# Hydrogels for Water Filters: Characterization and Regeneration

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**ABSTRACT:** Acrylic acid was crosslinked with *N*,*N*′-methylenebisacrylamide followed by a reaction with hexamethylenetetramine (HMTA) to form a new hydrogel, Gel (**2**). Water absorbance rate and retention of Gel (**2**) were characterized. At the same time, factors affecting absorbance rate such as pH, temperature, and ions concentration were studied. The rate decreased with decreasing pH and increasing ions concentration, whereas increased with raising temperature. The effect of the hydrogel on bacterial (*Staphylococcus aureus* and *Escherichia coli*) viability and growth rate was determined. Gel (**2**) has achieved a 5 log reduction on both *E. coli* and *S. aureus* in 2 h while no cells were detected after 3 h in case of *S. aureus* and 4 h in case of *E. coli*. Lifetime and regeneration of Gel (**2**) using both stirred flask and column (a model for a water filter) methods were determined. Identifying the lifetime using columns showed that the gel stays active up to 5 and 7 runs for both *E. coli* and *S. aureus* respectively. In addition, it was recycled successfully up to 10 times, using column and stirred flask methods, against both types of bacteria.  J Appl Polym Sci 123: 1889–1895, 2012

**Key words:** hydrogel; hexamethylenetetramine; water filters; recycling; bacteria

## INTRODUCTION

Polymeric materials such as *N*-halamine and poly Quat's were applied in water filters as disinfectants (Fig. 1).[1–12] *N*-halamine polymers exchange halogen ions with the cells of microorganisms resulting in their death.[1–9] Poly Quat's attracts these cells as the polymer carries positive charges while cells carry negative charges at certain physiological state.[1,2,12] This results in cells being adsorbed and expired.[1,2,12] Quaternary ammonium salts behave in a similar way like poly Quat's.[1,7,10]

New group of quaternary ammonium salts was prepared in a previous study based on crosslinked acrylic acid as a form of hydrogels. Acrylic acid was crosslinked with *N*,*N*′-methylenebisacrylamide followed by a reaction with five different amino containing compounds to form new hydrogels (Fig. 2).[7] The antibacterial activity of these hydrogels was evaluated against examples of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria using different methods; agar plates and columns.[7] The author has proved in this study that one of these hydrogels (Gel **2**, Fig. 2) is able to filter and kill bacterial cells in a column as a model for a water filter.[7] The hydrogel was allowed to absorb water to full saturation to close gabs in column so cells were filtered and expired due to the antibacterial action of the gel.[7]

In this current study, author is trying to determine some properties of Gel (**2**), water absorbance rate and water retention, to study its regeneration possibilities. Moreover, the effect of pH, temperature, and ions concentration on its rate of water absorbance was determined. The evaluation of antibacterial activity was extended by determining its effect on bacterial viability and growth rate using stirred flask method. This was followed by identifying Gel (**2**) lifetime in a column as a model for a water filter. Gel (**2**) regeneration (recycling possibilities) was studied using two different methods; stirred flask and column, to increase its lifetime and commercial properties. This full study will support applying such type of hydrogel in water filters on large scale. Moreover, the interaction between hexamethylenetetramine (HMTA) and crosslinked acid in addition to its releasing rate is currently under investigation and date will be published in due course.

## EXPERIMENTAL

### Materials

Acrylic acid, *N*,*N*′-methylenebisacrylamide, and hexamethelenetetramine (HMTA) were obtained from Sigma Aldrich, UK. Ammonium persulfate was

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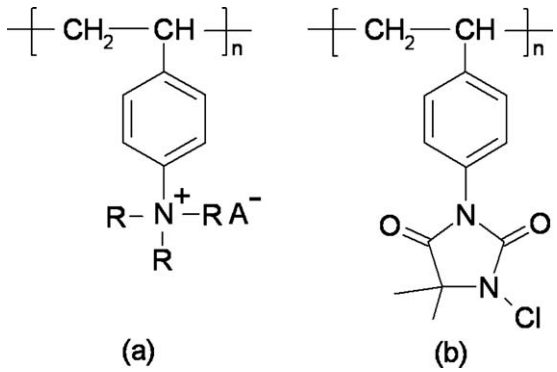


**Figure 1** Examples of antibacterial polymers: (a) poly Quat's and (b) *N*-halamine polymers.

obtained from El-Naser Co., 10 Ramadan Industrial Town, Sharkia, Egypt. Nutrient broth was supplied by Oxoid. Cultures of *S. aureus* and *E. coli* were obtained from the faculty culture collection. Primary cultures on nutrient agar slopes and subcultures on nutrient agar plates were stored at 4°C. All chemicals were used as received without any further purification.

