POSSIBILITY OF CROSSLINKING OF RAW XANTHAN WITH DIVALENT AND TRIVALENT METAL IONS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT: Biopolymers are biodegradable, biocompatible and non-toxic natural compounds with large molecular masses. Xanthan is a biopolymer produced by bacteria of genus Xanthomonas. Nowadays scientists are focused on examining the possibilities of crosslinking of xanthan using different agents. The aim of this paper was to investigate the possibility of crosslinking of xanthan with divalent and trivalent metal ions. During these studies, xanthan produced by reference strain Xanthomonas campestris ATCC 13951 on medium based on wastewater from white wine production was used. Xanthan solutions of different concentrations (1.0%, 2.0% and 2.5% (w/v)) were prepared in 0.1M sodium chloride. Salts of barium, calcium, magnesium, manganese, zinc, lead, as well as divalent and trivalent iron at different concentrations (0.025 M, 0.050 M and 0.075 M) and pH values (3, 5, 7 and 9) were used. The obtained results indicate that the success of crosslinking and the stability of the formed structures highly depend on the pH value of the salt solutions and the biopolymer concentration. The most stable, solid structures were formed by dropping 2.5% xanthan solution into a 0.075 M solution of FeCl3 at highly acidic pH value. The dry matter content of structures was 2.37%, and average dimensions were about 4.02 x 5.08 mm.

KEYWORDS: biopolymer, xanthan, crosslinking, metal ions

INTRODUCTION

The extensive industrialization results in a large resource exploitation and generation of significant quantities of waste effluents [1]. Taking into account the amount of waste streams generated by the food industry, as well as the content of organic and inorganic ingredients in them, it is clear that they cause high environmental problems. The emission of large volumes of effluents into natural recipient without adequate treatment is a big problem today, and with the evident trend of increasing the load in qualitative and quantitative terms, the inevitable issue will be active in the future also [2].

The pollution is mostly expanded by water flow, which is precisely the reason for the increase in the number of scientific research related to development of wastewater treatment [3]. In order to safely discharge wastewaters into the environment, it is necessary to purify those using appropriate mechanical, chemical and/or biological methods. Mechanical methods of purification of wastewater involve procedures for the removal of larger impurities and suspended particles, and are based on an action of physical forces such as gravity and pressure. Chemical methods are processes in which the removal of pollutants is carried out by performing certain chemical reactions or physicochemical phenomena. Biological processes of wastewater treatment are based on the application of microorganisms that adopt and metabolize numerous organic and inorganic substances from wastewater [4-6].

These methods have a number of advantages and disadvantages. Some of them require the use of toxic and expensive materials, while some are not completely effective and can lead to the accumulation of new waste [7]. Therefore, the attention of the scientific public is focused on testing the possibility of applying biocompatible sewage treatment materials, of which biopolymers have been most suitable [8].

Due to ability to absorb large amounts of water or biological fluids, with the formation of hydrogels [9], biopolymers have found a variety of applications, and among others they are used to remove individual metal ions from different wastewaters [10]. The most dangerous pollutants originating from industrial wastewater include toxic metals such as iron, lead, cobalt, copper, aluminum, sodium and others [3].

In a previous study, lead and iron ions have been successfully removed with xanthan, microbial biopolymer [3]. A large number of biopolymers of microbiological origin are known, but xanthan, produced by the *Xanthomonas* bacteria on semi-synthetic substrates, which are the basis of various industrial effluents [11], is the most important commercially. Xanthan has a unique structure and exceptional rheological characteristics [12] which are very appreciated in processes of purifying of wastewater. The aim of this paper was to examine the possibility of crosslinking of xanthan with divalent and trivalent metal ions. Based on the success of crosslinking of xanthan and the stability of formed structures, the potential for the use of this biopolymer for the removal of metal ions from wastewater was estimated.

EXPERIMENTAL

Preparation of xanthan solutions

1.0%, 2.0% and 2.5% (w/v) solutions of raw xanthan synthesized on wastewaters from white wine production using *Xanthomonas campestris* ATCC 13951, prepared in 0.1M sodium chloride, were used for a crosslinking procedure. Preparation of xanthan solution meant suspending the required amount of biopolymer in 0.1 M sodium chloride, and its long-term dissolution at constant mixing.

