VERICA MANOJLOVIĆ[1]
JASNA DJONLAGIĆ[1]
BOJANA OBRADOVIĆ[1]
VIKTOR NEDOVIĆ[2]
BRANKO BUGARSKI[1]

[1]Faculty of Technology and Metallurgy, University of Belgrade, Belgrade

[2]Faculty of Agriculture, University of Belgrade, Belgrade

SCIENTIFIC PAPER

664.644.7:537.2:532.135

EFFECTS OF CELL ADDITION ON IMMOBILIZATION BY ELECTROSTATIC DROPLET GENERATION

In this study, an attempt was made to assess the effects of cell addition and final concentration on the process of electrostatic extrusion as a method for cell immobilization in alginate microbeads. The electrostatic extrusion process is a complex function of several operating parameters, the system geometry, and the properties of the polymer solution, which is being extruded. The addition of cells results in the formation of a two–phase system, adding to the complexity of the process by the associated phenomenon of two–phase flow. This study especially focused on analyzing the effects of cell presence on each stage of the immobilization process. Specifically, the effects of the cell and alginate concentrations on the resulting microbead size and uniformity were assessed. Under the investigated conditions, microbeads, 50–600 μm in diameter, were produced and the increase in both alginate and cell concentrations resulted in larger microbeads with higher standard deviations in size. We attempted to rationalize the obtained findings by rheological characterization of the cell–alginate suspensions. H–NMR Spectroscopy of the alginate used in this study revealed a high content (67%) of guluronic residues and GG diad blocks (FGG = 55%). The mole fractions of the MM and GM diad sequences, F_{MM} and F_{GM}, were 21 and 12%, respectively. Rheological characterization revealed non–Newtonian, pseudoplastic behavior of the cell–alginate suspensions with an increase in viscosity as the alginate concentration was increased. However, the presence of cells even at high concentrations (5×10^8 and 1×10^9 cell/ml) did not significantly affect the rheological properties of Na alginate solution. Finally, the effects of the alginate and cell concentrations on the gelation kinetics and the dynamic–mechanical behavior of the obtained hydrogels were investigated. A molar ratio of G units to Ca^{2+} ions of 3.8 : 1 provided complete crosslinking, while an increase in the alginate concentration resulted in prolonged gelation times, but higher strength of the resulting gel. Cell presence decreased the rate of network formation, as well as the strength of the obtained Ca alginate hydrogel.

Key words: alginate, electrostatic extrusion, rheology, cell immobilization.

Alginate is one of the widely used hydrogels for cell immobilization due to biodegradability, biocompatibility and gentle immobilization procedure. It is a naturally derived linear copolymer of 1,4–linked β–D–mannuronic acid (M) and α–L–guluronic acid (G) residues [1–3]. Aqueous alginate solutions form hydrogels in the presence of divalent ions via ionic interactions between the acid groups on the G blocks and the chelating ions, generally Ca^{2+} [2]. As a result, calcium alginate gels are physically cross–linked systems with mechanical properties dependant on the proportion and length of the G blocks in a given alginate chain [1,2]. The gels are viscoelastic solids, with a network structure described by the "egg–box" model [4–7].

One of the promising techniques for the production of uniform alginate microbeads is electrostatic droplet generation, based on the use of electrostatic forces to disrupt a filament of alginate solution at the capillary/needle tip and form a charged stream of small droplets, which are collected in a hardening solution [8,9]. Under optimal operating conditions it was possible to obtain alginate microbeads down to 50 μm in diameter [9,10]. This technique was successfully applied for the immobilization of various cell types such as hybridomas [11,12], insect cells [11,13] islets of Langerhans [11,14] parathyroid cells [20] and brewing yeast cells [16–18]. Nevertheless, the process of electrostatic droplet formation is a complex function of a number of parameters such as the applied electrostatic potential, needle diameter, electrode distance and geometry, polymer solution flowrate, as well as the solution properties including surface tension, density and viscosity [19]. The situation becomes even more complicated when a cell suspension is introduced within the polymer, which can affect both the polymer properties and the extrusion process (i.e. the micro hydrodynamics within the capillary via electrostatic and physical interactions of the cells). We have previously investigated the effects of several operating and design parameters on the electrostatic extrusion of alginate–yeast suspension and the resulting microbead size such as the applied potential, needle size, electrode spacing, and suspension flowrate [20]. However, the effects of cells on hydrogel properties affecting the flow dynamics have not yet been examined.

