Pectin–Gelatin Complex Coacervates I: Determinants of Microglobule Size, Morphology, and Recovery as Water-Dispersible Powders

JEAN N. McMULLEN *x, DAVID W. NEWTON ‡, and CHARLES H. BECKER §

Received March 9, 1981, from the **Faculty of Pharmacy, University of Montreal, Montreal, Canada H3C 3J7;* the ‡*Department of Pharmaceutics, College of Pharmacy, University of Nebraska Medical Center, Omaha NE 68105;* and the § *College of Pharmacy, University of Florida, Gainesville, FL 32610.* Accepted for publication September 3, 1981.

Abstract □ The pectin–gelatin complex coacervate system was evaluated and characterized. The effects of final pH, mixing pH, colloid ratio, and solution concentration were investigated. A recovery procedure yielding microglobules of a controlled and uniform size in dry powder form which were readily revertible in water to a polydispersed suspension was developed. The effect of various conditions and additives on the recovery morphology and size of the microglobules was evaluated.

Keyphrases □ Coacervate system—pectin–gelatin complex, final pH, mixing pH, colloid ratio, solution concentration □ Microglobules—pectin–gelatin, suspension in water, dry powder recovery system □ Pectin–gelatin system—microglobules, effects of final pH, mixing pH, colloid ratio, solution concentration

The complex ionic relations of the gelatin–acacia coacervate system have been established and reported previously (1–3) and have been used successfully in the microencapsulation of solids as well as liquids (4–10). Until recently (9), it was difficult to control the morphological characteristics of and recover a free-flowing powder of unagglomerated microglobules which were spontaneously dispersible in aqueous solutions. Therefore, it seemed desirable to develop a complex coacervate system from pectin and gelatin capable of yielding spherical globules of a uniform size that could be controlled for possible application as a pharmaceutical delivery system in parenteral or other products.

Although complex coacervation of the gelatin–pectin system was initially reported by Bungenberg de Jong (1) and employed as the basis of one patent in 1966 (11), the system has apparently received no further attention until this study. Studies of pectin–albumin (12) and pectin–polyethylenimine (13) have, however, been reported. This study was, therefore, undertaken to evaluate and characterize the fundamental complex ionic relations between the amphoteric protein, type A gelatin, and the low equivalent weight anionic colloid, pectin, with special emphasis on the effects of pH of coacervation (pH_c), mixing pH (pH_m), colloid ratio, and solution concentration on microglobule morphology, size, percent yield, and recovery technique yielding a free-flowing powder which was redispersible in water.

EXPERIMENTAL

Materials—Type A gelatin[1] and pectin, NF[2], were used in solutions containing 1% (w/w) benzyl alcohol for preservation. Formaldehyde solution, USP, glycerin, 99.6%, and isopropyl alcohol, 99%, were used in the recovery of microglobules in dry powder form. These and other reagents were of analytical reagent grade and were used as received.

Preparation and Recovery of Coacervates—Coacervates were prepared at 45° from 40.0-g batches stirred by means of a magnetic stirrer and polytef-coated bar at a speed sufficient to produce a vortex without entrainment of air bubbles. Stock pectin and gelatin solutions of the same weight concentration were prepared by dispersing the colloids in cold water, stirring for 30 min in a water bath at 45° and storing at 5° for no more than 24 hr before use. Appropriate weights of stock solutions of the same concentration were individually adjusted[3,4] to the mixing pH with 1.0 *N* NaOH at 45° to give the desired pectin to gelatin ratio. The gelatin solution was added to the pectin solution with stirring, and after 2 min the pH was lowered by fast addition of 0.5 *N* HCl to pH 5, then by slower addition of 0.5 *N* HCl until the desired pH_c was reached. The batch was stirred for 30 min then 5 ml of 37% (w/w) HCHO was added with stirring. After 30 min of stirring under ambient conditions, the batch was covered with paraffin film and allowed to stand for 15–20 hr. The batch was then

