Electrophoresis of oil-containing edible microcapsules with protein-polyuronic shells

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Abstract

Introduction. The aim of this work is to determine the sign of the charge of microcapsules shells, containing oil composition and to estimate stability of microcapsules with different diameters in the electric field.

Materials and methods. The microcapsules were prepared by complex coacervation method. Remains of electrolytes were removed by dialysis or electro-dialysis. Purified microcapsules were subjected to electrophoresis at 100-400 V/m. Polydispersity was determined by means of our own method.

Results and discussion. Small microcapsules with protein-poliuronate shells moves from the cathode (-) to the anode (+) during electrophoresis. Microcapsules with a diameter much than 35μm are most susceptible to degradation in the cathode space, while remaining stable at low pH values at the anode surface.

Conclusions. Gelatin-Alginat and Gelatin-Hyaluronat shells have a negative electric charge. Electrophoresis can be used to obtain required diameter of coacervate microcapsules. High stability of the microcapsules in the anode space (acid) confirms the validity of their introduction into fermented dairy products.

Introduction

Food fortification with biologically active substances in microencapsulated form is of interest to improve their sustainability, alimentary and biological value [1]. Microcapsules suitable for incorporation into food products must have a fully edible shells consisting of biopolymers. Such microcapsules can be prepared by complex coacervation of differently charged polyelectrolytes [2]. Polyelectrolytes are important components of many foods. So, the fermented dairy products contain the positively charged protonated forms of the proteins, but fruit nectars and juices include negatively charged pectin [3]. Therefore, knowledge of the surface charge of the microcapsules is important for predicting their

stability in different types of foods. Electrophoresis is a reliable method to determine the charge of macromolecules of biopolymers [4]. The aim of this work is the experimental determination of the shell charge of microcapsules containing oil composition and sustainability assessment of microcapsules in the electric field.

Materials and methods

Oil composition. Microencapsulated oil composition was formulated from *Juglans regia* nut oil, oil concentrate of carotenes and sunflower seed oil in the ratio of 2:1:1. The concentration of carotene in the final composition was determined by spectrophotometric method [5] and is amounted to $0.209 \pm 0.001\%$ (m). That provides good visibility of microcapsules during their microscopy and photography.

Microcapsules. Edible microcapsules (fig. 1.) were obtained by complex coacervation method (Patent MD-557, BOPI 11/2012, p. 31-32.). Initially, an aqueous gelatine solution and oil composition was stirred to form O/W emulsion. The emulsion was cooled and the rate of stirring was reduced to create conditions for coacervation process. The dehydration of gelatine shells with sodium sulphate and fixing of the shells by means of salts of alginic or hyaluronic acids was followed.

Dialysis and electro-dialysis. Suspensions of microcapsules in supernatant solutions were purified by dialysis through cellulose membranes for 1-2 days. Good results were obtained using electro-dialysis at 100V/m during 30-60min until the resistance of the 1cm of supernatant solution reached $\approx 100k\Omega$, which corresponds to the conductivity of approx. $1 \cdot 10^{-3}$S/m. This is only one order of magnitude more than conductivity of distilled water, approx. $5 \cdot 10^{-4}$S/m [6], that talks about almost complete removal of sodium sulphate from the supernatant solutions.

