synthesized was cooled, crushed, ground, sieved, and washed repeatedly with hexane, and then with distilled water so as to remove residual monomers and traces of NVP homopolymer, if any, and then dried. The sieved copolymer in the particle size range 250–500 $\mu$m was treated with a weighed quantity (20% w/w) of molecular resublimed iodine (solid) at 37°C for 24 h. The copolymer was repeatedly washed with hexane for 48 h so as to remove even traces of free molecular iodine, if present. The copolymer was then washed with distilled water several times, dried, and weighed so as to assess the final iodide concentration in the copolymer. The iodinated copolymer was packed in a refill cartridge, flanked on either sides with layers of filter cloth. The column was connected to a water tap and a flow regulator. The schematic representation of the entire assembly is shown in Figure 1. Water was allowed to pass through the column at a rate of $50\pm2$ L per hour at $35\pm2$°C. A Zero-B filter, manufactured by Ion Exchange Ltd., India, that consisted of a tri-iodide quarternary ammonium styrenic anion exchange resin packed in a steel container, was attached to a separate tap and water passed through it at same flow rate and temperature. Water samples were collected after every 100 L water passed up to 2000 L and were eval-

uated for the presence of iodide ions. However, after 2000 L, because of considerable reduction in iodide ion release, the samples were collected after every 500 L of water passed.

### Characterization

The copolymer and its iodinated adduct was characterized using various techniques of polymer characterization. A weighed amount of copolymer and its iodine adduct were suspended in various polar and non polar solvents to assess the solubilities. The swelling behavior was monitored by suspending weighed pellets of copolymer in distilled water for 24 h. The pellets were weighed at regular intervals after blotting excess water and percent swelling was calculated using the equation:

$$\text{Percent Swelling} = (W_s - W_d)/W_d \times 100$$

Where $W_s$ and $W_d$ are the weights of the copolymer pellets in swollen and dry states.

Viscosity measurements were done in an AVS 310 Ubbelohde Viscometer (Schotte Gerate) in a constant temperature bath at 20°C using chloroform as solvent. Flow rates at various dilutions were measured and intrinsic viscosity was calculated by plotting $\eta_{sp}/C$ versus concentration and extrapolating the obtained curve to zero concentration.

The FT-IR spectra of the iodinated and noniodinated copolymers in KBr pellets were recorded on a Nicolet 5dx FT-IR spectrophotometer. The samples being hydrophilic in nature were dried completely before taking the spectra. $^1$H NMR spectra of the compolymer of MMA and NVP was recorded on a JEOL JNM-FX 100 FT-NMR spectrometer (JEOL, Tokyo) in $CDCl_3$. The spectra were run at room temperature using tetramethylsilane (TMS) as the internal standard.

### Release Studies

The concentration of iodide ions was evaluated using an Orion 290A iodide ion selective electrode. Solutions of 0.1M, 0.01M, and 0.001 M of sodium iodide were polished, and along with the reference electrode was placed in standard solutions and calibrated. A measured quantity of sodium nitrate was added to each sample (standard as well as test samples) as an Ionic Strength Adjuster (ISA). The iodide ion concentration in the experimental water samples (up to 8000 L) was then measured using the electrode.

### Evaluation of Antimicrobial Activity

#### *Zone of inhibition method*

Double layered nutrient agar plates and SDA plates (for *Candida*) were prepared and wells of 10 mm diameter were cut into them using a sterile pipette tip. A lawn of bacterial and fungi was laid over the plates using a sterile cotton swab. A weighed amount of copolymer granules (10 mg) was poured into the well using a sterile cut tip as a funnel. A noniodinated copolymer was placed in each plate as control. The test microbes taken for the present study were *E. coli, S. aureus* and *Candida spp*. The plates were incubated at 37°C for 24 h and the clear zone around the sample was measured the next day as a measure of antimicrobial activity of the iodinated copolymer granules.

