# Complex Coacervation of the Gelatin–Poly(acrylic acid) System

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Received 12 July 2005; accepted 29 November 2005
DOI 10.1002/app.23899
Published online in Wiley InterScience (www.interscience.wiley.com).

**ABSTRACT:** The system gelatin–poly(acrylic acid) (PAA) undergoes not only complex coacervation but also flocculation. The latter is incompatible with an encapsulation process. pH adjustment rate, ionic strength, temperature, and total macromolecular concentration have been studied to understand the origin of flocculation and to obtain a set of optimized parameters for coacervation using on-line turbidimetric titration. State diagrams were built, by varying gelatin/PAA mass ratio (R) and pH, for different PAA molar mass, which gave occurrence conditions of flocculation and coacervation. Flocculation can be avoided without significant yield decrease by pH adjustment. On the other hand, a modification of ratio (R) affects both coacervation yield and coacervate phase concentration. Spectrophotometric titration reveals a relative independence of the effective ratio within the coacervate and the initial mixing ratio before reaction. Conclusions are made concerning the use of this couple in an encapsulation process. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 708–714, 2006

**Key words:** complex coacervation; microencapsulation; phase diagrams; phase separation

## INTRODUCTION

The gelatin–gum arabic couple is known for a long time to undergo complex coacervation[1,2] and serve as a wall-forming material for microcapsules.[3]

However, to modulate the microcapsules properties, the use of synthetic polymer is of great importance to obtain controlled functions. Poly(acrylic acid) (PAA) has a simple structure that can be easily modified (copolymerisation or grafting) and can thus serve as a reference molecule. However, it is necessary to link knowledge of microscopic behavior in solution and during complexation with process parameters.

During the past decades, research has left the pure theoretical approach of coacervation.[1,2,4–6] Efforts have been focused on the microscopic description of the complexes formation between molecules or particles of opposite charge. The literature often mentions complexes built between polyelectrolytes and polyelectrolytes (PEC),[7] proteins (mostly globular),[8] micelles,[9] dendrimers,[10] etc. Through all those cases, some general rules can be formulated concerning the complex formation.

The reaction takes place in two steps. In the first one (called primary aggregation), a primary complex is formed between species of opposite charge. This step is mostly driven by the entropy gain due to the release of little counter ions and by enthalpy loss due to electrostatic interactions.[11] It is counter-balanced by the conformational entropy loss of the PEC involved in the complexes. The second step of the reaction takes place if the neutralization of the complex is sufficient. Hydrophobic interactions induce not only shrinkage of the primary complexes (intramolecular interaction) but also an aggregation of the complexes themselves (intermolecular interaction called secondary aggregation).

For PECs, the structure of the primary complex can be of different types, where extreme model cases are[11] ladder and scrambled eggs. In the ladder model, PEC are paired and form a double-strand complex. If one species is longer than the other, the molecules are respectively, called host and guest. In the scrambled eggs model, many chains of each type are integrated in one complex. Similar models can be derived if one species has a spherical shape (globular proteins, micelles, dendrimers).

Some defects on the primary complex induce a bad local neutralization and can disable the secondary aggregation. Among those defects, one can notice the following: an insufficient covering of a host by guest molecules, bad structural adequation between both species (difference in charged groups spacing, charges accessibility, etc.), lack of polyelectrolyte backbone flexibility, and loops (local disconnection between the polyelectrolyte and the counter-polyion).

As the so-called "secondary complexes" are growing, a new (dispersed) phase is arising; the continuous

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one being called equilibrium phase. Literature dedicated to those complexes often does not cover the phase separation following secondary aggregation.

In the case of coacervation, the dispersed phase (coacervate) is liquid. The size of the droplets (coacervates) ranges up to 1–10 $\mu$m. Compared with the initial solution, this phase is enriched in macromolecular species but still contains ~80% water.

Among the other types of phase separation, the terms of flocculation, gelation, and precipitation can be used. The flocculation often refers to a dense,[12] sticky,[13] or gelled solid.[14] Van Oss[15,16] pointed out the flocculation as being a densification of the coacervates and not totally reversible.

