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FORMATION OF MICROCAPSULES' BIOPOLYMERIC SHELLS: ELECTROCHEMICAL ASPECTS

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Abstract: Study of the formation of comestible bio polymeric shells of microcapsules is actually for food science. Electrochemical measurements were carried out using a working unit with digital multi-tester in ohm-metric mode as main device. The stages of formation of complex-coacervate shells, its neutralization and recharging, are accompanied by visible and informative changes in the resistance values. Dynamical electrochemical measurements can be proposed as the effective tool for investigating and controlling the processes of simple and complex coacervation of polymer electrolytes, taking place in the microencapsulation process.

Keywords: *microencapsulation, coacervation, water retaining agents (WRA), sodium sulfate, gelatin, alginate, electrochemistry*

Introduction

Microencapsulation is a relatively new method of preserving biological activity and targeted delivery of important bio compounds [1]. One of the areas of microencapsulation is the enrichment of products with polyunsaturated fatty acids. A feature of microencapsulation of lipids is that the formation of microcapsules occurs in an aqueous medium [2]. The microcapsules obtained in this way are extracted from the aqueous medium and introduced into food products. The most interesting and useful are the microcapsules, the shells of which consist of one or more comestible biopolymers, which are polyelectrolytes. There is a sufficiently large number of works devoted to the interaction of biopolymers in food systems [3...6]. However, the processes of electrochemical interaction of the components of the microcapsules' shells are not sufficiently clarified. So, an electrochemical study of the biopolymeric microcapsules' shell formation is actually and is of interest.

1. Materials and methods

Measurements of the resistance, pH, temperature, setting of the feed rate of the reagents and mixing were carried out using a working unit connecting the functions of the reactor and the control and measuring system (Figure 1). The main measuring instrument was the digital multi-tester "UT33C" (Shenzhen Sunkoo-Reid Electronic Co., China), used in the ohmmeter mode. The ohmmeter electrodes were rigidly fixed in the reactor to ensure reproducibility of the measurements. The pH of the solutions was adjusted by the addition of citric acid crystals. The pH and temperature were monitored throughout the experiment.

The introduction of the same volume of solutions and measurement of the system resistance was carried out with a periodicity of 60 seconds at a constant rate of mixing. The dependence of the resistance of the system under study was plotted in function of the real concentration of added reagents in the solution. In addition, the mole fraction of salt and the ionic strength of the solution were calculated.

2. Results and discussions

The MCs' shell obtained by coacervation of biopolymers from the aqueous solution are in a swollen "semi-liquid" state. This makes it difficult to separate the MCs from the supernatant liquid, which leads to their coalescence and destruction.

2.1. Water-absorbing agents for shells

The formation of the coacervate shell must be accompanied by its dehydration under the action of water-removing factors: anhydrous salts and alcohols. It should be noted that the term "desiccant" is not entirely correct for the process of coacervation occurring wholly in an aqueous medium in which the polymers are not in a "wet" state but in a swollen state. The role of the water-removing factor is to reverse the process of swelling of the coacervates. The water-withdrawing factors must ensure the process of syneresis (self-compression) of the gel, in other words, turn it into a more stable shell in the form of a dense thin film.

