Influence of core material in microparticles formation by complex coacervation

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Introduction

The phase separation between proteins and polysaccharides occur naturally in biological systems (De Kruif et al. 2004) and this phenomenon can be manipulated to confer desired properties in the food industry (Weinbreck et al. 2004). It can be also used in the purification of macromolecules (Wang al. 1996), production of fat substitutes (Bakker et al. 1994), meet analogous (Tolstoguzov et al. 1974) and films (Shih et al. 2001). In microencapsulation, the phenomenon is better known as complex coacervation and the success of the particles formation depends on the relation between the hydrophilic biopolymers and the hydrophobic core material.

Studies of the microencapsulation by complex coacervation are based on analysis of the behavior phases of the mixtures, limiting themselves to parameters of processes and characteristics of the involved polymers (Antonov et al. 1999). There are evidences about the influence of core material on the encapsulation efficiency (Rabiskova et al. 1994; Rabiskova et al. 1998) and in the present investigation, we verified the influence of the core material in coacervation process and consequently on the particles characteristics. We studied three oils with different physico-chemicals properties on coacervation yield, encapsulation efficiency and morphological characteristics of the particles.

Materials and Methods

Materials. Gelatin (type A, LF 21502/04, Gelita, Brazil) and gum Arabic (IRX49345, CNI, Brazil) were used as wall materials. Commercial vetiver oil (batch 0210222, Dierberger Óleos Essenciais, SP, Brazil), Nujol mineral oil (batch 303, Schering-Plough) and almond oil (0406291,Prunus amygdalus var. dulce, Bioessencia-Produtos Naturais Ltda) were used as core material.

Microparticles preparation. The microparticles were produced by complex coacervation and included (I) Emulsification of 2.5 g of oil in 100 mL gelatin solution at 50 ºC, 14000 rpm for 1 minute (Ultra turrax, IKA, Germany) followed by incorporation into 100 mL gum Arabic solution (2.5 %wt./v) at 50 ºC. (II) Slow reduction of pH solution until pH 4.0. (III) Gradual cooling (3 hours) of the system from 50 ºC to 10 ºC.

Process yield. The systems obtained were weighed after a minimum of 12 hours in a refrigerator at 4 ºC. After coacervation, the capsules produced and precipitated, were centrifuged at low velocity (5000 rpm/ 5 min - Damon/ IEC HN-S Centrifuge), separated and then weighed. The moisture content of the coacervated phases was analyzed using a vacuum oven at 60ºC during 24h. The microencapsulation process yield (MY) was calculated as the percent of dry material precipitate in relation to the initial dry mass (mass of polymers on a dry basis + the oily core). The polymer yield (PY) was calculated as dry polymer precipitate (Coacervate mass - encapsulation efficiency) over dry polymer initial.

Encapsulation efficiency. The total load of oil in the coacervates was determined gravimetrically by extracting the oil with dichloromethane after maceration of the dried microparticles. The encapsulation efficiency (EE) was defined as the oil content recovered per grame of the microparticles produced in relation to the original oil amount used to produce the particles.

Mean particle size. The mean particle sizes were determined using a laser Light Scattering (Mastersizer S, MAM 5005, Malvern, Germany) with sample suspension unit. Microparticles were suspended in distillate water.

Optical microscopy. The morphology of the moist particles was observed by optical microscopy (Nikon, Eclipse E800, Tokyo, Japan), using (x 20) objective. The images were captured using the Image Pro Plus 4.0 software.

Zeta potential determinations. The ζ-Potential measurements was used to follow the microparticles formation steps with a Zetasizer 2000 (Malvern, UK) and it was made: i) in the emulsion of different oils in 2.5 %wt./v gelatin at 50ºC; ii) after gum Arabic addition at 50ºC and adjust of pH of coacervation. The emulsions and coacervates were diluted (1:100) in distilled water at the same pH value (5.2 for the emulsion and 4.0, for the coacervates). The emulsification of water at pH of gelatin solution did not produced sufficiently accuracy (±2 mV).

Size emulsion characterization. The measurements of average size of emulsion droplets were carried out immediately after emulsion preparation. The images were obtained by optical microscopy (Jenaval, Carl Zeiss, Germany) - with objective of 12.5x and optovar 0.8x, without projective, connected to the chamber (Hitachi 45-752, Tokyo, Japan) and to the computer with Global software Lab Image. The image analyses were made through free software Scion (www.scioncorp.com) with the minimum number of 200 droplets.

Results and Discussions

Oil type	Precipitate (g)	PY (%)	MY (%)	EE (%)
Vetiver	41	99	97	92
Almond	33	98	91	73
Paraffin	58	52	47	38

Table 1 : Characteristics of coacervation process.

The Table 1 shows some indexes obtained to characterise the coacervation process efficiency. The production of particles by complex coacervation will depend on the phenomenon of complexation and also the affinity of the coacervates droplets by the oily phase.

Despite to observe a high quantity of coacervated mass to paraffin oil (58g), these particles presented the lesser microparticles yield- MY- (47%) compared to almond (91%) or vetiver oil microparticles (97%). Furthermore, almost half part (48%) of biopolymers initially in the process was not complexed when paraffin oil was used, unlike the other oils, where most gelatin and gum Arabic were precipitate.

It knows that the complex coacervation is induced by electrostatic interactions and anyone with properties to modify the charge balance can helps or not the complexation. The charged molecules interact first through electrostatic interactions and then aggregate. The neoformed aggregates slowly rearrange in time to form the coacervates (Mattison et al. 1999) and so, is possible that the oil superficial properties has influence in complexed polymer fixation.