Author address: V. Manojlović, Tehnološko metalurški fakultet, Univerzitet u Beogradu, Karnegijeva 4, Beograd
Paper accepted: May 20, 2005

The cell presence can also influence the gelation kinetics and mechanical properties of the final Ca alginate microbeads. Most studies of alginate bead production were carried out in excess of the hardening solution (i.e. $CaCl_2$) over prolonged times in order to ensure complete gelation [1,25]. The immobilization of highly sensitive mammalian cells (e.g. bone marrow cells) can require minimal exposure to $CaCl_2$ solution. Assessment of the gelation kinetics can therefore be essential for optimization of the immobilization techniques for these cells.

The objective of this study was to further investigate and rationalize the process of cell immobilization by the electrostatic droplet generation technique. Specifically, we aimed to assess the effects of the cell and alginate concentrations on the resulting microbead size and characterize the rheological properties of cell–alginate suspensions at different temperatures. Finally, we attempted to analyze the gelation kinetics at limited supplies of Ca^{2+} ions and the effects of cell addition on gelation kinetics.

EXPERIMENTAL

^{1}H NMR spectroscopy

The investigated polymer was low viscosity sodium alginate Protanal LF 20/40 (FMC Biopolymer). The mole ratio of mannuronate (M) to guluronate (G) residues (M/G) and the mole fraction of GG, MM and GM diad sequences F_{GG}, F_{MM} and F_{GM} were determined by ^{1}H NMR spectroscopy according to Grasdalen [21]. The alginate sample was partly degraded by very mild acid hydrolysis in order to decrease the viscosity of the solution. Hydrolyzed alginate sample was dissolved in D_2O at neutral pD. ^{1}H NMR spectra were run at 400 MHz on a Bruker AC 250 E NMR spectrometer.

Electrostatic extrusion

Polymer solutions of different concentrations in the range 1–4% were prepared by dissolving Na–alginate powder in distilled water. Polymer–cell suspensions were formed by mixing the prepared Na–alginate solutions with a suspension of brewing yeast cells Saccharomyces cerevisae at various volume ratios to obtain final cell concentrations in the range from 1 x 10^7 to 5 x 1 0^8 cell/ml. Spherical droplets were formed by extrusion of the polymer–cell suspension through a blunt stainless still needle using a syringe pump (Razel, Scientific Instruments, Stamford, CT) and a 10 ml plastic syringe. Electrode geometry with a positively charged needle and a grounded hardening solution was applied. The hardening solution was $CaCl_2$ at a concentration of 1.5%. The potential difference was controlled by a high voltage dc unit (Model 30R, Bertan Associates, Inc., New York) and was varied in the range 6.5 to 7.5 kV. The distance between the needle tip (20 gauge small or 22 gauge) and the hardening solution was 2.5 cm, while the flow rate of the polymer solution was 13.9 ml/h. A sample of 30 microbeads was taken from each experiment and the diameters of the microbeads were measured with an accuracy of 10 μm using a microscope (Carl Zeiss Jena). The average microbead diameter and standard deviations were then calculated from the measured data. The cell concentrations were determined by counting cells under the microscope using a Thoma chamber.

Dynamic rheological measurements

Dynamic rheological measurements of alginate samples before and during gelation were performed by using a Rheometrics RMS–605 mechanical spectrometer operating in the dynamic shear mode between parallel plates. The plate diameter was 25 mm, and the gap between the plates could be set between 1 and 3 mm. The frequency was varied from 0.1 to 100 rad/s. Pure Na alginate solutions in the concentration range 2–4% w/w, as well as the suspension of yeast cells at a concentration of 5 x 10^8 cell/ml were analyzed at the room temperature, 30 and 37^{o}C. The viscosities of the alginate solutions at lower concentrations (below 2%) could not be measured by the instrument because of the instrument lower limitation of 1 Pas. The liquid samples were analyzed at a constant strain of 30% and complex dynamic viscosities (η^*) as well as the storage (G') and loss (G") moduli were recorded. For each sample and temperature, up to 16 values could be recorded. Some values at lower concentrations and higher temperatures could not be obtained accurately because of inadequate sensitivities of the measurements.

