International Journal of Pharmaceutics, 27 (1985) 61–70
Elsevier

IJP 00901

Manufacture of gelatin/gelatin coacervate microcapsules

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(Received March 7th, 1985)
(Modified version received June 18th, 1985)
(Accepted July 5th, 1985)

Key words: complex coacervation – complex flocculation – gelatin – microelectrophoresis – microencapsulation – naproxen

Summary

Microelectrophoretic mobility profiles of two oppositely charged gelatins were used to determine the optimum pH and ionic strength conditions for coacervation to occur between the gelatins. Coacervation of aqueous mixtures of the two gelatins was only detected when the temperature was reduced below the gelation temperature of the gelatins. Coacervate yield was dependent on: the final temperature of the mixture; the time allowed for equilibration; the concentration of the gelatin solutions; and the pH and ionic strength of the media. Microcapsules containing naproxen were prepared by complex coacervation of the gelatins. Those variables which affected gelatin/gelatin coacervate yield had similar effects on microcapsule yield.

Introduction

Coacervation is a common method of microcapsule production and it is therefore important to understand the mechanisms controlling this process. Oppositely charged polyions in aqueous media may spontaneously coacervate to form two liquid phases; one concentrated in both polyions, the coacervate phase and the other dilute, the

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equilibrium phase (Bungenberg de Jong, 1949). The potential of encapsulation by coacervate droplets was first noted by Bungenberg de Jong (1949), who found that organic liquids or solid particles suspended in the equilibrium fluid of a coacervate system were taken up by coacervate droplets. Microencapsulation has found a variety of pharmaceutical uses. Harris (1981) achieved controlled release of potassium chloride by microencapsulation. Polli and Shoop (1976) prepared a palatable cholestyramine coacervate preparation using cellulose derivatives and alginates as complexing agents. Kitajima et al. (1976) have successfully microencapsulated aspirin to reduce irritancy. The most common method of coacervate microencapsulation used pharmaceutically involves complex coacervation between the polyions gelatin and acacia and has been extensively reported.

The aim of this study is to prepare microcapsules which release drug in a controlled and sustained manner. An investigation into the in vitro drug release from microcapsules prepared by the gelatin/acacia coacervation technique showed that these microcapsules did not meet the desired sustained release requirement (in vitro release studies; Burgess and Carless, 1985 and unpublished data). Drug release from microcapsules is dependent on the thickness of the microcapsule coat, and on the permeability of the drug molecules through this coat. The polyion coacervate coat is stabilized by the use of crosslinking agents such as glutaraldehyde and formaldehyde, which crosslink the gelatin molecules. The acacia molecules are not involved in this hardening process and it is therefore of interest to prepare complex coacervate microcapsules composed entirely of gelatin. Veis and co-workers (1960, 1961, 1963, 1967) prepared complex coacervates between solutions of oppositely charged gelatins. Veis could only achieve very low coacervate yields for gelatin/gelatin coacervation, but found that the yield increased on reducing the temperature of the mixture below the gelation temperature of the gelatins.

Complex coacervation is known to be dependent on a number of factors; pH, ionic strength, polyion concentration, polyion ratio, temperature (Bungenberg de Jong, 1949) and molecular weight (Overbeek and Voorn, 1957). The single most important factor is the charge of the two polyions. Veis (1967) showed that gelatin/gelatin coacervation was dependent on all these factors. Burgess and Carless (1984) described a method for the prediction and optimization of complex coacervation between two polyions, where microelectrophoresis was used to measure the charge on the polyions. It was hoped to achieve high gelatin/gelatin coacervate yields at temperatures above the gelation temperature of the gelatins, by optimizing the conditions as predicted by microelectrophoresis.

