Hydrogels for Water Filters: Preparation and Antibacterial Evaluation

Abd El-Shafey I. Ahmed

Department of Chemistry, Faculty of Science, University of Zagazig, Zagazig, Sharkia, Egypt

Received 22 December 2010; accepted 27 January 2011
DOI 10.1002/app.34228
Published online 20 May 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Acrylic acid was crosslinked with *N*,*N*′-methylenebisacrylamide and converted to bioactive hydrogels by neutralization with different amino containing compounds. Several amino containing compounds were used such as 2-aminopyridine, triethanol amine, hexamethylenetetramine (HMTA), pyridine, and imidazole. The best crosslinker ratio was determined in addition to the maximum absorbed water in different mediums. The antibacterial activity of the prepared gels were examined against examples of Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) using agar plate method. The study was extended by evaluating one of prepared gels in columns as models for water filters. All prepared gels showed antibacterial action in agar plate method against both bacterium and the column method using one of the prepared gels showed excellent filtration and biocidal action. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 1162–1167, 2011

Key words: antibacterial; gel; water filters; acrylic acid

INTRODUCTION

Poly Quat's is a type of biocidal polymers which carries positive charges due to the presence of quaternary nitrogen atoms (Fig. 1).[1–4] These positive charges enables the polymer to attract bacterial cells due to their negative charges, at certain physiological state; so cells adsorbed and expired.[1–4] Ammonium salts can behave like poly Quat's.[1,2]

Some types of water insoluble biocidal polymers were applied in water filters such as *N*-halamine polymers.[5] These polymers are charged with halogens that can be exchanged with bacterial cells resulting in their death.[1,5–7] Similarly poly Quat's was crosslinked to be applied in water filters.[8]

Hydrogels are a type of crosslinked polymers containing hydrophilic function groups that enables the polymer to absorb water.[9] Increasing the ratio of crosslinker increases water absorption until certain ratio where any further increase results in low absorbance. Hydrogels have many applications such as: drug delivery,[10] fast dissolving tablets,[11] scaffolds for tissue engineering,[12] modified fabrics for cosmetics or pharmaceuticals,[13] wound dressings,[14] sensors,[15,16] diet aid,[17] moisture trap,[18] and contact lenses.[19]

In this piece of work we are trying to apply a new approach by preparing hydrogels as a form of ammonium salts to act as disinfectants in water filters (Fig. 2). The idea was built on the fact that hydrogels absorb water to a certain level; gel maximum capacity. After this level the absorbance decreases and starts to be fixed. Based on this fact a new method was applied to produce a new water filter to treat drinking water from bacteria. Column (as a laboratory model for a water filter) was filled with hydrogel and the gel was treated with water to reach to its maximum absorbance while the column was closed. At the maximum capacity all the pores and spaces inside the column was blocked and any added water will pass without any further absorption by the gel. At this stage adding water contaminated with bacterial cells could result in their filtration on the top of the gel while the clean water passes through. The gel was designed to have some bactericidal action which enables the column to kill trapped cells.

For this purpose acrylic acid was crosslinked and neutralized with five different amino containing compounds to form five hydrogels. The structure of the prepared gels was followed using FTIR. The antibacterial activity of them (against both Gram-positive and Gram-negative bacteria) was evaluated using agar plate method[1,20,21] and one of them was applied in columns as a model for water filter.[5]

EXPERIMENTAL

Materials

Acrylic acid, *N*,*N*′-methylenebisacrylamide, imidazole, hexamethylenetetramine (HMTA), and 2-aminopyridine were obtained from Sigma Aldrich, UK.

Correspondence to: A. E.-S. I. Ahmed (a.i.ahmed@surrey.ac.uk) or (aiaibrahiem@zu.edu.eg).

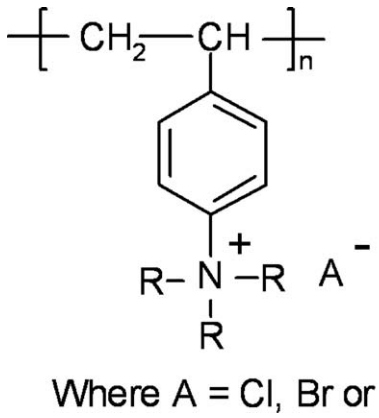

Figure 1 An example for poly Quat's.