### Hydrogel preparation

Acrylic acid (9.2 g) was dissolved in distilled water (45 mL). *N*,*N*′-methylenebisacrylamide (0.06 g) and ammonium persulfate (0.9 g) were added. The mixture was heated gently at 40°C with vigorous stirring until gelling. The resulting hydrogel was cooled and grounded to small pieces with average diameter of 4 mm followed by soaking in distilled water (120 mL) containing HMTA (17.9 g) for 16 h at 40°C. The resulting hydrogel, Gel (**2**), was dried at 90°C for 24 h (Fig. 2). The diameter of the Gel granules after this period of drying was 1 mm in average. Crosslinked acrylic acid, Gel (**1**), was prepared to be used as a control (Fig. 2).[7]

### Absorbance rate and water retention

Gel (**2**, 1 g) was allowed to swell in distilled water for 24 h. The hydrogel was filtered, dried with clean filter papers, and weighed at timed intervals for 24 h (Fig. 3). The samples were allowed to stay in distilled water for another 24 h to achieve maximum absorbance. The hydrogel was filtered, dried with clean filter papers and three samples were weighed out of it (50 g each) to start the identification of Gel (**2**) water retention. These samples were heated at different temperatures (40, 50, and 60°C) and weighted at timed intervals for 48 h (Fig. 4).

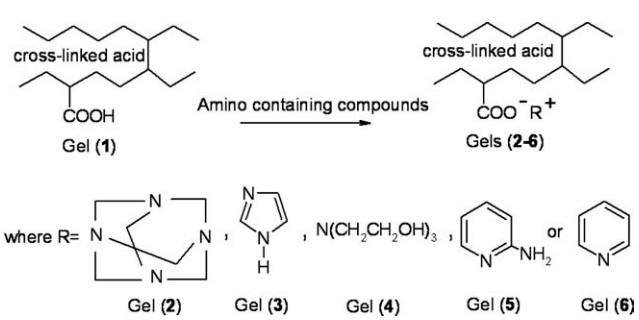


**Figure 2** The prepared hydrogels.

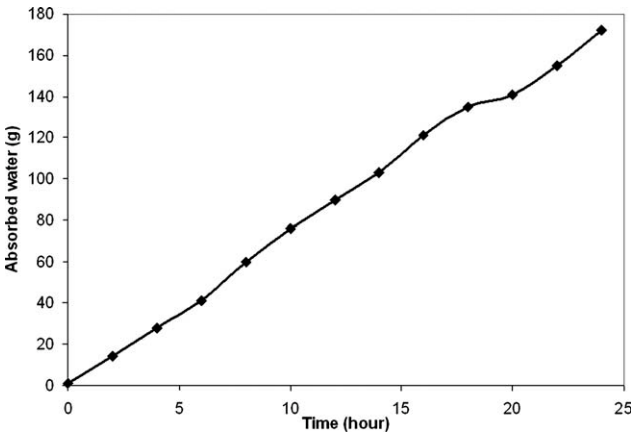


**Figure 3** Water absorbance rate with time.

### Effect of temperature, pH, and ions concentration on absorbance rate

Gel (**2**, 1g) was allowed to swell in distilled water at different temperatures (40, 50, and 75°C) for 24 h. The hydrogel was filtered, dried with clean filter papers, and weighed at timed intervals in each case [Fig. 5(a)].

Similar method was applied to determine the effect of pH on hydrogel absorbance rate. Gel (**2**) was soaked in water at different pH values; 5, 7, and 9 for 24 h [Fig. 5(b)]. The weight of the hydrogel