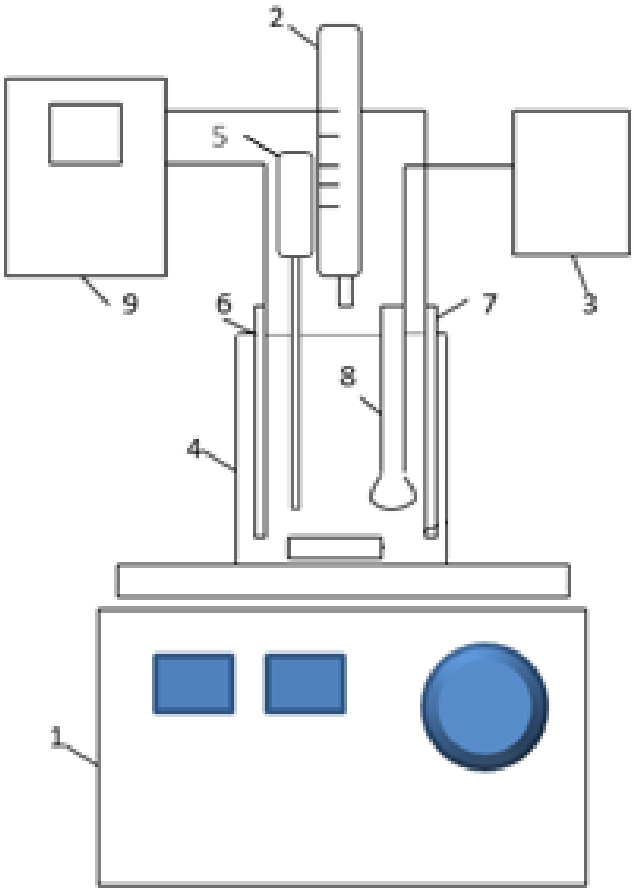

Figure 1. Installation. *1* - magnetic stirrer, heater; *2* - reagent dispenser; *3* - pH-meter; *4* - reactor; *5* - thermometer; *6*, *7* – ohm-metric electrodes; *8* - pH-sensitive electrode; *9* – ohmmeter

The drainage and water-withdrawal capacity can be estimated from the calculation of water's chemical absorption, calculated for 100 g of the corresponding reagent, the results of which are given in Table 1.

Salts of divalent metals ($CaCl_2$, $CuSO_4$, $FeSO_4$, etc.) give the corresponding hydroxides in alkaline media and therefore cannot be used as water-removing agents at pH > 7. Carbonates and alkaline acetates are not suitable for acid media (pH <7), where CO_2 or CH_3COOH will be released, respectively. These salts increase the pH of the medium during hydrolysis. The salts of Ca^{2+}, Cu^{2+}, Fe^{2+} and other polyvalent metals precipitate the anions of pectic substances and polyuronic acids. Metal ions have different effects on proteins, which are macromolecules with variable charge (zwitter-ions). Precipitation occurs when the protein molecules are negatively charged at pH > pI [3, 4].

At first glance, this property should only contribute to the formation of a denser shell.

Table 1

Water-absorption capacity of some common water-removing agents (WRA)

WRA formula	Hydratated WRA formula	Water per 100g of WRA, g.
$CaCl_2$	$CaCl_2 \cdot 6H_2O$	97.3
$CuSO_4$	$CuSO_4 \cdot 5H_2O$	56.2
$FeSO_4$	$FeSO_4 \cdot 7H_2O$	82.9
Na_2CO_3	$Na_2CO_3 \cdot 10H_2O$	170.0
Na_2SO_4	$Na_2SO_4 \cdot 10H_2O$	126.8
C_2H_5OH abs.	$C_2H_5OH \cdot 5H_2O$	195.7
CH_3COONa	$CH_3COONa \cdot 3H_2O$	65.9

However, the interaction of metal ions with polyanionic macromolecules leads to neutralization of the negative charge of a high molecular weight poly-anion, and hence to the coagulation of macromolecules.

Thus, this can lead to an excessive strengthening of the biopolymer shell, which will lead to the destruction of the microcapsule. Anhydrous sodium sulfate is devoid of many disadvantages of named dehydrating agents. This salt is easy to clean and to regenerate, it absorbs a large amount of water, and doesn't undergo hydrolysis.

Introduction of the sodium sulfate into the system under study doesn't affect the pH value directly. In addition, sodium sulfate can be used at any pH values, generated by other components of the mixture.

This property is particularly important for microencapsulation, since pH has a decisive role in the formation of protein-polysaccharide shells of microcapsules [5, 6].

Due to its features and being used in food industry, sodium sulfate is the most suitable dehydrating reagent for formation of biopolymer shells of microcapsules.

Sodium sulfate forms recently described heptahydrate [7, 8]. This causes its unique solubility in function of temperature (Figure 2), probably, affecting its properties as a WRA.

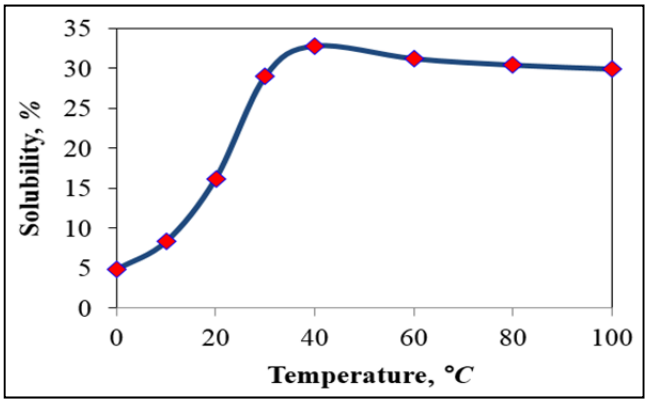

Figure 2. Unusual temperature dependence of sodium sulfate solubility [9]

