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INFLUENCE OF COMPRESSION SPEED AND DEFORMATION PERCENTAGE ON MECHANICAL PROPERTIES OF CALCIUM ALGINATE PARTICLES

Article Highlights

- Mechanical properties of alginate beads were investigated in distilled water and dry condition
- Young's moduli and corresponding forces for tested deformation scale were determined
- Compression tests were performed using Universal Testing Machine (AG-Xplus)
- Influence of varied range of deformation percentage and compression speed was examined

Abstract

Hydrogel particles are often used as carriers for the immobilization of enzymes, polyphenolic antioxidants, whole microbial, plant or mammalian cells. In many processes, the mechanical properties of alginate particles are essential due to their exposure to mechanical forces in production process. Determination and improvement of hydrogels mechanical properties is very important in prevention of the undesirable side effects during the manufacturing process and product application. The aim of this study was to define the mechanical properties of single particles submerged in water and in dry conditions using the compression method between two flat surfaces. The results indicated that the formulation of alginate beads and water loss during compression have significant influence on their mechanical behavior and stiffness. Calcium-alginate particles were produced using an electrostatic droplet extrusion technique, with an initial sodium alginate concentration of 1.5%, w/V, and calcium chloride (2.0%, w/V) as the gelling solution. The research findings were used to determine the influence of working conditions, sample deformation (10-50%) and different compression speeds (1-50 mm/min) on the mechanical strength of alginate beads. The Young's moduli and maximal forces for investigated deformation percentage of the alginate particles were determined from generated force-displacement and stress-strain curves during compression.

Keywords: alginate particles, compression method, mechanical properties.

Hydrogels are weakly cross-linked three-dimensional hydrophilic polymers which do not dissolve in water but are able to absorb large amounts of it. Hydrogels derived from natural polymers have a number of advantages over the synthetic ones, such as biocompatibility, biodegradability and non-toxicity, with a drawback of poor mechanical properties. Calcium alginate is a hydrophilic, biocompatible and commonly used carrier for immobilization of a wide range of molecules of biological significance, microbial, plant or animal cells, and polyphenolic antioxidants [1-6]. In addition, a special benefit is easy shape controlled production of alginate hydrogels, including the possibility for production of spherical particles and microparticles [7-9]. Colloidal carriers

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made of hydrophilic polysaccharides such as alginate particles which size less than 10 μm are a promising alternative for improving the bioavailability of insulin [10]. Significant interest has been seen in the use of hydrogels as biomaterials in various biomedical applications and devices [11,12]. In previous years, in order to study the mechanical properties of biomaterials exposed to mechanical load, a number of methods based on measurements of full-field displacement and strain have been used [13-15]. Blewett *et al.* [16] have described the basic methodology for compression testing of single microparticles. This methodology has been applied in determination of the mechanical properties of suspension-cultured cells and microspheres or microcapsules [17-20]. The compression method is also used in testing of mechanical properties of mammalian cells, yeast and bacterial cells [21-23].

The mechanical properties of alginate hydrogels are highly dependent on the characteristics of the polymer, the crosslinker, and the gelling environment [24,25]. Alginate beads made of higher guluronic acid contents and gelling cations of higher chemical affinity were found to have greater stiffness. The stress-strain behavior of alginate particles is time-dependent, which reflects a combination of the intrinsic mechanical properties of the hydrogel [26]. Young's modulus of beads formed with various divalent cations was measured to study the influence of the gelling type and conditions on the resulting bead's elasticity [27].

Deformation of solid-and liquid-filled spheres has been described in many studies [28-31]. Hertz (1882) first studied compression of elastic solid sphere between two flat rigid surfaces for the case of small deformations. The theory is supposed to be valid up to about 10% of strain, but researchers have modified and extended the Hertz theory to a greater compression ratio [30,32].