### Disinfection Efficiency Test

In order to evaluate the efficacy and longevity of the iodinated copolymer as a water disinfectant, a disinfection efficiency test was conducted. In this test, known concentrations of microbial cells composed of *E. coli, S. aureus,* and *Candida spp.* was inoculated into the water reservoir. This contaminated water was passed through the copolymer filled cartridge. The eluting water was collected at regular intervals and streaked over a nutrient agar plate and the plates incubated at 37°C for 24 h. The resulting number of microbes were counted using a hemocytometer and the exact number of living cells differentiated from dead cells using the agar streak method. The number of colonies visible the next day were taken as a count of microbes surviving after passing through the copolymer filled column.

## RESULTS AND DISCUSSION

### Preparation

The copolymer was weighed, after iodination and subsequent washing, and the amount of iodine incorporated was calculated. It was found that the total iodine incorporated into the copolymer was 11.5% (w/w). The white-colored copolymer acquired a brown color as a result of iodination.

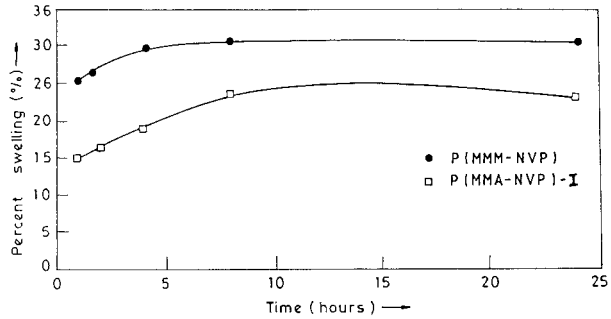


**Figure 2** Swelling behavior: P(MMA-NVP) copolymer and iodine adduct.

## Characterization

### *Solubility studies*

The copolymers of MMA and NVP were found to be soluble in most of the polar and nonpolar solvents. They were, however, insoluble but swelled to certain degree in water and methanol. On the contrary, the iodine adduct of the copolymer swelled in various solvents but failed to go into solution completely.

### *Swelling behavior*

The results of the water uptake capacity of the copolymer is indicated in Figure 2. As is evident, the copolymer swelled to about 25% in 1 h and attained equilibrium swelling of 30% in 24 h. The iodine adduct of the copolymer however swelled by 15% in 1 h, reaching a maximum value of 23% in 8 h then gradually declined with time probably due to leaching of iodide ions into the surrounding environment. The lesser swelling of the iodinated copolymer might be due to blocking of the water interacting sites by the hydrophobic iodine molecules.

### *Viscosity studies*

The intrinsic viscosity ($\eta$) of the copolymer was calculated by plotting $\eta_{sp}/C$ versus concentration and extrapolating the values to zero. The intrinsic viscosity was $4.9 \times 10^{-2}$ mL/g at 20°C in chloroform.

### *FT-IR studies*

The FT-IR spectra of the copolymer and its iodine adduct are shown in Figure 3. The peak at 1730 $cm^{-1}$ is characteristic of the C=O stretching of the ester group in MMA. Similarly, the peak for C=O stretching of tertiary amide appears at 1682 $cm^{-1}$. The prominence of these two peaks in the spectra confirms presence of both MMA as well as