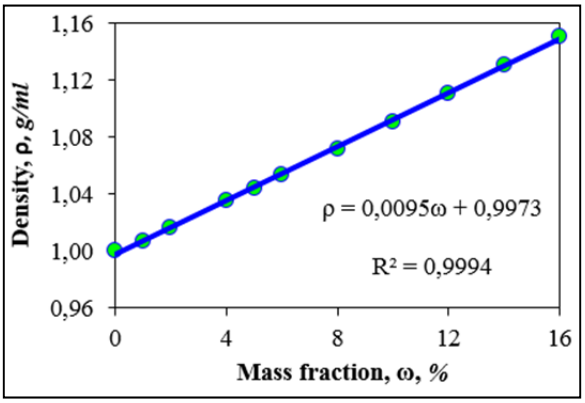

Figure 3. Densities of Na_2SO_4 solutions at 20**°C**

At the same time, in the temperature range from 10 to 35°C, which corresponds to the process of microencapsulation, this dependence is almost linear. To avoid precipitation of hydrates at 20…25°C, 16% sodium sulfate solutions were used. Advantage of this reagent is the good linear dependence of density in function of concentration (Figure 3).

2.2. Influence of sodium sulfate on the formation of the primary gelatin shell

When sodium sulfate is added to distilled water, three regions of resistance values are observed (Figure 4). When the concentration is increased to 4%, the solution resistance is sharply reduced. In the range from 4 to 6%, the resistance drops slowly. Finally, at concentrations greater than 6%, the resistance of the solution increases rapidly. A sharp increase in the resistance of the solution with concentrations bigger than 6% can be explained by a decrease in the mobility of the conductive ions caused by the lack of free water molecules. In other words, it is under such conditions that sodium sulfate begins to act as a water-removing agent.

Results of electrochemical measurements, (Figure 4), shown that solution resistance changes abruptly at pH = 3.5. Therefore, we believe that in these conditions reagent works most effectively.

The change in the resistance of solutions containing gelatin is more complex, that in case, described above (Figure 1, b). Of greatest interest is the section corresponding to 6.2 ... 6.8%, at which the resistance of the solution remains practically unchanged. The presence of this plateau can be explained by the gradual dehydration of gelatin molecules. The resistance of the solution remains practically unchanged, since sodium sulfate binds the released water molecules. Dehydration of gelatin leads to its sedimentation in the model system, which does not contain microcapsules. In a system containing phase of lipid drops, gelatin macromolecules are adsorbed on the oil/water interface. Therefore, in the process of microencapsulation, when concentrations of Na_2SO_4 equal to 6.2-6.8% are reached, the shell from adsorbed gelatin is compacted, that is, microcapsules with the solid shells are formed. These observations are consistent with our earlier gravimetric determination of the optimal concentration of Na_2SO_4 (5.4-7.2%), obtained at such pH values [10].

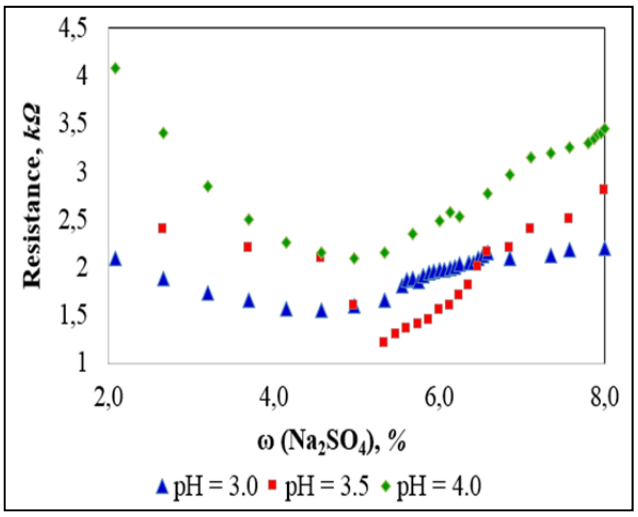

Figure 4. Resistance of Na_2SO_4 solutions at various pH values

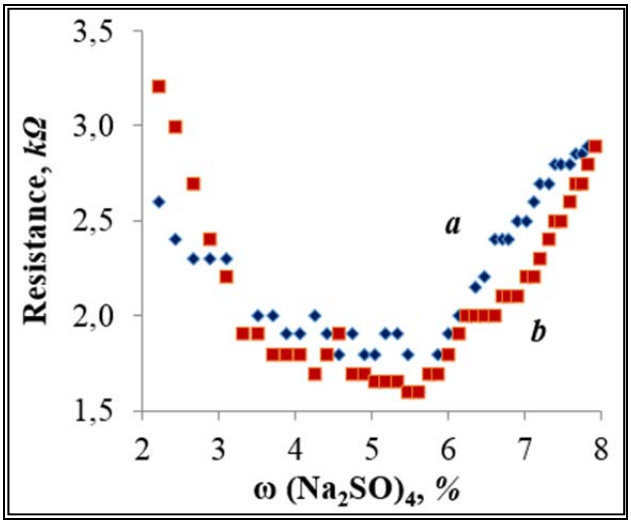

Figure 5. Resistanse of sodium sulfate solution (*a*); in the presense of gelatine (*b*)

