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# EFFECT OF GELATIN PROPERTIES IN COMPLEX COACERVATION PROCESSES

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## ABSTRACT

The effects of Bloom grade and iso-electric-point of gelatin on the complex coacervation with acacia and micro-encapsulation of theobromine were investigated. The zetapotential of various gelatins and acacia was measured at different pH values and compared with the optimal pH range for complex coacervation. Values for electrical equivalence pH of gelatins and acacia were in the same range as the maximal coacervate volumes. For the high Bloom grade gelatins the pH range in which most complex coacervate was formed was smaller for Alkaline-processed gelatin than for Acid-processed gelatins. The same small pH range was observed for the low Bloom grade gelatin of the Acid-type compared to high Bloom grade gelatins of the same type. The total amount of complex coacervate increased and the relative contents of theobromine in microcapsules decreased for gelatins with high Bloom grade. Microcapsules prepared with a high Bloom grade gelatin were irregular shaped and showed poor flow characteristics. No differences in theobromine release profiles from the various microcapsules were observed.

## INTRODUCTION

Complex coacervation is a well known method for micro-encapsulation of sparingly water soluble compounds (1). The method comprises formation of a complex coacervate by two poly-ions, like gelatin and acacia, with subsequent incorporation of core material. The experimental conditions largely determine the amount of complex coacervate and the properties of the microcapsules. Several papers have been published describing the effects of temperature, stirring rate, concentration of core material and crosslinking agent on the properties of microcapsules (2-6).

In complex coacervation processes gelatins with different Bloom grade (gelstrength) and iso-electric-point can be used. The aim of the present study was to investigate the effects of various properties of gelatin, like Bloom grade and iso-electric-point on the complex coacervation and micro-encapsulation efficiency. Theobromine was chosen as a model drug compound.

## MATERIALS

All materials were used as received. Theobromine (O.P.G., Utrecht, the Netherlands) met the requirements of the Ph. Eur. II. Different types of gelatins with various Bloom grade (see table 1) were obtained from Sanofi Bio-Industrie (Badhoevedorp, the Netherlands). Acacia was obtained from Osterman (Osnabrück, Germany). Isopropanol, glutaraldehyde (25%), hydrochloric acid (1N) and sodium hydroxide (1N) were of analytical grade.

## METHODS

### Determination of iso-electric-point (IEP)

The IEP was determined using a turbidity method. Gelatin solutions (1% w/v) were prepared and the pH was adjusted to predetermined values

(range 3.5-9.0). The pH of the sample with highest turbidity was considered the IEP of gelatin. Results are the mean of two measurements.



### Determination of complex coacervate volume

Gelatin/acacia coacervates were prepared by mixing aqueous solutions of gelatin (3% w/v) and acacia (3% w/v) at 40°C. The pH (range 3.0-5.0) was adjusted with 0.1 N hydrochloric acid. After mixing for 15 minutes samples were taken. The complex coacervate was allowed to settle for 2 hours at 40°C. The total sample volumes and the complex coacervate volumes were determined and the percentage of complex coacervate was calculated.

### Determination of Zeta-potential

A slightly modified version of the method described by Burgess and Carless (7) was used to determine the zeta-potential. The acacia or gelatin was adsorbed on Aerosil 200 (Nethe BV, Amsterdam, The Netherlands) in demineralized water. The zeta-potential of this dispersion was determined at 20°C using a Malvern Zetasizer 2C (Malvern, Instr. Ltd., Worcestershire, England). Voltage-current conditions were in accordance with the Zetasizer manual.

### Micro-encapsulation of theobromine

Optimum conditions for the micro-encapsulation process regarding yield and shape were determined using gelatin type A3 (see table 1) and acacia (based on a full factorial design). This resulted in a stirring rate of 200 rpm, a concentration of gelatin and acacia (ratio 1 : 1) of 1.5% w/v, and a temperature of 40°C. The initial concentration of theobromine was set at 2.5% w/v.