Alginate beads can be formulated using different types and concentrations of alginate and gelling cations. Results obtained using the Hertz theory after compression of samples at speed 40 mm/min, show that the bead's Young's modulus is dependent on the presence of gelling cations [33]. In this paper, mechanical behavior of wet alginate beads submerged in water, as well as at air (dry condition), was tested by compression of a single bead between two flat surfaces. The corresponding force imposed on the beads during the compression was measured. The results obtained in this study showed the influence of a wide range of compression speed and deformations on mechanical properties of calcium alginate beads.

EXPERIMENTAL

Alginate particles were produced using an electrostatic extrusion technique [34,35]. Commercially available medium viscosity powdered alginic acid sodium salt from brown algae (Sigma Aldrich, USA) was used in the particles production process. Alginic acid is composed of 61% mannuronic and 39% guluronic acid and M/G ratio is 1.56. The calcium alginate particles were made by extruding sodium alginate 1.5% solution, *w/V*, into 2.0%, *w/V*, of calcium chloride solution using a syringe pump (Racel, Scientific Instruments, Stamford, CT, USA). Alginate spherical beads were obtained by collecting distance of 3 cm between the blunt stainless steel needle tip (18 gauge) and the surface of the gelling solution of calcium chloride dehydrate, 99+% (Acros organics, USA). The beads were allowed to harden in the gelling solution for 30 min. Compression tests of single alginate beads submerged in distilled water (wet conditions) and in dry conditions were performed using a universal testing machine, AG-Xplus (Shimadzu, Japan) equipped with a 50 N force load cell (force range from 0.005 to 50 N). The force-detecting unit in the load cell is equipped with a strain gauge that develops appropriate deformation when force is applied. The load cell converts the magnitude of applied force into the change in the resistance of the strain gauge, and outputs are converted into an electric signal [36]. During the compression test in wet conditions, beads were submerged in distilled water in petri dish and placed on a flat plate. In dry conditions, the bead was placed on the stainless steel plate after gentle drying on filter paper. In order to guarantee good test reproducibility, due to the particulate nature of the beads, a cylindrical steel plate with a flat end with a large contact area (50 mm in diameter) was used to compress the bead and in both cases was moved continuously at the tested speeds. Measurements were repeated thirty times on alginate beads formed under identical conditions. The plate was set to return to its original position immediately after compression. Distance between the final plate position (position at the final, prescribed deformation) and the plate at the first contact (zero point) with the bead was specified *via* specimen dialog box in software and measured during the test. Zero point adjustment is controlled via smart controller and specialized equipment's software TRAPEZIUMX 1.13.

The compression was performed up to 10, 30 and 50% of sample deformation (ratio of displacement to initial bead diameter) at an ambient temperature of 25 °C. Since the response of some materials is

significantly affected by the loading type (*e.g.*, high speed loading [26,33], cyclic loading [37], etc.), the testing is performed at different compression speeds in this work – in the range of 1-50 mm/min. Thirty beads from each sample were compressed and automatic detection of the contact between the plate and sample was carried out with a contact force of 0.052 N.