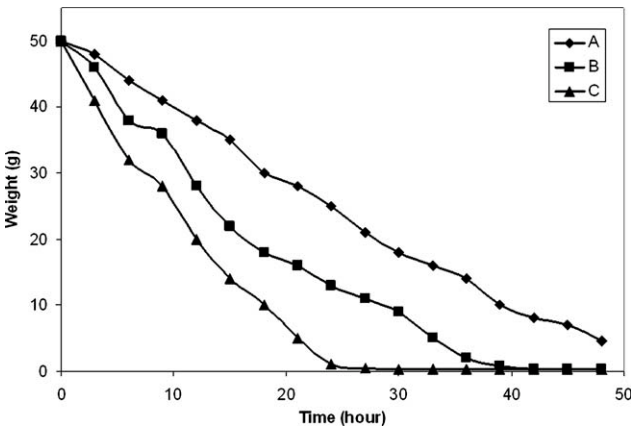


**Figure 4** Water retention at different temperatures; (A) 40, (B) 50, and (C) 60°C.

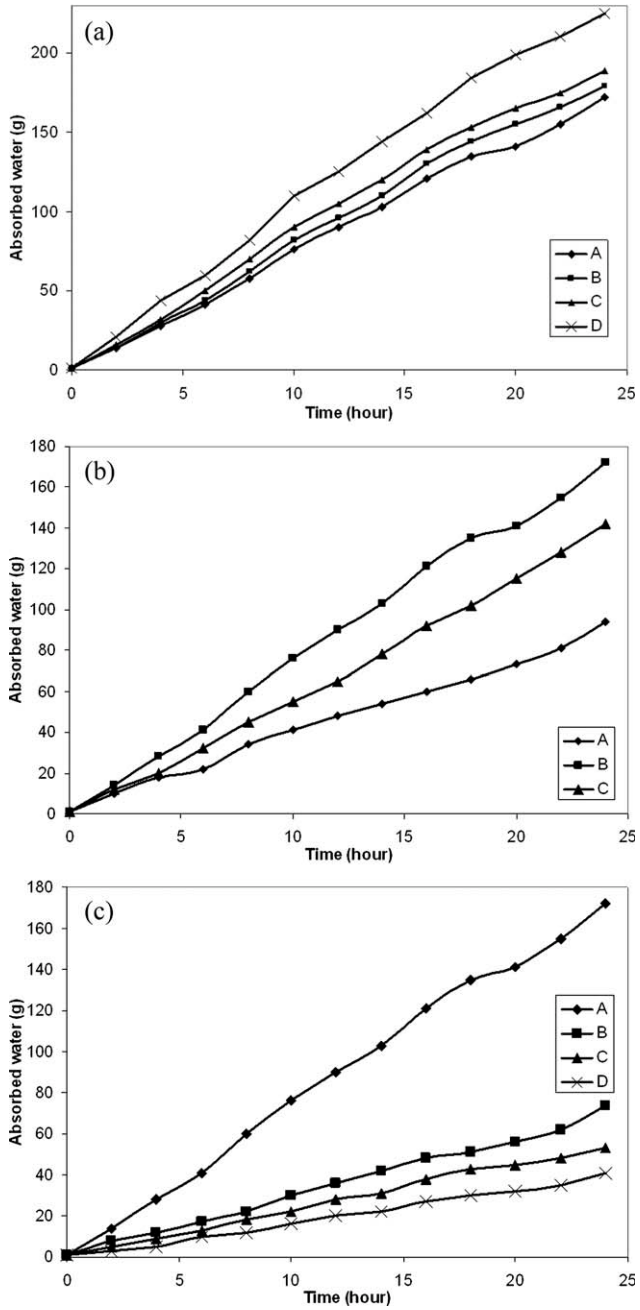


**Figure 5** Effect of (a) temperature, (b) pH, and (c) ions concentration on water absorbance rate. Where in (a) (A) = ambient temperature, (B) = 40°C, (C) = 50°C and (D) = 75°C. (b): (A) = pH 4, (B) = pH 7, and (C) = pH 9. (c): (A) = distilled water, (B) = 0.5%, (C) = 1%, and (D) = 3%.

was identified at timed intervals by filtering the hydrogel and drying its external surface using clean filter papers at these intervals.

The effect of ions concentration on absorbance rate was determined by soaking Gel (**2**, 1 g) in different saline solutions with different concentrations; 0.5, 1, and 3% for 24 h. The hydrogel were filtered, outer surface was dried with clean filter papers and weighed at timed intervals [Fig. 5(c)].

