Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.11407

64 (2017) 400–408
February

Influence of electric parameters on the alginate-polyethersulfone microcapsule structure

Dorota Lewińska*, Marcin Grzeczkowicz, Barbara Kupikowska-Stobba

Nalecz Institute of Biocybernetics and Biomedical Engineering, PAS, 4 Trojdena, 02-109 Warsaw, Poland, Tel. +4822 6599143 Ext 130; Fax: +4822 6597030; emails: dlewinska@ibib.waw.pl (D. Lewińska), mgrzeczkowicz@ibib.waw.pl (M. Grzeczkowicz), bkupikowska@ibib.waw.pl (B. Kupikowska-Stobba)

Received 3 April 2016; Accepted 22 July 2016

ABSTRACT

Alginate-polyethersulfone microcapsules obtained with the one-stage three-nozzle, electrostatic technique designed by our team can successfully be used to encapsulate biologically active material such as functional proteins, microorganisms, bacteria, fungi and mammalian cells. The paper presents the results of studies on the influence of electric parameters on microcapsule size and internal structure. During the manufacturing process, three different liquids, i.e., aqueous sodium alginate solution, separating liquid and membrane-forming solution, were forced through a three-nozzle head, placed in an electrostatic field. After gelling in a gelling bath, the multi-layer drops appearing at the tip of the three-nozzle head formed microcapsules. The electrostatic field was applied through electric impulses with varied values of: voltage (U), frequency (f) and impulse duration (τ). The results indicated, that an increase of all examined electric process parameters resulted in a decrease in average microcapsule diameter and lower uniformity of batches in terms of size. Average membrane thickness (parameter B) did not change significantly, but along with the increase of all electrical parameters, a significant decrease of membrane thickness at the thickest part (parameter N) was observed. The microcapsules that were the most symmetric in regard to membrane thickness were obtained in the series with variable voltage (U).

Keywords: Alginate; Microencapsulation of cells; Alginate-polyethersulfone microcapsules; Electrostatic technique; Immobilization

1. Introduction

Recently, hydrogel microcapsules [1] and microbeads [2,3] have been extensively studied in terms of their applications in broadly defined water treatment systems [4,5]. Both of them can be manufactured using an electrostatic method of forming alginate gel droplets introduced in the 1990s by Bugarski, Poncelet and their collaborators [6,7]. They used the phenomenon, studied by Lippmann, of the decrease in surface tension of a liquid flowing through a charged capillary, which occurred as a result of the application of an electrical charge. It causes both, shape and size of the meniscus of liquid flowing out from a metal nozzle, to change: it becomes longer and thinner, and droplets falling from its tip, are significantly smaller. Their diameter may even be reduced to tens of micrometers.

The three-nozzle electrostatic method of forming a three-layer droplet designed by our team [8,9] allows to form biocompatible hydrogel microcapsules surrounded by a semi-permeable membrane formed of high-molecular weight synthetic polymers in a single stage, using the wet phase inversion method. In this technique, three liquids are pressed through a three-nozzle head (nested nozzle type): the aqueous sodium alginate solution (natural polymer) through the inner; the glycerine (separating liquid) through the middle, and the solution of a synthetic polymer in an organic solvent through the external nozzle. As a result, a three-layer droplet forms at the tip of the head and then drops into a aqueous gelling bath containing calcium ions. As a result of the gelling, the microcapsule's gel core and the semi-permeable polymer membrane surrounding it are formed. To reduce microcapsule size, the head is placed in an electrostatic field applied in a pulse mode, with set, regulated voltage (U),

* Corresponding author.

Presented at the Conference on Membranes and Membrane Processes in Environmental Protection (MEMPEP 2016), Zakopane, Poland, 15–19 June 2016.

electric impulse frequency (f) and impulse duration (τ). The use of electrostatic method enables to produce microcapsules in gentle conditions (room temperature, physiological pH, no toxins), thus allows the encapsulation of sensitive, biologically active materials, such as functional proteins (hormones, enzymes), and even living cells [9–11].

Conditions inside the microcapsule depend mainly on properties of the semi-permeable membrane (porosity, hydraulic permeability, cut-off), but also on the structure of the microcapsule as a whole, including its diameter and membrane thickness [12–14].

Recently, we presented results of studies on the influence of core liquid (alginate) and membrane-forming solution flow on microcapsule structure [15]. We concluded, that flow rates of both liquids strongly influenced both, microcapsule size and the uniformity of batches in terms of size. The thickness of the polyethersulfone membranes and their symmetry also depend on the flow rates of both liquids.

In previous papers [9,15], we showed that the three-liquid set-up (aqueous alginate solution/glycerol/polyethersulfone solution in NMP) studied by us was also subjected to the phenomenon described above. The aim of the studies presented in this paper was to investigate how a change in each of the electrical parameters involve in the encapsulation process influenced on microcapsule size, batch uniformity in regard to size, thickness of formed semi-permeable membranes and uniformity of their thickness.