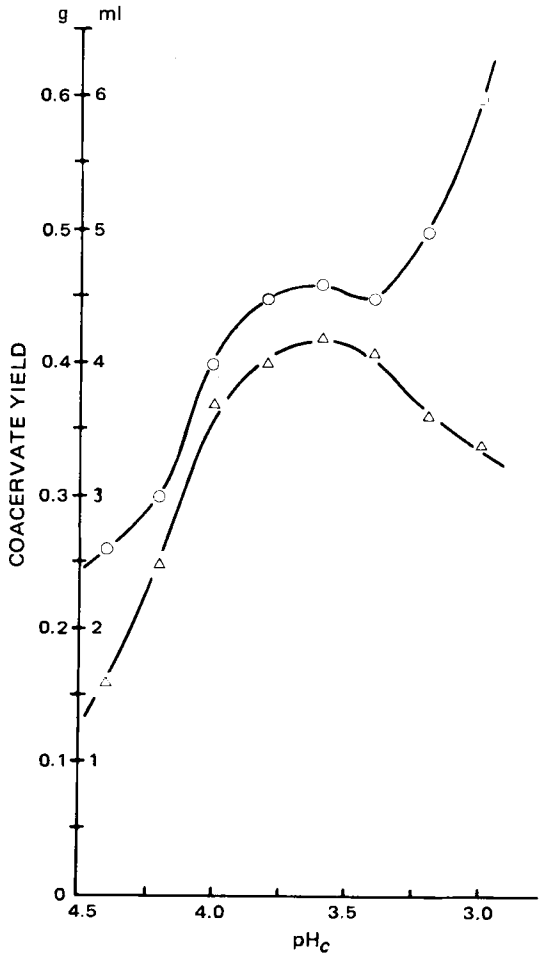


Figure 1—*Coacervate yields* versus pH_c *for isovolumetric (20 ml) 2% (w/w) gelatin and pectin solutions at* pH_m *9.0. Key:* O, *yield in milliliters after centrifugation at 2000 rpm for 60 min;* Δ, *yield of recovered dry microglobules, grams.*

[1] 275 Bloom, isoelectric point 8.6, Fisher Scientific Co., Fair Lawn, N.J.
[2] Sunkist Growers Inc., Ontario, Calif.
[3] Beckman Century SS-1 pH meter, Beckman Instruments, Fullerton, Calif.
[4] Miniature glass 476031 and calomel 476017 electrodes, Corning Scientific Products Co., Medfield, Mass.

0022-3549/82/0600-0628$01.00/0

Table I—Effect of pH_m on Diameter of Spherical Microglobules Produced from Isovolumetric Coacervates of 2% (w/w) Pectin (20 ml) and Gelatin (20 ml) Solutions Adjusted to pH_c 3.8 at 45°

pH_m	Diameter of Microglobules, ≤μm
3.80	0.5
5.85	1.1
6.51	1.4
6.98	2.0
8.06	3.9
9.01	5.4
9.49	7.3
10.04	10.0

stirred to resuspend any sedimented microglobules, and 40 g of the suspension was centrifuged at 1000–2000 rpm for 15–60 min, depending on microglobule size and morphology. The supernate was then decanted. The microglobules were resuspended in 5 ml of glycerin with a vortex mixer and 35 ml of isopropyl alcohol or other flocculating agent was slowly added while mixing. The flocculated microglobules were then filtered using a Buchner funnel and filter paper[5], washed with two 100-ml portions of isopropyl alcohol and dried for 15–20 hr in an oven at 36 ±1°.

Effect of Colloid Concentration—Pectin and gelatin solutions of 1.0, 1.5, 2.0, 2.5, and 3.0% (w/w) concentration were used. Batches were prepared by combining 20-g portions of each solution in order to give a colloid ratio of 1:1 (w/w). Coacervation was carried out for three pH_m values, 8.0, 9.0, and 10.0, over a pH_c range of 5.0–2.6, and evaluated for weight percent yield and microglobule morphology at 1000× under a microscope.

Effect of Colloid Ratio—Pectin to gelatin colloid ratios >1:1 were not studied because the average size of microglobules produced was <1 μm and, consequently, difficult to recover in dry form. The pectin–gelatin ratios studied, at a 2% (w/w) total colloid concentration, were 15, 25, 30, 33, 40, and 50% pectin fractions of total colloid. Coacervation was carried out according to the pH_m and pH_c conditions listed above. Microglobule morphology and weight percent yield were evaluated.