Ammonium persulphate, triethanolamine, and pyridine were obtained from El-Naser, 10 Ramadan Industrial Town, Sharkia, Egypt. Nutrient broth and nutrient agar were 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.

Preparation of the gels

Acrylic acid (9.2 g) was dissolved in doubly distilled water (45 mL). *N*,*N*′-methylenebisacrylamide (0.187 g) and ammonium persulphate (0.1 g) were added to the reaction vessel. The mixture was heated gently to 40°C with vigorous stirring until the formation of a white precipitate. The resulting gel was cooled and grounded to small pieces and then soaked in a solution of doubly distilled water (120 mL) contained HMTA (17.9 g) and the reaction vessel was heated for 16 h at 40°C. The resulting gel (I), Figure 2, was dried at 90°C for 24 h to give yellow granules. The previous experiment was repeated using other amino compounds (imidazole, 8.7 g, triethanol amine, 16.9 g, 2-aminopyridine, 1.2 g and pyridine, 1.0 g) instead of HMTA to give gels II–V, respectively (Fig. 2). The structures of the prepared gels were followed using FTIR. Crosslinked acrylic acid (Gel 0, Fig. 2) was prepared as well without any further additives (amino containing compounds) to be used as a control.

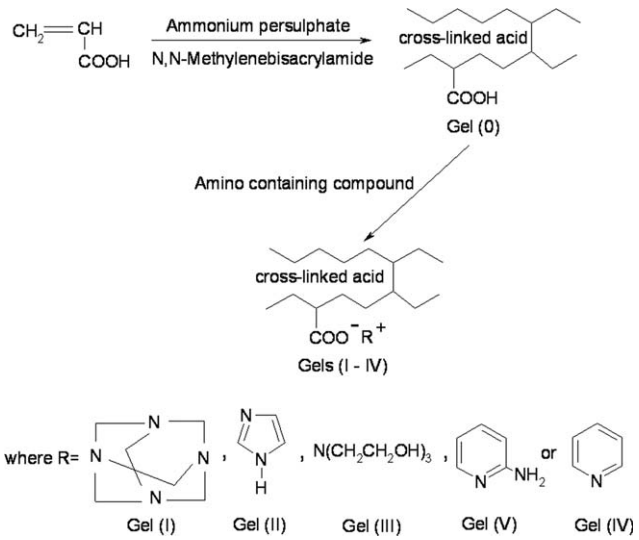

Figure 2 Preparation of different gels.

Effect of crosslinker ratio on water absorbance

Gel (I) was prepared with different ratios of crosslinker (*N*,*N*′-methylenebisacrylamide); 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5, and 6% using the method described above. At each ratio the amount of absorbed distilled water by 1 g of the dry gel was determined by immersing the dried gel granules in distilled water (500 mL) for 12 h followed by drying the outer surface with a clean filter paper and weighing (Fig. 3). The same experiment was repeated in saline solution (1%).

Effect of changing the amino containing compounds on water absorbance

Gels (I–V) were prepared using best crosslinker ratio (found from the previous experiment) and their dry granules (1 g) were immersed in distilled water (500