The gelation kinetics were analyzed by rheological examinations of mixtures of Na alginate (1–4% w/w) and $CaCl_2$ (1.5%) solutions at a strain of 5% in order to ensure good contact between the hydrogel and the rheometric plates. The volume ratios of Na alginate to $CaCl_2$ solutions were varied to obtain ratios of G units to Ca^{2+} ions in the range from 15:1 to 1:1 in order to determine the quantity of Ca^{2+} ions necessary for complete gelation. Considering that the reaction of ion exchange was almost instantaneous and than the gelation process was diffusion controlled, the addition of $CaCl_2$ was performed under vigorous mixing. Discs fitting the size of the measuring compartment were stamped out from the obtained homogenous mixtures and subjected to rheological measurements. The storage (G') and loss (G") moduli of the hydrogel were recorded over time at 20^{o}C and a constant frequency of 6.28 rad/s.

RESULTS AND DISCUSSION

^{1}H NMR spectroscopy

The chemical characterization results of Protanal LF 20/40 obtained by H–NMR spectroscopy revealed that the alginate used in this study had a high content

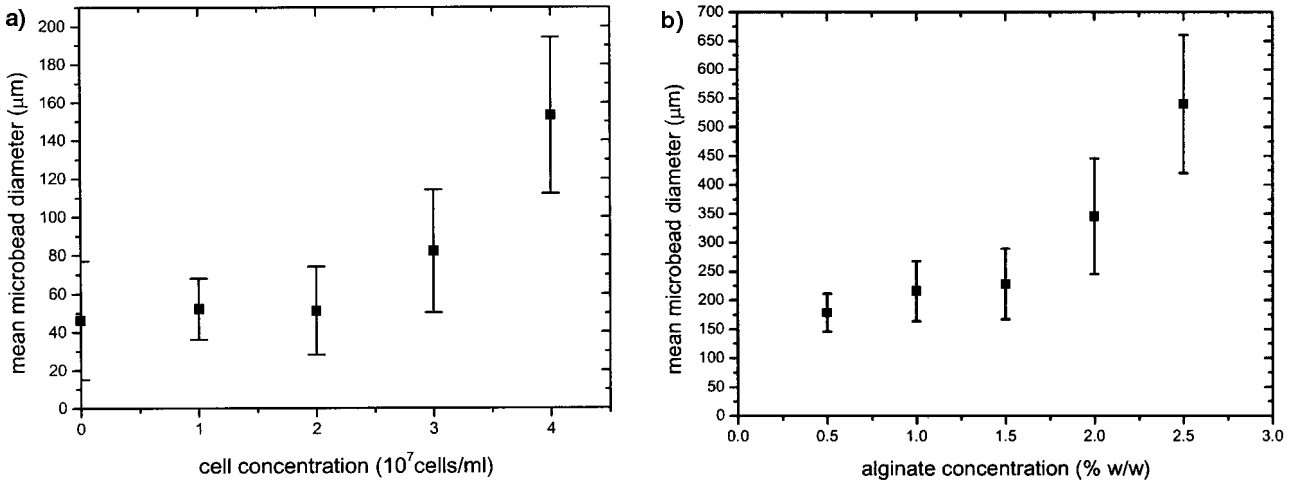

Figure 1. Mean microbead diameter as a function of: a) cell concentration (1.5% w/w Na alginate concentration, 7.5 kV applied potential, 22 G needle, 13.9 ml/h flowrate, 2.5 cm electrode distance); b) Na alginate concentration (5 x 10^8 cell/ml, 6.5 kV applied potential, 20 Gs needle, 13.9 ml/h flowrate, 2.5 cm electrode distance).

(67%) of guluronic residues and was rich in GG diad blocks (F_{GG} = 55%). The mole fractions of MM and GM diad sequences, F_{MM} and F_{GM}, were 21 and 12%, respectively. The H NMR spectra showed no significant peaks originating from proteins or other impurities.

Electrostatic extrusion

The first aim of this study was to assess the effects of cell and alginate concentrations on the mean microbead size obtained by the electrostatic extrusion technique. The effects of cell concentration in the range 1 x 10^7 – 4 x 1 0 cell/ml of 1.5% w/w alginate are presented in Figure 1a. The addition of cells up to a concentration of 2 x 10^7 cell/ml had a negligible effect on the microbead diameter. However, when the cell concentration was increased to 4 x 1 0 cell/ml, a 3–fold increase in the microbead diameter was observed (i.e. from 51±17 μm to 1 53±51 μm). At the given operating conditions (needle size 22 G), further increase in the cell concentration resulted in the non–uniform outflow of the cell–polymer suspension and a trimodal distribution of the microbead sizes (data not shown).