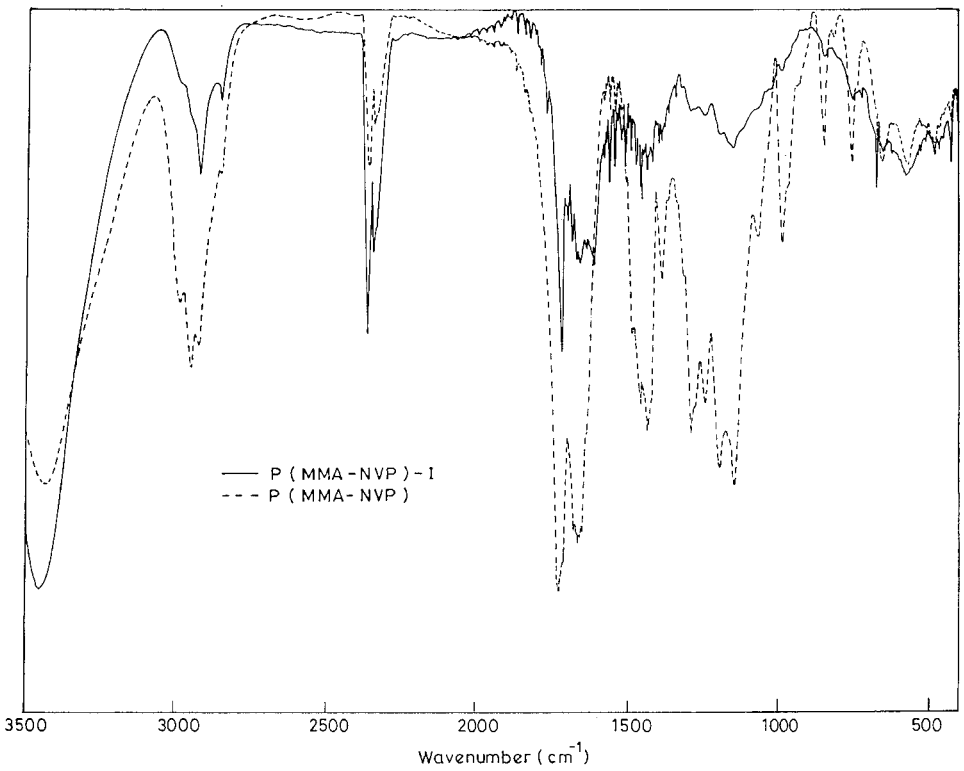


**Figure 3** FT-IR spectra of P(MMA-NVP) copolymer and iodine adduct.

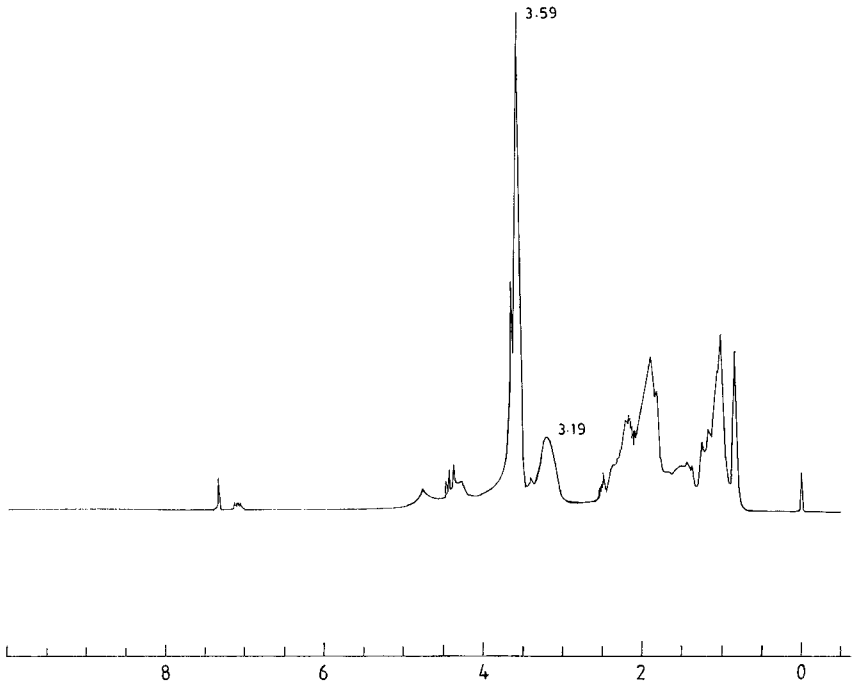


**Figure 4** $^1$H NMR spectra of PMMA-NVP copolymer.

NVP in the copolymer, thus indicating the copolymerization of MMA and NVP. However, the band at 1682 $cm^{-1}$ decreases as a result of iodination indicating attachment of iodine to carbonyl groups of NVP. Moreover, disappearance of peaks 1121 $cm^{-1}$ and 1319 $cm^{-1}$ specific to the C-N bond, as a result of iodination, indicates that iodine probably also interacts with the N-atom of the pyrrolidone ring.