Through the literature, some parameters influencing the complex formation can be isolated. Few articles[17] deal with influencing parameters of the coacervation itself. The problem is now to evaluate how much a parameter can possibly control the encapsulation process. As a first part, we will focus here on the coacervation reaction in the case of PAA and gelatin. Different physicochemical parameters will be studied by on-line turbidimetric titration allowing a pH adjustment rate control. Different wall-forming material can be obtained by varying gelatin/PAA mass ratio (R) and PAA molar mass. These parameters will thus be treated jointly with pH, which permits reaction control. On-line turbidimetric method, spectophotometry, and dry matter content evaluation will be used to characterize the reaction and the coacervate. Attention will be paid to clarify the distinction between coacervation, precipitation, and flocculation.

## METHODS

### Materials

All the PAA samples were a kind gift from Röhm and Haas European Laboratories (Sophia Antipolis, France), given in solution and used as such. PAA molecular weight is 2, 4.5, 10, 60, and 200 kDa. Gelatin was purchased from PB gelatins (Vilvoord, Belgium). It is a pig skin origin, type A with a pHi of 6.5, and a bloom of 260. Gum arabic was purchased from Panreac Quimica (Barcelona, Spain). pH adjustments are done with NaOH and HCl of analytical grade.

All the experiments were done in a 1 L double wall reactor so as to control temperature. The turbidimeter is a "turbiscan on-line," kindly lended by Formulaction (Toulouse, France).

### Coacervation reaction

Gelatin solution was prepared 1 h before use. For PAA, solution was prepared just before use. pH was adjusted to 9 in each solution before mixing. A HPLC volumetric pump then delivered HCl at controlled flow to lower the pH.

In the absence of any indication, a set of default parameter was used as a reference:

- pH drop from 8 to 5 in 7 min
- Temperature: 40°C
- Total concentration of macromolecules: 2.4%
- Gelatin/PAA mass ratio: 1/1
- PAA molar mass: 10 kDa
- The ionic strength is considered to its "default value" once coacervation pH is reached, when pH is adjusted at 9 before mixing and no NaCl is added. It can be lowered by adjusting pH before mixing at 6 or increased by adding NaCl.

### On-line turbidimetric method

For turbidimetric titration, reactionnal bath was pumped with a peristaltic pump through the turbidimeter. pH was controlled with a Schott pH meter. Combining both signals of turbidity and pH as a function of time, one could obtain turbidity versus pH. All the data were recorded during the pH descent from pH 9 (after mixing).

For the state diagrams, the onset and end of the phase separation domains are defined by abrupt rise or fall of turbidity.

### Yield and concentration of the coacervate phase

After formation of the coacervate phase at the desired pH, the system was put at 4°C overnight. Equilibrium phase was then extracted. Gelled coacervate phase was dried in a vacuum oven until constant mass.

The coacervate yield is the ratio between the mass of dried coacervate and total mass of macromolecules initially introduced. The concentration of coacervate is the ratio between dried and wet mass of the coacervate phase.

### Gelatin dosage–effective ratio

To 1 mL of equilibrium phase, 1 mL of Bradford reagent is added. The absorbance at 595 nm is compared to that of a reference curve established with solutions of known concentration.

According to the residual gelatin concentration and the overall coacervation yield, the concentration of gelatin and PAA inside the coacervate phase is calculated. The effective ratio is defined by the gelatin/PAA mass ratio inside the coacervate (also called microstoichiometry); the mixing ratio being the same but with the quantities initially introduced (macrostoichiometry). Without further indication, "ratio" indicates the mixing one.

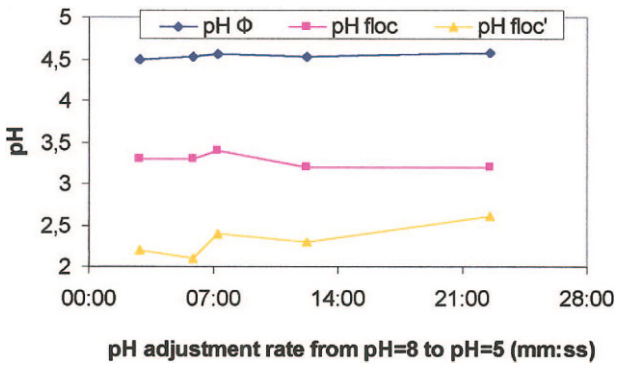


**Figure 1** Variation of the position of $pH_\phi$ as well as flocculation domain according to the rate of pH adjustment. Mw = 10 kDa and R = 1/1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

## RESULTS

### Macroscopic aspect of mixtures

In addition to the well-known states of solution and coacervation, the mixtures present a third one that will be qualified as flocculation.

