O. Sachuk, V. Zazhigalov, O. Kobulei

MECHANOCHEMICAL ACTIVATION AND PHOTOCATALYTIC ACTIVITY OF OXIDE ZINC-MOLYBDENUM COMPOSITION

The study focused on the mechanochemical treatment (MChT) influence (during 2, 4 and 8 hours in air) on the properties of oxide zinc-molybdenum composition Zn/Mo = 25 : 75. It established the formation of nanodispersed particles of raw oxides and their structure changes, which facilitate the formation of a new compound: zinc molybdate. This crystallization phase at the next thermal treatment leads to complex composition formation which contains nanoparticles of zinc and molybdenum oxides, and zinc molybdate, and demonstrates a very high activity (more than traditional photocatalyst TiO_2) in the liquid phase of T safranin degradation in the UV irradiation.

Modification of zinc-molybdenum oxide system $ZnO\text{-}MoO_3$ = 25 : 75 by mechanochemical treatment in air during 2, 4 and 8 hours leads primarily to a change in the crystal structure of the initial composition, its substantial milling, and formation of new compounds. Interaction of nanopowder particles, which stimulates the MChT, leads to the formation of a new phase: a product of interaction between two oxide–zinc molybdate, which has a nanoscale particle of 12–16 nm size and synthesized at significantly lower energy compared to traditional solvation or the thermal method. The newly formed phase, which is formed by mechanochemical activated reactions, leads to the formation of zinc molybdate structure that is not really in the traditional methods of obtaining this compound. These changes and the formation of a new active surface increases their photocatalytic activity in the degradation safranine T water with UV irradiation. The sample after MChT during 2 hours and further heat treatment, leading to crystallization of zinc molybdate phase, shows the maximum activity higher than traditional TiO_2 (P-25) photocatalyst.

Keywords: mechanochemical treatment, zinc-molybdenum oxide composition, photocatalytic reaction.

Матеріал надійшов 12.01.2015

УДК 544.777

D. Bilko, Y. Borodulin, N. Antoniuk, I. Kolesnyk, A. Burban

ENTRAPMENT MODIFICATION OF THE POLYACRYLAMIDE GEL WITH CHITOSAN POLYMERS FOR THE PURPOSES OF SURFACE ACTIVATION FOR TISSUE ENGINEERING

In recent years hydrogels have become attractive targets for development of the controlled release rate substances that are characterized by suitable chemical and physical properties.

Recent advances in the tissue engineering and cell therapy technologies have introduced additional value to the biologically compatible hydrogels that could be chemically modified with specific factors, such as cell microenvironment proteins, allowing for shaping and controlled growth of the cells in the 3D formations, suitable for further tissue engineering and cell therapy. This paper reports a mechanism of the entrapment modification of the polyacrylamide based gels with chitosan polymer. Such modification allows for the further covalent crosslinking of the protein-based growth factors and other extracellular matrix proteins that fully support proliferation and maintenance of the adhesion dependent and adhesion independent cells, including hematopoietic and mesenchymal stem cells.

Keywords: hydrogel, chitosan, flowdown, tissue engineering.

Object of the study: formation and modification of polyacrylamide hydrogels with chitosan.

Subject of research study: characteristics and properties of the unmodified and modified chitosan polyacrylamide hydrogels.

Objectives: Development of the methodology for hydrogels obtaining based on chitosan-polyacrylamide and their modifications with proteins for biomedical purposes.

Tasks:

- To develop methods of formation and modification of polyacrylamide and chitosan-polyacrylamide gels.
- To investigate the sorption and desorption of bovine serum albumin (BSA) on unmodified polyacrylamide gels and modified with chitosan ones.
- To explore the possibility of covalent binding of BSA to the modified chitosan-polyacrylamide hydrogels.
- To test biocompatibility, cell adhesion and proliferation on the chitosan-polyacrylamide-protein gels in the *in vitro* systems.

Polyacrylamide hydrogels are quite popular for studying cell-substrate interaction and are easiest to obtain [1–3]. Polyacrylamide gels possess good mechanical, chemical and optical characteristics, making them suitable for use in biotechnological industry [4]. Gel deforms linearly in response to applied force and restores the tension after the termination of the force. In addition, gel strength can be changed by changing the concentration of the crosslinking agent methylenebis(acrylamide) [5]. Such properties are difficult to achieve for other gels making polyacrylamide a very attractive candidate for modification [6; 7].