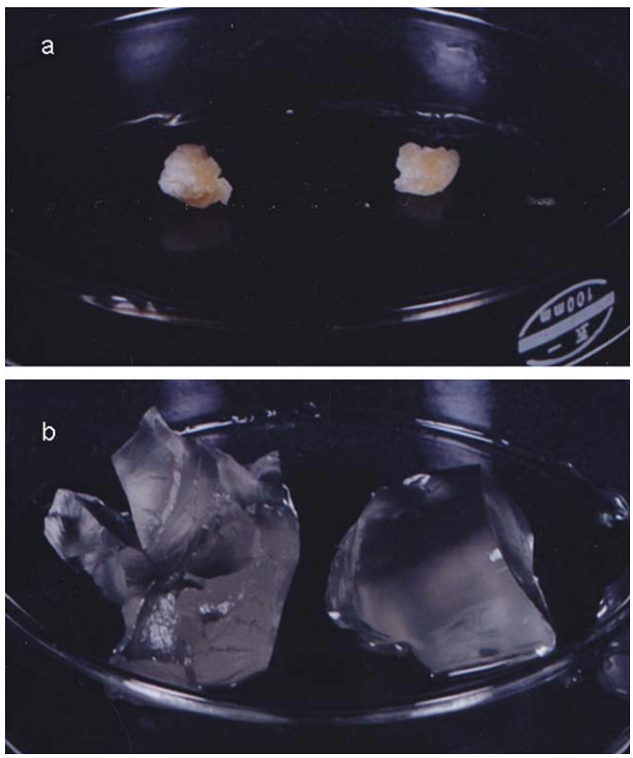

Figure 3 The gel before (a) and after (b) soaking in distilled water for 12 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

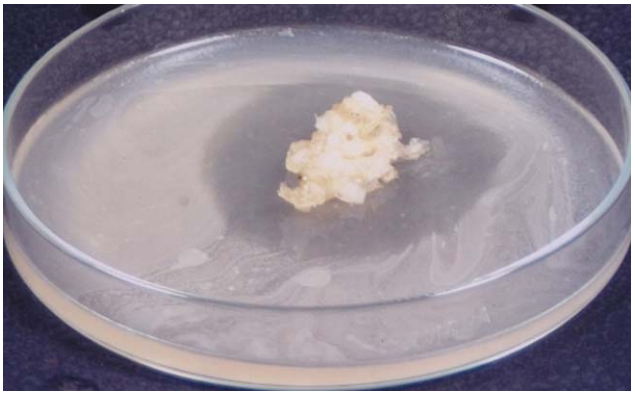

Figure 4 An example for the agar plate method for the evaluation of antibacterial activity of one of the gels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mL) for 12 h. Each gel was removed from water; outer surface was dried with filter paper and weighed.

Determination of maximum absorbance of the gel

Gel (I, 1 g based on the dry granules weight) was immersed in distilled water (1 lit). The water absorbance was followed at timed intervals; each 24 h until the gel weight become stable.

Determination of the biological activity of the prepared gels

Agar plate method

Nutrient agar (Oxoid) was prepared (250 mL), held molten at 50°C and 1.0 mL of a 17-h nutrient broth culture of either *S. aureus* or *E. coli* was added. The seeded agar was poured into Petri dishes; three dishes for each sample (triplicates) per bacterium type (*E. coli* or *S. aureus*). Wells (5 mm) were cut into the agar in the middle of each plate. Small amounts of each sample (Gels I–V, 0.1 g) were placed at the corresponding well while gel (0) was used as a control. The plates were incubated for 24 h at 37°C and the inhibition zones (Fig. 4) around samples were recorded, Table I.[1,7]

Column method

Column preparation. Gel (I, 1 g based on the dry weight of the granules) was backed into glass column (5-cm diameter and 40-cm length). The column was closed from the bottom while distilled water was added. The water absorbance was observed until the gel reached to its maximum absorbance. The column was then opened and became ready for experiment. Two different columns were prepared per bacterium; one contains gel (0) to work as a control while the other contains gel (I) to be investigated.

Bacterial suspensions. The suspensions were prepared by inoculating nutrient broth (50 mL) using single colony of either Gram-positive (*Staphylococcus aureus*) or Gram-negative (*Escherichia coli*) bacteria. The suspensions were incubated at 37°C for 17 h.[1–5]

The prepared suspensions were perfused through the columns 50 mL each time and per column. The number of bacterial colonies was counted before and after perfusion from each column using Miles and Misra method.[22] At the same time the suspension turbidity was determined using spectrophotometer (wave length = 560 cm^{-1}) before and after perfusion.[5] The experiment was repeated several times using fresh bacterial suspensions in each run.

Cells viability on the surface of the column

The viability of the filtered cells on the top of each column was examined at timed intervals. A sample was taken from these cells at each time interval and used to inoculate a fresh amount of nutrient broth. The suspension was incubated at 37°C for 17 h and the growth was followed by counting using Miles and Misra method.[22] In addition the turbidity of the solution was determined using spectrophotometer. The experiment was achieved for the four columns including the control columns.