A 25%-solution of glutaraldehyde was used to harden the microcapsules. Possible differences in reactivity between various gelatins and

glutaraldehyde were monitored previously; glutaraldehyde was added to coacervate films and allowed to react for 0.25 - 2 hours before washing and drying. The dissolution behavior of the hardened films was studied qualitatively. Differences in dissolution of the hardened films were only observed at reaction times up to 20 minutes. Hence, with a hardening time of over 20 hours any difference in reactivity was eliminated.

In the micro-encapsulation process of theobromine the pH was adjusted for each type of gelatin to the midpoint of the pH-range of maximum coacervate volume. After 15 minutes of coacervation the solution was cooled to 10°C (0.5°C/min) and the microcapsules were crosslinked by adding 5 ml (25%) glutaraldehyde-solution and mixing for 22 hours.

After washing with demineralized water (2 x 400 ml) and treatment with isopropanol (2 x 400 ml) the microcapsules were centrifuged and dried at 40°C during 12 hours.

## Particle size analysis

A microscope (Zeiss, Oberkochen, Germany) was used to analyze the size of the microcapsules. Samples were mounted on slides and the Feret-diameter of the microcapsules was determined ($n > 200$). The mean Feret-diameter was calculated from frequency-probability plots.

## Dissolution test method

The dissolution profile of micro-encapsulated theobromine was determined in an USP XXII dissolution test apparatus 2. Rotational speed of the paddle was 100 rpm. Hydrochloric acid (900ml, pH = 1.2, 37°C) was used as the dissolution medium. The curves represent the mean of six measurements (s.d. $\leq$ 1.2%). The amount of theobromine released from the microcapsules was determined using an automated spectrophotometer (Uvikon 710, Kontron AG, Zürich, Switzerland) at 273 nm; $A^1_1 = 554$. Sink conditions prevailed.

The total drug content was determined as described by Newton et al (8). In short, 20 mg microcapsules were digested in 25 ml artificial intestinal fluid. After 60 minutes the medium was diluted with demineralized water and the absorption was measured at 273 nm using an Uvikon 810 double-beam spectrophotometer (Kontron AG, Zürich, Switzerland).

### Flow characteristics

A standard hopper with an 7.5 mm orifice was used to determine the flow rate. The time necessary for 30 ml microcapsules to flow out of the hopper was determined (n = 10).

## RESULTS AND DISCUSSION

### Effect of pH on electrophoresis and coacervate volume

The effect of gelatin type (see table 1) on the micro-encapsulation process of theobromine was studied. First, optimum pH values for complex coacervation of gelatin and acacia were determined. In figure 1 the coacervate percentage for 1:1 mixtures of gelatin and acacia at various pH values are plotted. For high Bloom grade (HB) gelatins (A3, A4 and B) the total coacervate volume was higher than for low Bloom grade (LB) gelatin (A1). This can be explained since a LB gelatin will remain dissolved to a larger extent, resulting in an incomplete and less stable complex with acacia.

The pH-ranges with highest yield of complex coacervate are presented in table 1. For acid processed gelatins the pH-range with highest yield of coacervate is broader for a HB gelatin than for a LB gelatin. This range seems to be narrower for the alkaline processed gelatin.

For the various gelatins and acacia zeta-potential values were determined. In figure 2 the results are plotted as a function of pH. For type A gelatins zetapotential values were not significantly different. Hence, Bloom grade did not affect the zetapotential.

**Table 1.** Characteristics of various types of gelatin used in the micro-encapsulation process

| Gelatin type | Bloom grade | IEP[a] | EEP[b] | Coacervate pH range[c] |
|---|---|---|---|---|
| A1[d] | 75 (LB)[f] | 7.9 | 3.9 | 3.4 - 3.9 |
| A2 | 150 (MB)[g] | 8.4 | 3.8 | 3.3 - 4.3 |
| A3 | 232 (HB)[h] | 7.7 | 3.9 | 3.4 - 4.6 |
| A4 | 250 (HB) | 8.4 | 3.8 | 3.4 - 4.7 |
| B[e] | 260 (HB) | 4.7 | 3.9 | 3.5 - 4.3 |