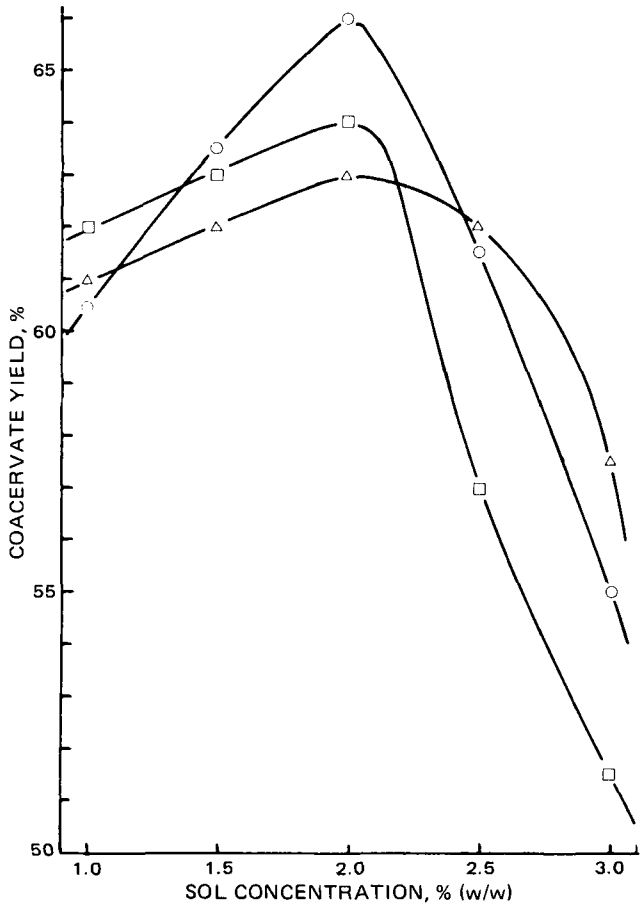


Figure 2—*Coacervate yield* versus *solution concentration for isogravimetric gelatin and pectin solutions adjusted to pH_m values of 8.0, 9.0, and 10.0 and coacervated to pH_c 3.5. Key: ○, pH_m = 8.0; □, pH_m = 9.0; △, pH_m = 10.0.*

[5] Whatman No. 3, Whatman, Inc., Clifton, N.J.

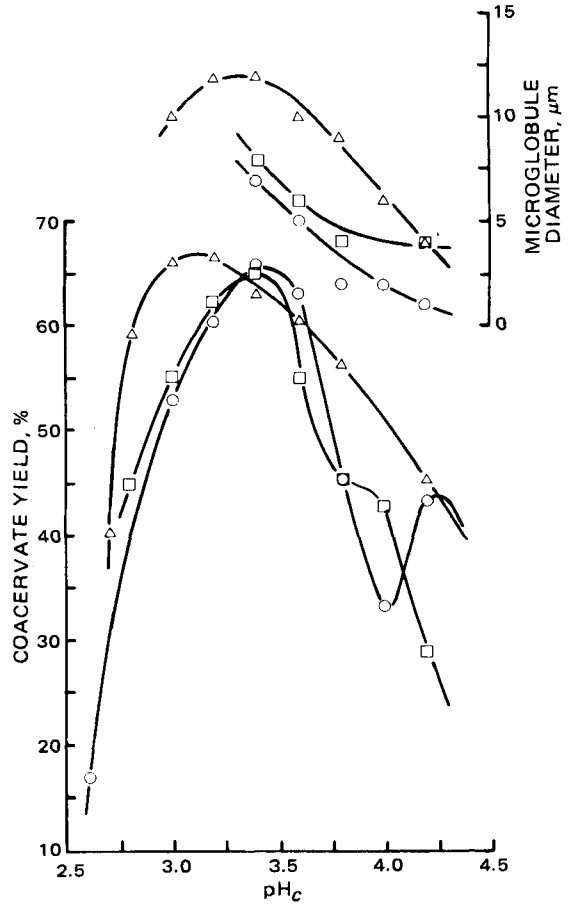


Figure 3—*Coacervate yield (left ordinate) and microglobule diameter (right ordinate)* versus *pH_c for isogravimetric (20 g) 2% (w/w) gelatin and pectin solutions adjusted to pH_m values of 8.0, 9.0, and 10.0. Key: ○, pH_m = 8.0; □, pH_m = 9.0; △, pH_m = 10.0.*

Effect of Additives on Recovery—Batches weighing 40.0 g were prepared from 2.0% (w/w) solutions to give a pectin–gelatin mixing ratio of 33:67%, and adjusted to a pH_m of 10.0 and pH_c of 4.5.

Glycerin volumes of 5, 10, 15, 20, 25, and 30 ml were slowly added to individual batches while mixing, with stirring at 45° after achieving pH_c 4.5.

Volumes of 37% (w/w) HCHO (adjusted to pH 5.0) of 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 ml were added to individual batches at 20° after achieving pH_c 4.5. The effect of formaldehyde treatment time was determined by adding 15 ml of 37% (w/w) HCHO and stirring for 1, 3, 7, 9, 12, 24, 36, and 54 hr.