During coacervation, the medium is turbid, but homogeneous on the scale of the millimetre or centimetre. Flocculation is mainly differentiated by the appearance of visible clusters, whose size is in the range of millimetre or centimetre. Flocs, once isolated, appear to be small sticky gelled particles. Flocculation can be detected using the following:

- A time instability of the turbidity signal on the scale of the second
- A reduction in mean turbidity (average on 15 s) related to the contrast between flocs and continuous phase.
- Similar to the common notation ($pH_\phi$, $pH_\phi'$)[18] used for coacervation, we will adopt pH floc and pH floc′ to limit the flocculation domain.

### Effect of the physicochemical parameters

#### pH adjustment rate

The effect of pH adjustment rate is weak. The principal effect is the broadening of the flocculation as kinetics increases (Fig. 1). When an acid droplet falls into the reaction bath, the pH is locally sufficiently lowered to induce a turbid zone, which is dissipated quickly (less than 1 s). Whenever the pH of the bath is close enough to the coacervation domain, the system can turn locally into flocculation. If the redissolution kinetics of the flocs are rather slow, their accumulation in solution can induce an increase of the extent of the flocculation domain.

#### Ionic strength

With the four tests carried out with PAA 4.5 kDa, the ionic strength taken as reference corresponds to the widest coacervation and flocculation domains (Fig. 2). Flocculation disappears for ionic strength higher than the reference level. Tests carried out with a molar mass of 10 kDa results in same conclusions.

According to the variations operated, the ionic strength reference value is at least equal to $10^{-1}$ mol/L. In this range, increasing ionic strength discards the complexes.[19] For the highest ionic strength, this suppression can be partially screened by the precipitation of the macromolecular species. An increase of ionic strength could be used to be freed from flocculation. But in the same time, the coacervation yield would be decreased. Instead, pH can be limited to the zone where only coacervation is occurring.

#### Temperature

Bringing the temperature up to 60 or 70°C makes the detection of the flocculation unclear. However, a turbidity reduction remains in a pH range close to that of flocculation at 40°C. An increase of temperature induces a decrease of coacervation domain width and a decrease of the turbidity plateau level (Fig. 3). The temperature is a factor supporting solubilization and hence limiting phase separation. Once again, flocculation can be avoided to the detriment of the coacervation yield. On the other hand, the use of low temperatures in the encapsulation process is limited by the gelation of gelatin.

#### Total concentration of macromolecules

The flocculation appears only for total concentrations higher than 0.3%. The precipitation of the PAA at high concentrations prevents from measuring $pH_\phi'$. The extent of the coacervation domain must thus be eval-

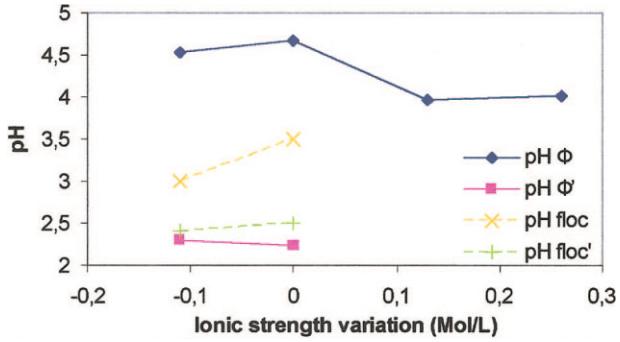


**Figure 2** Variation of the position of $pH_\phi$ and of the flocculation domain according to the variation of ionic strength as compared to a reference level (corresponding to the default physicochemical parameters). Mw = 4.5 kDa and R = 1/1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

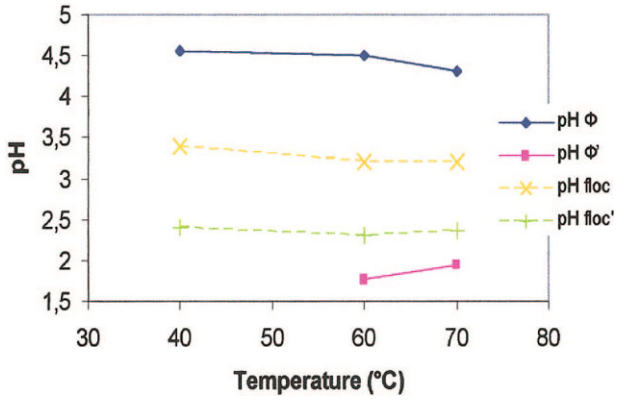


**Figure 3** Position variation of the coacervation and flocculation domains according to the temperature. Flocculation is visually detectable only at 40°C. Mw = 10 kDa and R = 1/1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