### *$^1$H NMR studies*

$^1$H NMR spectroscopy was used to determine the copolymer composition. Figure 4 shows the $^1$H NMR spectra of PMMA-NVP copolymer. The characteristic -$OCH_3$ signal of MMA appears at $\delta$ = 3.59. Similarly, the signal at $\delta$ = 3.2 appears due to the characteristic –$CH_2N<$ group in NVP.[18] The copolymer composition was determined from the intensities of the -$OCH_3$ protons of the MMA unit. The percentage of MMA was calculated to be 63.7%. The results indicate that the copolymer was richer in MMA as compared to its comonomer, NVP. This anomalous behavior is due to the higher reactivity ratio of MMA ($r_1$ = 4.7) as compared with that of NVP ($r_2$ = 0.005) during copolymerization.[19]

### *Release studies*

The results of the release behavior of iodide ions from the iodinated matrix in comparison to the Zero-B Filter are given in Figures 5 and 6, where the concentrations of iodide ions have been plotted against the volume of water passed through

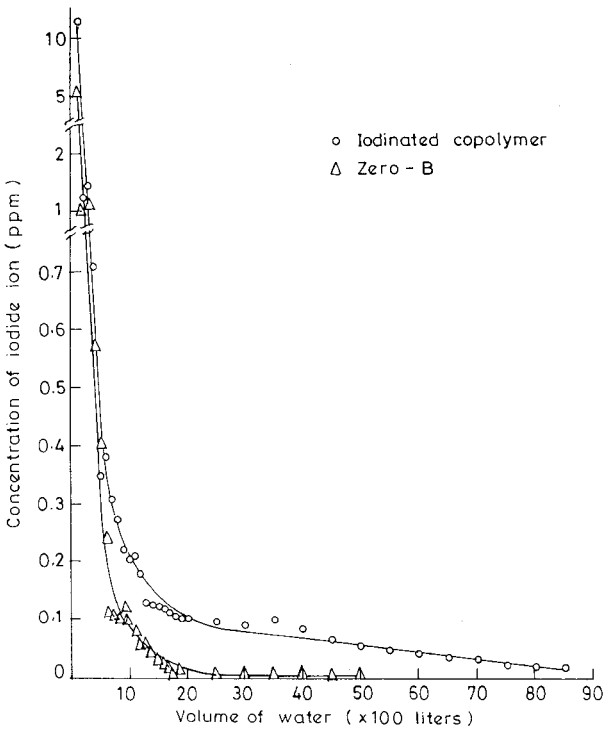


**Figure 5** Release profile of iodinated P(MMA-NVP) copolymer vs Zero-B.

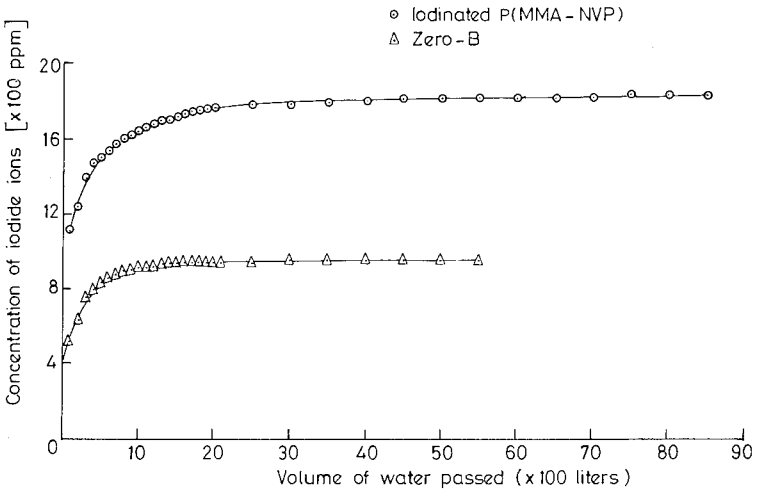


**Figure 6** Cumulative release profile: Zero-B vs iodinated copolymer.

the column. The release pattern in Figure 5 shows that initially there was a burst release up to 11.2 ppm and 5.3 ppm, which declined to 0.7 ppm and 0.5 ppm as 300 L of water passed through the copolymer and Zero-B matrix, respectively. As evident from the Figure 6, the release of iodide ions from the copolymer matrix was sustained even up to 8000 L of water passed through it whereas with Zero-B there was no measurable release after 4500 L of water. The total iodine content, the loss in weight of the copolymer after release, the total iodide ion concentration and total iodine released from the copolymer are listed inTable I. The data indicate that release was sustained for a prolonged duration and 1.8 g of iodide ions were released from the matrix as 8000 L of water passed through the column, which is 64.26% of the total iodine packed in the filter and 96.2% of the total iodine released from the filter.