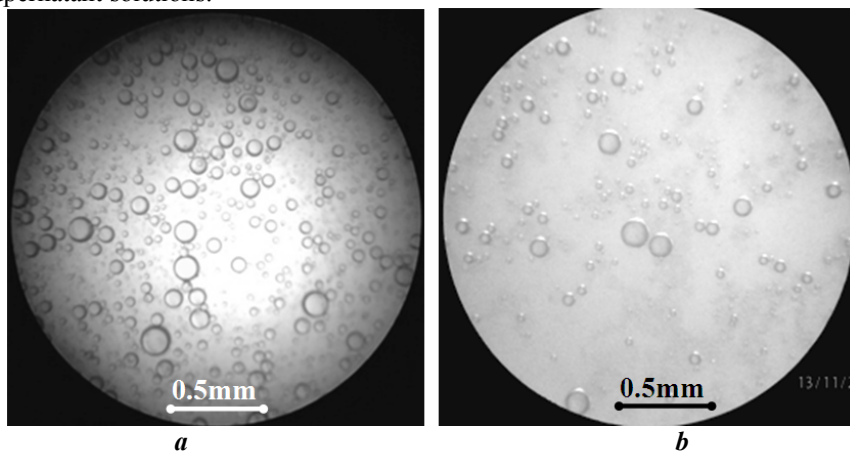**Fig. 1. Microcapsules with Gelatine-Alginate (GelAlg) shells (*a*), and with Gelatine-Hialuronat shells (*b*) - "*in status nascendi*".**

Electrophoresis. Microcapsules, purified by dialysis, were subjected to electrophoresis with graphite or stainless-steel electrodes, introduced in the U-shaped tubes. Parameters for graphite / steel electrodes: distance between cathode and anode surfaces: 10/25cm; Voltage: 10/100V; field intensity: 100/400V·m^{-1}.

Polydispersity measurements. This was determined by our own method. Samples of microcapsules were photographed by the high resolution camera (3-12 megapixels) in the optical microscope in transmitted or lateral light (Figure 1). Real diameter of the observed field of view was 2.1mm. Microcapsules of certain sizes were manual counting in the images, zoomed to 15-20cm. Then, specific volume of the microcapsules with average diameter, the volume of fraction and the volume fraction of microcapsules of a certain size were calculated, using formulas:

$$V_{MC,i} = \frac{\pi <d_i>^3}{6}; V_i = N_{MC,i} \cdot V_{MC,i}; \varphi_i = \frac{V_i}{\sum V},$$

in which: $V_{MC,i}$ - volume of single MC with average diameter $<d_i>$; V_i - volume of fraction i, N_i - number of certain species of microcapsules; φ_i - volume fraction of microcapsules of certain diameter $<d_i>$; $\sum V$ - summary volume of all fractions.

Table 1

Example: calculation of polydispersity for GelHur MC "in nascendi"

$d_{i,min.}$, μm	$d_{i,max.}$, μm	$<d_i>$, μm	$N_{MC,i}$	$V_{MC,i}$, μm^3	V_i, μm^3	φ_i, %
0	11.9	5.95	77	110	8493	0.16
11.9	23.8	17.80	20	2953	59060	1.09
23.8	35.7	29.65	37	13648	504980	9.35
35.7	47.6	41.55	25	37559	938970	17.4
47.6	59.5	53.40	14	79730	1116222	20.7
59.5	71.4	65.25	7	145459	1018212	18.8
71.4	83.3	77.15	2	240440	480880	8.90
83.3	95.2	89.00	2	369121	738242	13.7
95.2	107.1	100.95	1	538664	538664	9.90