2. Materials and methods

2.1. Microcapsule production

Alginate-polyethersulfone microcapsules, manufactured in order to study the influence of process electric parameters on microcapsule structure, were prepared in a set-up of three liquids: 1.5% (w/w) sodium alginate of medium viscosity (Sigma) in saline (BASF, Poland); glycerol (POCh); and 13% (w/w) polyethersulfone (PES), type E 2020 P (M_W = 42 kDa, BASF) in 1-methyl-2-pyrrolidone (NMP, Sigma) with the addition of 9.5% (w/w) polyvinylpyrrolidone (PVP M_W = 40 kDa, Merck), of dynamic viscosity 790 mPas (25°C). For gelling, 1.1% (w/v) aqueous solution of calcium chloride, with the addition of 0.5% (w/v) Tween 80 surfactant (POCh), was used.

Microcapsule diameters were measured using a crystallographic microscope with a micrometer screw. The average diameter (D_A) of microcapsules prepared in each batch was calculated basing on measurements of 20 capsules, as was standard deviation (SD) and variation coefficient of diameter ($VC = (SD \times 100)/D_A$).

2.2. Microcapsule structure analysis

The internal structure of the microcapsules was analysed with an optical microscope, using a method described in [9]. After dehydration, microcapsules were embedded in metacrylate resin Historesin Embedding Kit 7022 31731 (Leica), and subsequently cut into 10 to 25 μm thick sections with an RM 2265 (Leica) microtome. For analysis, sections selected from the geometric centre of the microcapsule, were taken. After gluing on slides, they were examined and photographed under an optical microscope (Olympus CKX 41) equipped with a digital camera Camedia 5050 Zoom.

Membrane structure was described numerically using the following structural parameters:

A – membrane thickness at the thinnest part of the microcapsule cross-section

B – average membrane thickness (thickness over most of the membrane circumference)

N – membrane thickness at the thickest part (polymer bulge)

N/B – ratio of parameter N to parameter B. It describes the symmetry of the membrane in regard to its thickness

N/A – ratio of parameter N to parameter A, also describes the symmetry of microcapsule membranes.

The parameters were measured using photographs of the relevant parts of microcapsule cross-sections, taken under high magnifications (using photos of the Olympus measurement standard) with the ImageJ computer program (estimated maximum measurement error 10 μm). The given values are average ones and were calculated for three microcapsules of each batch (σ was calculated for critical t-value 1.3210).

Analysing, how the value of individual electrical process parameters influenced on size and internal structure of microcapsules, we examined, whether and in what way the values of the structural parameters mentioned above were dependent on the process parameter,which were simultaneously modified during their formation process.

3. Results and discussion

3.1. Study of the influence of voltage (U) on AGP microcapsule size and structure

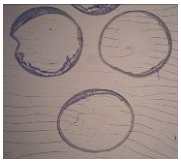
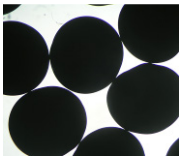
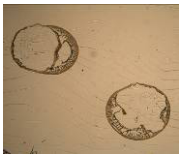
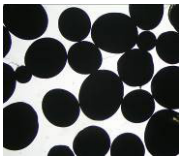
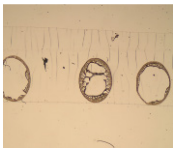
In the first experimental series, six batches of microcapsules at electrical voltage (U) varied from 0 to 13 kV, were obtained. The remaining electric parameters were kept constant at following levels: electric impulse frequency f = 50 Hz and impulse duration τ = 5 ms. All batches were obtained at constant values of overpressures of the gasses forcing out all three liquids: for aqueous alginate solution P_1 = 20.3 kPa; glycerol, P_2 = 30.4 kPa and polyethersulfone solution, P_3 = 10.1 kPa. The distance of the head tip from the surface of the gelling liquid (L) was 50 mm.

Table 1 presents photographs of selected microcapsule batches obtained in this series, the appearance of the preparations made with them, the voltage at which the given batch was formed (U), average microcapsule diameter (D_A) together with the variation coefficient of the diameter (VC) and values of structural parameters of membranes (A, B and N).

As it may be observed in presented photographs, microcapsule batches obtained at voltages of up to U = 8 kV were spherical in shape, and variation coefficients of their diameters were very low and varied from 2% to 20%. Further increase of voltage resulted in the formation of elongated microcapsules, which were egg- or spindle-shaped or cylindrical. Some of them were considerably elongated and resemble deformed pipe fragments.