## Evaluation of Antibacterial Activity

### *Zone of inhibition studies*

The antibacterial activity of the iodinated copolymer is quite evident from the photographs in Figures 7–9. A clear zone of 15 mm, 25 mm, and 20 mm diameter was observed around the iodinated sample in the plates inoculated by *E. coli, S. aureus,* and *Candida spp.,* respectively, whereas the rest of the nutrient agar/SDA plate was covered by a lawn of bacteria/fungus. A noniodinated copolymer placed on the nutrient agar plate as control was completely surrounded by bacterial/fungal colonies. The antimicrobial activity of the iodinated copolymer, after the passing of 8000 L water, is indicated in Table II. It is clear from Table II that the copolymer retains its antimicrobial activity even after the passing of 8000 L of water although reduced to a certain degree.

The appearance of a clear zone around the iodinated copolymer indicates absence of bacterial/fungal colonies around the iodinated copolymer. This indicates the microbiocidal role of the iodinated copolymer because the microbial cells, initially laid over the plate by the cotton swab, were killed and thus could not proliferate to produce a lawn. The microbiocidal role of the iodide ions could be attributed to the fact that these ions bind with important biomolecules in the microbial cells and thus inhibit various cell processes.[20] The

**Table I Concentration of Iodide Ion and Molecular Iodine Released from the Iodinated Copolymer Matrix**

| | |
|---|---|
| Weight of copolymer filled into the cartridge | 25.0 g |
| Total iodine incorporated into the filter | 2.875 g (11.5%) |
| Total weight of the copolymer after release | 23.08 g |
| Total iodine released from the filter | 1.92 g (66.78%) |
| Cumulative iodide ion released from filter along with 8000 L of water | 1.8476 g (96.2%) |
| Total iodine released as species other than $I^-$ ion | 0.0724 g (3.77%) |

This data is valid for 8000 L volume of water passed through the filter.