Results and discussions

Nearly all known methods of obtaining a coacervate microcapsules with polyelectrolyte membranes involve the use of dehydrating agents, particularly sodium sulphate [1, 2]. It in turn causes vigorous electrolysis of water, which distorts the results of electrophoresis. Therefore there is a need of purification of the supernatant solution from the excess of sodium sulphate, maintaining microcapsules intact. We noticed that soaking in water causes destabilization of the microcapsules shells. This phenomenon may have at least two reasons. First, this is the removal of sodium sulphate from polyelectrolyte membranes. Second, slowly hydrolysis of polyelectrolyte complexes is possible. Filtration or centrifugation caused partial coalescence of the microcapsules in the irregular dodecahedrons and the further destruction of the microcapsules, similar to the destruction of foams [7]. The best results among all tested purification methods showed the dialysis of microcapsules suspensions through cellulose membranes. The dialyzed suspension was stirred, and the microcapsules were subjected to electrophoresis. A destruction of microcapsules at the anode wasn't observed even after 10-20min after the start of electrophoresis. But in the small time interval, the microcapsules were deformed and even broken at the cathode (fig. 2, right). In the cathode space appeared free oil, indicating extensive destruction of shells of the microcapsules.
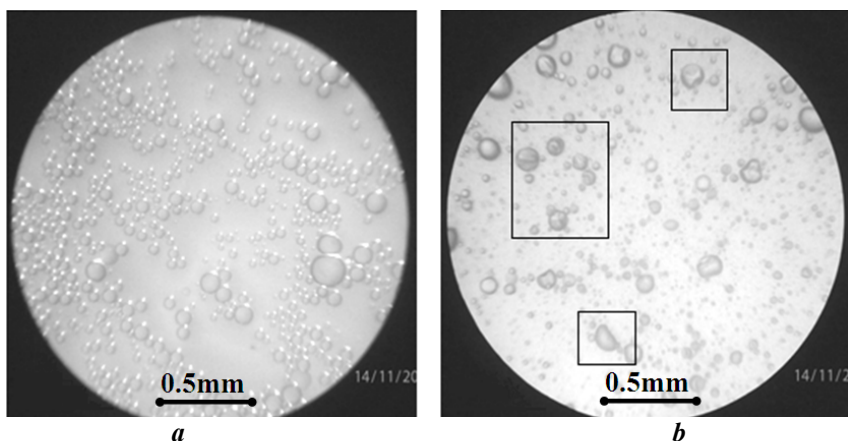
**Fig. 2. The microcapsules coated with GelHur after electrophoresis:
at the anode (*a*); at the cathode (*b*); broken MCs are contoured.**

We explain the destruction of the microcapsules in the cathode space as follows. Electrolysis of water takes place rapidly in the presence of even small, trace amounts of electrolytes, such as sodium sulphate, and leads to a change pH near electrodes.

Cathode (−): $2H_2O \rightarrow H_2 + OH^- - 2e^-$; *water reduction*

Anode (+): $2H_2O \rightarrow O_2 + 4H^+ + 2e^-$; *water oxidation*

Electrolysis: $6H_2O + 2Na_2SO_4 \rightarrow (2H_2 + 4NaOH)_{cathode} + (O_2 + 2H_2SO_4)_{anode}$

It is known that the complexes of protein with polyuronic acids and their salts are particularly stable only in a narrow range of pH, namely, 2-5 [8]. It can be assumed that this interval is not respected on the cathode, whereby the microcapsules become unstable. We note that, despite apparent simplicity, is very difficult to measure the pH values in the immediate vicinity of the electrode, but it is possible to do in an indirect way, using electrochemical model. Thus, when experimentally determined current strength is 100μA, in 15 minutes of electrolysis must be formed approx. $1 \cdot 10^{-6}$ mol of ions, according to Faraday laws of electrolysis. Naturally, near the cathode and the anode are formed the equal number of OH^- and H^+ ions equivalents, respectively. Assuming that the volume of the near-electrode space is about 1ml or 0.001L, the molar concentrations of ions constitute approx. 0.001mol/l. Therefore:

$[H^+]_{anode} \approx 0.001$ mol/L => pH_{anode} =3.

$[OH^-]_{cathode} \approx 0.001$ mol/L => $pOH_{cathode} = 3$ => $pH_{cathode} = 14 - 3 = 11$