Preparation of metal ion solutions

Ions of divalent and trivalent metals were used in this experiment as crosslinking agents. Salts (chlorides, sulphates and nitrates) of barium, calcium, magnesium, manganese, zinc and lead were used as metal ion sources. For the purposes of this research, aqueous solutions of the mentioned metal ions of different concentrations (0.025 M, 0.050 M and 0.075 M) and pH values (3, 5, 7 and 9) were prepared.

Crosslinking procedure

The crosslinking of raw xanthan was carried out at ambient temperature by dropping xanthan solution into a solution of metal ions with a peristaltic pump at a rate of 1 drop/s (tube diameter: 0.3 mm) with a constant stirring of 150 rpm provided by placing a vessel for crosslinking on a magnetic stirrer. Figure 1 shows a schematic view of the used apparatus.

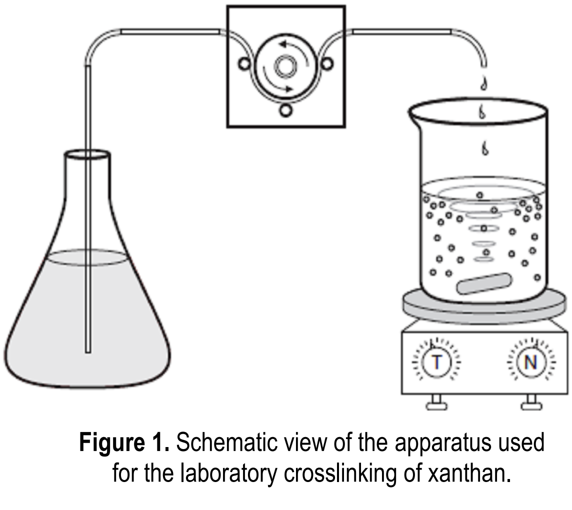

Figure 1. Schematic view of the apparatus used for the laboratory crosslinking of xanthan.

Hardening, i.e. the mechanical stability of the formed xanthan structures, was achieved by their aging in ionic metal solution for 10 minutes with constant mixing.

Characterization of formed structures

At the end of the hardening, characterization of the formed xanthan structures was carried out. For this purpose, dimensions of the cross-linked structures were measured, and the content of the dry matter was determined.

Dimensions of the cross-linked xanthan structures were determined by measuring using a nonius. A randomly selected sample of ten cross-linked structures was used for measurement.

The dry matter content of cross-linked xanthan structures was determined gravimetrically, by drying them at a temperature of 105ºC until a constant mass was achieved [13]. A randomly selected sample of twenty cross-linked structures was used for measurement.

RESULTS AND DISCUSSION

In accordance with the defined aim, during this research, the possibility of crosslinking of xanthan with divalent (Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Fe^{2+}) and trivalent (Fe^{3+}) metal ions was investigated. Crosslinking was performed by dropping the xanthan solution into the metal ion solutions as described above. In order to examine the influence of the pH value and the concentration of metal ions on the success of crosslinking, the characterization of formed structures was carried out, which, in addition to visual characterization, included measurement of the size, as well as determining the dry matter of the structures. The visual characterization was performed in order to estimate formation and stability of cross-linked structures.

Influence of pH of metal ion solution on crosslinking efficiency

In order to examine the influence of the pH value of the metal ion solution on the crosslinking efficiency, 1.0% (w/v) xanthan solution was added to 0.050 M metal ion solution whose pH value was adjusted to 3, 5, 7 or 9. The possibility of crosslinking, appearance and stability of formed structures for all investigated ions, other than lead and iron, is described and summarized in Table 1.

The results shown in Table 1 indicate that the formation of solid and stable structures could not be achieved in all the analyzed conditions. The influence of pH value of the lead and iron ion solution on the crosslinking of xanthan was not tested because it was impossible to correct this parameter in solutions of the mentioned ions. It was impossible to adjust pH value of the iron ion solution above 3, while in the solution of the lead ions a precipitate was formed when adding both acids and bases. Therefore, the solutions of the mentioned metal ions without correction of pH values were used in further experiments for the crosslinking of xanthan.