In the presented series, as the applied voltage (U) increased, the average diameter (D_A) of obtained microcapsules obtained decreased rapidly from 2.85 mm (U = 0) to

Table 1
Alginate-polyethersulfone microcapsules formed at variable electric voltage U

Batch symbol	General view of the batch	Microcapsule cross-section	U kV	D_A mm	VC D_A %	A μm	B μm	N μm
AGP-120			0	2.85 ± 0.08	3	48 ± 10	69 ± 3	234 ± 50
AGP-122			5	2.44 ± 0.05	2	16 ± 4	170 ± 64	620 ± 105
AGP-123			8	1.32 ± 0.26	20	15 ± 5	120 ± 21	244 ± 47
AGP-125			13	0.97 ± 0.35	36	34 ± 11	96 ± 16	141 ± 14

0.97 mm (AGP-125, U = 13 kV), in accordance with the curve presented in Fig. 1, which may be well approximated by a straight line with a comparatively high correlation coefficient (R = 0.9661).

3.2. Analysis of internal structure of first-series microcapsules

To illustrate the effect of the voltage (U) change on internal microcapsule structure, membrane parameters as a function of increasing voltage are presented in Fig. 2. In this series, microcapsules had very thin membranes. Average membrane thickness (parameter B) changed within a narrow range, rising initially from 70 to 170 μm (for U = 5 kV), and then falling to 96 μm for batch AGP-125. Such a course of changes may be approximated by a second-degree polynomial (dotted line), the equation of which is presented in Table 2. Parameter A (dashed line) decreased gradually as voltage increased, from 48 to 14 μm. The greatest changes were observed for parameter N (continuous line), which initially grew and then decreased gradually from 312 to 140 μm, except for a sample obtained at a voltage of U = 5 kV, for which it was 620 μm.

All three sets of data could be approximated by second-degree polynomials. The equations for individual curves and the values of determination coefficients (R^2) are presented in Table 2.

Using the presented data, one can conclude that a correlation between the increase in voltage and the size of a structural parameter exists only in the case of parameter A – membrane thickness at the thinnest part. In the other two cases the values of the correlation coefficient (R^2) are too low.

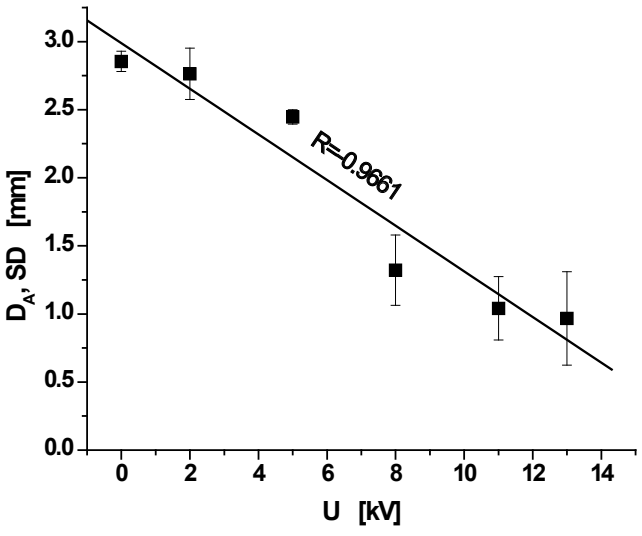


Fig. 1. Change in average microcapsule diameter D_A as a function of voltage U change.

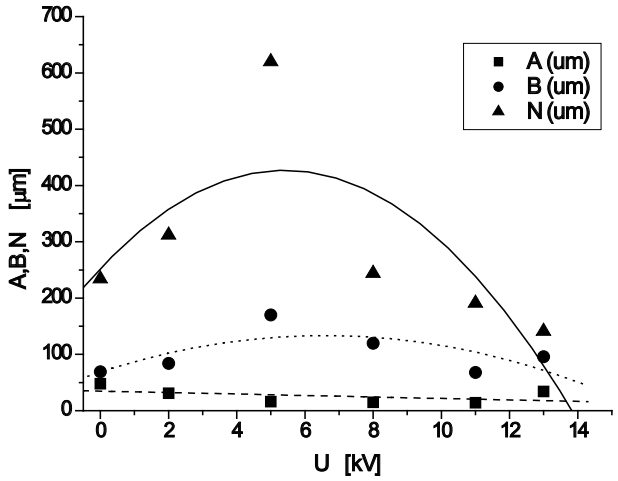


Fig. 2. Changes in microcapsule structural parameters: A, B and N as a function of increasing electric voltage *U*.

3.3. Study of the influence of electric impulse frequency (f) on AGP microcapsule size and structure

The experiments were conducted at following conditions: voltage U = 15 kV, electric impulse duration τ = 3 ms, distance of the head tip from the surface of the gelling liquid L = 50 mm, overpressure of gas forcing out glycerol P_2 = 0.4 kPa, overpressure of gas forcing out the polyethersulfone solution P_3 = 10.1 kPa and overpressure of gas forcing out the alginate solution P_1 = 20.3 kPa (in the first two batches), and then 25.3 kPa. Nine batches of microcapsules were obtained, at applied impulses frequencies increase from 10 to 60 Hz. Table 3 presents photographs of selected batches, the applied frequency (*f*), average diameter of microcapsules (D_A) together with the variation coefficient (*VC*), and membrane structure parameters.