In order to assess the effects of polymer concentration in cell suspensions with a high cell concentration (5x10^8 cell/ml) on the microbead size, we used a larger needle (20 Gs) and varied the Na alginate concentration in the range from 0.5 to 4% w/w. Under the operating conditions used, uniform spherical microbeads were obtained up to the concentration of 2.5% w/w (Figure 1b) and additional increase resulted in non–spherical microbeads. The increase in alginate concentration up to 1.5% w/w had little effect on the average microbead diameter, whereas further increase up to 2.5% w/w resulted in an approximately two–fold increase in the microbead size (Figure 1b). It should be mentioned that an earlier study demonstrated that the mean size of the droplets increased by as much as four to five fold as the polymer viscosity was increased from 1 to 10 Pas [22]. In the present study, spherical droplets were obtained using a narrow range of alginate concentrations (0.5–2.5% w/w) exhibiting viscosities of the order of magnitude of 1 Pas so that the observed effects were less pronounced.

It should be noted that an increase in alginate concentration resulted in an increase in the standard deviations of the microbead diameters (Figure 1b). The observed results were in agreement with the mechanisms of droplet formation under the action of an electrostatic field [23,24]. As the electrostatic field is applied, the almost spherical shape of the liquid meniscus at the tip of the needle is deformed into a conical shape. Consequently, the alginate solution flows through this weak area at an increasing rate causing the formation of a neck. In the experiments with pure Na alginate solutions, the neck formation was more pronounced as the alginate concentration was increased from 0.8 to 1.5% w/w so that at the latter concentration the neck elongated up to 1 mm before detachment [19]. Furthermore, detachment of the drop was, in that case, accompanied by detachment of the linking filament, which then broke up into a large number of smaller droplets resulting in non–uniform size distribution. The results obtained in this study implied a similar mechanism of droplet formation at higher alginate concentrations in the cell suspension.

Electrostatic extrusion experiments revealed an increase in microbead size with increasing both cell and alginate concentrations implying changes in the fluid flow and droplet formation mechanism.

Rheological measurements

Rheological characterization of cell–alginate suspensions

In order to get an insight in the process of electrostatic extrusion of cell–polymer suspensions we

performed rheological characterizations of pure Na alginate solutions and suspensions of yeast cells at high concentrations ($5x10^8$ and $1x10^9$ cell/ml). The experiments were performed at room temperature representing the usual conditions for electrostatic extrusion. However, in order to simulate the conditions for the immobilization of temperature–sensitive cells (e.g. mammalian cells), rheological characterizations were also performed at 30 and 37°C.

The complex dynamic viscosities (η^*) of cell suspensions with a cell concentration of $5x10^8$ cell/ml and Na alginate at concentrations in the range 2–4% w/w at all investigated temperatures did not significantly differ from those measured in pure Na alginate solutions. The results indicated non–Newtonian, pseudoplastic behavior for all the samples. A representative set of results for 4% w/w Na alginate at 30°C is presented in Figure 2. In addition, similar results were obtained for suspensions with a higher cell concentration ($1.0x10^9$ cell/ml), indicating that the cell presence at the investigated concentrations did not have any significant influence on the rheological parameters in the experimental range of frequencies, probably due to cell elasticity and orientation in the shear stress field.

However, the effects of cell concentration on the mechanism of electrostatic droplet formation and the microbead size (Figure 1a) still need to be explained, possibly by modifications of the fluid flow in the capillary or electrostatic interactions.

Rheological determination of gelation kinetics

In order to optimize the $CaCl_2$ concentration and duration of alginate exposure to $CaCl_2$, we investigated the gelation kinetics of Na alginate at different molar ratios of G units to Ca^{2+} ions, at different Na alginate concentrations, as well as in the presence of cells.

In the first experimental series we investigated the gelation kinetics of Na alginate of different concentrations at the ratio of G units to Ca^{2+} ions of 3.8:1, since it was previously determined that a molar ratio of 3.8:1 allowed physical crosslinking (data not shown). With an increase in alginate concentration from 1 to 4% w/w the rate of the gelation process decreased due to higher mass transfer resistances for the diffusion of Ca^{2+} ions. In the investigated geometry of the system, about 20 min. was needed to form a gel using 1% w/w Na alginate solution. The time needed for the gelation of 2% w/w alginate was approximately twice as much. Finally, the 4% w/w alginate exhibited elastic behavior as much as viscous during the first hour of gelation and after about 60 min. the values of the storage modulus (G') became slightly higher thant the values of the loss modulus (G") (Figure 3).