RESULTS AND DISCUSION

The principle of the measurement was to impose a strain on the alginate particles by compression and to measure the corresponding force and the resulting stress, as well as to analyze the generated force-displacement curves. Pre-testing conditions - force, compression speed and percentage of a sample deformation, were pre-defined in the materials testing software TRAPEZIUMX 1.13. The advantage of this type of equipment is possibility of compression at high speed which minimized influence of time dependent particles behavior. The diameter of the beads varied between 2.6 and 3.0 mm. The results obtained at speed compression of 1 mm/min and 10, 30 and 50% of sample deformation showed that the measured maximal forces for submerged alginate beads are in the range of 0.083±0.002, 0.179±0.016 and 0.317±0.031 N, respectively, as it is schematically presented in Figure 1. The particles were compressed up to 50% of deformation and during this period, the bead height was determined at suitable time intervals (1-45 min). It was found that the beads of alginate recovered almost instantaneously to their initial height after being compressed up to 30% of deformation, which is not the case with the beads compressed beyond 30% of deformation; this is presumably due to the failure of the polymer network with higher percentage of deformation, which led to irreversible deformation of the beads. From Figure 2, it can be seen that higher measured forces of the particles are obtained in dry conditions, due to loss of liquid during compression. The values of Young's modulus were determined using the force-displacement and engineering stress-strain curves (in the remaining text – stress-strain curves). The resulting force-displacement data pairs were converted into corresponding stress and strain values, based on the initial bead diameter. Elastic modulus was calculated from the slope using least squares regression of the plot of the stress versus strain data obtained during compression. Curves fitting for stress-strain experimental data and quality of the fit ($R^2 > 0.99$) are shown in Figure 3. Shape of the samples has effect on the presence of the tail at the beginning of the force-displacement and the sress-strain curves. Since alginate particles are viscoelastic, slopes of the tangents to elastic parts of the stress-strain curves are used for determination of the modulus. Elastic parts of the stress-strain curves (for most of the tests: from 10 to 20% strain) are selected according to analyzed experimental recovery degree of alginate particles after compression and elastic limits studied in literature data by compressing single beads to various final deformations [33]. The values of the modulus were in the range of 44.9-66.8 kPa, for the compression speed 1 mm/min, depending on submerged alginate bead's final deformation up to 50%. The logarithm of the force-displacement experimental data was plotted and linear relationship with slopes close to 1.5 indicates that the results up to 30% of sample deformation could be analyzed using Hertz theory. The influence of speed on the modulus value will be discussed at the end of this section.`

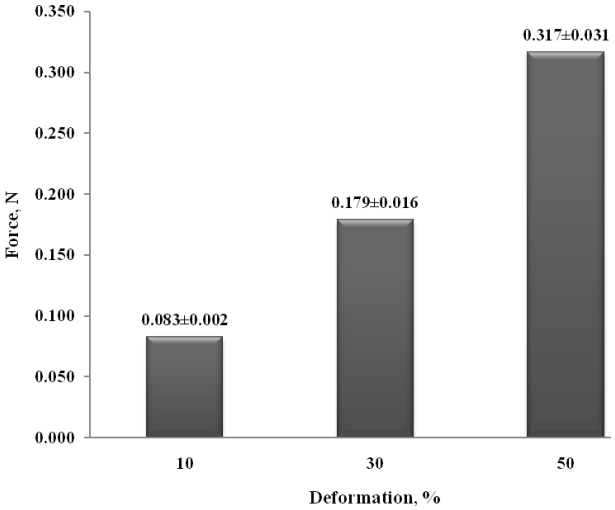

Figure 1. Determined maximal force as a function of deformation of the investigated submerged samples (means±SD, n = 30).

Alginate hydrogels are two-phase systems, certain amount of the water (typically more than 90%) is bound to the polymer network, and the rest is free. The stress-strain relation observed during measurement is an indication of a complex influence of the intrinsic mechanical properties of the matrix itself and its permeability on beads stiffness [38,39]. It was found that the modulus values of alginate beads were dependent on the mechanical load because the alginate beads are generally considered to be viscoelastic and may lose liquid under compression [26].

As mentioned previously, in order to analyze the influence of hydrogel permeability and liquid flows on