uated by $pH_{\phi}$. Both coacervation and flocculation domains are widest for a concentration of 1.2% (Fig. 4). According to the total concentration, the width of the domains follows the evolution commonly observed for the coacervation yield.[1] As it has been said about the pH adjustment rate, the kinetics can explain the collision of the secondary aggregates, their aggregation to form coacervates, and the formation of the flocs. The reduction in the total concentration will decrease the number of complexes formed in the reaction bath (primary complexes) and thus their probability of collision (secondary complexes). This phenomenon can explain the shift of the flocculation domain towards lower pH at 0.3%

### Coupled effects of pH, ratio, and PAA molecular mass

The PAA molar mass and the gelatin–PAA ratio can be modified so as to obtain various materials. The goal of this part is to determine, for each molar mass/ratio couple, a pH range corresponding to coacervation and flocculation.

#### State diagrams

The state diagrams are obtained through the turbidity measurement by means of isoturbidity curves. Outside the domain, the system is in the solution state, and inside, coacervation takes place. On these diagrams, points are added to set the pH of appearance of flocculation. These state diagrams are established for each PAA molar mass (Fig. 5).

The coacervation domain width increases with an increase of the PAA molar mass. Some results have already shown that the incorporation in the complexes of high molecular mass polymers was favored.[20] Our results indicate that the molar mass influence on coacervation is not limited to kinetic or competition effects. Molecular weight affects solubility of lone chains and can also affect the solubility of a complex. Ratios like 1/16 or 16/1 still permits detection of coacervation but appear to be unfavourable. These ratios induce a discrepancy of the structural adequation with generation of locally nonneutralized zones in the complex.

Jiang and Zhu[21] carried out similar experiments using a PAA of 20 kDa, a mass ratio of 1.4/1, and a macromolecule total concentration of 1.4%. Several experiments carried out with R = 1/1 are comparable (the closest is PAA 10 kDa, 1.2%). The correlation between the values of $pH_{\phi}$ is good (less than 0.3 pH unit). The shape of the turbidity curve is also in good agreement. However, in their case, no flocculation is detected. The difference between both works is that we used a gelatin of higher pHi (6.5 versus 4.9), inducing stronger interactions between the oppositely charged molecules.

In our experiments, flocculation appears for molecular masses higher than 10 kDa. In that case, flocculation is reversible, i.e., below a certain pH, the flocs disappear. On the other hand, with 200 kDa, the flocs are not redissolved. For an intermediate molar mass (60 kDa), the flocs are partially redissolved; small particles remain visible, even if those are much smaller and less numerous than the flocs corresponding to the maximum of flocculation.

According to the molecular mass, the flocculation domain grows up (along the pH and ratios axis) faster than the coacervation domain does. Thus, for a molar mass of 200 kDa and R = 1/1, coacervation is exploitable on a range of 0.2–0.3 pH units. It is probable that for higher molar mass, coacervation is not exploitable any more, as flocculation covers entirely the coacervation domain. Moreover, the reversibility of the formation of the flocs depends on the intensity of the interactions. Finally, we can supplement the previous vision concerning the relationship between states of the

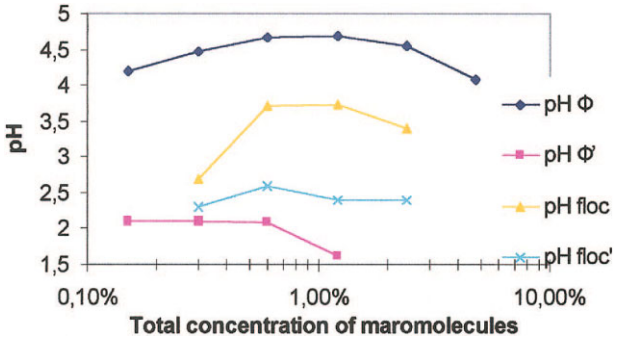


**Figure 4** Variation of position of the coacervation and flocculation domains with the total concentration in macromolecules. Mw = 10 kDa and R = 1/1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