Table 1. Influence of the pH value of 0.050 M metal ion solution on the success of the crosslinking of 1.0% (w/v) raw xanthan solution

Metal ion source	**Initial pH value**	**Characteristics of formed structures**			
		pH=3	**pH=5**	**pH=7**	**pH=9**
			CHLORIDES		
$BaCl_2\cdot2H_2O$	6.66	very unstable, disintegrated structures	unstable, disintegrated structures	unstable structures that lasted for a few seconds	very unstable, disintegrated structures
$CaCl_2$	6.94	unstable structures that in first few seconds tend to spherical form, but after a few seconds, they elongate and disintegrate	unstable spherical, long lasting structures	unstable structures, decay after a few seconds	completely unstable structures
$MgCl_2\cdot6H_2O$	7.29	unstable spherical structures	in first few seconds structures tend to spherical form, but after a few seconds, they elongate and disintegrate	unstable spherical structures	completely unstable structures
$MnCl_2\cdot4H_2O$	6.86	*	*	unstable structures that dissolve very quickly	*
$FeCl_3\cdot6H_2O$	1.95	*	*	*	*
			SULPHATES		
MgSO4	7.05	unstable structures that disintegrate very quickly	unstable structures	unstable structures	unstable structures, last for a long period of time
$MnSO_4\cdot H_2O$	6.84	elongated spherical structures, which decay after a long time	unstable elongated spherical structures, last for a short period of time	unstable elongated spherical structures, last for a short period of time	*
$ZnSO_4\cdot7H_2O$	6.21	unstable ellipsoidal structures, last for a long period of time	smaller unstable ellipsoidal structures, last for a short period of time	*	*
$FeSO_4\cdot7H_2O$	4.34	unstable structures of indefinite forms, eventually disintegrate and dissolve	*	*	*
			NITRATES		
$Pb(NO_3)_2$	4.62	spherical structures that are very unstable, disintegrate on touch	*	*	*

*- It was impossible to adjust pH value.

Based on the results in Table 1, it can be noticed that if chlorides of barium, magnesium and manganese ions are used as crosslinking agents, the most stable structures are formed at a pH value of 7. The most defined shaped structures are formed in the calcium ion solution at a pH value of 5.

The results of crosslinking of xanthan with sulphates solutions show the possibility of application of solution with different pH values. According to the obtained results, it can be found that if solutions of ions of zinc and iron are used as crosslinking agents, the most stable structures are formed at a pH value of 3.

Further, if manganese ion solutions are used as crosslinking agents, very durable, elongated spherical structures are formed at a pH value of 7. The most durable structures can be formed by crosslinking of xanthan with solution of magnesium ions at a pH value of 9. The most defined spherical structures in this experiment were formed by crosslinking of xanthan with nitrate solution of lead ions at a pH value of 3. Bearing in mind that xanthan crosslinking is possible with solutions of the mentioned metals ions, but it does not result in a stable and solid structure, it can be said that these metals do not crosslink xanthan well enough at a concentration of 0.05 M.

Table 2. Influence of the concentration of metal ions in solution on the success of the crosslinking of 2.0% (w/v) raw xanthan solution

Metal ion source	**pH value of metal ion solution**	**Characteristics of formed structures**		
		c=0.025 M	**c=0.050 M**	**c=0.075 M**
		CHLORIDES		
$BaCl_2\cdot 2H_2O$	7.00	unspecified structures, disintegrate on the touch, very unstable, dissolves after standing in solution	rod shaped structures, unstable, disintegrate on the touch	very unstable ellipsoidal structures, disintegrate on the touch
$CaCl_2$	5.00	very unstable ellipsoidal structures, disintegrate on the touch	ellipsoidal structures, unstable on the touch, disintegrate after few seconds	ellipsoidal structures, very unstable, disintegrate fast on the touch
$MgCl_2\cdot 6H_2O$	7.00	unspecified structures, disintegrate on the touch, very unstable	ellipsoidal structures, look stable but disintegrate fast on the touch	unstable structures that tend to spherical form, disintegrate on the touch
$MnCl_2\cdot 4H_2O$	7.00	ellipsoidal structures, break down on the touch, very unstable, dissolve after standing in solution	ellipsoidal structures, look stable but disintegrate on the touch	ellipsoidal structures, unstable, disintegrate on the touch
$FeCl_3\cdot 6H_2O$	1.95	very stable ellipsoidal structures, remain in solution for a long time, do not dissolve	stable solid structures that do not disintegrate on touch	ellipsoidal stable structures, do not disintegrate
		SULPHATES		
MgSO4	9.00	rod shaped structures, unstable, disintegrate on the touch	rod shaped structures, more stable and long-lasting, but disintegrate on the touch	ellipsoidal structures, unstable, disintegrate on the touch
$MnSO_4\cdot H_2O$	7.00	ellipsoidal structures, very unstable, disintegrate on the touch	ellipsoidal structures, unstable, disintegrate on the touch	unspecified structures, disintegrate on the touch, unstable
$ZnSO_4\cdot 7H_2O$	3.00	rod shaped structures, unstable, disintegrate on the touch	rod shaped structures, unstable, disintegrate on the touch	ellipsoidal structures, unstable, disintegrate fast on the touch
$FeSO_4\cdot 7H_2O$	3.00	structures almost do not form, difficult to see due to the colour of the solution	ellipsoidal structures, more stable and long-lasting, but disintegrate on the touch	supersaturated solution
		NITRATES		
$Pb(NO_3)_2$	3.00	unspecified structures, disintegrate on the touch, very unstable	ellipsoidal stable structures, disintegrate on the touch	ellipsoidal, stable but very soft structures