These estimations shows that microcapsules remain near the anode in the pH values, "friendly" to them. At the same time, at the cathode is very easily achieved the conditions for their destruction. It is quite obvious for us, that the reason is the local pH at the cathode, equal to 11, much greater, than that of the isoelectric point of the gelatin, equal to 4.8-5.0 [8]. From the above it follows that the destruction of gelatin-alginate membranes in the cathode space occurs by the following mechanism:
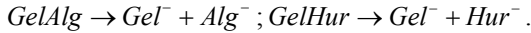
1.Neutralization of gelatine macromolecules at near its isoelectric point (pI ≈ 4.8):

$$Gel^+ + OH^- \rightarrow Gel^0 + H_2O \, ;$$
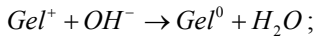
2.Acquisition of negative charge by gelatine molecules at pH > pI:

$$Gel^0 + OH^- \rightarrow Gel^- + H_2O \, ;$$
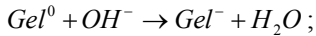
3.Unbundling of polyelectrolyte shells because of repulsion of negatively charged macromolecules of gelatin and polyuronic salts :

$$GelAlg \rightarrow Gel^- + Alg^- \, ; GelHur \rightarrow Gel^- + Hur^- \, .$$

As a matter of fact, the described process is not pure electrophoresis. Rather, this process can be called as "destruction of the microcapsules, caused by electrolysis", but is not can be called as "electrolysis of microcapsules".

High stability of the microcapsules in the anode space is further supported by statistical data for polydispersity of different samples (fig. 3.).
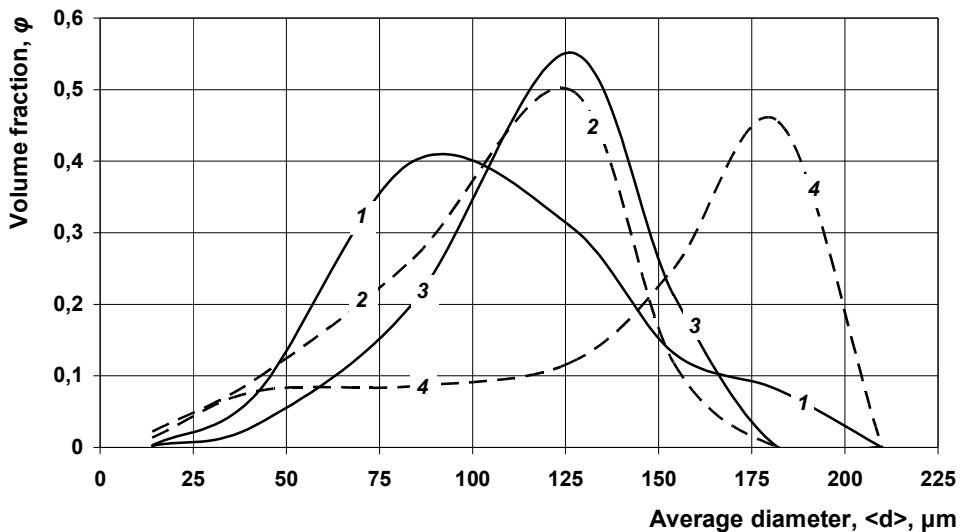

**Fig.3. Statistical distributions of microcapsules coated with GelAlg:**
1 – "in nascendi"
2 – on surface
3 – cathode (-)
4 – anode (+)

Maximum of the distribution curve of microcapsules with different diameter, taken directly from the reactor, "in (status) nascendi", is located at 75-100μm. In comparison with the other curves, this curve is shifted towards the smaller microcapsules. At the same time, the polydispersity of the microcapsules in the anode space is practically identical to polydispersity of the microcapsules which were not subjected to electrophoresis, from the surface of the dialyzed suspension. In these cases, the maximum of the distribution curve is situated at <d> ≈ 125μm. Such shifts towards larger diameters can be explained due to different densities, which cause flotation of large microcapsules and deposition of the small ones [9]. At the beginning of the DC passing, a very intensive migration of the

microcapsules from the negative electrode (cathode) to the positive (anode) was observed. The process speed was 0.5-1.0mm/min. Movement of the microcapsules in the opposite direction, from the anode to the cathode, was much slower (near 0.1mm/min). Migratory MC-flows from both electrodes gradually slows down, forming incoherent suspensions in the U-shaped tube. These were separated from the near-electrode spaces, and always were displaced noticeably toward the anode (+).