The results obtained in the present study imply much longer gelation times (20 to 70 minutes) as compared to the earlier reports [20,25]. However, it should be emphasized that the time to achieve complete gelation strongly depends on the system geometry, that was used. For beads with diameters in the range from 2 to 6 mm in an excess of $CaCl_2$, the times to achieve complete gelation varied in the range 3 to 25 min. (the molar ratios of G units to calcium ions were up to 1:1) [20]. In studies of alginate bead production the duration of bead exposure to the hardening solution varied between 30 minutes and 12 hours in order to ensure complete gelation [26]. In the present experiments, the gelation kinetics were investigated under conditions of limited Ca^{2+} ion supply in a parallel plate geometry in which the diffusion rate of Ca^{2+} ions influenced the rate of gelation and the time needed to achieve the gel point.

The obtained values of G' and G" for 4% w/w alginate gel were about 1000 Pa and approximately 5–fold higher as compared to the values measured for 1% w/w alginate gel (Figure 3). These results are consistent with earlier studies [1], which indicated an increase in gel strength as the alginate concentration was increased. However, as higher alginate concentrations result in higher fluid viscosities leading to changes in the droplet formation mechanism and

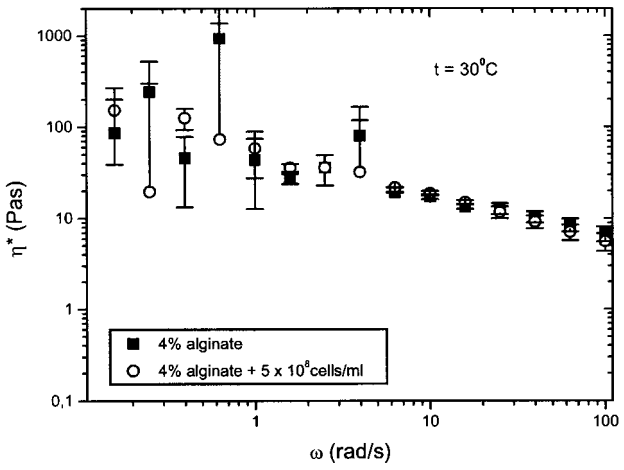

Figure 2. The effect of cells on the rheological properties: complex dynamic viscosities (η^*) of pure 4% w/w Na alginate solutions and with added cells at a concentration of $5x10^8$ cell/ml as a function of frequency at 30°C

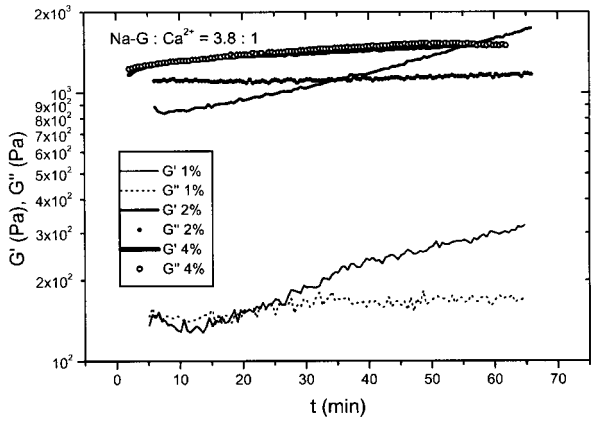

Figure 3. Gelation kinetics of Na alginate of different concentrations: storage (G') and loss (G") moduli at 6.28 rad/s and at 20°C vs. time, after adding $CaCl_2$ in the molar ratio of G units to Ca^{2+} ions of 4:1 (the data present an average of n = 3–5; STD = ±9%)

non–uniformity of the produced microbeads (Figure 1b), a compromising solution has to be determined for each application.

The effects of cell addition (5 x 10^8 cell/ml) on the gelation kinetics of 2% w/w alginate at the ratio of G units to Ca^{2+} ions of 3.8:1 were investigated in the second experimental series (Figure 4). The presence of cells caused gelation to occur at a prolonged time as compared to the pure alginate solution due to the reduction of space available for Ca^{2+} ion diffusion. Consequently, the value of G' had not reached the value of G" even after an hour of gelation. Considering the tendency of increase in G', it can be expected that the crossover of G'–t and G"–t is reached at about t = 70 min.

Previous investigations have also shown that the presence of yeast cells slowed down the gelation process [20]. The presence of 10% yeast (dry weight basis) caused an increase in the gelation time of 31% yeast compared to similar yeast–free beads [20].