Materials and Methods

Two types of gelatin were obtained from Gelatin Products Ltd., U.K., Type A (acid processed) gelatin and Type B (alkali processed) gelatin. The gelatins had the following characteristics: Type A, Bloom No. 256, isoelectric pH 8.3, $M\bar{n}$. 4.7×10^4 and ash content 0.2% w/w.; Type B, Bloom No. 250, isoelectric pH 4.8, $M\bar{n}$. 4.6×10^4, and ash content 1.1% w/w. The isoelectric pH values were measured by

microelectrophoresis and by ion exchange. The $\overline{Mn}$. was measured by membrane osmometry using a Wescan Model 231 membrane osmometer, Wescan Instruments Inc.

Micronized Naproxen (BP) was obtained from Co. Farmaceutica Milanese. The naproxen powder was ball milled in an agate ball mill, to give a powder with a geometric weight mean diameter of 5.9 μm, and a standard deviation of 0.47 μm (Coulter Counter).

Microelectrophoresis

A Zeta-Meter was used in conjunction with a Plexiglas cell. Microelectrophoresis was conducted at 1 mM NaCl unless otherwise stated and in order to maintain constant ionic strength as the pH was varied, 1 mM NaOH and 1 mM HCl solutions were used. The polyions were adsorbed onto Minusil (colloidal silica), of particle size 2.7 μm (geometric weight-mean diameter, with a geometric standard deviation of 0.72 μm) prior to microelectrophoresis (Burgess and Carless, 1984). A 0.02% w/v polyion solution and a 0.01% w/v Minusil suspension were used. The electrophoretic mobility was the mean of at least 20 readings and the coefficient of variation was less than 5%.

Preparation and recovery of gelatin / gelatin microcapsules

This method is an adaptation of that of Nixon and Nouh (1978) for the production of gelatin/acacia microcapsules. Optimized conditions are described here and the range of conditions used is given in the results section. 250 ml each of 1% w/v deionized solutions of Types A and B gelatin were mixed together at 45°C with constant stirring for 1 h, after which the temperature was reduced to 25°C, which was maintained for 4 h. At the end of this period 10 ml of a 16% Formaldehyde Solution B.P. was added to harden the walls of the microcapsules, prior to rapid cooling of the suspension to 4–5°C (stirring was continued throughout the process). Where drug was added, this was first dispersed with glycerol, before adding to the Type B gelatin solution.

The microcapsules were centrifuged at low speed 1000–2000 rpm for 10 min at 5°C. The equilibrium fluid was decanted and the microcapsules were washed twice with cold water (5–10°C), once with a 1 : 1 mixture of isopropanol and water (5–10°C), and finally in cold isopropanol (5–10°C). The microcapsules were collected in silicone-coated evaporating dishes and dried over a stream of nitrogen gas to produce a free flowing powder.

Particle size analysis of gelatin / gelatin coacervates and microcapsules

Gelatin/gelatin coacervates and microcapsules were particle sized by Coulter Analysis, using Isoton II as a counting medium.

Results and Discussion

Investigation of gelatin / gelatin coacervation

The pH range where the two gelatins bear opposite charges and should therefore

form complex coacervates is, pH 4.8 to pH 8.3 (Burgess and Carless, 1984). The predicted optimum pH being, pH 5.4 (the electrical equivalence point, that is the pH where the two gelatins carry an equal and opposite charge). At pH 5.4 the electrophoretic mobility of both gelatins is low. 0.5×10^{-8} $m^2 \cdot s^{-1} \cdot V^{-1}$ and this relatively small charge may be insufficient to bring about coacervation (Burgess and Carless, 1984). The electrophoretic mobilities of both the gelatins increase significantly when the ionic strength is decreased below 1 mM from 0.5×10^{-8} $m^2 \cdot s^{-1} \cdot V^{-1}$ at 1 mM to 0.9×10^{-8} $m^2 \cdot s^{-1} \cdot V^{-1}$ at 0.25 mM (Burgess and Carless, 1984) and gelatin/gelatin coacervation should then occur more readily. This is in agreement with the studies of Veis and co-workers (1960, 1961, 1963, 1967).