The totality of all observed phenomena, demonstrates that the charge of microcapsule shells, which were not subjected to electrophoresis, is negative. Negative charge is also conserved in the initial stages of the electrophoresis. Some "runaway" of microcapsules from the anodic space suggests that recharging of MC-shells occurs at the anode surface, probably, because of two possible ways:

1. Neutralisation of polyuronic anions by H^+, liberated during the electrolysis:

$$Alg^- + H^+ \rightarrow HAlg \ ; Hur^- + H^+ \rightarrow HHur \ .$$
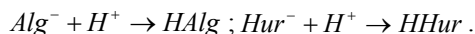
2. Further high-positive charging of gelatin molecules.

Interestingly, that the first presumed phenomenon should not lead to the destruction and destabilization of the microcapsule shell, and can even strengthen them thanks to the formation of insoluble polyuronic acids at the pH < 3 [10].

As for second phenomenon, the high positive charging of gelatin can cause the intra-molecular repulsion and critical straightening of macromolecules. This in turn should lead to the destruction of the shells. However, this straightening is possible only at very low pH values [11], which can not be achieved on the basis of electrochemical model, discussed above.

Insofar as the destruction of the microcapsules at the anode on the investigated electrophoresis conditions are not observed, the first process is most probable.

Statistical analysis shows that the mobile and stable fraction (fig. 4., left) consists of microcapsules having a diameter less than 35µm. A polydispersity of this fraction obeys the law of normal distribution (fig. 4., right).
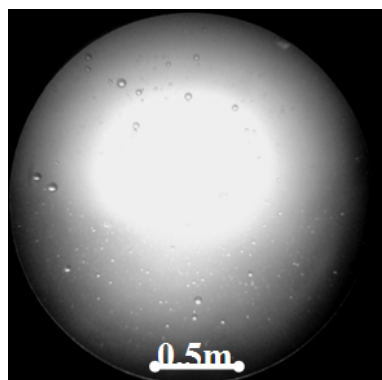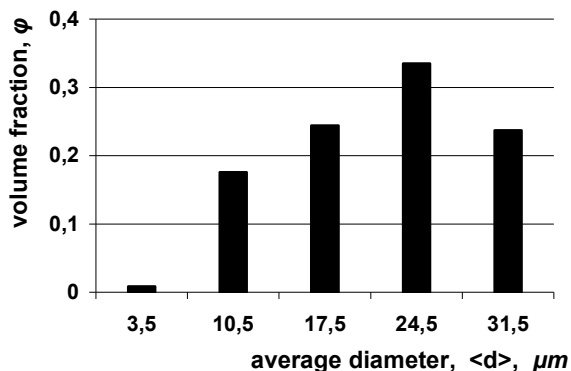
<center>*a*   *b*</center>

**Fig. 4. Stable fraction with GelAlg shells (*a*);
its statistical distribution (*b*).**

The effect of migration of the microcapsules that do not suffer destruction in the electrode spaces is of interest for further practical applications. In particular, it will be possible to separate unstable large microcapsules and stable small ones, which are most valuable for food and cosmetic industries.

Conclusions

1. In an electric field the microcapsules with gelatine-polyuronic shells moves from the cathode (-) to the anode (+). This electrophoresis clearly confirms the negative charge of MC-shells, and further suggests the mechanism of their neutralization and recharge.
2. Microcapsules with diameter > 35µm undergo the electrochemical destruction at the cathode, constantly remaining in the cathode space because of their low density. At the same time, a fraction of the microcapsules with a diameter less than 35µm is practically not destroyed by the electric field, leaving the cathode space during electrophoresis. This effect can be used for separation of microcapsules of different diameters.
3. At the anode, the destruction of microcapsules at the investigated parameters of electrophoresis does not occur, because is not reached the critical level of pH, smaller than 2. High stability of the microcapsules in the anode space (containing H^+) confirms the validity of their introduction into dairy products.

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