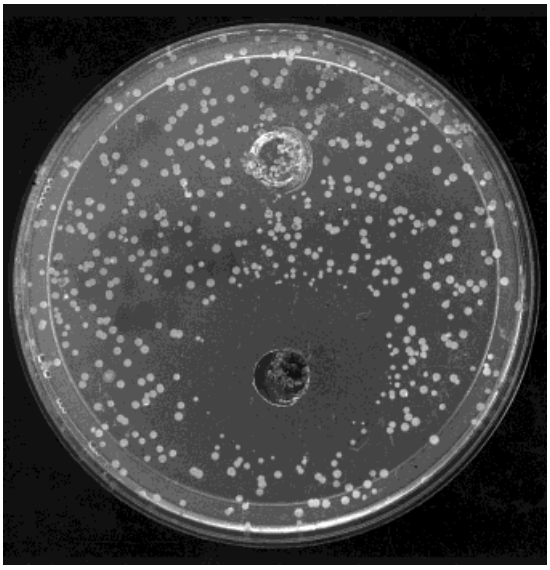

**Figure 7** Zone of Inhibition around iodinated copolymer in *E. coli*.

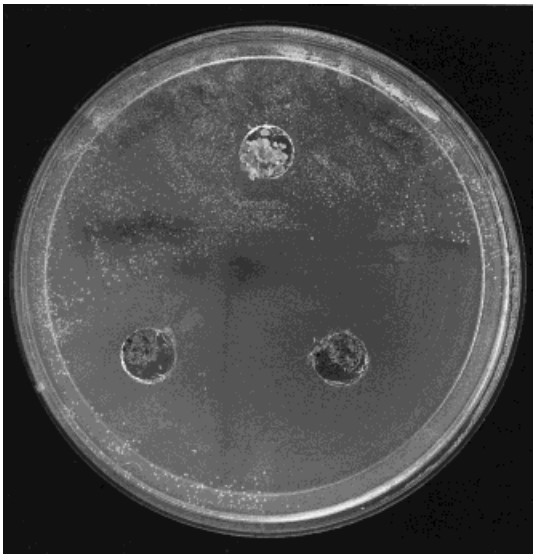

**Figure 9** Zone of Inhibition around iodinated copolymer in *Candida*. (The well with smaller zone indicates the activity of washed copolymer).

clear zone around *S. aureus* and *Candida* are wide as compared to *E. coli*. This can be explained by the fact that *E. coli*, being a Gram-negative bacterium, has an outer membrane in addition to a cell membrane, which resists the entry and subsequent microbiocidal action of iodine.

### *Disinfection efficiency test*

The results of the disinfection efficiency tests are given in Table III. The data indicate that there appears to be no growth up to passage of 5000 L of water through the column as there is no growth of microbes on the culture plates. After 5000 L, one or two microbes seem to escape the column as is clear from the microbial count indicated in the table.

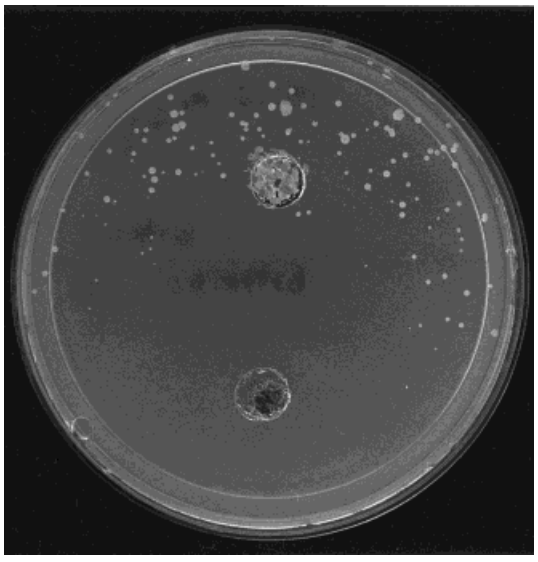

**Figure 8** Zone of Inhibition around iodinated copolymer in *S. aureus*.

## CONCLUSION

As evident from the release studies, the iodinated MMA-NVP copolymer matrix proves to be more effective and remains effective for a longer duration in comparison with the Zero-B filter that is currently available in the Indian market for in-house treatment of drinking water. The iodinated copolymer has been found to be viable, stable, insoluble, and an active disinfectant against a

**Table II Antimicrobial Activity of the Copolymer Before and After Passing 8000 L of Water**

| P(MMA-NVP) | Zone of Inhibition (mm) in Species | | |
|---|---|---|---|
| Copolymer | *S. aureus* | *E. coli* | *Candida* |
| Fresh (before 8000 L) | 25 ± 2 | 15 ± 2 | 20 ± 2 |
| Washed (after 8000 L) | 15 ± 2 | 7 ± 2 | 10 ± 2 |

**Table III Microbial Count in Respect to Water Flow Through the Column**

| | Microbial Density/100 L of Water Passed | | |
|---|---|---|---|
| Species | Initial | Up to 5000 L | 5000–8000 L |
| *S. aureus* | $2 \times 10^5$ | Nil | $1 \pm 1$ |
| *E. coli* | $2 \times 10^5$ | Nil | $5 \pm 2$ |
| *Candida* | $1.5 \times 10^5$ | Nil | $1 \pm 1$ |

variety of microbes as well as fungal species as indicated in the antimicrobial studies. This copolymer thus holds a promising future as an efficient disinfectant for potable drinking water for prolonged duration.

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