Gelatin/gelatin coacervation was investigated at the predicted optimum coacervation conditions, i.e. pH 5.4, and zero ionic strength. A concentration range of 0.2–4.0% w/v (1 : 1 mixtures) was investigated at 40°C. However, neither macroscopic nor microscopic coacervation was evident over this concentration range and so the following variables were investigated: pH (range, pH 4.8–8.3); ionic strength (range, 0–10 mM); final gelatin concentration (0.5, 1.0 and 2.0% w/v); and mixing ratio (0.25–2.50, Type A to Type B gelatin). Although coacervation was not evident in any of these mixtures at 40°C, some of the mixtures did show unusual behaviour when the temperature was reduced below the gelation temperature of the gelatins. Within specific limits: pH (5.2–5.8); ionic strength (0–3 mM); total gelatin concentration (0.2 to 2.0); and polyion mixing ratio (0.75 to 1.50, Type A to Type B gelatin) the Type A/Type B gelatin mixtures flocculated on cooling. The time lapse prior to the onset of flocculation, as determined by visual examination, for Type A/Type B gelatin mixtures under a range of conditions are given in Tables 1 to 4.

Bungenberg de Jong (1949) reported that coacervation, flocculation, and precipitation are all closely related phenomena. He introduced the term 'complex flocculate' to describe flocculation which occurs on mixing two oppositely charged polyion solutions where flocculation is readily reversible, occurs over a particular pH range for a particular mixing ratio of polyions, and is suppressed by the addition of salt or

TABLE 1

THE EFFECT OF pH ON THE TIME REQUIRED FOR FLOCCULATION TO OCCUR IN TYPE A/TYPE B GELATIN MIXTURES

pH	Time (h)
4.8	– (gel formed)
5.0	– (gel formed)
5.2	– (gel formed)
5.3	1.5
5.4	0.3
5.5	1.0
5.6	3.0
5.8	– (gel formed)
6.0	– (gel formed)
6.5	– (gel formed0

TABLE 2

THE EFFECT OF IONIC STRENGTH ON THE TIME REQUIRED FOR FLOCCULATION TO OCCUR IN TYPE A/TYPE B GELATIN MIXTURES

Ionic strength (mM)	Time (h)
0.0	0.3
0.5	1.5
1.0	2.5
2.0	20.0
3.0	– (gel formed)
5.0	– (gel formed)
10.0	– (gel formed)

TABLE 3

THE EFFECT OF TOTAL GELATIN MIXING CONCENTRATION ON THE TIME REQUIRED FOR FLOCCULATION TO OCCUR IN TYPE A/TYPE B GELATIN MIXTURES (1 : 1)

Total gelatin concentration (% w/v)	Time (h)
4.0	– (gel formed)
3.0	– (gel formed)
2.0	20.0
1.6	2.5
1.2	1.0
1.0	0.3
0.8	1.0
0.5	2.5
0.4	4.5
0.2	20.0

TABLE 4

THE EFFECT OF TYPE A/TYPE B GELATIN MIXING RATIO ON THE TIME REQUIRED FOR FLOCCULATION OF THESE MIXTURES TO OCCUR

Mixing ratio Type A gelatin : Type B gelatin	Time (h)
0.25	– (gel formed)
0.50	20.0
0.75	0.5
1.00	0.3
1.25	0.5
1.50	0.5
2.00	20.0
2.50	– (gel formed)

by high polyion concentration. The flocculation observed in the gelatin mixtures studied may therefore be considered 'complex flocculation'.

Slow cooling of the gelatin should increase the chance of collagen fold occurring (Josse and Harrington, 1964) and therefore more ordered gelation should occur, promoting coacervation rather than disordered flocculation. Gelatin/gelatin mixtures as described above were cooled below their gelation temperature in a slow, controlled manner and what appeared to be very viscous coacervate droplets formed. Under microscopic examination these droplets were seen to flow and coalesce with one another, showing liquid properties which the flocculates described above did not possess. Gelation is presumably responsible for the viscous nature of these coacervate droplets.