### Effect of Gel (2) on bacterial viability and growth rate using stirred flask method

A culture of *E. coli* was prepared by inoculating one bacterial colony into 20 mL of nutrient broth. The solution was incubated for 24 h at 37°C. From this bacterial suspension 0.1 mL was transferred to a 20 mL bottles containing 10 mL of fresh medium. A further five bottles were prepared, so the total number was six; three used for testing the effect of Gel (**2**) on bacterial growth and the other three to study its effect on bacterial viability.[2]

To study its effect on *E. coli* growth rate, Gel (**2**, 1 g, allowed previously to swell for 12 h) was added to the first bottle while Gel (**1**, 1 g, allowed to swell for 12 h) was added to the second bottle to act as gel control and the third was left as a bacterial control. The three bottles were stirred at 37°C and sampled at timed intervals for viable count [Fig. 6(a)]. Similar method was applied against *S. aureus* [Fig. 6(b)].[2]

To study the effect of the hydrogel on *E. coli* viability, the other three bottles were incubated for 17 h at 37°C, and the number of bacteria was determined by viable count using the "Miles and Misra technique."[13] Then Gel (**2**, 1 g, allowed previously to

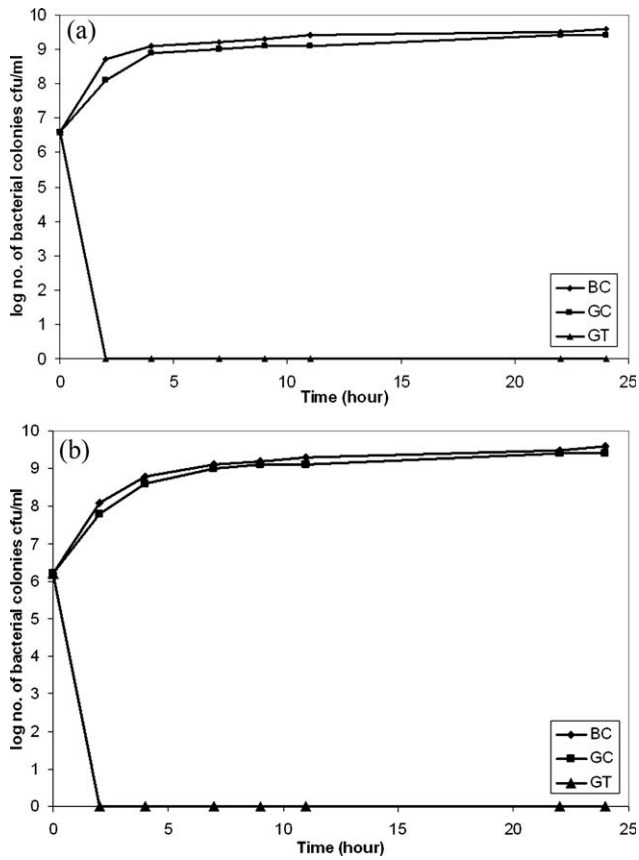


**Figure 6** Effect of Gel (**2**) on growth rate of (a) *E. coli* and (b) *S. aureus*. Where BC = bacterial control, GC = hydrogel control (Gel **1** effect) and GT = investigated hydrogel (Gel **2** effect).