RESULTS AND DISCUSSION

When the hydrogel reaches its maximum water absorbance, any excess water can go through. During this study hydrogel was backed into column and allowed to absorb water to reach its maximum capacity to close the gaps between the gel pieces. At this stage bacterial suspensions were perfused through the column to investigate the filtration and the disinfection activity of the column.

To achieve this idea new hydrogels were prepared using crosslinked poly acrylic acid as a substrate. Acrylic acid monomer was polymerized in presence

TABLE I
Inhibition Zone Around Each Gel in a Challenge Against Both *E. coli* and *S. aureus*

Hydrogel number	Inhibition zone by cm	
	E. coli	*S. aurous*
C	0	0
I	3.2 ± 0.4	4.0 ± 0.3
II	2.6 ± 0.2	3.1 ± 0.1
III	1.6 ± 0.3	1.7 ± 0.2
V	2.8 ± 0.2	3.3 ± 0.2
IV	1.4 ± 0.4	1.8 ± 0.1

Where *C* is the control; gel (0).

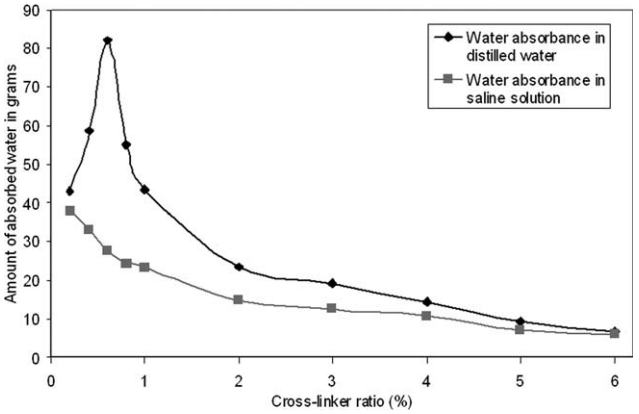

Figure 5 Amounts of absorbed water in grams by Gel (I) prepared using different ratios of crosslinker in distilled water and saline solution.

of *N*,*N*′-methylenebisacrylamide as a crosslinker and ammonium persulphate as initiator. The acid was neutralized with nitrogen containing compounds to form salts and to have some antimicrobial activity. Five nitrogen containing compound were used; hexamethylenetetramine (HMTA), 2-aminopyridine, pyridine, imidazole and triethanol amine (Fig. 2). All selected nitrogen containing compounds contain basic nitrogen atoms which can react easily with the crosslinked acid to give a form of poly Quat's.[1,2] The antibacterial activity of the prepared gels can be attributed to positive charges on the polymer which enables them to attract bacterial cells as they carry negative charges at certain physiological state.[1,4] As soon as the cells adsorbed to the polymer surface an exchange can happen between the cells and the polymer. Nitrogen containing moieties (counter ions) on the polymer which carry the positive charge can replace hydrogen atoms in equilibrium around the cell membrane. This can disturb this equilibrium and may results in perfusing of the counter ions inside the cells to change their nature resulting in their death. The polymer in this case restores the hydrogen ions to replace the nitrogen containing counter ions so it will back to its original structure; crosslinked acrylic acid. This will encourage a further study for the recycling possibilities of this type of gel as it can be charged several times with amino containing compounds. The recycling possibilities of such polymer is currently under investigation, data will be published in due courses.

The neutralization reaction was followed using FTIR. The carbonyl groups in all cases appeared around 1735 cm^{-1}. In case of using imidazole and 2-aminopyridine in the reaction a peak for NH was reported at 3151 and 3180 cm^{-1} respectively, and at 3358 cm^{-1} in case of using triethanol amine representing the hydroxyl group.

The ratio of the crosslinker can affect the water absorbance of the gel. For this reason the ratio of the crosslinker was changed during the preparation of one of the hydrogels and the water absorbance was examined. Gel (I) were prepared using different crosslinker ratios; 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5, and 6%. It was noticed that increasing the ratio of the crosslinker increases the amount of absorbed distilled water until certain ratio where the absorbance start to decrease (Fig. 5). From Figure 5 it can be seen that the best crosslinker ratio was 0.6% which enables the gel to absorb maximum amount of distilled water at this definite period of time. The reverse was noticed in case of soaking the gel in saline solution; the amount of absorbed water decreased with increasing the crosslinker ratio (Fig. 5).