The effect of variables on gelatin / gelatin coacervate yield

As described by Bungenberg de Jong, coacervate yield is likely to be dependent on: pH; ionic strength; polyion concentration; and polyion mixing ratio. Type A/Type B gelatin coacervate yield has been shown to be dependent on the final temperature of the mixture and on the time allowed for equilibration at this temperature, since this coacervation process is induced by gelation forces which take time to develop (Burgess and Carless, 1985).

The effect of total gelatin concentration on coacervate yield was investigated over the concentration range 0.2% w/v to 4.0% w/v, at a final temperature of 15°C, allowing a 6-h equilibration period (Fig. 1). Maximum coacervate yield (86.5% w/w) occurred at a total gelatin concentration of 0.5% w/v. No yield was detected at a concentration of 2.5% w/v or above. As the total gelatin concentration is increased the concentration of polyions in the equilibrium phase is increased and as a result the charges on neighbouring molecules may neutralize one another by coulombic attraction to form a large stable gel type network, reinforced by hydrogen bonding. As a result the energy gain on coacervate phase separation will be reduced and coacervation suppressed (self-suppression). A concentration of 2.5% w/v is, however, a comparatively low concentration for self-suppression to occur. Self-suppres-

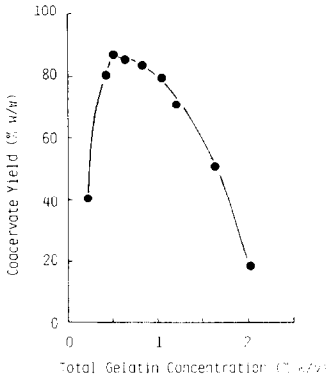

Fig. 1. The effect of total gelatin concentration on gelatin/gelatin coacervate yield.

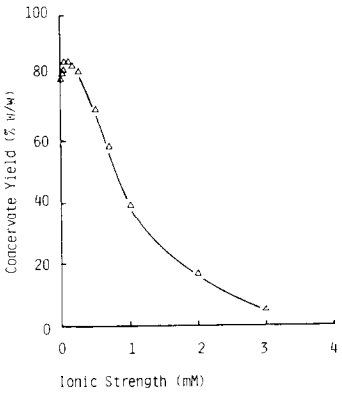

Fig. 2. The effect of ionic strength on gelatin/gelatin coacervate yield.

sion in gelatin/acacia coacervation occurs at much higher total concentration values (Bungenberg de Jong, 1949; Burgess, 1984). This is probably a consequence of the great extent of interaction possible between the two types of gelatin molecules in the equilibrium phase, which have similar structures and are in the expanded form.

The effect of ionic strength on coacervate yield over the range zero to 10 mM added NaCl is shown in Fig. 2. Coacervate yield increased slightly as the ionic strength increased from zero to 0.1 mM, further increase in ionic strength caused the coacervate yield to decrease rapidly and coacervation was completely suppressed at an ionic strength of 5.0 mM. This effect is therefore in agreement with the predictions from the microelectrophoresis data. Due to the dramatic effect of ionic strength on coacervate yield it was not possible to make an accurate assessment of the effect of pH on coacervate yield, since alteration of the pH involves introducing ions into the system. It was noted, however, as predicted from microelectrophoresis, that alteration of the pH away from pH 5.4 significantly reduced the coacervate yield. It appears that both electrostatic attraction and gelation forces are necessary for the formation of gelatin/gelatin coacervates.

Morphology of gelatin / gelatin coacervate droplets

Individual gelatin/gelatin coacervate droplets were produced at final temperatures of 30, 25 and 15°C by stirring the mixtures at 300 rpm. Following a 6-h equilibration period at the final temperature samples were viewed under an optical microscope. The samples equilibrated at 30°C were ellipsoidal, those at 25°C were either ellipsoidal or amorphous, and those at 15°C tended to be in an aggregated state and resembled the complex flocculi obtained when the gelatin mixtures are cooled rapidly. It appeared that in order to produce individual liquid coacervate droplets, the final temperature had to be 25°C or above.