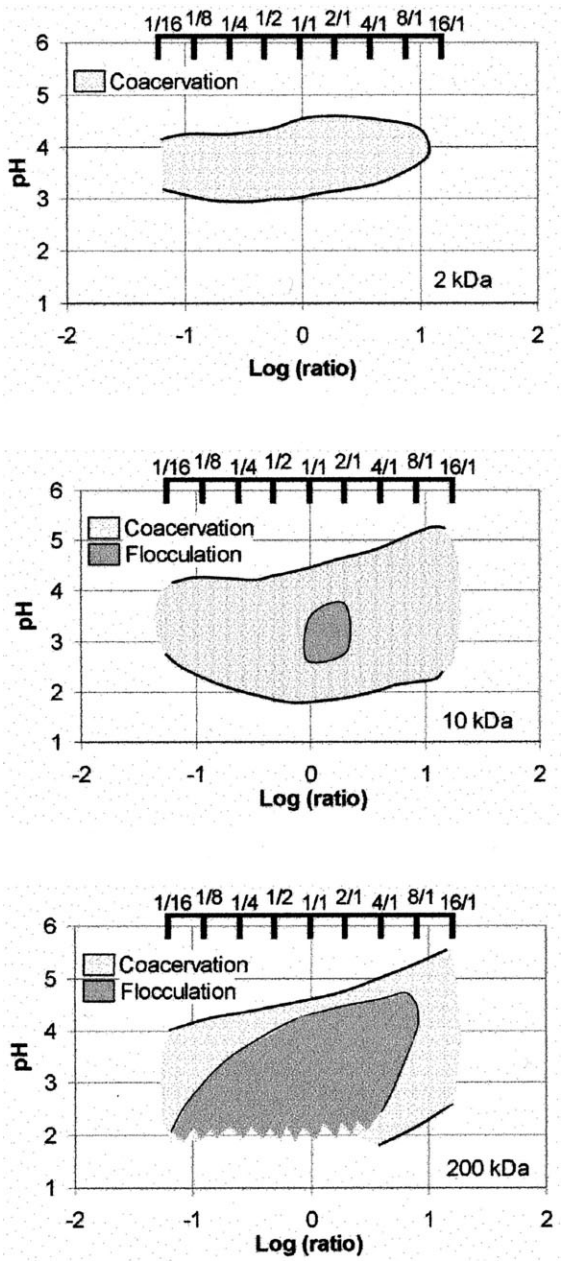


**Figure 5** Examples of state diagrams according to the ratio and pH, for a PAA molar mass of 10 and 200 kDa. Right and left coacervation domain edge position is out of the studied range. The domains limit at low pH could not be determined due to irreversible floculation (notched edge).

system.[11,22] Their sequence, although the interactions between molecules of opposite charges are intensified, is thus the following:

- Solution
- Soluble complex
- Insoluble complex (coacervation)
- Reversible flocculation
- Irreversible flocculation (precipitation gelation).

### Comparison with gelatin–gum arabic

Among the three ratios tested, only the ratio 1/1 made it possible to observe a coacervation (Fig. 6). This fact can be seen as a greater sensitivity to structural inadequation. The encapsulation using gelatin and gum arabic is generally done for similar physicochemical parameters and a pH of 3.8, that is, in the middle of the domain.[23]

The system does not give rise to flocculation and the width of the domain is comparable to that obtained with the lowest PAA molar mass.

These results can be related to the branched structure and the lower quantity of charges present on the gum arabic as compared to the PAA.[24,25] The first factor induces a bad structural adequation, with the appearance of "loops." The second factor implies interactions of lower intensity. These defects enhance the solubilization of the complexes and hinder phase separation.

## Coacervation yield

### Effect of pH

If the pH range is included in the phase separation domain, the yield appears to be almost constant (Fig. 7). It decreases to reach a null value when the pH is brought outside this domain. On the other hand, the higher the molar mass of the PAA, the higher is the maximum concentration within the coacervate. For the three most important molar masses, it results in a clear densification of the coacervate phase, as the pH is decreased.

During the passage from coacervation to flocculation, the yield does not undergo a sharp discontinuity. When the pH approaches the flocculation domain, the concentration of the coacervate increases and flocculation appears when the concentration exceeds ~30%. At the end of the study, the two lowest molecular masses will not be maintained, as they induce low yields and weak domain extents.

