P-053	**Study of variables in complex coacervation process**	

A. S. Prata[1*], C.R.F. Grosso[1]
[1*] University of Campinas – Campinas, Brazil
(ana.prata@fca.unicamp.br)

INTRODUCTION AND OBJECTIVES

Complex coacervation, as encapsulation technique, has been exploited by the promising use of biopolymers and the absence of organic solvent. The association of the biopolymers is termodinamically favorable and it occurs through electrostatic interactions. Despite to the probability and extent of complexation to depend mainly on the polymers (nature and concentration) and the pH range, the interaction associative is more complex than a simple charge balance and even to the classical pair gelatin/goma arabic, the mechanisms of interaction has not yet been fully explained. This process can be followed by several techniques: gel permeation chromatography, differential scanning calorimetry, spectrophotometry but a simplest manner is correlate the incompatibility between the polyions complexed with turbidimetric measures. The purpose of this work was to investigate the influence of variables on complex coacervation using the classical system to facilitate further microencapsulation process with another polymers.

MATERIALS AND METHODS

Materials. Gelatine type-B (LF 21502/04, Gelita, Brazil) and gum Arabic (ref. IRX49345, CNI, Brazil), Almond oil (0406291, Prunus amygdalus var. dulce, Bioessencia-Produtos Naturais Ltda).

Coacervation estimatives. *S*olutions of gelatine (GE) and gum Arabic (GA) were prepared at 2.5% w/w and mixtures of different volumetric proportions were acidified (HCl 1N) or alcalinized (NaOH 1N) in several pHs (range of 3 to 8) for the analysis: *Zeta potential.* The superficial load of ternary water-GE-GA systems was investigated with Statitic zeta potential (Zetasizer 2000, Malvern Inst., UK). The mixtures were diluted with deionized water to the equipment detection range, at the lecture pH. *Optical turbidimetry.* The absorbance of biopolymers in coexisting phases were determined by spectrophotometry (Specord UV VIS, Carl Zeiss) at λ=590nm (Antonov & Zubova, 2001). All measurements were performed after equilibrating the phases for 30min after pH adjustment. As the zeta potential, the readings were make rapidly to avoid gelation of GE.

Matrix-Screening of Process Factors. A fractional factorial design (2^{7-3}) was used to verify the variables effects on the yield coacervation with almond oil (Statistica, Statsoftware, v. 5.0). A total of 19 trials including 3 repetitions at central point experimental were prepared according to design shown in Figure 2. *Microparticles Preparation.* Microparticles were prepared according a experimental matrix, as follows: (I) Emulsification of x7 g of oil in 100 mL GE solution at x_3ºC, x_2 rpm for x_1 minute (Ultra turrax, IKA, Germany) followed by incorporation into x_6 mL GA solution (x5 %w/v) at x_3 ºC. (II) Slow reduction of pH solution until pH x_4. (III) Gradual cooling (3 hours) of the system from x_3ºC to 10 ºC. *Process yield.* The precipitated particles obtained, after a minimum of 12 hours in a refrigerator at 4 ºC, were centrifuged at low velocity (5000 rpm/ 5 min; Damon/ IEC HN-S), separated and weighed. The moisture content of the coacervates was analyzed using a vacuum oven at 60°C/24h. The microencapsulation process yield 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).

RESULTS AND DISCUSSIONS

The GA-GE solutions were subjected to turbidity and zeta potential measurements as function of pH (Figure 1). At the diluted phase and in appropriate pH, the positively charged GE is attracted to the negatively charged GA to form coacervates droplets. The isolated polymers showed no dependence with the pH (data not shown). The cloudy of the system increases with the concentration of complexed polymers. For the ratio 1:1, the higher absorbance of polymeric pairs occurred between pH 3.5 and 4.5, near isoelectric point of GE (type B) which includes the commonly pH used in literature (Lamprecht et al., 2000).

The width of the peakes obtained for all combinations of polymeric pair studied was reduced at higher polysaccharide concentrations. Two regions of inhibition could be observed. One, previously mencioned, when the pH is above of the isoelectric point (I.P.) of the GE. The GA has majoritarially more negative charges and the GE must unfold and interact yourself. However, this protein has positive charges below of its I.P., limiting the range of interaction. Another inhibition region could be observed at pH extremally acid (below 3.5) due to inhibition of the ionization of some groups of gum Arabic (NH_2 and COOH), reducing therefore the intensity of interaction with the positively charged protein.

The experiments with zeta potential were investigated in order to predict the most appropriate pH interval for the formation of the electrostatic complexes. The electroneutrality of compounds formed can be seen when the line crosses the x-axis. The combined picture that emerged using the mixtures of the polymeric pairs, as the turbidity assays, showed considerable pH dependence. The neutral point of the complexes with 1:1

GE:GA was near pH 4.0. At this point, there is no excess of neither positive or negative charges. Data obtained by zeta potential agree with those obtained by turbidimetry indicating the optical turbidimety is a potential tool to estimate the coacervation formation.