In addition, the presence of yeast cells in our studies caused reductions in G' (between 8% and 34%) and G" (~7%) as compared to the values obtained for alginate systems without cells. Other investigations also indicated a decrease of gel strength by the presence of microorganisms [20,27]. The obtained results imply that immobilized microorganisms could cause irregularities in the network structure and reduce gel elasticity. Furthermore, these effects could be even more pronounced with cell growth in the culture. This phenomenon should be considered before exposing immobilized cell systems to severe environmental stresses in industrial applications.

CONCLUSIONS

In this study an attempt was made to obtain an insight into the process of electrostatic extrusion by determining and relating the effects of cell and alginate concentrations as two operating parameters to the rheological characterization of the cell–alginate suspensions. Both parameters affected the size and distribution of the microbeads such that larger microbeads with higher standard deviations in size were produced at higher cell and alginate concentrations. The rheological characterization of pure Na alginate solutions and alginate/cells suspensions revealed non–Newtonian, pseudoplastic behavior. The presence of cells even at high concentrations (5 x 10^8 and 1 x 10^9 cell/ml) did not significantly influence rheological properties of Na alginate indicating that the observed effect of cells on the microbead size should be further explored and elucidated. However, the presence of cells affected the kinetics of alginate gel formation, as well as the final properties of Ca alginate. The network formation of Ca alginate was slower with lower crosslinking density causing lower hydrogel strengths as compared to the hydrogel without cells. The results of this study revealed some of the phenomena during cell immobilization by electrostatic extrusion and indicated some of the directions for process optimization for the production of alginate microbeads with the desired properties. Further studies of two phase flow in the capillary, electrostatic, and physical interactions of cells, as well as the development of mathematical models relating all the phenomena in the electrostatic extrusion process to the operating parameters and the properties of the resulting microbeads are needed to fully describe and optimize the electrostatic droplet generation technique.

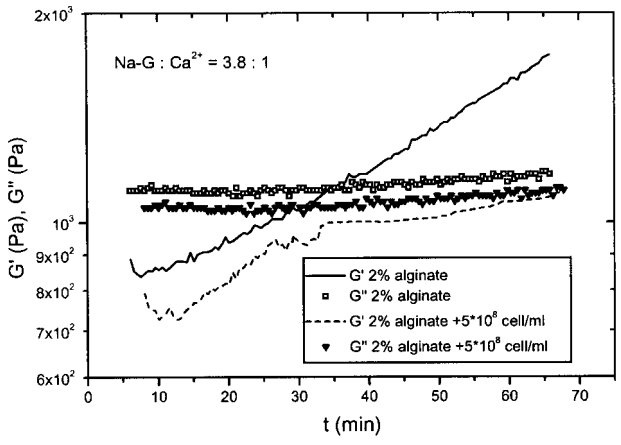

Figure 4. The effect of cell addition (5 x 10^8 cell/ml) on the gelation kinetics of 2% w/w Na alginate: storage (G') and loss (G") moduli at 6.28 rad/s vs. time, after adding $CaCl_2$ in the molar ratio of G units to Ca^{2+} ions of 3.8:1 (the data present an average of n = 4; STD = ±14%)

REFERENCES

[1] A. Martinsen, G. Skjak–Braek, O. Smidsrod, Alginate as Immobilization Material; I. Correalation between Chemical and Physical Properties of Alginate Gel Beads, Biotechnol Bioeng **33** (1989) 79–89.

[2] W.R. Gombotz, S.F. Wee, Protein release from alginate matrices, Adv Drug Delivery Rev **31** (1998) 267–285.

[3] S.T. Moe, K.I. Draget, G. Skjak–Braek, O. Smidsrod, Alginates in Food Polysaccharides and Their Applications, ed by Stephen AM. Marcel Dekker, New York **67** (1995) 245–286.

[4] P. Eiselt, J. Yeh, R.K. Latvala, L.D. Shea, D.J. Mooney, Porous carriers for biomedical applications based on alginate hydrogels, Biomaterials **21** (2000) 1921–1927.

[5] G.T. Grant, E.R. Morris, D.A. Rees, P.J.C. Smith, D. Thom, Biological interactions between polysaccharides and divalent cations: The egg–box model, FEBS Lett **32** (1973) 195–198.