During the production of the second batch of capsules (P_1 = 20.3 kPa, f = 20 Hz) a rapid decrease in the flow of alginate solution was observed, hence the overpressure of the gas forcing out the core liquid was raised to 25.3 kPa. The remaining batches presented in Table 3 were obtained using the higher overpressure (P_1). In some batches, alginate cores not covered by a membrane, appeared.

When the frequency of applied electrical impulses (*f*) raised, the diameter of obtained microcapsules decreased significantly (Fig. 3).

In the frequency range of 10 to 60 Hz, average capsule diameter (D_A) decreased from 2.70 to 0.96 mm. Simultaneously, the variation coefficient of average diameter (*VC*) raised significantly from 5.7% to 30.4%, and the shape of obtained capsules changed from spherical to oval, while some microcapsules contained polymer strands.

3.4. Analysis of the internal structure of second-series microcapsules

The data presented in Fig. 4 shows, that microcapsules obtained in this series had very thin membranes, and their thickness (apart from the polymer bulge) changed to a small extent, when electric impulse frequency increased. Their average thickness (parameter B) was from 24 to 106 µm. Membrane thickness at the thinnest part varied from 7 to 32 µm.

Table 2
Equations of curves fitting the changes in microcapsule structural parameters as a function of voltage and values of determination coefficient R^2

Structural parameter	Fitting curve equation	Determination coefficient R^2
A	$y = 48.14459 - 9.68916x + 0.64493x^2$	0.9477
B	$y = 68.84774 + 19.64989x - 1.4946x^2$	0.4759
N	$y = 252.40844 + 64.7128x - 5.99543x^2$	0.5480

Parameter N (polymer bulge) was the most sensitive one in regard to changes in electric impulse frequency, and was the highest at the lowest values of f (10 and 20 Hz). Other seven batches had a significantly thinner polymer bulge with thickness below 250 µm. These are batches obtained at f over 20 Hz.

It may be concluded that the increase of electrical impulse frequency (*f*) caused the polymer bulge (N) to diminish, what resulted in the formation of more symmetric membranes. Changes in the size of parameter N as a function of electric impulse frequency (*f*) could be described quite well by a second-degree polynomial of equation presented in Fig. 4.

3.5. Study of the influence of electric impulse duration

In the third experimental series, the influence of changes in the duration of electrical impulses (τ) on microcapsule size and structure was studied. The remaining process parameters were kept constant, at: voltage U = 17 kV, impulse frequency f = 20 Hz, distance of the head tip from the surface of the gelling liquid L = 50 mm, overpressure of the gas forcing out alginate solution P_1 = 25.3 kPa, glycerol, P_2 = 30.4 kPa, polyethersulfone solution, P_3 = 10.1kPa. Impulse duration was increased in consecutive batches from 1 to 9 ms (by 1 ms each time).

Photographs of selected batches, the values of applied τ, average microcapsule diameter sizes (D_A) together with their variation coefficients (*VC*) and membrane structure parameters (A, B and N) are shown in Table 4.

In this series, seven microcapsule batches were obtained. During the production of the batch at τ = 7 ms, problems with the flow of core liquid occurred, so the experiment was not continued. Starting with batch AGP-140, small quantities of alginate cores, which were not covered by the membrane, were found in the gelling bath of each batch.

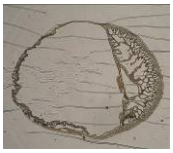
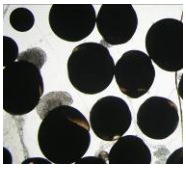
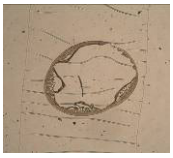
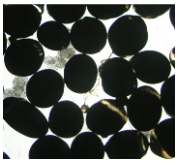
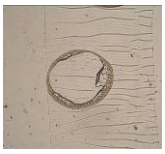
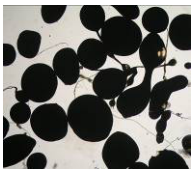
As shown in Fig. 5, average microcapsule diameter (D_A) decreased gradually from 2.28 to 1.37 mm as τ increased. The variation coefficient of microcapsule diameter (*VC*) changed from 22% to 6%, with the lowest values for batches obtained at τ = 4 and 5 ms.

The decrease in average microcapsule diameter (D_A) as a function of the duration of electrical impulses (τ) may be approximated well by a second-degree polynomial with the equation shown in Fig. 5. The determination coefficient is quite high: R^2 = 0.9467.

3.6. Analysis of the internal structure of third-series microcapsules

Changes in internal structure parameters (A, B, N) of alginate-polyethersulfone microcapsules obtained in the series with variable electrical impulse duration (τ) are shown in Fig. 6.