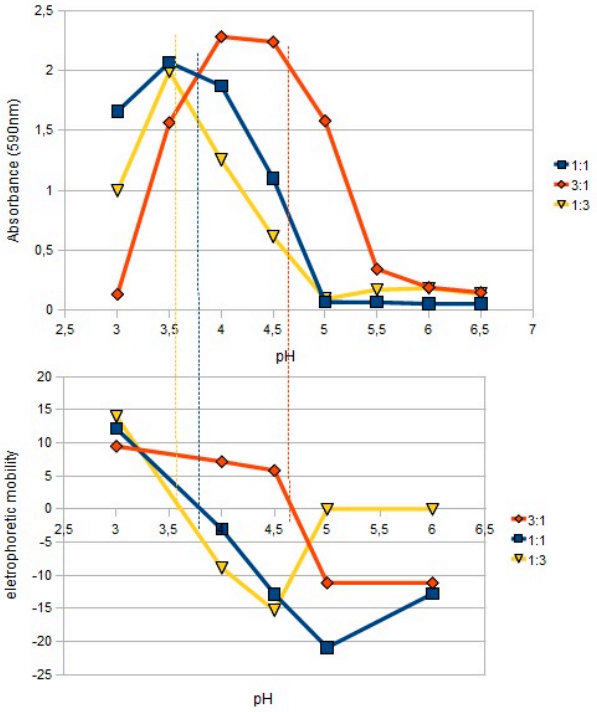

Figure 1:Estimative of coacervates formation by absorbance and zeta potential. GE:GA (2.5% w/w).

The thermodynamic phenomena of coacervation associated to emulsification parameters result in formation of crosslinked structures around the droplet oil. The probability and intensity of these processes depend on the properties of the polymers and oil core material, including the load conditions before analyzed, and also, the mechanical force added to make the emulsion. The complexes formed had been presented conformation differences.

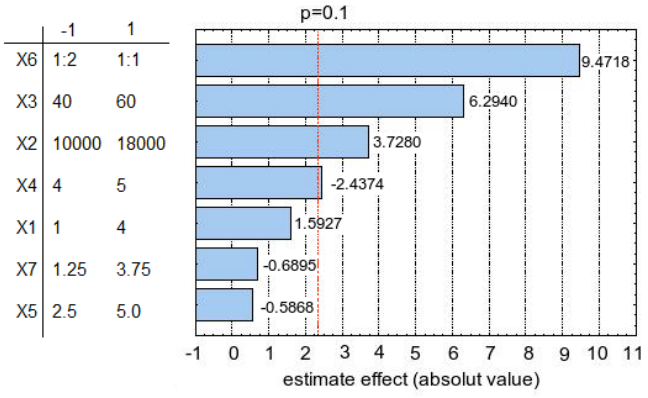

Figure 2:Analysis of cocaervates yield with almond oil of the factorial experiment . R^2= 0,6424. x1:Homogenization time (min); x2:Homogenization velocity (rpm); x3: Temperature (oC); x4: pH; x5: Concentration of polymer (%w/v); x6: GE:GA; x7:Almond oil core material (g).

These factors influencing the probability of microencapsulation were evaluated observing the yield of coacervate with almond oil and the relative importance was graphically represented by Figure 2. As assumed by Figure 1, an unfavorable pH condition determines fundamentally the compatibility between the polymers and at pH 5.0, it was impossible to prepare microparticles, even if the successful choice of the others parameters also important in particles formation it was made. At this pH, the oil droplets do not have a polymer covering (Figure 3C).

The best assays represented by the higher yield assay and visually best microparticles had presented two parts of protein for one of polysaccharide (Figure 3B) and contrarily, the lowest yields were obtained with higher proportion in GA (Figure 3D). Therefore, the limiting factor is gelatin concentration because this protein do not develop an enough positive charge to associate in the same proportion with the polysaccharide.

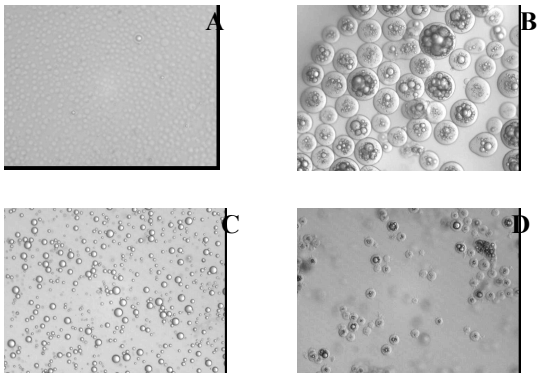

Figure 3. Micrographies obtained of factorial experiment.

Another important factor is the temperature of coacervation, being reported that lowest temperature are more effective to provide phase separation. Therefore, the increase of temperature solution provides an improved solubility of the protein due to exposition of hydrophilic groups, as well as to favor electrostatic linkage and in this work, increasing temperature of 40 to 60 ^{o}C have improved the complexation of the polymers.

CONCLUSIONS

The complex coacaervation can be estimated by the use of simple technique. It was observed the importance of charge balance and conformation of the polymers on particle formation.

ACKNOWLEDGEMENTS

The authors are grateful to Capes, CNI and Gelita.

REFERENCES

- Lamprecht et al. (2000). Europ. J.of Pharm and Biopharm., 49, 1-9.