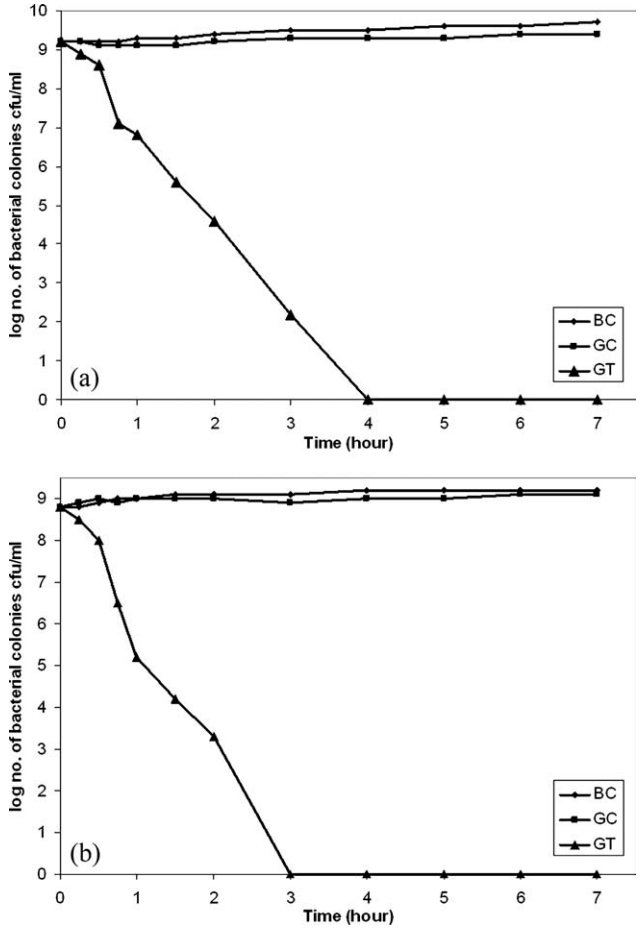


**Figure 7** Effect of Gel (**2**) on (a) *E. coli* and (b) *S. aureus* viability. Where BC = bacterial control, GC = hydrogel control (Gel **1** effect) and GT = investigated hydrogel (Gel **2** effect).

swell for 12 h) was added to the first bottle; Gel (**1**, 1 g, allowed previously to swell for 12 h) was added to the second, to act as a gel control, and the third vessel was left as a bacterial control. The three bottles were stirred at ambient temperature and sampled for viable counts at timed intervals, Figure 7(a), using "Miles and Misra" method.[13] Similar procedure was repeated to investigate the effect of Gel (**2**) on *S. aureus* viability [Fig. 7(b)].[2]

### Gel (2) regeneration using stirred flask method

The experiment of determining the effect of Gel (**2**) on bacterial (*E. coli* or *S. aureus*) viability was repeated. After the end of the experiment, HCl (1*N*) was added until pH 3. Gel (**2**) was filtered and dried to full dryness. Gel (**2**) was recharged with HMTA using the method described before and allowed to swell in distilled water for 12 h. The refreshed hydrogel was examined again against fresh amount of bacterial suspension. The same method was repeated up to 10 times [Fig. 8(a,b)] and the results were compared to hydrogel control, Gel (**1**), and bacterial control.

### Gel (2) lifetime as a disinfectant in columns (models for water filters)

Gel (**2**, 1 g) was backed in a column, 5 cm diameter and 20 cm length, and allowed to swell by adding distilled water for 24 h. Bacterial suspension (50 mL), either *E. coli* or *S. aureus*, was perfused through the column. The filtered cells were left on the top of the column for 4 h followed by transferring a sample to a fresh amount of nutrient broth (10 mL). The broth was incubated for 17 h at 37°C and the viability was determined by counting using Miles and Misra technique.[13] Several runs (up to 10) were performed through the same column using fresh bacterial suspension each time. The same procedure was repeated to investigate the end of