The effect of changing the type of nitrogen containing compound on water absorbance was examined. It was noticed that by neutralizing the acid with nitrogen containing compound the water absorbance has been increased (Fig. 6). Changing the nature of the bond from covalent bond in the crosslinked acid to ionic bond in the prepared salts increased the absorbance. In addition presence of extra amino or hydroxyl groups enables the hydrogel to form hydrogen bonds with water which increases the absorbance. Also by changing the nature of the amino containing compounds the absorbance has been changed. The maximum absorbance was recorded for gel (III) prepared using triethanol amine. This can be explained on the base that triethanol amine contain extra three hydroxyl groups that can form hydrogen bonds with water and increase the absorbance. At the same time it has aliphatic chains in branched shape which increases the absorbance. The second one was gel (I); prepared using HMTA which after formation has three tertiary nitrogen atoms able to form hydrogen bonds with water in addition to its aliphatic skeleton. Gels

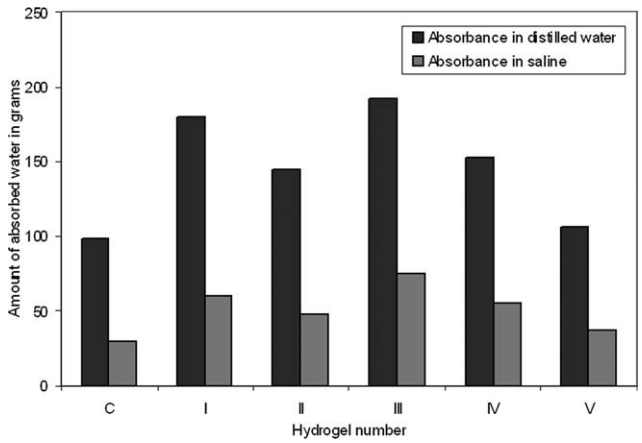

Figure 6 Amount of absorbed water in grams using different prepared gels compared to nontreated crosslinked acrylic acid. While C is the crosslinked acrylic acid without treatment (control).

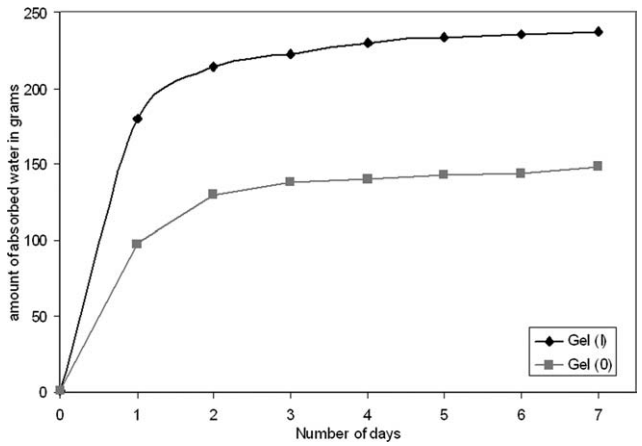

Figure 7 The absorbance of distilled water by 1 g of gel (I) at different time intervals.

prepared using imidazole and 2-aminpyridine record close results. The lowest gel in absorbance was that prepared using pyridine as it has no extra amino or hydroxyl groups (Fig. 6). Similar behavior was noticed in saline solution but the amount of absorbed water was lower than that in distilled water as expected (Fig. 6).

To start identifying the antimicrobial activity of the prepared gels it was very important to determine at what time the gel will reach to its maximum absorbance. This is very important for evaluating the gel in column as a model for water filters. Gel (I) was selected to this job due to the known chemotherapeutic effect of HMTA[23] and due to its high level of water absorbance found form previous experiments. Gel (I, 1 g based on the dry weight) was soaked in distilled water. The gel was taken out of water and the external surface was dried with a peace of filter paper and weighted. The gel was restored back to water and followed until fixed weight was determined. It was noticed that the maximum absorption of water was during the first 24 h. During the next 48 h the absorption was increased with small amounts while after the fourth day the absorbance begin to be fixed (Fig. 7). Similar behavior was noticed for the control gel but it was fixed after the second day (Fig. 7).