Polyacrylamide gel is optically pure and fluorescently neutral. Such properties allow direct microscopy visualization of cellular processes under the microscope *in situ* through the gel. One of the important characteristics of the polyacrylamide gel is non-adhesive surface properties of the gel that prevents formation of the connective tissue capsule upon implantation *in vivo*. Polyacrylamide hydrogel can be polymerized in to any shape, which is useful for many fields of science and industry [8–10].

Methods

Hydrogels based on polyacrylamide were obtained by radical polymerization following a typical chain mechanism in exothermic process. If cross-linked polymer is synthesized in an aqueous solution, we get structured hydrogel system. For reactions of polymerization, an optimal ratio of acrylamide: N,N'-methylenebis(acrylamide) of 37.5 : 1 was used. Ammonium persulphate and TEMED were used as catalysts for the reactions [11]. Resulting gels were mechanically strong, transparent and optically pure.

For cross-linked gels in the form of a thin film with varying final gel, solutions of acrylamide in concentration of 5, 10, 15, 20 % have been prepared. For the purposes of gel modification, 2 % w/v solution of chitosan was added before polymerization in order to entrap chitosan polymers within the hydrogel structure. Entrapment of the chitosan and its chemical properties allow possibility for further covalent modification with various molecules, including proteins.

To determine the kinetics of adsorption, a series of solutions containing 0.01 % of bovine serum albumin (BSA) was prepared. The change in the concentration of bovine serum albumin during the sorption and desorption on the hydrogels was determined with UV spectroscopy in 200–400 nm region. Quantitative analysis (e.g. intensity of absorption of UV radiation), including the registration of concentration changes over time, prove the existence of the substance in specific groups of molecules (chromophores). Maximum absorption solution of bovine serum albumin is observed at 215 nm. To determine the appropriate concentration of solutions, a calibration curve was prepared, based on standard solutions of BSA.

Hydrogels, modified with chitosan, can be considered as composite material in which there are interpenetrating polymer networks. High molecular weight chitosan does not allow any desorption from the gel structure during gel washing from the unpolymerized monomers and polymerization by-products, as well as low molecular weight compounds [12]. Therefore included chitosan served as a suitable molecule for covalent modification by polypeptides, opening up new opportunities for modeling of the extracellular matrix environment, in a 3D structure.

The cells cultivation on polyacrylamide hydrogels, modified with chitosan and covalently grafted bovine serum albumin. Erythroleukemia K562 cell line was used for testing of the biocompatibility of the polyacrylamide gels modified with chitosan inclusions. Biocompatibility testing was performed in 24-well plates in DMEM growth media, supplemented with 10 % fetal calf serum. Cells were plates at 1.5×10^5 per ml without addition of supplementary growth factors. Cell viability was accessed with trypan blue staining in hemocytometer.

Cultivation of K562 cell line in suspension culture *in vitro* was done in 24-well plates in DMEM

growth medium, supplemented with 10 % DMEM without adding growth factors. Cells were plated at a concentration of 1×10^5 of viable cells per ml and incubated in 5 % CO_2 atmosphere at 37 °C.

Control of the cells was carried out under an inverted light microscope with 100–400x magnification that allowed direct observation of the cells on the surfaces of the gels.

Results

Modified with chitosan polyacrylamide hydrogels can be considered as composite material in which there are two phases: one is cross-linked polyacrylamide, a second phase is linear chitosan. High molecular weight chitosan, in predictions, does not allow its diffusion from the polyacrylamide, while allowing free diffusion of the unpolymerized reactants, therefore gels can be made non-toxic and free from low molecular weight compounds.

Infrared spectroscopy of the gels

IR spectra of hydrogels, obtaining from acrylamide solution at concentrations 5, 10, 15, 20 % with the ratio of acrylamide: N,N'-methylenebis(acrylamide) of 37.5 : 1 with and without addition of chitosan were obtained (Fig. 1–5).