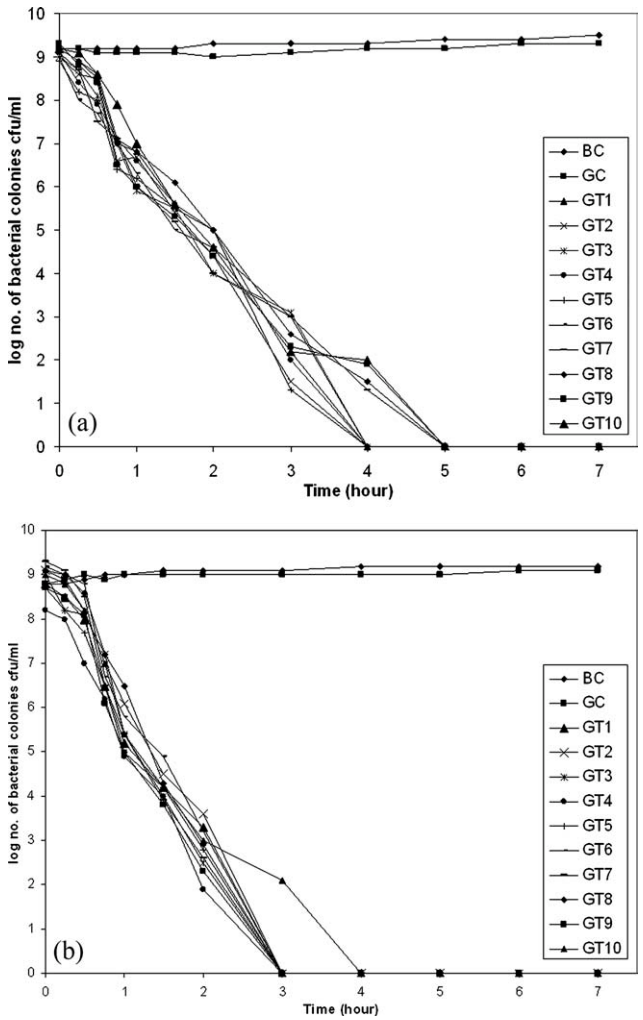


**Figure 8** Regeneration possibilities of Gel (**2**) using stirred flask method against (a) *E. coli* and (b) *S. aureus*. Where BC = bacterial control, GC = hydrogel control (Gel **1** effect) and GT1-GT10 = investigated hydrogel for 10 times of recycling trails (Gel **2** effect).

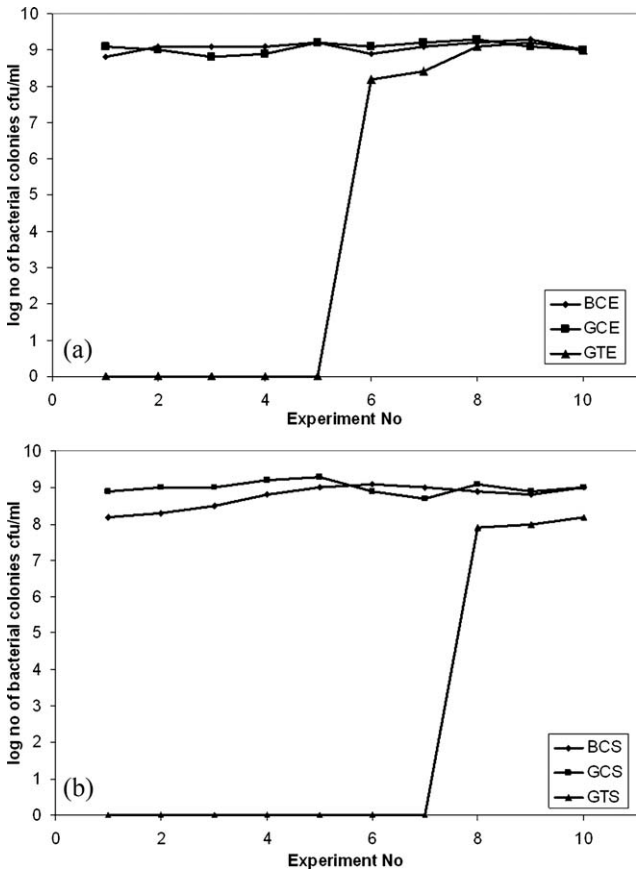


**Figure 9** Gel (**2**) lifetime in columns against (a) *E. coli* and (b) *S. aureus*. Where BCS = *S. aureus* bacterial control, GCS = Gel (**1**) effect on *S. aureus* (hydrogel control column) and GTS = Gel (**2**) effect on *S. aureus* (investigated hydrogel column). BCE = *E. coli* bacterial control, GCE = Gel (**1**) effect on *E. coli* (hydrogel control column), and GTE = Gel (**2**) effect on *E. coli* (investigated hydrogel column).

antibacterial activity of the column (Fig. 9). Column control was performed using Gel (**1**). Bacterial control was applied by inoculating fresh nutrient broth using bacterial cells that have no contact with any hydrogels.

### Gel (2) regeneration

The previous experiment, lifetime determination against both *E. coli* and *S. aureus*, was repeated. After the end of antibacterial activity of the column, the column was washed with HCl (1*N*) and the hydrogel was dried to complete dryness. The dry gel was recharged with HMTA as described before. The hydrogel was transferred back to the column and the previous experiment (lifetime determination protocol) was repeated. This procedure was repeated up to 10 times to investigate the possibility of hydrogel recycling. The results were compared to a hydrogel control, Gel (**1**), and bacterial control.