Table 3
Alginate-polyethersulfone microcapsules formed at different electric impulse frequencies *f*

Batch symbol	General view of the batch	Microcapsule cross-section	*f* Hz	D_A mm	VC D_A %	A μm	B μm	N μm
AGP-136			10	2.70 ± 0.15	6	16 ± 2	81 ± 16	712 ± 218
AGP-129			30	1.43 ± 0.29	20	25 ± 12	96 ± 33	235 ± 28
AGP-130			35	1.14 ± 0.27	24	32 ± 2	87 ± 16	195 ± 17
AGP-133			50	0.88 ± 0.26	30	16 ± 6	106 ± 46	198 ± 16
AGP-135			60	0.96 ± 0.25	26	21	58	119

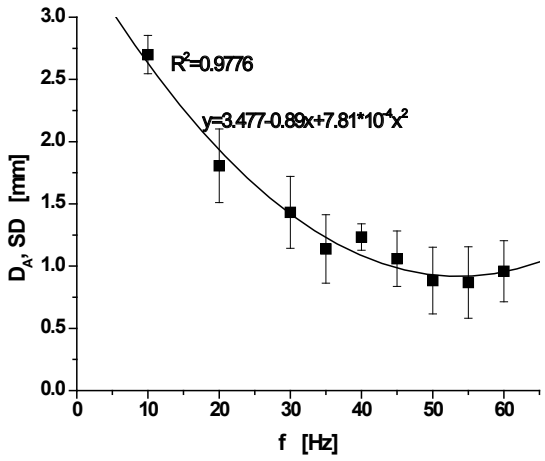


Fig. 3. Relation between electric impulse frequency *f* and average microcapsule diameter D_A.

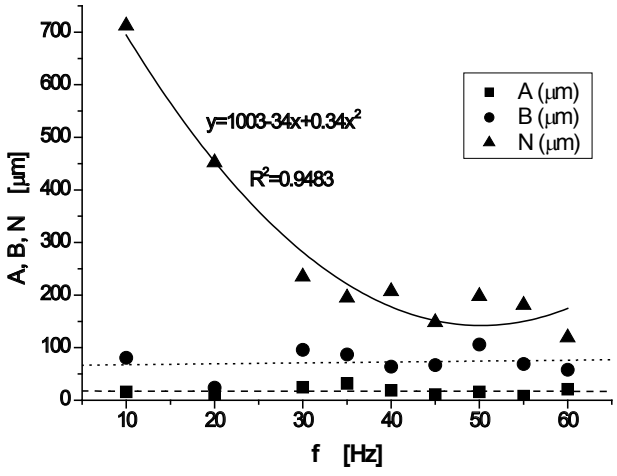
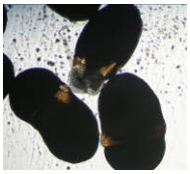
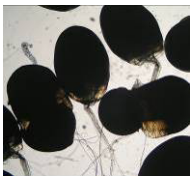
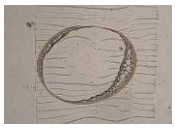
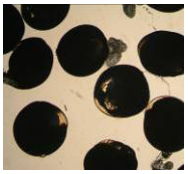
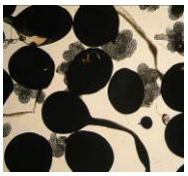
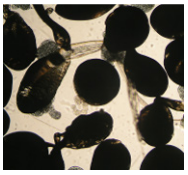


Fig. 4. Changes in membrane thickness of microcapsules obtained at different values of electric impulse frequency *f*.

Table 4
Microcapsules formed at different durations of electrical impulses τ, external appearance of batches, average diameter of batch D_A, internal microcapsule structure and structural parameters

Batch symbol	General view of the batch	Microcapsule cross-section	τ ms	D_A mm	VC D_A %	A μm	B μm	N μm
AGP-137			1	2.28 ± 0.43	19	5 ± 1	22 ± 19	394 ± 75
AGP-138			2	1.96 ± 0.39	20	7 ± 4	146 ± 102	319 ± 242
AGP-140			4	1.51 ± 0.09	6	14 ± 9	78 ± 29	180 ± 123
AGP-142			6	1.37 ± 0.15	11	7 ± 3	52 ± 16	178 ± 21
AGP-143			7	1.40 ± 0.31	22	8 ± 3	39 ± 2	117 ± 25

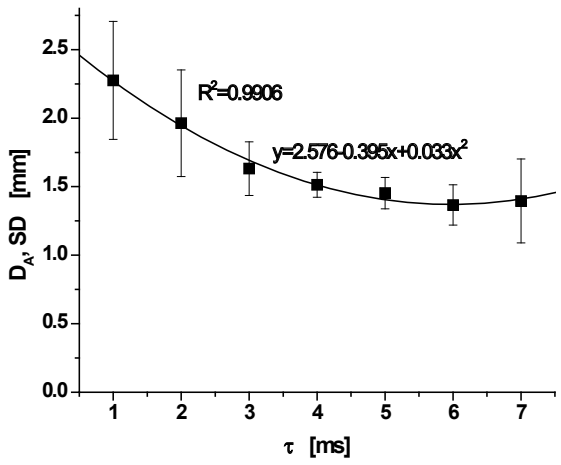


Fig. 5. Changes in average microcapsule diameter D_A as a function of changes in electric impulse duration τ.