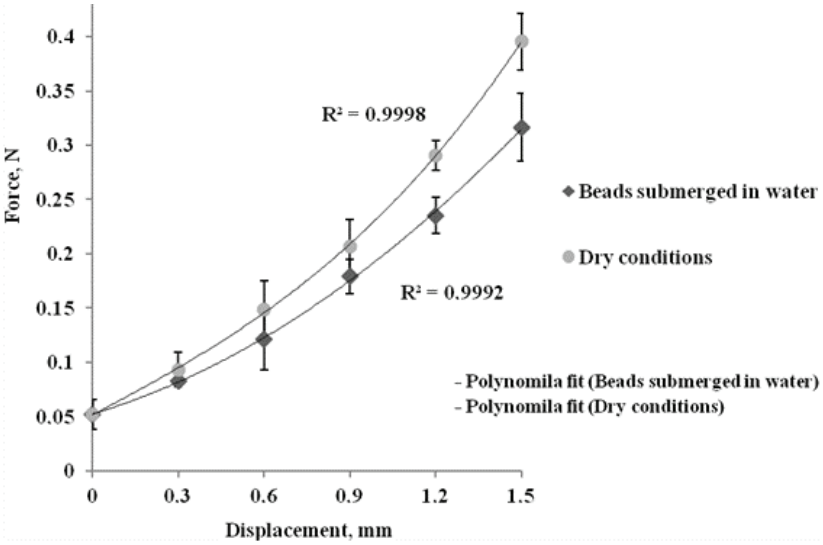

Figure 2. Force in function of displacement for alginate beads compressed to 50% of initial beads diameter at 1 mm/min for different testing conditions.

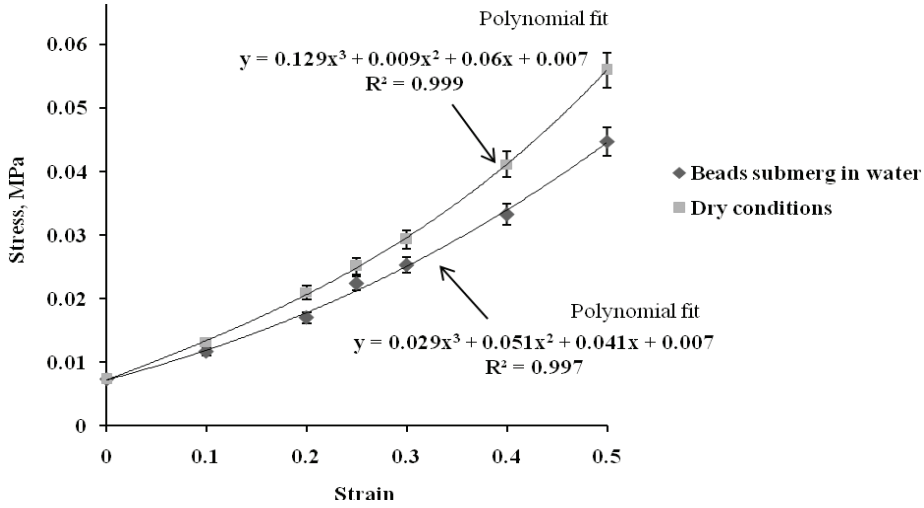

Figure 3. Stress-strain curves for alginate beads fitted to the experimental data for different testing conditions (means±SD, n = 30).

particles mechanical properties, alginate beads were tested between two parallel plates (see Figure 4).

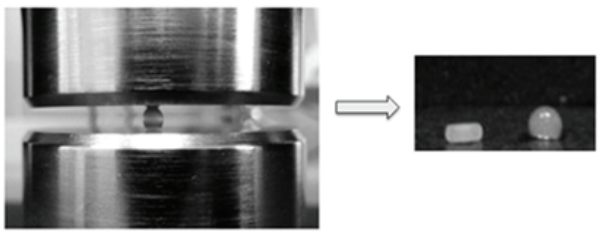

Figure 4. Image of single alginate bead compressed up to 50% of initial diameter.

Experimentally obtained force-displacement curves showed that the hydrogel's highly water-swollen nature has influence on their mechanical properties. The obtained results indicated that alginate beads in dry conditions have higher values of maximal forces in comparison with beads submerged in water and exposed to the same deformation; this can be explained by the influence of water loss and material permeability on the mechanical properties of beads. The value of the elastic modulus of alginate beads examined in dry conditions and for the tested scale of final deformation up to 50% and compression speed 1 mm/min varied between 59.3-89.0 kPa. Similar results for the modulus calculated according to Hertz theory in dry conditions at low compression speed and same range of deformation of Ca alginate beads (made of medium viscosity alginate, M/G ratio 1.56) were obtained in the literature [27].