Gelatin/gelatin droplets were prepared at various stirring rates with a final temperature of 25°C. The particle size of the coacervate droplets was determined by Coulter analysis at selected time intervals after the mixture had reached the equilibration temperature. The effect of time allowed for coacervation to occur on

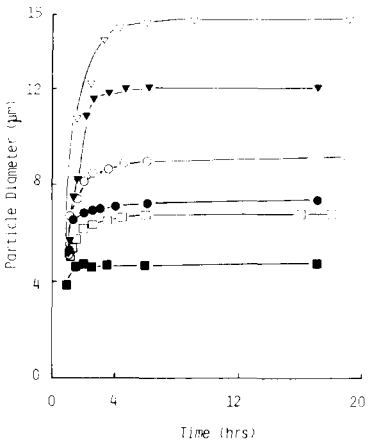

Fig. 3. The effect of time allowed for coacervation to take place on the particle size of gelatin/gelatin coacervate droplets prepared at different stirring rates. Key: ▽, 220 rpm; ▼, 300 rpm; ○, 400 rpm; ●, 450 rpm; □, 500 rpm; ■, 900 rpm.

the droplet size is shown in Fig. 3 for the various stirring speeds studied. At all the stirring rates investigated the particle size increased with time. This increase was rapid initially and then plateaued. The number of particles counted also increased with time, particularly over the initial period.

The morphology of the gelatin/gelatin coacervate droplets produced at 25°C is probably a consequence of the viscosity of the coacervate phase. The stirring forces may deform viscous droplets since the stabilizing forces within these droplets are insufficient to return the droplets to their original shape. Ellipsoidal droplets therefore result and when two or more of these droplets collide, agglomeration and partial coalescence may occur giving rise to amorphous droplets. Similar-shaped coacervate droplets have been reported by McMullen et al. (1982), prepared from viscous gelatin/pectin complex coacervates. Slower stirring speeds resulted in increased droplet size and in an increase in the proportion of amorphous droplets. Coacervate droplets prepared at 15°C resemble flocculi and are in a highly aggregated state. At 15°C 'complex flocculation' rather than complex coacervation must occur, since the temperature is too low for the complex to be in a liquid state.

Production of naproxen microcapsules

Microcapsules were prepared over a temperature range of 5–30°C, by the method given in the Materials and Methods section. The variables which affected gelatin/gelatin coacervate yield had similar effects on microcapsule yield. Ball-milled naproxen was used as the core material (drug-to-colloid ratio, 1 : 5). The gelatin/gelatin coacervate microcapsules were difficult to isolate due to the excess gelatin present in the equilibrium fluid which was of the order of 40% w/w of the total gelatin added for microcapsules prepared at 25°C.

The percentage yields of recovered microcapsules are shown in Table 5 for three different batches prepared at each temperature together with the average drug

TABLE 5

THE EFFECT OF FINAL TEMPERATURE ON PRODUCTION OF NAPROXEN MICROCAPSULES (DRUG : GELATIN RATIO = 1 : 5)

Temperature (°C)	Microcapsule yield (% w/v)	Average drug content per 500 mg of microcapsules (mg)	Drug encapsulated as a percentage of the total drug added (% w/w)
30.0	12, 10, 15	107.2	15.4
25.0	35, 57, 45	87.5	47.9
20.0	55, 67, 63	42.7	31.6
15.0	68, 72, 59	11.9	9.5
10.0	72, 78, 62	9.8	8.3
5.0	46, 75, 71	10.5	8.9

content of these microcapsules. The amount of drug encapsulated as a percentage of the total drug added is also shown. The variation in microcapsule yield at each final temperature was due to loss of sample at the isolation/recovery stage. A general increase in microcapsule yield as the final temperature was increased was still apparent, in agreement with the effect of final temperature on gelatin/gelatin coacervate yield. The percentage of colloid available to coat the drug particles decreased as the final temperature was increased since the coacervate yield decreased with temperature. This explains the higher drug content of microcapsules prepared at 30°C. The total amount of drug encapsulated was lower at higher temperatures possibly due to the lower percentage of colloid available to coat the drug. The low drug content in microcapsules prepared at low final temperature may be a result of loss of drug from microcapsules with defective walls. At final temperatures of 30°C and 25°C the coacervate droplets could be seen to form around the drug particles (viewed by optical microscope). At lower final temperatures however, complex flocs tended to form on and aggregate around the drug particles, forming an incomplete covering, and as a result most of the drug was lost when these 'microcapsules' were washed with isopropanol.