As presented in Fig. 6, membrane thickness at the thinnest part (parameter A) was practically constant, varying from 5 to 9 μm: only for batch AGP-140 (τ = 4 ms) it was 14 μm. Average membrane thickness (B) decreased systematically from 147 to 27 μm, apart from the first batch (τ = 1 ms), for which it was very low and equal to 22 μm. Attempts to correlate changes in parameter B as a function of increasing electrical impulse duration (τ) with a polynomial or straight line were unsuccessful. As shown in Table 5, obtained determination coefficients are very low.

After excluding from analysis the batch of microcapsules obtained at the lowest value of τ = 1 ms (AGP-137), the relation between electric impulse duration and average membrane thickness B may be described with a second-degree polynomial.

The greatest differences were observed for parameter N (polymer bulge), which gradually decreased from 394 μm (AGP-137) to 117 μm. The nature of the relation between N and impulse duration (τ) may be described with a second-degree polynomial with the equation presented in the chart (Fig. 6).

3.7. Analysis of results

Cumulative charts were prepared to illustrate, how changes in individual electric parameters of the electrostatic triple droplet formation process affected average diameter and internal structure of AGP microcapsules.

The parameter E is proposed as the independent variable described by Eq. (1):

$$[\text{V min ml}^{-1}] \quad (1)$$

where v_1, v_2 and v_3 represent flows of alginate, glycerol and membrane-forming solution, respectively.

The course of changes in average diameter (D_A) as a function of the increase in parameter E was very similar for all examined electrical parameters involved in microcapsule production process and was quite well described using second-degree polynomials. The equations are contained in Table 6. The lowest correlation was observed for the series of microcapsules obtained at variable values of electric impulse frequency (f). In other two cases, determination coefficients were over 0.9.

Analysis of the chart (Fig. 7) demonstrates, that ranges of changes in the average diameter size (D_A) in the series with a variable U and f were very similar. The smallest range of changes in D_A was noted for the series obtained with a variable duration of electric impulses (τ), while the rate of changes was similar in the series with a variable f. It indicates that, precise regulation of microcapsule dimensions may be possible.

It is possible to describe internal microcapsule structure quite well using the following membrane symmetry parameters: N/B and N/A, which show, how the polymer membrane varies in terms of thickness. Fig. 8 shows the values of average symmetry coefficients for individual microcapsule series.

The value of parameter N/B_A was comparatively low in all experimental series and varied within a narrow range from 3.03 for batches with variable U to 5.19 in batches with variable τ. The second parameter of symmetry – N/A_A – had significantly higher values: from 14.60 (U series) to 23.05 (τ series). The most symmetric membranes were obtained in the batch with a variable voltage (U), and the least symmetric, in the experimental batches with a variable duration of electrical impulses (τ).

Changes in values of both symmetry parameters resulted primarily of large differences in the size of the polymer bulge (Fig. 9) occurring in the first two, or three (in the case of the series with variable U), chronologically obtained batches

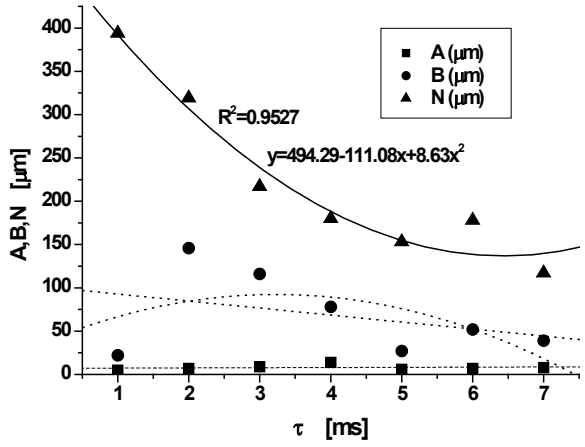


Fig. 6. Changes in membrane thickness of third-series microcapsules as a function of the duration of electric impulses τ.