[a])Iso-electric point; [b])Electrical equivalence pH; [c])Data based on maximum coacervate volume measurements; [d])Acid-processed and [e])Alkaline-processed gelatins; [f])Low Bloom grade; [g])Medium Bloom grade; [h])High Bloom grade;

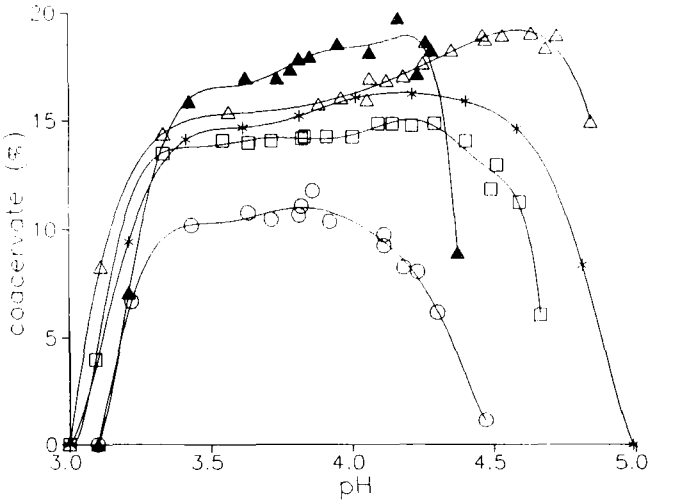



**FIGURE 1**

Effect of pH on coacervate volume of acacia and various types of gelatin (1:1). ·-· Gelatin A1, —gelatin A2, -·- gelatin A3, ·-· gelatin A4, ▲ gelatin B

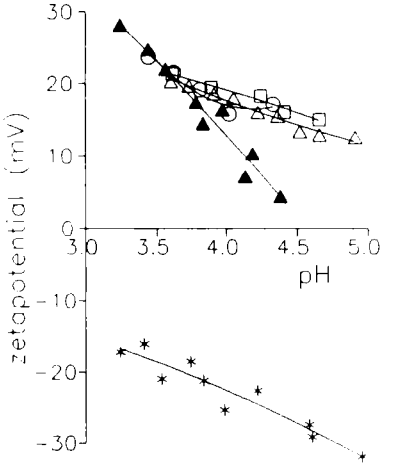
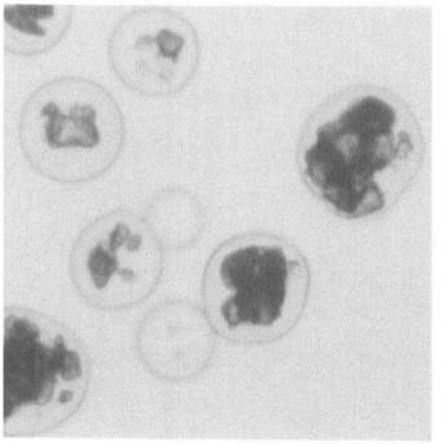



**FIGURE 2**

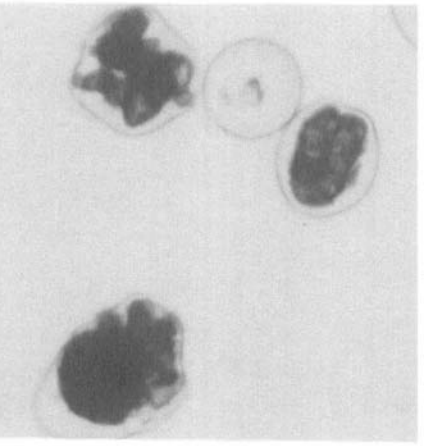
Zeta-potential of acacia and various types of gelatin at different pH values. ○ Gelatin A1, ⊣ gelatin A2, △ gelatin A4, ▲ gelatin B, * acacia.

Increasing the pH resulted in a more rapid decrease in zeta potential for type B than for type A gelatin. The same observations were made by Burgess and Carless (7). They ascribed these differences to the different ways of preparation and hence different amounts of $-NH_2$ and -COOH groups of the two gelatin types.