The results for compression speed influence on material behavior were investigated and maximal

force values at deformation of 30% for different compression speeds (1-50 mm/min) are shown in Figure 5. Namely, with the increase of speed, values of maximal forces increased as a response of material on the corresponding strain rate. The differences in force values decrease for the speeds higher than 10 mm/min.

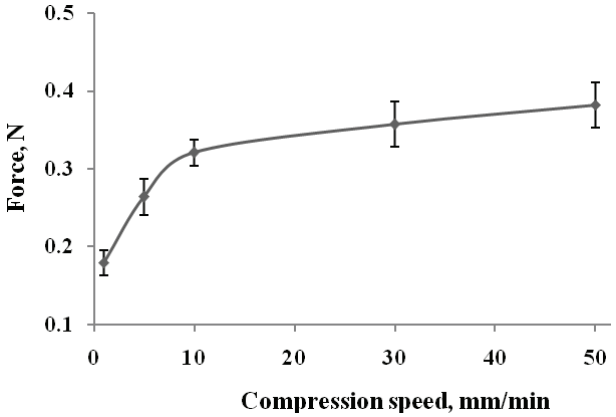

Figure 5. Force in function of compression speed for submerged alginate beads compressed to 30% of initial beads diameter at different speeds. The error bars mean standard deviation of the mean values.

The results from stress-strain curves for the wet beads compressed to 30% of deformation indicate that the modulus values significantly increase (in the range from 53.2 to 131.0 kPa) for compression speeds 1-30 mm/min. On the other hand, for speeds exceeding 30 mm/min, the differences in modulus values decrease and calculated values of modulus were in the range between 143.7 and 168.8 kPa. Literature data confirm that the Young's modulus values of alginate beads became constant when the compression speed was greater than 36 mm/min [33].

CONCLUSION

Calcium alginate is one of the most widely used alginate hydrogels. This study presents the obtained results of the determined maximal forces for analyzed deformation percentage (10-50%) and Young's modulus of alginate beads compressed between two plates at different speeds (1-50 mm/min). The results of the presented examinations indicate that the speed compression has pronounced influence on mechanical properties of alginate particles – the faster the compression, the higher the force, implying that there was time-dependent behavior of alginate particles. At lower compression speeds (1 mm/min), water could smoothly flow from the bead, but at the higher speeds (30-50 mm/min) the loss of the water is negligible, leading to the approximately constant values for the modulus. The study of mechanical properties of beads submerged in water and in dry conditions showed that during a compression test part of the interstitial water flows out of the particle solid matrix, which leads to the change in the volume of the particle and affects the particle behavior during the exposure to the force.

The procedure of examining hydrogel mechanical stability in aqueous medium (wet conditions) brings more accurate results regarding bead stiffness in real conditions of use. The presented results bring an improvement in testing procedure of hydrogel stiffness during production, storage and product application. The compression method has potential for testing the time-dependent behavior of hydrogel beads, particularly liquid loss during compression by further modeling of the compression process.

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REFERENCES

[1] G. Pfister, M. Bahadir, F. Korte, J. Controlled Release **3** (1986) 229-233

[2] J.E. Melvik, M. Dornish, in Focus on Biotechnology: Fundamentals of Cell Immobilisation Biotechnology, V. Nedović, R.G Willaert (Eds.), Kluwer Academic Publishers, Dordrecht, 2005, p. 33-53

[3] A. Belščak-Cvitanović, R. Stojanović, V. Manojlović, D. Komes, I. Juranović Cindrić, V. Nedović, B. Bugarski, Food Res Int. **44** (2011) 1094-1101