Influence of metal ion concentration on crosslinking efficiency

In order to investigate the effect of concentration of metal ions on crosslinking process, a xanthan solution of different concentrations (1.0%, 2.0% and 2.5% (w/v)) was dropped into 0.025 M, 0.050 M and 0.075 M solutions of metal ions whose pH value was set to the value that was found to be the most suitable in the previous set of experiments except for the solutions of lead and iron ions whose pH value was not corrected. By crosslinking of 1.0% (w/v) xanthan solution, no stable structures were formed (Table 1), so no further analyses were performed. The possibility of crosslinking of 2.0% and 2.5% (w/v) xanthan solutions, appearance and stability of formed structures for all investigated ions, is described and summarized in Table 2 and Table 3, respectively.

The results represented in Table 2 show that the concentration of metal ions in the solution significantly affects the success of crosslinking, but also the stability of the formed structures. From the examined bivalent ions of metals (Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, $Pb^{2\ +}$, Fe^{2}), the best crosslinking was accomplished with lead ions, while the solutions of other ions did not show positive crosslinking results in the applied experimental conditions. By using xanthan with a solution of lead ions at all investigated concentrations stable structures were formed.

Nevertheless, it was noticed that the stability of the formed structures increased with the increase of the concentration of lead ions in the solution. The most stable, ellipsoidal shaped structures were formed at concentration of lead ions of 0.075 M.

Table 3. Influence of the concentration of metal ions in solution on the success of the crosslinking of 2.5% (w/v) raw xanthan solution

Metal ion source	pH value of metal ion solution	Characteristics of formed structures		
		c=0.025 M	c=0.050 M	c=0.075 M
CHLORIDES				
$BaCl_2\cdot 2H_2O$	7.00	unstable ellipsoidal structures, disintegrate on the touch	ellipsoidal structures, very unstable, disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch
$CaCl_2$	5.00	unstable ellipsoidal structures, disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch	ellipsoidal structures, very unstable, disintegrate on the touch
$MgCl_2\cdot 6H_2O$	7.00	unstable unspecified structures, disintegrate on the touch	unspecified structures, look stable but disintegrate fast on the touch	unstable unspecified structures, disintegrate on the touch
$MnCl_2\cdot 4H_2O$	7.00	unstable structures, disintegrate on the touch, dissolves after standing in solution	unstable structures, disintegrate on the touch	unstable unspecified structures, disintegrate fast on the touch
$FeCl_3\cdot 6H_2O$	1.95	very stable ellipsoidal structures, remain in solution for a long time, do not dissolve	stable solid structures, do not disintegrate on the touch	stable structures that tend to spherical form, do not disintegrate
SULPHATES				
MgSO4	9.00	rod shaped structures, unstable, disintegrate on the touch	rod shaped structures, more stable and long-lasting, but disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch
$MnSO_4\cdot H_2O$	7.00	ellipsoidal structures, very unstable, disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch
$ZnSO_4\cdot 7H_2O$	3.00	unstable ellipsoidal structures, disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch	unstable ellipsoidal structures, disintegrate fast on the touch
$FeSO_4\cdot 7H_2O$	3.00	very unstable structures, disintegrate after a few seconds	unstable structures, disintegrate on the touch	supersaturated solution
NITRATES				
$Pb(NO_3)_2$	3.00	ellipsoidal structures, disintegrate on the touch, very unstable	stable ellipsoidal structures, disintegrate on the touch	ellipsoidal, stable but very soft structures

Crosslinking of 2.0% (w/v) solution of xanthan with trivalent iron ions was very successful. Crosslinked structures were ellipsoidal and stable, and they did not break apart, unlike most structures crosslinked with divalent ions. The most stable structures were formed by using 0.075 M solution of trivalent iron ions.