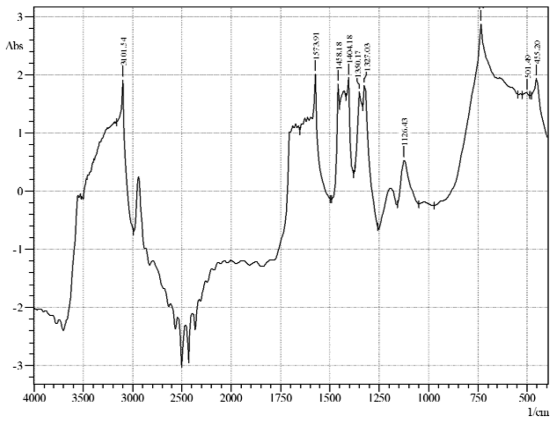

Fig. 1. IR spectrum of a hydrogel containing 20 % of acrylamide with a ratio acrylamide: N,N'-methylenebis(acrylamide) of 37.5 : 1

Absorption band at 3510–3100 cm^{-1} corresponds to valence vibration of N-H-bond, max values at 3101 cm^{-1} is valence vibration of CH_2-group. The band at 2900 cm^{-1} is due to valence vibrations of N-H in the plane of the amide group. In the frequency range of 1700–1450 cm^{-1} strong absorption bands of amide I and amide II are predicted. The first is responsible for stretching vibrations of C = O groups of the amide bond and has a peak at 1581 cm^{-1}. Amide II band is due to deformation vibrations of N-H, observed at 1458 cm^{-1}. In the spectra the Amide III band at 1350 cm^{-1}, is responsible for the covalent C-N and plane deformation vibrations of N-H. The bands at 750–455 cm^{-1} correspond amide bands IV and V, which are caused by deformation oscillations within the non-planar deformations of the C = O and N-H bonds [13].

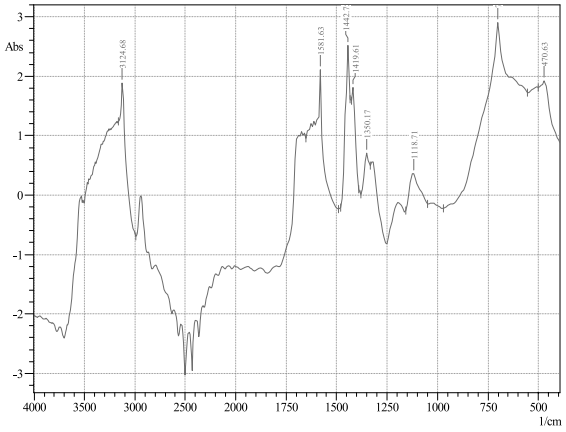

Fig. 2. IR spectrum of a hydrogel, obtained from 5 % acrylamide solution with a ratio of acrylamide: N,N'-methylenebis(acrylamide) 37.5 : 1 with 0.2 % chitosan

The spectral patterns of the chitosan modified polyacrylamide gels generally identical to the patterns of the pure unmodified hydrogel, however in the absorption spectra of the amide III band at 1350 cm^{-1}, which is responsible for the covalent C-N and plane deformation vibrations of N-H are less intense that can be explained by screening these groups by ionic groups of chitosan.

It is likely that 5 % acrylamide gel has a large pore size and therefore only a limited entrapment of the chitosan is observed within the gel structure.

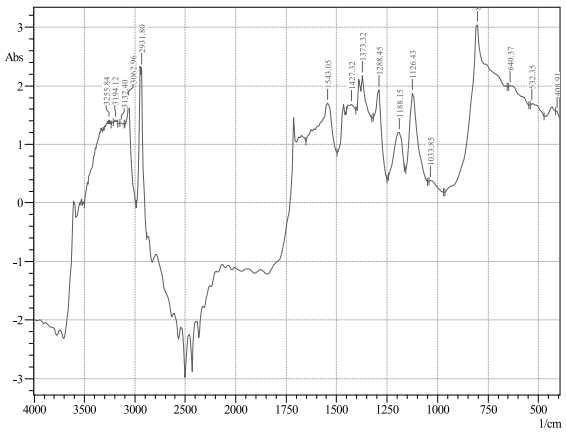

Fig. 3. IR spectrum of a hydrogel, obtained from 10 % acrylamide solution with a ratio of acrylamide: N,N'-methylenebis(acrylamide) 37.5 : 1 with 0.2 % chitosan

Adsorption bands at 3255, 3194, 3132 cm^{-1} are corresponding to the hydroxyl and amino groups of chitosan molecule, trapped within the structure of

the hydrogel. In addition, the band, corresponding to deformation vibrations of hydroxyl groups of the chitosan, appeared at 1288 cm^{-1}.