[4] B. Obradović, A. Osmokrović, B. Bugarski, D. Bugarski, G. Vunjak-Novaković, Mater. Sci. Forum **555** (2007) 417--422

[5] R. Stojanović, A. Belščak-Cvitanović, V. Manojlović, D. Komes, V. Nedović, B. Bugarski, J. Sci. Food Agric. **92** (2012) 685-696

[6] M. Rakin, Lj. Mojović, S. Nikolić, M. Vukasinović, V. Nedović, Afr. J. Biotechnol. **8** (2009) 464-471

[7] B. Bugarski, B. Amsden, R. Neufeld, D. Poncelet, M.F.A. Goosen, Can. J. Chem. Eng. **72** (1994) 517-522

[8] D. Poncelet, V.G. Babak, R.J. Neufeld, M.F.A. Goosen, B. Bugarski, Adv. Colloid Interface Sci. **79** (1999) 213--228

[9] V. Manojlović, B. Obradović, V. Nedović, I. Leskosek-Cukalović, B. Bugarski, Hem. Ind. **58** (2004) 62-64

[10] C.P. Reis, R.J. Neufeld, A. Riberio, F. Veiga, Chem. Ind. Chem. Eng. Q. **12** (2006) 47-52

[11] A.D. Debeljković, L.R. Matija, Đ. Lj. Koruga, Hem. Ind. **67** (2013) 861-870

[12] T. Andersen, B.L. Strand, K. Formo, E. Alsberg, B.E. Christensen, Carbohydr. Chem. **37** (2012) 227-258

[13] I. Donati, Y.A. Morch, B.L. Strand, G. Skjak-Bræk, S. Paoletti, J. Phys. Chem., B **113** (2009) 12916-12922

[14] N. Mitrović, M. Milosević, A. Sedmak, A. Petrović, R. Prokić-Cvetković, FME Trans. **39** (2011) 55-60

[15] M. Ahearne, Y. Yang, K-K Liu, in Topics in Tissue Engineering: Mechanical characterisation of hydrogels for tissue engineering applications, N. Ashammakhi, R. Reis, F. Chiellini (Eds.), 2008, p. 3-14

[16] J. Blewett, K. Burrows, C. Thomas, Biotechnol. Lett. **22** (2000) 1877-1883

[17] J.T. Chung, K.D. Vlugt-Wensink, W.E. Hennink, Z. Zhang, Int. J. Pharm. **288** (2005) 51-61

[18] A.E. Smith, Z. Zhang, C.R. Thomas, Chem. Eng. Sci. **55** (2000) 2031-2041

[19] E. Dintwa, P. Jancsók, H.K. Mebatsion, B. Verlinden, P. Verboven, C.X. Wang, C.R. Thomas, E. Tijskens, H. Ramona, B. Nicolaï, J. Food Eng. **103** (2011) 265-272

[20] M. Mancini, M. Moresi, R. Rancini, J. Texture. Stud. **30** (1999) 639-657

[21] Z. Zhang, M.A. Ferenczi, A.C. Lush, C.R. Thomas, Appl. Microb. Biotechnol. **36** (1991) 208-210

[22] J.D. Stenson, C.R. Thomas, P. Hartley, Chem. Eng. Sci. **64** (2009) 1892-1903

[23] C. Shiu, Z. Zhang, C.R. Thomas, Biotechnol. Tech. **13** (1999) 707-713

[24] K.H. Bouhadir, D.S. Hausman, D.J. Mooney, Polymer **40** (1999) 3575-3584

[25] C.K. Kuo, P.X. Ma, Biomaterials **22** (2001) 511-521

[26] C.X. Wang, C. Cowen, Z. Zhang, C.R. Thomas, Chem. Eng. Sci. **60** (2005) 6649-6657

[27] C. Ouwerx, N. Velings, M. Mestdagh, M. Axelos, Polym. Gels Networks **6** (1998) 393-408