## RESULTS AND DISCUSSION

New group of hydrogels were prepared by reacting crosslinked acrylic acid with some amino containing compounds such as imidazole, triethanolamine, 2-aminopyridine, pyridine, and HMTA (Fig. 2).[7] The antibacterial activity of these hydrogels was determined and the action of one of them as a disinfectant in columns (models for water filters) was studied.[7] Hydrogel absorbs water to full saturation closing gabs between particles in the column.[7] Any excess water will pass through the column resulting in filtration to any contamination present in this water; bacterial cells.[7]

The current study is aiming to investigate the recycling possibilities of this hydrogel, Gel (**2**), to increase its lifetime in water filters. To achieve this goal some properties of Gel (**2**), such as water absorbance rate and water retention, were examined to understand its swelling behavior in water. In his previous study, author had proved that the absorbance did not increase with a significant amount after 48 h of soaking the hydrogel in distilled water. However it was very important to identify the exact absorbance rate during the first 24 h. So Gel (**2**) was allowed to expand in distilled water for 24 h and the weighing was performed for each 2 h. It was noticed that the absorbance rate was around 7 g per hour (Fig. 3). The samples were left for another 24 h in distilled water to reach to full saturation with water. Three different samples from the fully saturated hydrogel were heated at different temperatures (40, 50, and 60°C) to identify their water retention. It was noticed that increasing temperature has increased water loss with time. Raising temperature up to 60°C enables the gel to reach its full dryness in 30 h (Fig. 4). The temperature (60°C) and time, required to full drying, were used as a base in the recycling procedure of Gel (**2**) during the regeneration trails.

Some other factors that control water absorbance rate such as temperature, pH, and ions concentration were studied. Gel (**2**) was soaked in distilled water at three different temperatures. The weight of Gel (**2**) was determined at timed intervals. It was noticed that increasing the temperature has increased water absorbance rate [Fig. 5(a)]. Similarly, pH was changed to investigate its effect on water absorbance. Gel (**2**) was soaked in solutions at different pH values. It was noticed that the highest level of absorbance was recorded at pH 7 while the lowest was recorded at pH 4 [Fig. 5(b)]. This could be explained on the base that in acidic medium a neutralization reaction converts the hydrogel to its corresponding crosslinked acid. This may restrict such hydrogel at low pH values. Moreover, the concentration of ions present in water affects rate of absorbance. It was

noticed that increasing ions concentration has decreased the swelling ratio [Fig. 5(c)].

It can be seen from the previous data that water with high level of ions and low pH value will not be suitable to work with Gel (**2**) as it will restrict its expansion. Full dryness of Gel (**2**) was achieved at 60°C for 30 h. Soaking Gel (**2**) in water for 24 h is enough to close gabs between gel particles. Raising temperature may support fast swelling in distilled water.

Taking these results in mind, recycling possibilities of Gel (**2**) was performed using two different methods; stirred flask and columns. Using stirred flask method has required determining the effect of Gel (**2**) on bacterial viability and growth rate. This was achieved by stirring Gel (**2**) with bacterial suspensions (either *E. coli* or *S. aureus*, previously grown cells). It was noticed that Gel (**2**) achieved a 5 log reduction in 2 h against both types of bacteria while complete disinfection was reported after 3 h in case of *S. aureus* and 4 h in case of *E. coli* [Fig. 7(a,b)]. At the same time, determining the effect of Gel (**2**) on growth rate was performed by growing bacteria in presence of Gel (**2**). It can be seen from Figure 6 that no bacterial growth was reported for both types of bacteria in presence of Gel (**2**).

To determine the recycling possibilities using stirred flask method, Gel (**2**) was stirred with bacterial suspension (either *E. coli* or *S. aureus*, previously grown cells) until the end of its antibacterial action followed by decreasing pH to 3 using 1*N* HCl. Addition of HCl helps in disinfecting any bacterial cells that may still be alive and remove any remains of HMTA that may still be connected to the crosslinked acid. Gel (**2**) was filtered, dried to full dryness, and recharged again with HMTA followed by repeating the stirring process with another amount of fresh bacterial suspension. It was noticed that after repeating the previous method several times (up to 10 times) Gel (**2**) is still able to kill bacterial cells [*E. coli* or *S. aureus*, Fig. 8(a,b)]. This encourages further studies to the regeneration possibilities using other methods; column.

To identify the regeneration possibilities of Gel (**2**) using column method the lifetime of the gel in columns was determined [Fig. 9(a,b)]. The gel was placed inside a closed column and allowed to swell for 24 h in distilled water. The column was opened to remove excess water. Bacterial suspensions of either *E. coli* ($1.3 \times 10^3$ cfu/mL) or *S. aureus* ($2.4 \times 10^3$ cfu/mL) were perfused through the column. The filtered cells on the top of the column were allowed to have a contact time up to 4 h with the gel. Samples of the filtered cells were transferred to a fresh amount of nutrient broth. The broth was incubated at 37°C for 17 h and the growth was determined by counting. After the end of the contact time (4 h) another run through the column was performed using fresh amount of bacterial suspension and the method was repeated again. It was noticed that the column stay active in case of *E. coli* up to 5 runs and for 7 runs in case of *S. aureus*.