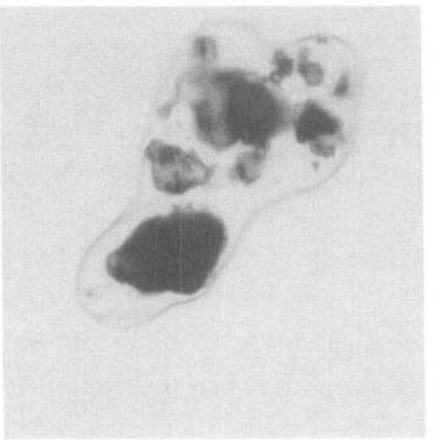
At the electrical equivalence pH (EEP) the net charge of the gelatin and acacia is zero. At this point the attracting forces between the oppositely charged components are the highest. At lower pH values the net charge of the poly-ions is positive and at higher pH values the net charge is negative. Intense binding and hence a high degree of coacervation was anticipated for acacia and the gelatin types at their respective EEP values. The EEP points of the various gelatins and acacia are presented in table 1. No significant differences in EEP values were observed. Comparison of the EEP values and pH range for highest yield showed that for all gelatins the EEP values are within the pH range of maximum coacervate volume, but not necessarily in their midpoint.



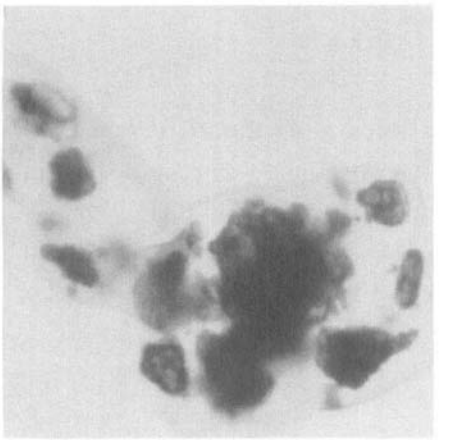
Gelatin A1



Gelatin A2



Gelatin A4



Gelatin B

**FIGURE 3**

Photographs of theobromine-containing microcapsules produced with various gelatin types ⊢—— 50 μm ——⊣.

**Table 2.** Characteristics of theobromine-containing microcapsules

| Gelatin type | Content of theobromine (%) | Mean particle diameter ($\mu$m) | Shape | Flow (s) |
|---|---|---|---|---|
| A1 | 69.1 (0.3) | 20 (5) | spherical | 5.8 (1) |
| A2 | 61.6 (0.4) | 30 (6) | spherical | 6.5 (0.5) |
| A4 | 57.3 (0.2 | 130 (11) | irregular | ∞ |
| B | 65.4 (0.3) | 100 (9) | irregular | ∞ |

Standard deviation in parentheses; ∞= infinite flow time.



This is in contradiction with observations from Burgess and Carless (7), who found that the pH value with highest degree of coacervation coincided with the EEP values.

Since one of the aims of this study was to determine the effect of different gelatin properties on the efficacy of the micro-encapsulation process, the experimental pH values were set at the midpoint of maximum coacervate volume.

## Micro-encapsulation of theobromine

Microcapsules of theobromine were formed according to the method described in the experimental section. Apart from gelatine type and pH value of the solution during complex formation all experimental conditions were kept constant.

In figure 3 photographs are shown of the microcapsules formed with the gelatins A1, A2, A4 and B. Microcapsules formed with HB gelatins (A4 and B) were irregular shaped, whereas LB-microcapsules were spherical. An explanation for this phenomenon may be the generally higher viscosity of colloid solutions containing HB-gelatins (9).

It was more difficult to recover individual microcapsules formed with HB gelatins than microcapsules produced with LB gelatin. In the drying step the HB-microcapsules agglomerated to clusters. Probably the