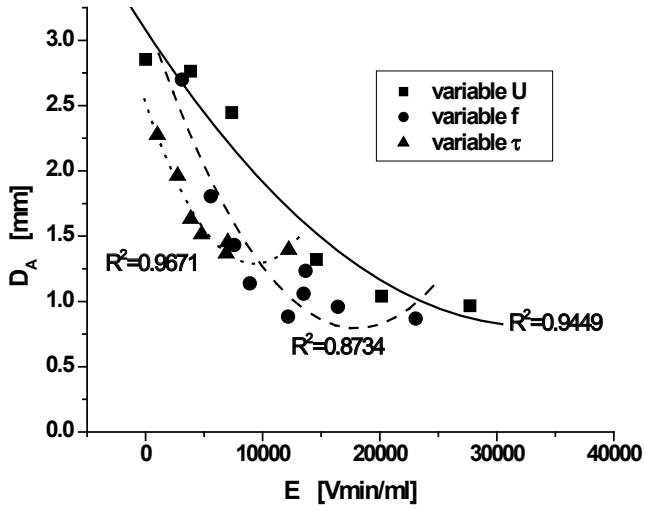


Fig. 7. Changes in average AGP microcapsule diameter D_A in the series with variable voltage U (continuous line), electric impulse frequency f (dashed line) and impulse duration τ (dotted line).

Table 5
Equations of curves fitting the changes in parameter B as a function of the duration of electric impulses τ and values of determination coefficients R^2 for all obtained batches, and excluding the batch obtained at τ = 1 ms

Fitting curve equation	Determination coefficient R^2
$y = 38.57 + 33.45x - 5.19x^2$	0.3055
Fitting curve equation without AGP-137	Determination coefficient R^2 without AGP-137
$y = 280.67 - 76.39x + 6.02x^2$	0.9216

Table 6
Equations of curves fitting changes in average microcapsule diameter D_A as a function of parameter E estimated for values of electric parameters of the encapsulation process and value of the determination coefficient R^2

Electric parameter	Fitting curve equation	Determination coefficient R^2
U	$y = 3.07776 - 1.36789E\text{-}4x + 2.06355E\text{-}9x^2$	0.9449
F	$y = 3.18421 - 2.65841E\text{-}4x + 7.39195E\text{-}9x^2$	0.8734
T	$y = 2.51738 - 2.64088E\text{-}4x + 1.41839E\text{-}8x^2$	0.9671

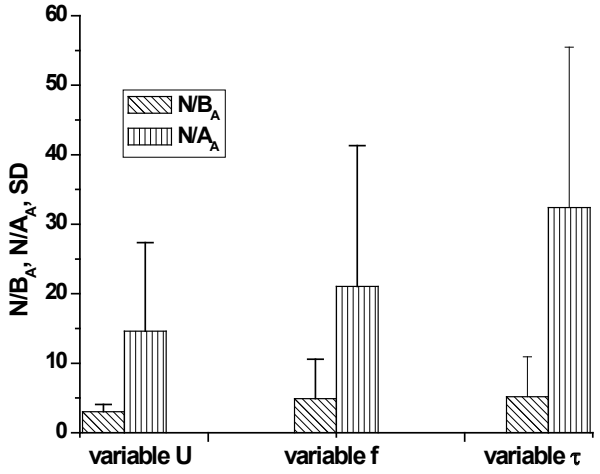


Fig. 8. Average values of membrane symmetry parameters N/B_A and N/A_A for microcapsules obtained in all series with variable electric parameters.

of each series. As the value of each of electric parameters of the encapsulation process raised, a significant decrease in the size of the polymer bulge occurred. In the area marked in the chart, the value of parameter N for the obtained microcapsules changed within a narrow range from around 120 to 200 μm.

4. Conclusions

As a result of the conducted studies, it may be concluded, that values of all examined electric parameters involved in the three-nozzle process of microcapsules formation significantly influenced on the size of obtained microcapsules, the uniformity of their batches in terms of size, and their internal structure.

Average microcapsule diameter (D_A) decreased as each of studied electric parameters increased; however, the range of changes of D_A was similar in series with variable voltage (U) and frequency (f), and smaller in the case of the series, where impulse duration (τ) was varied.

As average diameter (D_A) decreased, the uniformity of obtained microcapsules in terms of size also decreased, as demonstrated by the increase of the variation coefficient (VC) of the average diameter (D_A). The series with a variable electric impulse duration (τ) was an exception to this pattern, as the highest values of VC D_A were noted at the start and the end of the series (VC increased from 19% to 22%), and the lowest, in the middle of the batch at τ = 4 and 5 ms (VC = 6 and 8%, respectively). Similar results have previously been recorded in studies on electrostatic formation of alginate microspheres [2,11,16].

The influence of the value of individual electric parameters on microcapsules shape was ambiguous as cylindrical or elongated microcapsules were obtained at both, after the application of high voltages (U), and for low values of impulse duration (τ). Deformed microcapsules, including microcapsules, which contained polymer strands or were coated by a very thin membrane, seemed to occur significantly more often in the series with variable impulse duration (τ); however, this conclusion still requires confirmation.