Conclusions

Microelectrophoresis data was successfully used to predict the effect of pH and ionic strength on gelatin/gelatin coacervation. This coacervate system was shown to be strongly dependent on temperature, temperature reduction being necessary to induce coacervation. This is probably a consequence of the small charges carried by the gelatins, these being too low to bring about phase separation by electrostatic interaction alone. Both electrostatic and gelation forces appear to be involved in gelatin/gelatin complex coacervation. Very slight changes in the manufacturing conditions may result in 'complex flocculation' rather than complex coacervation.

The gelatin/gelatin microencapsulation process described here takes several hours to carry out and is labour intensive. The critical limits of pH, ionic strength and polyion concentration are narrow and the percentage yields of microcapsules and the percentage drug encapsulated are both relatively low. The temperature at which the microcapsules are formed at is also very critical, as only a 5°C alteration in temperature may result in either a dramatic reduction in yield or in the production of microcapsules with defective walls.

Acknowledgements

The authors thank: Gelatin Products Ltd. for samples of gelatins; and the Nicholas Drug Consortium for financial assistance.

References

Bungenberg de Jong, H.G., Crystallisation-coacervation-flocculation. Complex colloid systems. In Kruyt, H.R. (Ed.), Colloid Science, Vol. II, Elsevier, Amsterdam, 1949, pp. 232–255, 335–429.

Burgess, D.J., Physico-chemical properties of complex coacervates of gelatin, Ph D Thesis (London), 1984, pp. 47–101.

Burgess, D.J. and Carless, J.E., Microelectrophoretic studies of gelatin and acacia for the prediction of complex coacervation. J. Colloid Interface Sci., 98 (1984) 1–8.

Burgess, D.J. and Carless, J.E., Complex coacervate formation between acid and alkali processed gelatins. In Bailey, F.E., and Eisenberg, A. (Eds.), Coulombic Interactions in Macromolecular Systems, ACS Symposium Series, American Chemical Society, Washington, DC, 1985, in press.

Harris, M.S., Preparation and release characteristics of potassium chloride microcapsules. J. Pharm. Sci., 70 (1981) 391–394.

Josse, J. and Harrington, W.F., Role of pyrrolidine residue in the structure and stabilization of collagen. J. Mol. Biol., 9 (1964) 269–287.

Kitajima, M., Kondo, A. and Arai, F., Process of producing aspirin-containing capsules. U.S. Patent, 3, 951, 851 (1976).

McMullen, J.N., Newton, D.W. and Becker, C.H., Pectin–gelatin complex coacervates I: Determinants of microglobule size, morphology, and recovery as water-dispersible powders. J. Pharm. Sci., 71 (1982) 628–633.

Polli, G.P. and Shoop, C.E., Palatable cholestyramine coacervate compositions. U.S. Patent 3, 974, 272 (1976).

Overbeek, J.TH.G. and Voorn, M.J., Phase separation in polyelectrolyte solutions. Theory of complex coacervation. J. Cell. Comp. Physiol., 49, Suppl. 1 (1957) 7–26.

Veis, A., Phase separation in polyelectrolyte solutions. II. Interaction effects. J. Phys. Chem., 65 (1961) 1798–1803.

Veis, A., Phase separation in polyelectrolyte systems. III. Effects of aggregation and molecular weight heterogeneity. J. Phys. Chem., 67 (1963) 1960–1964.

Veis, A. and Aranyi, C.J., Phase separation in polyelectrolyte systems. I. Complex coacervates of gelatin. J. Phys. Chem., 64 (1960) 1203–1210.

Veis, A., Bodor, E. and Mussell, S., Molecular weight fractionation and self-suppression of complex coacervation. Biopolymers, 5 (1967) 37–59.