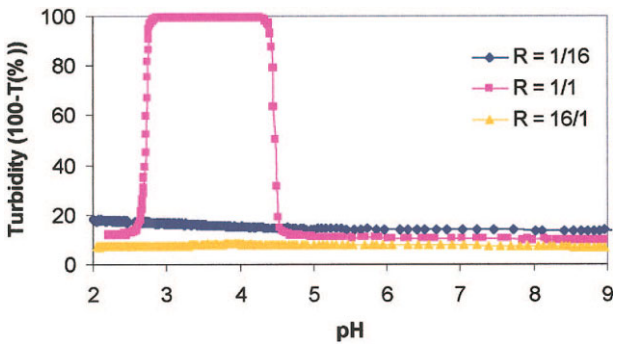


**Figure 6** Turbidity curves according to the pH for the gelatin–gum arabic couple. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

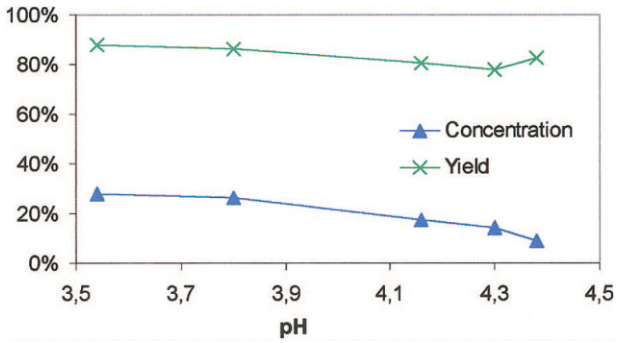


**Figure 7** Characterization of the coacervate phase obtained with PAA 200 kDa and R = 1/1. Flocculation occurs for pH lower than 4. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

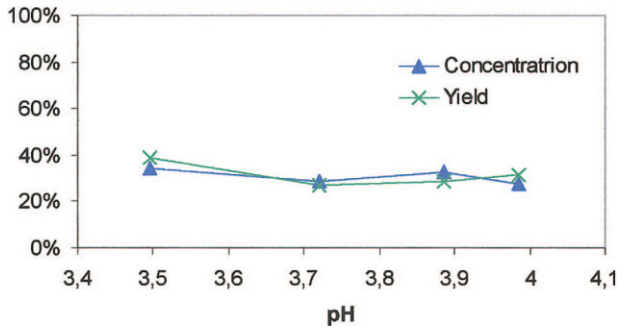


**Figure 9** Characterization of the coacervate phase obtained with PAA 200 kDa and R = 1/4. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

### Effect of mixing ratio

For the three remaining molar masses, the study was limited to the ratios lower than 2/1 so that the wall-forming material contains a great part of PAA. The pHs are taken in the coacervation zone (between $pH_\phi$ and the beginning of the flocculation zone).

The coacervate obtained with PAA 200 kDa presents a strong dependence of the yield with the ratio, being maximum for ratios 1/1.2.1 (Fig. 8).

The concentration of the coacervate (dry matter content) is maximum for ratio 1/4. With this ratio, the concentration remains stable in the studied pH range, and increases slightly when the pH approaches the flocculation limit (Fig. 9).

For the other molar masses, the evolutions are similar. Only the yield and coacervate concentration values do change (Table 1).

In spite of a poor coacervation yield, ratio 1/4 seems attractive because of its high concentration. One can expect that the high concentration allows to induce enough structural changes to modify the properties of the capsules built with that material.

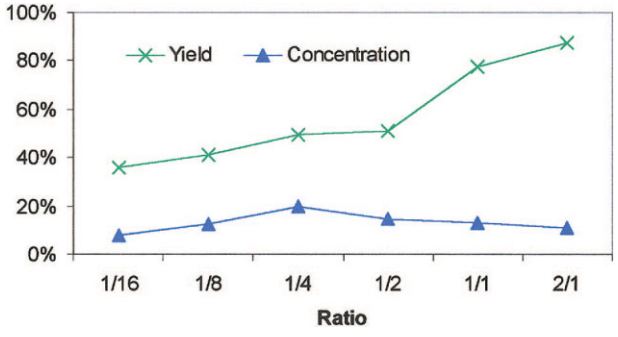


**Figure 8** Characterization of the coacervate phase obtained with PAA 200 kDa, with various ratios. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

### Evaluation of the effective ratio

The measured quantity of residual gelatin is always very weak, that leads, by calculation, to an effective ratio always higher than the mixing ratio. Moreover, it should be noticed that this shift depends on the initial ratio. The effective ratio is closest to the initial one when the latter is 1/1. The consequence on the coacervate is important: in the case of the PAA 60 kDa for example, the composition of the coacervate is almost unchanged for mixing ratios from 1/1 to 1/16 (Fig. 10).