We have verified that the oil contributes with charges on the system, and it can interfere directly on process formation of the particles. The ζ- potential can be used as a measure of the electrostatic repulsive forces, which indicates the potential stability of the colloidal system. These measurements were made to each step of complex coacervation for the different oils.

Coacervation steps	almond	mineral	vetiver
water	*	*	*
gelatin/oil emulsion	20±1	3±0.8	21±1.5
addition of gum Arabic	12±1	13±0.6	19±1.8

Table 2 : Zeta potential in the steps of complex coacervation. *Measure not achieved.

Although the superficial charge of all emulsion droplets in gelatin to be positive, those produced with paraffin oil had presented a lower value. The charges at this point of process indicate the adsorption and as higher charge, as higher the amount of linking sites in gum Arabic (negatively charged at this pH). After polysaccharide addition, all particles presented an excess of positive charges (Table 2).

These results suggest that the oil properties interfere in the complexation of polymers, avoiding in some way, that the balance of charges establishes itself.

The encapsulation efficiency, 92% and 73% with vetiver and almond oils respectively, was also higher than with paraffin oil (38%). This difference could be due to the better hydrophilic characteristics of vetiver oil components compared both to almond and paraffin oil. The vetiver oil is a complex mixture of compounds with severals functional groups with approximately a half part on hydrophilic compounds like alcohols, acids and ketones, which promotes a balance with the lipophilic substances. On the other hand, the paraffin oil is basically constituted by a long chain of hydrocarbons, and therefore, this hydrophilic-lipophilic balance does not occur. In accord with this, Rabiskova et al. (1998) investigated a large range of materials and they had perceived that the properties of oils surface, in particular its relative hydrophobicity, influences significantly its insertion in the coacervates. In another paper, the authors had verified that tensoactifs with higher ratio of hydrophobic chains (low HLB) helped the squalene encapsulation, producing particles of gum Arabic/gelatin with high efficiency, varying from 93.2% to 99.6% (Rabiskova et al. 1994). Also in the presence of surfactant, the encapsulation efficiency using capsaicin was increased (Xing et al. 2005).

Morphological characteristics of the microparticles showed marked differences between the oils tested. Figure 1 shows the microparticles obtained: paraffin microparticles are composed of a reservoir-type core phase, entrapped in a shell material of a fairly constant thickness. Those produced with vetiver and almond oil, are multinucleous like a matrix-type. Most of microparticles produced with paraffin oil were practically empty, being able to touch the oil in the surface of particles, as a result of low encapsulation efficiency. In these systems, flotation of particles was not observed.

The emulsification is a preliminar step on coacervation process and the characteristics of emulsion droplets can be maintained after polymer coacervation. Particles produced with vetiver oil were higher (43.5 μm) followed by those with paraffin oil (35.0 μm) and almond oil (19.2 μm). The diameters of these emulsion droplets presented almost the inverse relation. The emulsion droplets produced with vetiver oil presented the lower mean diameter, 5.9 μm, followed by 9.2 μm with almond oil and 33.9 μm with paraffin oil. As smaller the size of emulsion droplets as higher the number that it can be put on matrix polymer (multinucleous particles). Also, with higher emulsion droplets, a film is formed around only one droplet each time, and the particles present the tendence to be mononucleous (microcapsule) (Figure 1).

The same behavior was observed by Weinbreck et al. (2004), in a coacervate system with gum Arabic and whey proteins. Droplets of orange oil were individually covered by coacervates when produced by a magnetic stirrer (higher emulsion droplets) and when ultra turrax homogenizer was used, the smaller droplets were encapsulated in a coacervated matrix.

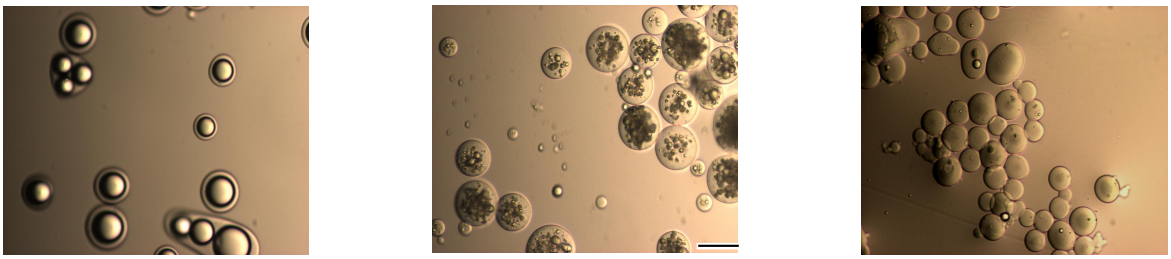

Figure 1: Particles produced with A. Mineral; B. Vetiver; C. Almond oils. Bar represents 40µm.

Conclusions

To produce microparticles by complex coacervation means to optimize the interaction between the polymers and to create the surface to adhesion these coacervates. The oil composition may has a minimum hydrophilic-lipophilic balance to create the adhesion surface to the coacervates polymers and to use an oil with high hydrophobicity avoid the complexation between the polymers. The zeta measurements is a potential tool to estimate the probability of microencapsulation by complex coacervation. The experiments realised in this work, in concordance with the literature, can suggest the microcapsules (mononucleous) or microparticles (multinucleous) formation depends on mainly the emulsion droplets size.

Acknowledgements

The authors are grateful to Capes-Brazil for the scholarship to the first author and to the following donations: Colloides Naturels (gum Arabic) and Gelita (gelatin).

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