Determination of the antimicrobial activity of the prepared gels started with agar plate method. The experiment was performed for all prepared gels compared to nontreated crosslinked poly acrylic acid, gel (0), as a control. Both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria were included in this study, Table I. Both bacterium were grown on plates contains the gels in wells at the middle of each plate. The experiment was performed in triplicates. It can be seen from Table I that all gels have antibacterial action against both *E. coli* and *S. aureus.* It was noticed as well that Gel (I) achieved the maximum effect. In addition, the gels succeeded in absorbing some water from the agar present in the plate and start to swell (Fig. 4). This help in liquid exchange between the agar and the gel which increases the antimicrobial activity of the gel. From Table I one can conclude that gels containing more than one nitrogen atom have antimicrobial effect higher than that containing only one nitrogen atom even in presence of oxygen atoms.

Gel (I) was selected for further antimicrobial investigations using columns as a model for water filters. The gel was backed into the column and allowed to reach to its maximum absorbance by soaking in distilled water for 2 days while the column was closed from the bottom. It was noticed that as the particles start to absorb water they tend to bush each other up to the top of column. As soon as the particles reach to its final size the column was opened to remove excess water. Two columns were prepared in the same way one for *E. coli* and the other for *S. aureus*. Similar columns were prepared for gel (0) to work as a control.

Bacterial suspensions (*E. coli* or *S. aureus*) were perfused through columns in portions; 50 mL each time. The suspensions were diluted so the final number for *E. coli* was 1.6×10^3 and for *S. aureus* was 1.2×10^3. The bacterial viability was determined before and after perfusion through the column. In addition the turbidity was determined before and after perfusion using spectrophotometer.

It was noticed that using spectrophotometer was not that good way to judge the bacterial turbidity as nutrient broth was usually diluted by water originally absorbed by the gel. So the evaluation of the viability was determined by counting only using Miles and Misra method. It was noticed that bacterial cells (both *E. coli* and *S. aureus*) are trapped on the upper layers of the gel and can not proceed further. The experiment was repeated for 10 runs using fresh bacterial suspensions each time and similar behavior was reported for both types of bacteria. But similar behavior was reported for the control column as well. This behavior was expected as the spaces between the gel particles were very small to pass any cells. The gel particles have been grown together in a very tight and small space in comparison with their big size which gives no spaces after their growth.

As the same behavior was reported for all columns; control, gel (0) and samples under investigation, gel (I), the column experiment was repeated again. After perfusing bacterial suspension (50 mL), samples were collected from the bacteria trapped on the top of each column at timed intervals (each 1 h). The samples were used to inoculate fresh amount of nutrient broth and the solution were incubated at 37°C for 17 h and the growth was followed by

counting and also by spectrophotometer. It was noticed that after 7 h and up to 12 h *E. coli* cells trapped on the top of gel (I) column was unable to grew in case of transferring to fresh broth. The same was recorded for *S. aureus* but after 5 h. The difference in behavior of the two types of bacteria may be attributed to the fact that *S. aureus* is not a motile bacterium.[5] So it can settle on the surface of the gel which enables maximum contact between both of them. This contact time allowed transferring high concentration of nitrogen containing moieties to *S. aureus* so it was affected more than *E. coli*. At the same time all samples collected from the control columns (*E. coli* or *S. aureus*) succeeded in growing at all time intervals. These results showed that gel (I) column is able to filter and kill bacterial cells of both Gram-positive and Gram-negative bacteria.

The previous results indicated that hydrogel can be used in water filters as a disinfectant if it has antimicrobial activity. Physical properties, recycling possibilities, and the effect of different factors such as pH and temperature on this type of hydrogel are under investigation; data will be published in due course. In addition some other applications are under investigation.

CONCLUSIONS

New group of hydrogels were prepared. Their ability to absorb water in different mediums was examined. The best crosslinker ratio to enable the gel to absorb maximum amount of water was determined; 0.6%. It was found that using amino containing compounds to neutralize the crosslinked acid enables the gel to absorb more water. Amino containing compounds with aliphatic parts and high number of hetero atoms enable the gel to absorb more water. All prepared hydrogels showed antimicrobial activity in the agar plate method. Gel (I) were used in columns to filter and kill both *E. coli* and *S. aureus.*

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