The origin of this deviation of the ratio is not deferred in the literature. Only a few articles are interested in the effective ratio. Van Oss[15] stipulates that the ratio remains unchanged during phase separation. Mattison et al.[22] specify that the pK of the functions varies according to the environment. Especially, this one can be very affected in a complex. This modification of the charges can disturb the equilibrium established between the coacervate and the equilibrium phase. In conclusion, the driving forces of the coacervation phenomenon are not enough known to understand the phenomenology related to the coacervate composition. Unfortunately, measurements could not be confirmed by other methods.

## DISCUSSION

As expected, domain width and yield are increased by parameters known to be favorable to the complexes formation.

**TABLE I**
**Yield and Concentration of the Coacervate Obtained for Several Molar Masses and Ratios**

| | R = 1/2 | | R = 2/1 | |
|---|---|---|---|---|
| PAA (kDa) | Yeild (%) | Conc. (%) | Yeild (%) | Conc. (%) |
| 10 | 27 | 33 | 79 | 20 |
| 60 | 27 | 31 | 77 | 16 |
| 200 | 34 | 31 | 82 | 11 |

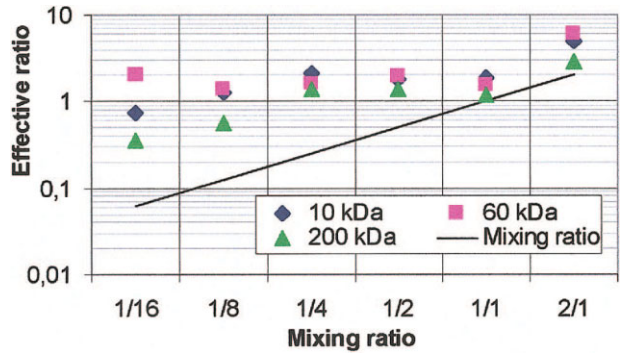


**Figure 10** Effective ratio measured in the coacervate according to the molar mass. The straight line points out the ratio obtained if the mixing ratio would be preserved. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

An increase in the ionic strength and the temperature disables flocculation, whereas it supports precipitation of the PAA. Moreover, flocculation only intervenes for pH higher than 3, i.e., when the charge of the PAA is nonnull. The flocculation and the precipitation of the PAA must thus be distinguished.

The preceding results have shown that once modifying the physicochemical parameters, the coacervation and flocculation domains follow the same evolutions. Moreover, flocculation always occurs within the coacervation zone, for the conditions where the interactions are strongest. Flocculation thus seems to be a phenomenon having the same physicochemical origins as coacervation, but taking place for higher values of the interactions between macromolecules. Van Oss[15] already made a distinction between phenomena that he qualified as coacervation and flocculation, but the links between the phase separations remained unclear. Especially, Van Oss did not mention whether the occurrence of flocculation is closely related to the nature of the macromolecules or whether it depends on the physicochemical parameters.

Because of their stickiness, flocs induce an aggregation of capsules when used in an encapsulation process (results not shown). It seems to be obvious that the floc size, and hence their macroscopic appearance, is dependent on the agitation. However, their sticky character is of physicochemical origin and thus independent of the agitation. As agitation can not be maintained over the whole encapsulation process, the capsules agglomeration will eventually occur. Thus, better than trying to compensate consequences of the flocs stickiness, it seems better to avoid stickiness itself.

## CONCLUSIONS

The appearance of flocculation is unfavorable for the formation of nonagglomerated capsules.

If one tries to control the flocculation with parameters affecting complex solubility (temperature, molecular mass, ionic strength, total macromolecules concentration) or complex defects (mass ratio), a compromise must be made with the coacervation yield. However, the residual charge of the complex can control (by way of pH) flocculation without significant yield decrease. The positions of the domain compatible with effective encapsulation process have been compiled in state diagrams.

This domain seems to collapse for PAA molar mass higher than 200 kDa. Moreover, PAA molecular weight must be at least 10 kDa to comply with acceptable yield. Moreover, the effective ratio is weakly affected by mixing ratio. Thus, different materials can only be obtained, because of a higher coacervate concentration for mixing ratios around 1/4. This could indicate structural changes inside the coacervate phase. Hence, to modulate capsule shell properties, ratio and PAA molar mass seem to offer only a narrow range of modifications.

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