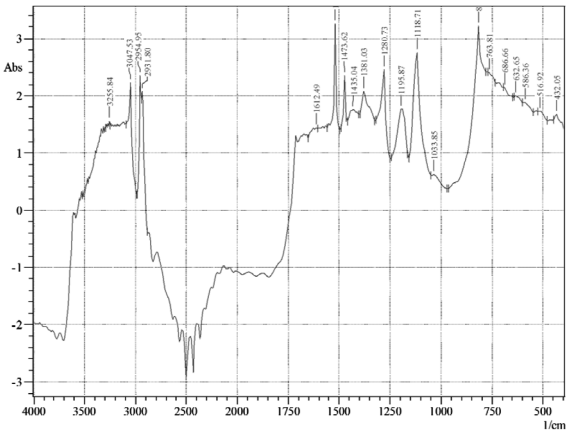

Fig. 4. IR spectrum of a hydrogel, obtained from 15 % acrylamide solution with a ratio of acrylamide: N,N'-methylenebis(acrylamide) 37.5 : 1 with 0.2 % chitosan

The bands at 3255, 2954 cm^{-1} are corresponding hydroxyl and amino groups of chitosan and a band at 1280 cm^{-1} is corresponding to deformation vibrations of hydroxyl groups of chitosan. The band 2931 cm^{-1} is due to deformation vibrations of N-H in the plane of the amide group and has greater intensity, which can be explained by the higher content of acrylamide. It is fair to say that 15 % gel contains entrapped chitosan within its structure.

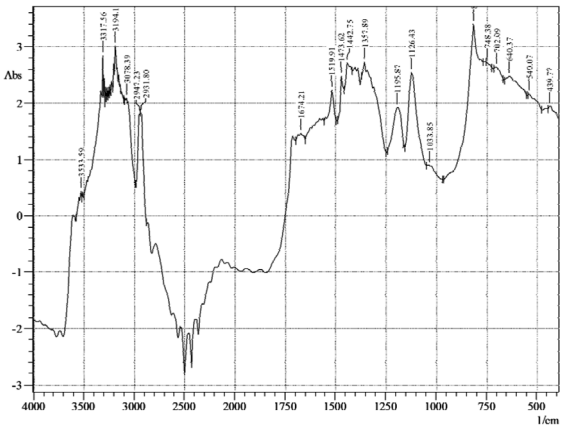

Fig. 5. IR spectrum of a hydrogel, obtained from 20 % acrylamide solution with a ratio of acrylamide: N,N'-methylenebis(acrylamide) 37.5 : 1 with 0.2 % chitosan

Peaks of the aliphatic OH-C-H bonds vibration are superimposed in to a broad peak in the range 3533–3000 cm^{-1}. A broadening of the bands at 3500–3100 cm^{-1} with a maximum at 3078 cm^{-1} is responsible for symmetric stretching vibrations N-H and -CH_2-groups. In addition, the band, appeared at 3533, 3317, 3194 cm^{-1} is corresponding to hydroxyl and amino groups of chitosan. The band 2931 cm^{-1} due to deformation vibrations of N-H in the plane of the amide group, and has greater intensity, which can be explained by the higher content of acrylamide. In the frequency range of 1700–1435 cm^{-1} the strong absorption bands of amide I and amide II could be observed. The first is responsible for stretching vibrations of C = O groups of amide bonds and has a peak at 1519 cm^{-1}. Amide II band due to deformation vibrations of N-H, observed at 1473 cm^{-1}. Absorption band of amide III at 1381 cm^{-1} is responsible for the covalent C-N and plane deformation vibrations of N-H. In addition, the band, appeared at 1195 cm^{-1}, is corresponding to deformation vibrations of hydroxyl groups of chitosan. The bands at 750–439 cm^{-1} are amide IV and V, which are caused by deformation oscillations of the C = O and N-H out of plane, respectively.

It is safe to say that 20 % polyacrylamide gel shows significant entrapment of the chitosan.