[6] B.T. Stokke, K.I. Draget, O. Smidsrod, Y. Yuguchi, H. Urakawa, K. Kajiwara, Small–Angle X–ray Scattering and Rheological Characterization of Alginate Gels, Macromolecules **33** (2000) 1853–1863.

[7] N.E. Simpsona, C.L. Stablerb, C.P. Simpsona, A. Sambanisc, I. Constantinidis, The role of the $CaCl_2$–guluronic acid interaction on alginate encapsulated βTC3 cells, Biomaterials **25** (2004) 2603–2610.

[8] T. Keshavartz, G. Ramsden, P. Phillips, P. Mussenden, C. Bucke, Application of electric field for production of immobilized biocatalysts, Biotechnol Tech **6**(5) (1992) 445–450.

[9] B. Bugarski, Q. Li, M.F.A. Goosen, D. Poncelet, R. Neufeld, G. Vunjak, Electrostatic droplet generation: mechanism of polymer droplet formation, AIChE J 40, **6** (1994) 1026–1032.

[10] V. Manojlovic, B. Obradovic, V. Nedovic, B. Bugarski, Electrostatic generation of alginate microbeads loaded with brewing yeast, Chemical Industry, **58** (2004) 62–64.
[11] M.F.A. Goosen, E.S.C. Mahmud, A.S. M–Ghafi, H.A. M–Hajri, Y.S. Al–Sinani, M.B. Bugarski, Immobilization of cells using electrostatic droplet generation, in: Immobilization of Enzymes and Cells, ed by Bickerstaff GF. Humana Press, Totowa, NJ, Chapter 20, 1997, p. 167–174.
[12] B. Bugarski, G. Vunjak, M.F.A. Goosen, Principles of Bioreactor Design for Encapsulated Cells in Cell Encapsulation Technology and Therapeutics, ed by Kuhtreiber WM, Lanza RP and Chick WL. Birkhauser–Springer Verlag, Chapter 30 1999, p. 395–416.
[13] B. Bugarski, J. Smith, J. Wu, M.F.A. Goosen, Methods for animal cell immobilization using electrostatic droplet generation, Biotechnol. Tech. **7–9** (1993) 677–683.
[14] B. Bugarski, L. Sajc, M. Plavsic, M. Goosen, G. Jovanovic, Semipermeable alginate – PLO microcapsules as a bioartifical pancreas, in Animal Cell Technology: Basic & Applied Aspects, Vol. 8 ed by Funatsu K, Shirai Y and Matsushita T. Kluwer Academic Publishers, Dordrecht 1997, p 479–486.
[15] S. Rosinski, D. Lewinska, M. Migaj, B. Wozniewicz, A. Werynski, Electrostatic microencapsulation of parathyroid cells as a tool for the investigation of cell's activity after transplantation, Landbauforschung Völkenrode SH 241 2002, 47–50.
[16] V. Nedovic, B. Obradovic, I. Leskosek–Cukalovic, O. Trifunovic, R. Pesic, B. Bugarski, Electrostatic generation of alginate microbeads loaded with brewing yeast, Proc Biochem **37**(1) (2001) 17–22.
[17] V.A. Nedovic., B. Obradovic, I. Leskosek–Cukalovic, G. Vunjak–Novakovic, Immobilized yeast bioreactor systems for brewing, in Focus on Biotechnology, Vol. IV: Engineering and Manufacturing for Biotechnology, ed by Thonart Ph and Hofman M. Kluwer Academic Publishers, Dordrecht 2001, p 277–292.
[18] V. Nedovic, B. Obradovic, D. Poncelet, M.F.A. Goosen M., I. Leskosek–Cukalovic, B. Bugarski, Cell immobilisation by electrostatic droplet generation, Landbauforschung Volkenrode SH **241** (2002) 11–17.
[19] D. Poncelet, V. Babak, R.J. Neufeld, M. Goosen, B. Bugarski, Theory of electrostatic dispersion of polymer solution in the production of microgel beds containing biocatalyst, Advances in Colloid Interface Science **79** (2–3) (1999) 213–228.
[20] C.D. Gilson, A. Thomas, F.R. Hawkes, Gelling Mechanism of Alginate Beads with and without Immobilized Yeast, Process Biochemistry (1990) 104–108.
[21] H. Grasdalen, B. Larsen, O. Smidsrod, A.P. M.R. Study of the Composition of Uronate Residues in Alginates, Carbohydrate Research, **68** (1979) 23–31.
[22] S.N. Jayasinghe, M.J. Edirisinge, Electrically forced jets and microthreads of high viscosity dielectric liquids, Aerosol Science **35** (2004) 233–243.
[23] B. Bugarski, B. Obradovic, V.A. Nedovic, D. Poncelet, Immobilization of cells and enzymes using electrostatic droplet generator, in Focus on Biotechnology, Volume 8a: Fundamentals of Cell Immobilisation Biotechnology, ed by Nedovic V and Willaert RG. Kluwer Academic Publishers, Dordrecht 2004, p 277–294.
[24] B. Bugarski, B. Obradovic, V.A. Nedovic, M.F.A. Goosen, Electrostatic droplet generation technique for cell immobilization, in Finaly dispersed systems, ed by Shu JP and Spasic A. CRC Press, Boca Raton., Chpt 31 in press 2005.
[25] R.E. Webber and K.R. Shull, Strain Dependence of the Viscoelastic Properties of Alginate Hydrogels, Macromolecules **37** (2004) 6153–6160.
[26] D. Williams and D.M. Munnecke, The production of ethanol by immobilized yeast cells, Biotechnol Bioeng **23** (1981) 1813–1825.
[27] I. Camelin, C. Lacroix, C. Paquin, H. Prevost, R. Cachon, C. Divies, Effect of Chelatants on Gellan Gel Rheological Properties and Setting Temperature for Immobilzitaion of Living Bifidobacteria, Biotechnol Prog **9** (1993) 291–297.