[28] H. Hertz, Zeitschrift fur Reine Angewandte Mathematik **92** (1882) 156-171

[29] T.J. Lardner, P. Pujara, Mechanics Today **5** (1980) 161--176

[30] Y. Tatara, J. Eng. Mater. Technol. **113** (1991) 285-291

[31] D. Andrei, B. Briscoe, P. Luckham, D. Williams, J. Chim. Phys. **93** (1996) 960-976

[32] Y.L. Lin, D.M. Wang, W. M. Lu, Y.S. Lin, K.L. Tung, Chem. Eng. Sci. **63** (2008) 195-203

[33] E.S. Chan, T. Lim, W. Voo, R. Pogaku, B. Ti Tey, Z. Zhang, Particuology **9** (2011) 228-234

[34] I.T. Kostić, B.D. Isailović, V.B. Đorđević, S.M. Lević, V.A. Nedović, B.M. Bugarski, Hem. Ind. **66** (2012) 505-517

[35] B. Bugarski, Q. Li, M.F.A. Goosen, D. Poncelet, R.J. Neufeld, G. Vunjak, AIChE J. **40** (1994) 1026-1032

[36] Shimadzu Autograph AG-Xplus Series Instruction Manual, Analytical & Measuring Instruments Division, Shimadzu Corporation, Kyoto, 2010, p.3-13

[37] D. Šumarac, B. Medjo, N. Trišović, Theor. Appl. Mec. **35** (2008) 287-304

[38] G. Grigorescu, S. Rosinski, D. Lewinska, L. G.Ritzén, H. Viernstein, E. Teunou, D. Poncelet, Z. Zhang, X. Fan, D. Serp, I. Marison, D. Hunkeler, J. Microencapsul. **19** (2002) 641-659

[39] V.B. Nguyen, C.X. Wang, C.R. Thomas, Z. Zhang, Chem. Eng. Sci. **64** (2009) 821-829.

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NAUČNI RAD

UTICAJ BRZINE KOMPRESIJE I PROCENTA DEFORMACIJE NA MEHANIČKE KARAKTERISTIKE KALCIJUM ALGINATNIH ČESTICA

Hidrogel čestice su najčešće korišćeni nosači prilikom imobilizacije enzima, polifenolnih jedinjenja, biljnih i životinjskih ćelija. U mnogim procesima čestice hidrogela su izložene mehaničkim silama koje mogu izazvati deformaciju, čime se ograničava njihova praktična primena. Definisanje i unapređenje mehaničkih svojstava hidrogelova je veoma važno u cilju sprečavanja neželjenih efekata u toku samog proizvodnog procesa, kao i dalje primene proizvoda. Cilj ovog rada je bilo ispitivanje mehaničkih svojstava pojedinačnih čestica potopljenih u vodi, kao i na vazduhu (suvi uslovi) pomoću metode kompresije između dve ravne površine. Rezultati su ukazali da sastav alginatnih čestica i gubitak vode tokom procesa kompresije imaju značajan uticaj na njihove mehaničke karakteristike i čvrstoću. Kalcijum-alginatne čestice su dobijene postupkom elektrostatičke ekstruzije, potiskivanjem natrijum-alginata (1.5%) kroz špric pomoću infuzione pumpe u rastvor kalcijum-hlorida (2.0%). Dobijeni rezultati su iskorišćeni za ispitivanja uticaja radnih uslova, procenta deformacije (10-50%) i različitih vrednosti brzine sabijanja (1-50mm/min) uzorka na mehanička svojstva nosača. Modul elastičnosti kao i maksimalna sila za ispitivani procenat deformacije alginatnih čestica su određeni pomoću dobijenih eksperimentalnih krivih zavisnosti sile od pomeranja i napona od deformacije tokom procesa kompresije.

Ključne reči: alginatne čestice, metoda kompresije, mehanička svojstva.