The results show that chitosan content in the structure of cross-linked polyacrylamide hydrogel slightly decreases hydrophilic nature of the gel. This can be explained by the fact that chitosan is shielding ionic groups of the polyacrylamide and the fact that pure chitosan film has a contact angle within 45–50°. With the increase of the acrylamide concentration in the forming solution (from 10 % to 20 %), hydrophilic nature of the gel is somewhat reduced, due to space filling macromolecules and reduction of bound water (Fig. 6). The values of the contact angles for hydrogels obtained in the first approximation meets the requirements for biocompatible materials (for biocompatible molecules wetting angle up to 60°).

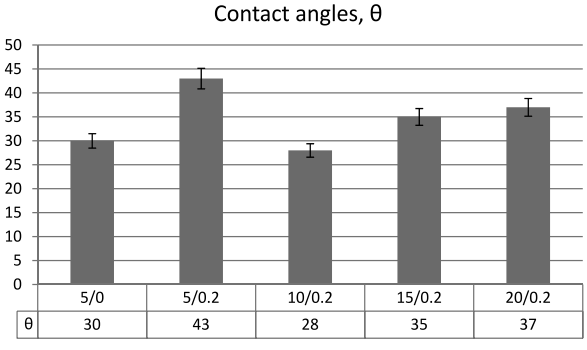

Fig. 6. Contact angles of water on polyacrylamide hydrogels

Swelling kinetics experiments indicated that addition of the 0.2 % chitosan to the gel structure during polymerization only slightly decreases the swelling kinetics of the gels, most noticeable for the 5 % and 10 % gel and near physiological pH values (Fig. 7). More concentrated gels display a limited increase of the swelling kinetics with inclusion of the chitosan molecules.

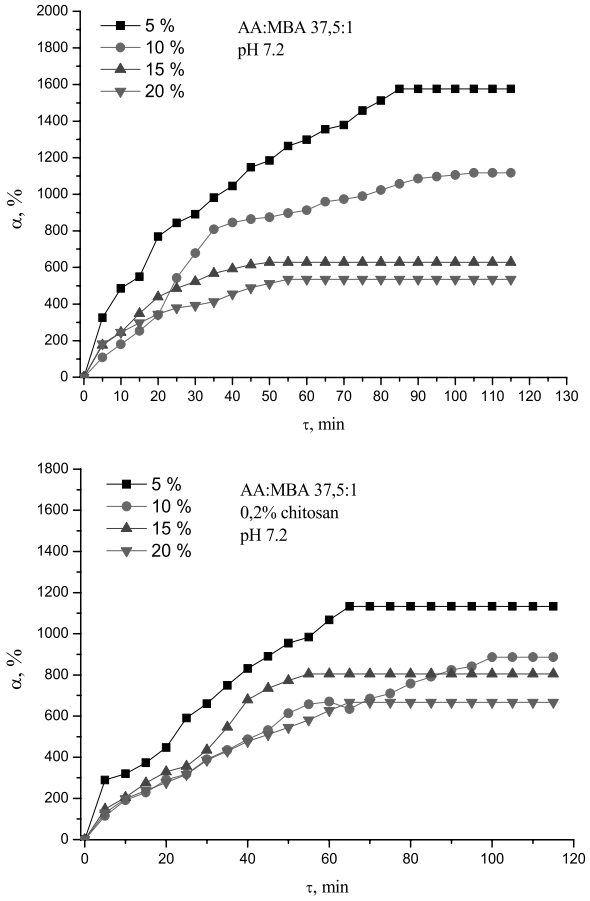

Fig. 7. Swelling kinetics of polyacrylamide hydrogels with different acrylamide concentration with and without chitosan inclusion at pH 7.2