2.3. Electrochemistry of coacervation

Addition of a 0.5% solution of potassium alginate to the system leads to a visible increase of its resistance. This indicates an abrupt decrease in the number of ions that carry the charge when small amounts of alginate are added (Figure 6).

Such an electrochemical signal corresponds to the formation of protein-polysaccharide, namely, gelatin-alginate complex, $GelAlg^+$. With the further addition of a certain amount of

alginate, the resistance of the solution increases slowly. In our opinion, this corresponds to a gradual increase in the molecular weight of the $GelAlg^{+}$-complexes while maintaining a positive charge. Finally, with the addition of about 35 ml of the alginate solution, an abrupt reduction in the resistance of the solution occurs. This corresponds to the achievement of a mass ratio of m(Gel) / m(Alg), which corresponds to the formation of neutral compounds $GelAlg^{0}$ and its further precipitation [2]. This process is accompanied by decrease in the system's viscosity, and by the synerhesis of the biopolymer phase, leading to the expulsion of sodium sulfate into the solution:

$$GelAlg^{+} + Alg^{-} \rightarrow GelAlg^{0}$$

$$Na_2SO_{4(GelAlg)} \rightarrow Na_2SO_{4(Aq)}$$

Both factors can contribute to reducing of resistance. Finally, with the addition of even larger amounts of alginate, the formation of negatively charged biphasic micelles ($GelAlg^{-}$) takes place and the viscosity of the solution increases, and as a consequence, the resistance increases:

$$GelAlg^{0}_{(S)} + Alg^{-}_{(Aq)} \rightarrow GelAlg^{-}_{(S+Aq)}$$

Conclusions

1. Salting out of gelatin from the solution, corresponding to the formation of primary gelatinous shells of microcapsules, occurs when the gelatin molecules are dehydrated by means of sodium sulfate and correspond to its content in the solution above 6%.
2. The stages of formation of complex-coacervate shells (GelAlg-complexes), its neutralization and recharging, are accompanied by visible changes in the resistance values. We consider that the drop in resistance at the recharging point is caused both by a decrease in the viscosity of the liquid phase and by the synerhesis of the polymer phase, which causes the transfer of sodium sulfate from the shell to the solution.
3. Dynamical measurements of resistance can be proposed as the effective tools for investigating and controlling the phenomena of simple and complex coacervation of polymer electrolytes occurring in the process of microencapsulation.

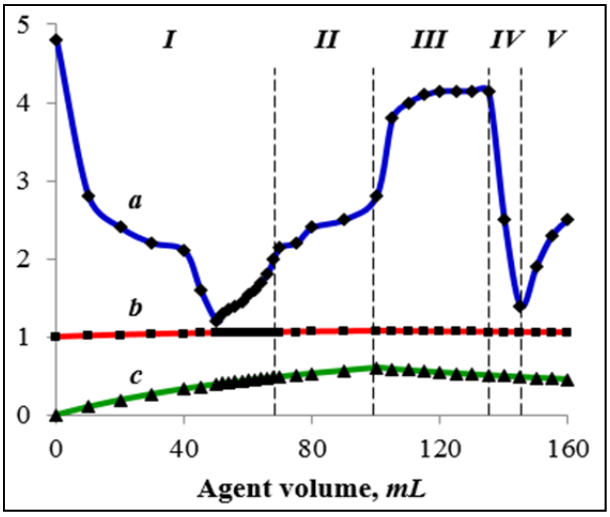

Figure 6. Electrochemical features of microencapsulation: *a* – resistance, *k*Ω; *b* – density, *g/ml*; *c* –molarity of Na_2SO_4, *mol/l*; *I* – no ccv.; *II* – simple ccv (Gel^{+}); *III* – complex ccv ($GelAlg^{+}$); *IV* – neutral shells ($GelAlg^{0}$); *V* – recharging of shells ($GelAlg^{-}$)

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