IZVOD

UTICAJ PRISUSTVA ĆELIJA U PROCESU ELEKTROSTATIČKE EKSTRUZIJE ALGINATA

(Naučni rad)

Verica Manojlović[1], Jasna Djonlagić[1], Bojana Obradović[1], Viktor Nedović[2], Branko Bugarski[1]

U ovom radu izvršeno je ispitivanje fenomena elektrostatičke ekstruzije. Proces elektrostatičke ekstruzije je vrlo složen, jer obuhvata pojedinačne uticaje različitih fenomenoloških parametara (jako spoljašnje električno polje, mikrohidrodinamičke pojave unutar kapilare, reološko ponašanje polimera), kao i međusobne uticaje ovih faktora. Prisustvo ćelija u sistemu čini ga još složenijim, što do sada fenomenološki nije razjašnjeno. Istraživanja su obuhvatila ispitivanje pojedinih značajnih fenomena koji se javljaju u ovoj metodi imobilizacije ćelija, polazeći od karakteristika samog nosača i suspenzije alginat/ćelije, preko parametara elektrostatičke ekstruzije, do kinetike geliranja alginata. Uz to, posebno je analiziran uticaj prisustva ćelija na sve faze ovog procesa. Detaljno je proučen uticaj promene koncentracije alginata i koncentracije ćelija u polaznoj suspenziji alginat/ćelije na veličinu i uniformnost proizvedenih mikročestica. Pri ispitivanim operativnim uslovima, proizvedene su mikročestice prečnika od 50-600 μm i porast koncentracije bilo alginata, bilo ćelija uzrokuje nastajanje većih čestica neuniformnije raspodele veličina. U pokušaju da se objasne dobijeni rezultati i ispita reološko ponašanje suspenzije kao jedan od parametara koji utiču na dinamiku dvofaznog strujanja u toku ekstruzije, izvršeno je reološko karakterisanje suspenzija alginat/ćelije. Reološka ispitivanja su pokazala da prisustvo ćelija čak i pri visokim koncentracijama ($5x10^8$ i $1x10^9$ ćelija/ml) ne utiče značajno na reološke osobine rastvora natrijum alginata. Na kraju, ispitan je uticaj koncentracije alginata i ćelija na kinetiku geliranja u uslovima ograničenog prisustva Ca^{2+} jona, s obzirom da imobilizacija izuzetno osetljivih ćelija sisara može da zahteva minimalno izlaganje rastvoru $CaCl_2$. Rezultati su pokazali da molski odnos guluronskih jedinica prema Ca^{2+} jonima od 3,8:1 omogućuje fizičko umrežavanje i da porast koncentracije alginata uzrokuje duže geliranje, ali i nastajanje jačeg gela. Prisustvo ćelija dovodi do sporijeg geliranja i prouzrokuje nastajanje slabijih hidrogelova što upućuje na zaključak da imobilisani mikroorganizmi uzrokuju nepravilnosti u strukturi mreže.

Ključne reči: alginat, elektrostatička ekstruzija, reologija, imobilizacija ćelija.