Rod shaped structures of xanthan, cross-linked with barium and zinc ions were unstable. They disintegrated few seconds after staying in solution. Structures cross-linked with calcium, magnesium and manganese ions were ellipsoidal but also unstable. 0.075 M solution of iron ions did not crosslink xanthan, and at smaller concentrations, structures were very unstable and difficult to see due to the colour of the solution.

The results represented in Table 3 are very similar to the results in Table 2. It is confirmed that the concentration of metal ions in the solution affects the success of crosslinking, but also the stability of the formed structures.

From the examined bivalent metals ions (Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Fe^{2+}), the best crosslinking was accomplished again with lead ions, while the solutions of other ions did not show positive crosslinking results in the applied conditions. Compared to crosslinking of 2.0% (w/v) xanthan, structures of 2.5% (w/v) xanthan cross-linked with the mentioned ions are a little bit harder but still unstable.

Both, structures of 2.5% (w/v) xanthan cross-linked with lead and iron ions showed similar characteristics like in previous research [3].

By using xanthan with a solution of lead ions at all investigated concentrations stable structures were formed. Nevertheless, it was noticed that the stability of the formed structures increased with the increase of the concentration of lead ions in the solution. The most stable, ellipsoidal shaped structures were formed at concentration of lead ions of 0.075 M. One fraction of stable structures cross-linked with 0.075 M solution of Pb^{2+} ions is shown in Figure 2.

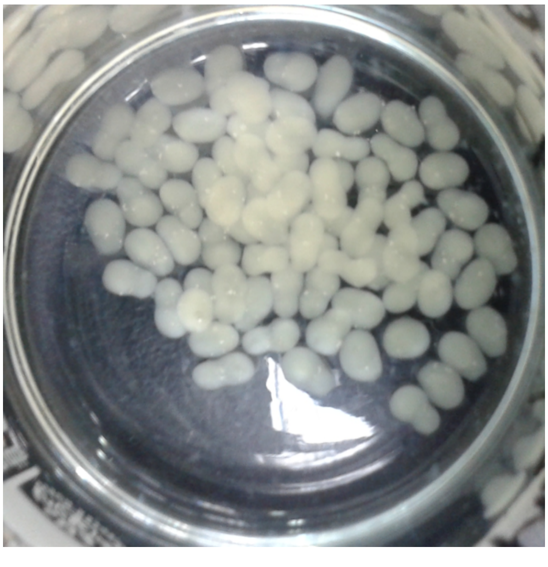

Figure 2. Structures formed with 2.5% (w/v) xanthan and 0.075 M lead ions solution

Crosslinking of 2.5% (w/v) solution of xanthan with trivalent iron ions was very successful. Cross-linked structures were ellipsoidal and stable, and they did not break apart. Structures of 2.5 % (w/v) xanthan were a bit harder than structures formed with 2.0% (w/v) xanthan solution. The most stable structures were formed by using 0.075 M solution of trivalent iron ions. The interior of these structures was filled, and their long persistence in the solution was noted.

Dimensions of formed structures

Based on the results given in Tables 2 and 3, it is clear that by uniformly dropping xanthan solution in a solution of different metals ions, the structures with different characteristics and sizes are formed, due to the presence of metallic ions, its source and concentration in the solution, as well as the pH value of the solution. Considering that the most stable structures are formed in the presence of iron and lead ions, continuation of this research is devoted to the determination of their dimensions. Results shown in Table 4 represent dimensions of structures cross-linked with solution of lead and iron ions of different concentrations.

Table 4. The dimensions of structures cross-linked with lead and iron ions of different concentrations

Structures	Average size: width x length (mm)		
	0.025 M	0.050 M	0.075 M
2.0% (w/v) xanthan cross-linked with Pb^{2+}	structures were not formed	4.46 x 5.40	4.42 x 5.66
2.0% (w/v) xanthan cross-linked with Fe^{3+}	3.40 x 4.98	4.00 x 5.54	3.72 x 5.22
2.5% (w/v) xanthan cross-linked with Pb^{2+}	4.14 x 6.20	4.04 x 5.84	4.04 x 5.56
2.5% (w/v) xanthan cross-linked with Fe^{3+}	3.44 x 5.50	3.98 x 5.22	4.02 x 5.08

The results shown in Table 4 indicate that the dimensions of the structures formed in the presence of different metal ions depend mostly on concentration of solution of metal ions. There is a difference between structures formed with 2.0% and 2.5% (w/v) xanthan solution, but it is insignificant. The results shown in Table 4 indicate that the dimensions of the structures formed in the presence of different concentrations of lead ions are reduced with the increase of their content in the solution. It is noticed that addition of 2.0% (w/v) solution of xanthan to 0.025 M solution of lead ions does not result in the formation of any structures. The structures with the largest dimensions were formed in the 0.025M solution of lead ions, while the smallest structures were formed in 0.075 M solution of the lead ions.