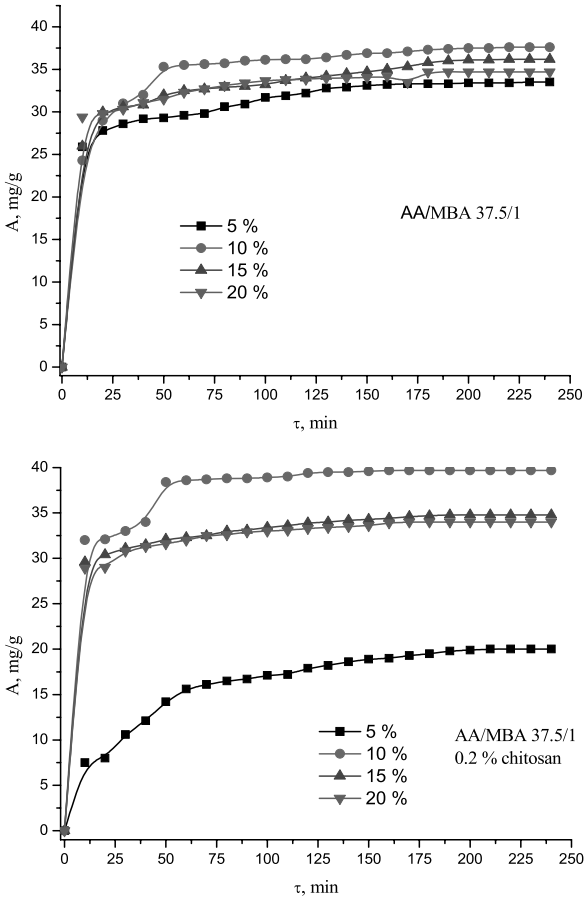

Fig. 8. Sorption kinetics of BSA on polyacrylamide hydrogel, obtained from acrylamide solution with different concentration, with a ratio acrylamide: N,N'-methylenebis(acrylamide) 37.5 : 1 with and without 0.2 % chitosan

It was established that the 10x increase of the gel strength in forming solution reduces the degree of swelling by 2–2.5 times, but remains at the level of 300–400 %, the presence of a chitosan modifier has a limited influence on the swelling kinetics.

Notice that 5 % hydrogel with chitosan modification shows significant decrease on the sorption kinetics. Graphs indicate that polyacrylamide gels possess high tropism for the BSA protein, a trait that is valuable for bioactivation of the gels by means of inclusion of the biologically active proteins (Fig. 8).

As shown, BSA desorption is insignificant (1.5–3 % within 3 hours) (Fig. 9). Most probably due to ionic interactions between charged amino acid groups of the protein and the gel matrix. This is also considered as a useful trait for the development of biocompatible hydrogels.

Testing of the cytotoxicity of the chitosan-modified polyacrylamide gels of different gel concentrations indicated that it was possible to wash out unpolymerized toxic constituents from the hydrogels to the extent that *in vitro* cell propagation could be achieved its surfaces.

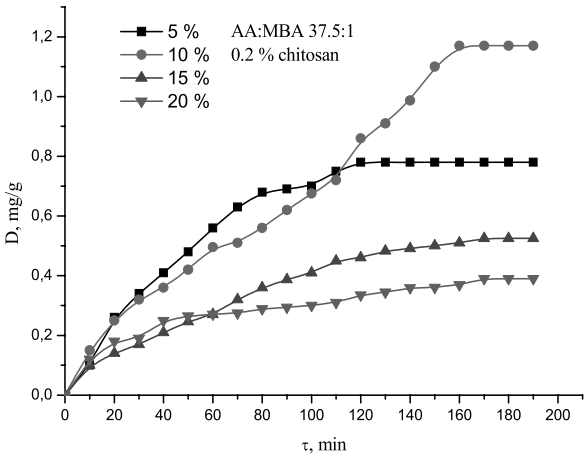

Fig. 9. BSA desorption kinetics from polyacrylamide hydrogels, obtained from acrylamide solution with different concentration, with a ratio acrylamide: N,N'-methylenebis(acrylamide) 37.5 : 1 with 0.2 % chitosan after grafting BSA

The results of growing cells indicate that polyacrylamide modified chitosan remains biologically neutral as based on the viability and proliferation assays on the K562 ell line. Gels do

not support cell adhesion to the surface and a suitable for non-adhesion dependent cell types. The cells were characterized by proliferation intensity and viability that corresponded control, indicating a biocompatible nature of the gel (Fig. 10, 11).