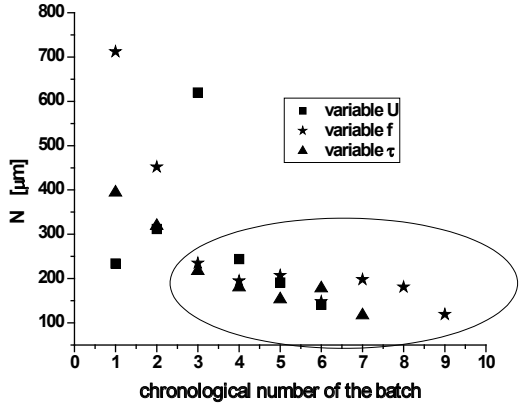


Fig. 9. Changes in polymer bulge size in microcapsule batches obtained with variable U, f and τ.

The electric parameters also affected the internal structure of the microcapsules. Average membrane thickness (parameter B) changed slightly in series with variable U and f, and in the series with variable (τ), it increased slightly as this electric parameter increased. The greatest changes could be observed in the case of the size of the polymer bulge (N), which decreased in all experimental series as the variable electric parameter increased.

The membranes with the most symmetrical structure were formed in the series with variable electric voltage (U).

References

[1] D.S. Yoon, K. Won, Y.H. Kim, B.K. Song, S.J. Kim, S.-J. Moon, B.S. Kim, Continuous removal of hydrogen peroxide with immobilised catalase for wastewater reuse, Water Sci. Technol., 55 (2007) 27–33.

[2] S. Siripattanakul-Ratpukdi, T. Tongkliang, Municipal wastewater treatment using barium alginate entrapped activated sludge: adjustment of utilization conditions, Int. J. Chem. Eng. Appl., 3 (2012) 328–332.

[3] S. Chikhi, F.Z. Ferradji, A. Badis, B. Bouzid, Microbial removal of xylene using free and immobilized *Streptomyces* sp. AB1: bioreactors application, Desal. Water Treat., 57 (2016) 6148–6156.

[4] L. Vacková, R. Stloukal, J. Wanner, The possibility of using encapsulated nitrifies for treatment of reject water coming from anaerobic digestion, Water Sci. Technol., 65 (2012) 1428–1434.

[5] C.Y. Chen, C.M. Kao, S.C. Chen, H.Y. Chien, C.E. Lin, Application of immobilized cells to the treatment of cyanide wastewater, Water Sci. Technol., 56 (2007) 99–107.

[6] B. Bugarski, Q. Li, M.F.A. Goosen, D. Poncelet, R.J. Neufeld, G. Vunjak, Electrostatic droplet generation, AIChE J., 40 (1994) 1026–1031.

[7] D. Poncelet, R.J. Neufeld, M.F.A. Goosen, B. Bugarski, V. Babak, Formation of microgel beads by electric dispersion of polymer solutions, AIChE J., 45 (1999) 2018–2023.

[8] D. Lewińska, A. Chwojnowski, J. Jankowska-Śliwińska, Microcapsules containing biologically active substances, especially living cells and/or microorganisms, alternatively genetically modified or natural or synthetic for biomedical applications, the way of their manufacturing and the device for this purpose, PATENT PL 208383 (2007).

[9] D.Lewińska,A.Chwojnowski,C.Wojciechowski,B.Kupikowska-Stobba, M. Grzeczkowicz, A. Weryński, Electrostatic droplet generator with 3-coaxial-nozzle head for microencapsulation of living cells in hydrogel covered by synthetic polymer, Sep. Sci. Technol., 47 (2012) 463–469.

[10] J. Xie, C.H. Wang, Electrospray in the dripping mode for cell microencapsulation, J. Colloid Interface Sci., 312 (2007) 247–255.

[11] A. Jaworek, Electrostatic micro- and nanoencapsulation and electroemulsification: a brief review, J. Microencapsulation 25 (2008) 443–468.
[12] M. DeCastro, G. Orive, R.M. Hernandez, Comparative study of microcapsules elaborated with three polycations (PLL, PDL, PLO) for cell immobilization, J. Microencapsulation 22 (2005) 303–315.
[13] J.R. Hwang, M.V. Sefton, The effect of polymer concentration and a pore-forming agent (PVP) on HEMA-MMA microcapsule structure and permeability, J. Membr. Sci., 108 (1995) 275–268.
[14] A. Chwojnowski, C. Wojciechowski, K. Dudziński, E. Łukowska, L. Granicka, New type of hollow fiber membrane for cell and microorganisms cultivation and encapsulation, Desalination, 240 (2009) 9–13.
[15] D. Lewińska, B. Kupikowska-Stobba, M. Grzeczkowicz, Influence of Flow Rates on Alginate-polyethersulfone Microcapsule Structure, K. Konieczny, I. Korus, (Eds.) Membranes and Membrane Processes in Environmental Protection, Monographs of the Environmental Engineering Committee PAS, Warsaw-Gliwice 2014, Vol. 118, pp. 367–380.
[16] D. Lewińska, S. Rosiński, A. Weryński, Influence of process conditions during impulsed electrostatic droplet formation on size distribution of hydrogel beads, Artif. Cells, Blood Substitutes Biotechnol., 32 (2004) 41–53.