Similar behaviour was observed with structures formed in the presence of iron ions. The dimensions of these structures also reduce with the increase in the concentration of metal ions in solution. Thus, the largest structures were formed by crosslinking with solution of iron 0.025 M solution of iron ions, while the smallest structures were formed in 0.075 M solution of the mentioned ions.

The largest structures were formed by crosslinking of 2.5% (w/v) solution of xanthan with 0.025 M solution of lead ions. This result is in accordance with the results from the previous research, where the structures with dimensions in the range from 3.4 to 6.8 mm were formed [3]. Reduction of dimensions of cross-linked xanthan structures with increase in concentration of metal ions in solution may be due to the fact that, in the presence of a higher concentration of ions, better crosslinking is performed, less porous structures are formed and the interior is filled better. Structures formed at lower concentration of ions are more porous, allowing diffusion of the solution into their interior, which results in the formation of larger-dimensional structures.

Dry matter content of formed structures

The characterization of the structures cross-linked with iron and lead ions of different concentrations also implied the determination of their dry matter. The results of the dry matter content of the structures cross-linked with lead and iron ions of different concentrations are shown in Table 5.

The results represented in Table 5 show the increase of dry matter content of structures formed with lead ions with the increase of concentration of mentioned ions and concentration of xanthan, both. Hence, if the lead ions are used as crosslinking agents, dry matter content of structures formed with 2.5% (w/v) xanthan solution is higher than for structures formed with 2.0% (w/v) xanthan solution. Structures cross-linked with 0.075 M solution of lead ions had the highest content of dry matter, while structures cross-linked with 0.025 M solution of lead ions had the lowest value of this parameter. The reason for the increase of dry matter content of structures by increasing of lead ions concentration may be a result of crosslinking of a large number of xanthan molecules with a large number of metal ions.

Table 5. Dry matter content of xanthan structures cross-linked with iron and lead ions of different concentrations

Structures	Dry matter content (%)		
	0.025 M	0.050 M	0.075 M
2.0% (w/v) xanthan cross-linked with Pb^{2+}	structures were not formed	2.2157	2.6809
2.0% (w/v) xanthan cross-linked with Fe^{3+}	1.4225	2.2466	3.8798
2.5% (w/v) xanthan cross-linked with Pb^{2+}	2.1175	2.9013	4.0461
2.5% (w/v) xanthan cross-linked with Fe^{3+}	1.5876	1.9249	2.3737

If the iron ions are used as crosslinking agents, dry matter content of structures formed with 2.5% (w/v) xanthan solution is a bit smaller than the same parameter for structures formed with 2.0% (w/v) xanthan solution. A reason for this kind of behaviour may be a result of different characteristics of this ion. Structures cross-linked with 0.075 M solution of iron ions had the highest content of dry matter, while structures cross-linked with 0.025 M solution had the lowest value of this parameter. The values of dry matter content of structures cross-linked with lead ions are insignificantly smaller than the values of dry matter content of structures cross-linked with iron ions.

CONCLUSION

In accordance with the defined aim, this paper examined the possibility of crosslinking of raw xanthan with divalent and trivalent metal ions in order to form stabile cross-linked structures. Based on the obtained results, it can be concluded that the success of the crosslinking of raw xanthan depends on the concentration of biopolymer solution, the concentration of metal ions solution, as well as on its pH value. It has been proved that the crosslinking of xanthan with Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, $Zn^{2+}$and Fe^{2+} ions in the applied experimental conditions does not result in formation of stable cross-linked structures. Xanthan can be cross-linked with divalent lead and trivalent iron ions in concentrations of 0.025 M to 0.075 M and not adjusted pH value. The most stable ellipsoidal structures, with average size of 4.02x5.08 mm, are formed with 2.5% (w/v) xanthan solution and 0.075 M iron ions solution. In the same conditions, somewhat softer, ellipsoidal structures of average dimensions of 4.04x5.56 mm are formed with 0.075 M lead ions solution. Based on the successful crosslinking and stability of the formed structures, it can be concluded that xanthan has a high potential for the application in the removal of iron and lead ions from wastewater.

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