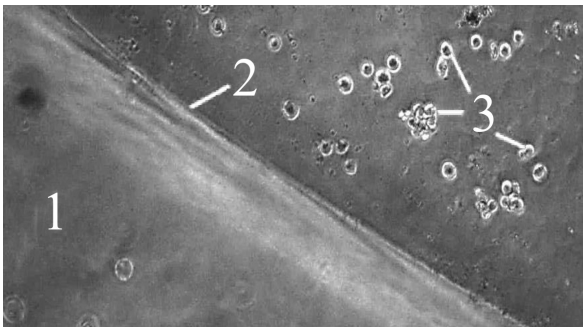

Fig. 10. K562 cells on the 10 % polyacrylamide gel with 0.2 % chitosan and covalently grafted bovine serum albumin at the time of application. × 400:
1 – hydrogel; *2* – gel edge; *3* – cells

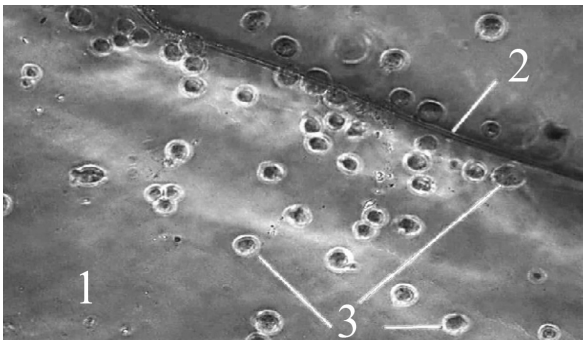

Fig. 11. Culture of K562 cells on the 10 % polyacrylamide gel with 0.2 % chitosan modification and covalently bound bovine serum albumin after 72 hour of cultivation. × 400:
1 – hydrogel; *2* – edge of the hydrogel; *3* – cells

The results indicate that the polyacrylamide gels modified with chitosan are biologically neutral and suitable for non-adhesion dependent cell lines. The cells were characterized by proliferation and viability intensity that corresponded control. Toxic effects at the cellular level were not observed.

Conclusions

The technique of formation and modification of chitosan-polyacrylamide hydrogel under optimal conditions: radical polymerization at room temperature in the presence of ammonium persulfate initiator, catalyst TEMED, MBA agent and chitosan modifier, was designed. We confirmed the presence of the functional groups of chitosan in the polyacrylamide hydrogels by IR spectroscopy. We proved suitable biocompatible nature of the modified hydrogels with contact angles less than 60 degrees. We showed that modified hydrogels readily adsorb proteins with a limited desorption, confirming the useful biocompatible characteristics of the obtained hydrogels. It was established that the sorption capacity PAA hydrogels modified by chitosan is 30–40 mg/g BSA; after grafting BSA covalently desorption poorly expressed – 2.3 % (pH 7.2), which allows for the cultivation and growth of cells. No cytotoxic effects at the cellular level could be observed for the K562 cell line propagated on the surface of the chitosan modified polyacrylamide proving the biocompatible nature of the obtained gels.

References

1. Park K. Biomedical Applications of Hydrogels : Handbook / K. Park. – NY : Springer, 2010. – 432 p.
2. Atta A. M. Effect of crosslinker functionality on swelling and network parameters of copolymeric hydrogels / A. M. Atta, A. Abdel-Azim // Polym. Adv. Technol. – 1998. – Vol. 9. – P. 340–348.
3. Дослідження кінетики вивільнення лікарських препаратів з термочутливих гідрогелів на основі N-ізопропілакриламіду та аліламіну / В. В. Коновалова, Ю. М. Самченко, Г. А. Побігай [та ін.] // Магістеріум. – 2013. – Вип. 51 : Хімічні науки. – С. 3–7.
4. Saima A. Hydrogels as potential drug delivery systems / A. Saima, R. Saeid, K. Kanchan // Scientific Research and Essay. – 2009. – Vol. 3 (11). – P. 1175–1183.
5. Самченко Ю. М. Оптимизация отмывки полиакриламидного геля от остаточных мономеров / Ю. М. Самченко, М. Н. Герцюк // Журнал хроматографічного товариства. – 2005. – Т. 5, № 3. – С. 11–18.
6. Кополімерні гідрогелеві мембрани для іммобілізації та культивування стовбурових клітин людини / О. О. Косенко, Л. Л. Лукаш, Ю. М. Самченко [та ін.] // Біополімери і клітина. – 2006. – Т. 22, № 2. – С. 143–148.
7. Самченко Ю. М. Сорбционные свойства дисперсных сополимерных гидрогелей / Ю. М. Самченко, В. В. Цирина, М. А. Альтшулер // Катализ и нефтехимия. – 2006. – № 14. – С. 112–115.
8. Отримання та дослідження гідрогелевих мембран на основі кополімерів акриламіду для електрофорезу / Г. А. Побігай, Ю. М. Самченко, В. В. Коновалова [та ін.] // Наукові записки НаУКМА. – 2012. – Т. 131 : Хімічні науки і технології. – С. 65–68.
9. Duchene P. Comprehensive biomaterials / P. Duchene. – Elsevier, 2011. – 3672 p.
10. Biomaterial Science : An Introduction to Materials in Medicine / ed. by Buddy D. Ratner, Allan S. Hoffman, Frederick J. Schoen, Jack E. Lemons. – 2nd ed. – Elsevier, 2004. – 864 p.
11. Synthesis and characterization of hydrogels based on grafted chitosan for the controlled drug release / H. H. Sokker, A. M. Abdel Ghaffar, Y. H. Gad [et al.] // Carbohydrate Polymers. – 2009. – Vol. 75 (2). – P. 222–229.
12. Wichterle O. Hydrophilic gels for biological use / O. Wichterle, D. Lim // Nature. – 1960. – Vol. 185, № 4706. – P. 117–118.
13. Ratner B. D. Synthetic hydrogels for biomedical applications / B. D. Ratner, A. S. Hoffman // Hydrogels for Medical and Related Applications, ACS Symposium Series. – Washington, DC, 1976. – Vol. 31. – P. 1–36.