To determine the recycling possibility of the column, it was run to the end of its lifetime in both cases; *E. coli* and *S. aureus*. Gel (**2**) was then washed with 1*N* HCl followed by drying to full dryness. The hydrogel was recharged with HMTA and lifetime experiment was repeated again. This protocol was repeated up to 10 times. It was noticed that the column was recycled successfully for both *E. coli* and *S. aureus* without a significant change in all trails which encourages applying Gel (**2**) in water filters and increases its commercial values.

The previous results indicated that Gel (**2**) is able to kill and stop bacterial growth. It could be recycled several times without significant change in its activity which increases its commercial value and encourages applying it on large scale. Further efforts are currently running to extend the research about this type of hydrogels, data will be published in due course, such as the interaction between crosslinked acid and HMTA in addition to its releasing rate. Moreover, porosity and surface area of the hydrogel and their effect on cells filtration were determined in addition to the interface between the cells and the hydrogel.

## CONCLUSIONS

The effect of some factors (pH, temperature, and ions concentration) on swelling behavior of a new prepared, Gel (**2**), was identified. The swelling rate of the hydrogel has decreased with decreasing pH and increasing ions concentration. At the same time increasing temperature has increased the swelling rate. The antimicrobial activity of the hydrogel was identified using stirred flask method. Gel (**2**) has achieved a 5 log reduction on both *E. coli* and *S. aureus* in 2 h while complete disinfection was reported in 3 h for *S. aureus* and 4 h for *E. coli*. The effect of Gel (**2**) on bacterial growth was investigated and it was found that no bacterial growth was reported in presence of Gel (**2**). Moreover, the lifetime of the gel in columns was identified. The column stands for up to 5 runs for *E. coli* and 7 runs for *S. aureus.* The gel was recycled successfully up to 10 times for both types of bacteria using two different methods; stirred flask and column encouraging its application in water filters on large scale.

## References

1. Ahmed, A. E. I.; Hay, J. N.; Bushell, M. E.; Wardell, J. N.; Cavalli, G. React Funct Polym 2008, 68, 248.

2. Ahmed, A. E. I.; Hay, J. N.; Bushell, M. E.; Wardell, J. N.; Cavalli, G. React Funct Polym 2008, 68, 1448.
3. Ahmed, A. E. I.; Hay, J. N.; Bushell, M. E.; Wardell, J. N.; Cavalli, G. J Appl Polym Sci 2009, 113, 2404.
4. Ahmed, A. E. I.; Hay, J. N.; Bushell, M. E.; Wardell, J. N.; Cavalli, G. J Appl Polym Sci 2010, 116, 2396.
5. Ahmed, A. E. I.; Wardell, J. N.; Thumser, A. E.; Avignone-Rossa, C. A.; Cavalli, G.; Hay, J. N.; Bushell, M. E. J Appl Polym Sci 2011, 119, 709.
6. Ahmed, A. E. I.; Cavalli, G.; Bushell, M. E.; Wardell, J. N.; Pedley, S.; Charles, K.; Hay, J. N. Appl Environ Microbol 2011, 77, 847.
7. Ahmed, A. E. I. J Appl Polym Sci 2011, DOI: 10.1002/app.34228. http://onlinelibrary.wiley.com/doi/10.1002/app.34228/abstract.
8. El-Masry, A. M.; Moustafa, H. Y.; Ahmed, A. I.; Shaaban, A. F. Pigment Resin Technol 2004, 33, 75.
9. El-Masry, A. M.; Moustafa, H.Y.; Ahmed, A. I.; Shaaban, A. F. Pigment Resin Technol 2004, 33, 211.
10. El-Masry, A. M. Pigment Resin Technol 2005, 34, 265.
11. Beyth, N.; Yudovin, F. I.; Bahir, R.; Domb, A. J.; Weiss, E. I. Biomaterials 2006, 27, 3995.
12. Tashiro, T. Macromol Mater Eng 2001, 286, 63.
13. Miles, A. A.; Misra, S. S. J Hygiene (London) 1938, 38, 732.