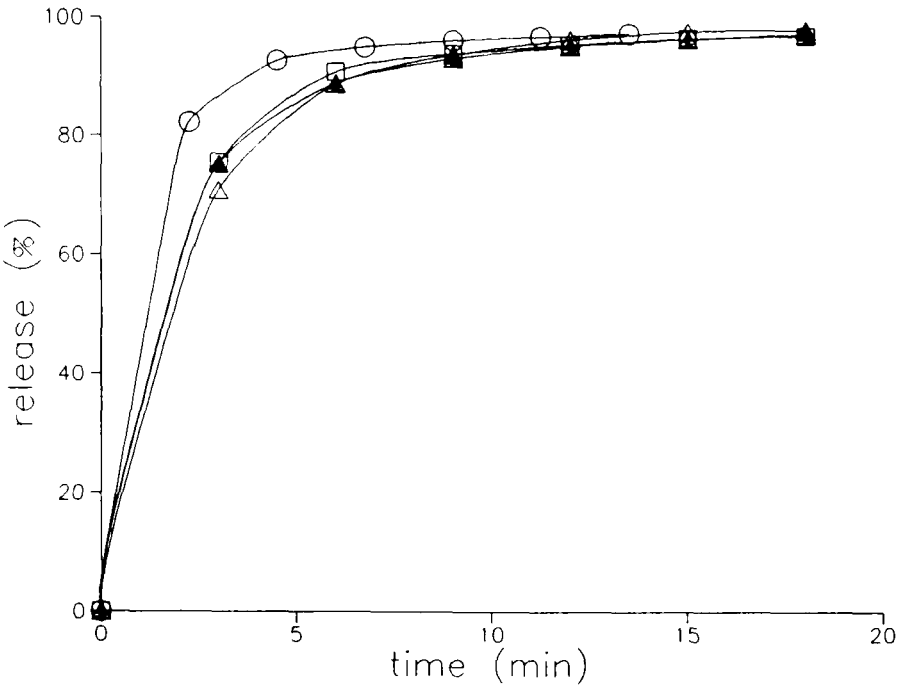



**FIGURE 4**

Release of theobromine from various types of microcapsules as a function of time. ○ Gelatin A1, □ gelatin A2, △ gelatin A4, ▲ gelatin B.

dehydration of the HB-microcapsules was incomplete and water acted as binding material.

In table 2 results of particle size analysis, flow property measurement and drug content determination of the microcapsules are summarized.

The irregular shaped HB-microcapsules, prepared with gelatins A4 and B did not flow at all, as was the case for pure theobromine. Encapsulation of theobromine with LB-gelatins resulted in small, spherical microcapsules with better flow characteristics compared to pure theobromine.

The Feret-diameter of the microcapsules increased with increasing Bloom grade for type-A gelatins. It was shown previously that the amount of coacervate increased with increasing Bloom grade (figure 1). These

higher amounts of coacervate may have resulted in microcapsules with thicker walls. Given a constant initial concentration of theobromine, this results in higher values for the diameter of the microcapsule.

The release rate of drug from microcapsules is an important issue. From the data obtained so far it was expected that the theobromine release rate would be lower for HB gelatin due to their higher wall thickness. In figure 4 the release profiles of theobromine from the various types of microcapsules are shown. Within 10 minutes 90%-release of drug was obtained for all batches which is comparable with the release profile of pure theobromine (90%-release within 6 minutes). Hence, within the described experimental set-up to manufacture the microcapsules, gelatin properties did not affect the release process, despite the presumed higher wall thickness for the HB-gelatins. Electron microscopic photographs showed the presence of cracks in microcapsules. It remains to be investigated whether these cracks caused the fast release of theobromine from the microcapsules.

## CONCLUSIONS

Optimum pH conditions for micro-encapsulation can be determined from measurements of total coacervate volume and from electrophoretic measurements. With the experimental set-up used in this study some discrepancies for the optimum pH range between the two methods were observed.

Gelatin properties clearly affected the complex coacervation and micro-encapsulation process. The alkaline-processed gelatin was more susceptible to pH changes compared with acid-processed gelatins. Given the experimental conditions used in this study, gelatins with high gel strength formed irregular shaped microcapsules with bad flow characteristics but with a high total yield of complex coacervate. The use of LB gelatins resulted in spherical microcapsules with better flow characteristics and a higher drug content.

The dissolution profiles of pure and encapsulated theobromine were superimposable, indicating that the microcapsule walls may not have been

perfectly formed. Cracks may have been present which facilitated drug release.



## ACKNOWLEDGEMENT

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