Білько Д. І., Бородулін Ю. В., Антонюк Н. Г., Колесник І. С., Бурбан А. Ф.

АКТИВАЦІЯ ПОВЕРХНІ ПОЛІАКРИЛАМІДНОГО ГЕЛЮ ХІТОЗАНОМ ДЛЯ ПОДАЛЬШОГО ВИКОРИСТАННЯ В ТКАНИННІЙ ІНЖЕНЕРІЇ

Доведено можливість модифікування структурного каркаса полімерних гідрогелів хітозаном, унаслідок чого відбувається покращення їхніх механічних властивостей без погіршення сорбційної ємності та здатності до набрякання, що робить можливим використання їх у тканинній інженерії як матеріалів біомедичного призначення.

Ключові слова: гідрогель, хітозан, набрякання, тканинна інженерія.

Матеріал надійшов 15.01.2016

УДК 662.997

Коломієць О. В., Сухий К. М., Бєляновська О. А., Вакулюк П. В., Томіло В. І., Прокопенко О. М.

ОСНОВНІ ЕКСПЛУАТАЦІЙНІ ХАРАКТЕРИСТИКИ КОМПОЗИТНИХ СОРБЕНТІВ «СИЛІКАГЕЛЬ/СУЛЬФАТ НАТРІЮ» ТА «СИЛІКАГЕЛЬ/АЦЕТАТ НАТРІЮ» ДЛЯ АДСОРБЦІЙНИХ ХОЛОДИЛЬНИКІВ

Досліджено кінетику адсорбції парів води композитними сорбентами «силікагель/натрій сульфат» і «силікагель/натрій ацетат», а також сорбційні властивості цих матеріалів. Показано вплив матеріалу на конструктивні параметри адсорбційного холодильника та холодильний коефіцієнт.

Ключові слова: композитний сорбент, адсорбційний холодильник, холодильний коефіцієнт.

Протягом останнього десятиріччя, коли економія енергоресурсів посідає провідне місце в усіх галузях науки і техніки, а тенденція монотонного зростання вартості первинного палива призводить до здорожчання електроенергії, розробка ефективних теплоакумулювальних матеріалів для акумулювання низькопотенційного тепла та пристроїв для опалення та охолодження на їхній основі є актуальним завданням для енергетиків усього світу. У результаті роботи Паризького саміту було ухвалено рішення про стимулювання розвитку новітніх технологій та зменшення викидів в атмосферу CO_2, NO_x та інших газів, які спричиняють парниковий ефект. Одним із технічних рішень, яке б дозволило одночасно розв'язати як екологічну, так і енергетичну та економічну проблеми, є конструювання і використання сонячних адсорбційних холодильників [1, с. 252–262; 2, с. 102–123], що споживають переважно теплову енергію. Джерелом теплової енергії найчастіше є сонце [3, с. 1714–1720; 4, с. 172–178]. Базовими