Glycerin, propylene glycol, polyethylene glycol, 50% (v/v) isopropyl alcohol in distilled water, and distilled water were evaluated as redispersing media. Volumes of 5 ml of each were added to individual batches after centrifugation and decantation of the supernate.

The effect of various water miscible flocculating agents was determined by adding to individual batches 35 ml of isopropyl alcohol, 1-propanol, ethanol, methanol, and acetone.

All batches studied for the effect of additives on recovery were evaluated as to their dry product characteristics, such as coherence and friability, and redispersibility in distilled water.

RESULTS AND DISCUSSION

Until the effect of pH_m was elucidated, previously reported complex coacervation procedures, particularly for the gelatin–acacia system, were applied to the gelatin–pectin system with little success. Some results of this exploratory phase are summarized in Table I. As reported previously (14), mixing of isohydric solutions (pH 3–4) does not change the system pH significantly, a fact demonstrated in this study but also one that

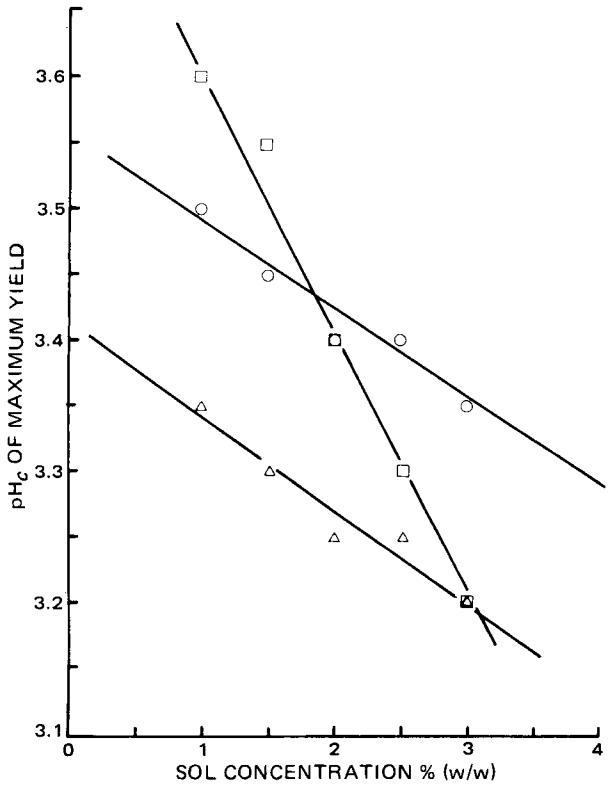


Figure 4—*pH_c of maximum microglobule yield* versus *total colloid concentration in pectin–gelatin (1:1) coacervates prepared from solutions adjusted to pH_m values of 8.0, 9.0, and 10.0. Key: ○, pH_m = 8.0; □, pH_m = 9.0; △ pH_m = 10.0.*

yielded such small globules as to be hardly resolvable under the microscope at 1000×. The method of Luzzi (4), mixing of isohydric (pH 6.5), isovolumetric solutions then adjusting the pH of the mixture with acid to the desired endpoint (pH 3.8), resulted in a marked increase in globule diameter to ~1 μm. The method of Newton (9), mixing of isovolumetric solutions, gelatin (pH 10) and pectin (pH 3.6), then adjusting the pH of the mixture with acid to the desired endpoint (pH 3.8), gave comparable results. These results suggested the possible involvement of the mixing pH in the control of microglobule size which was confirmed in a series of experiments where isohydric, isovolumetric solutions were adjusted to pH 7.0, 8.0, 9.0, 9.5, and 10.0 before mixing and coacervation to pH 3.8. The mean globule size increased from 2 to 10 μm as the mixing pH was increased from 7–10 (Table I).

Volume measurements of coacervate phases were impractical due to the noncoalescence of the globules into a continuous phase. A comparison of sediment volume of microglobules after centrifugation with yield based on dry weight as a function of pH_c is given in Fig. 1. The increasing sediment volume at pH_c <3.4 is attributed to a change in morphology from globular to elliptical particles having a lower packed density and greater hydration. Therefore, dry weight recovery was adopted to measure microglobule yields in all subsequent experiments.

Effect of Colloid Concentration—The results of studies to determine the effects of total colloid concentration on coacervate yield for solutions having equal concentrations of pectin and gelatin showed that maximum yield was obtained at a total colloid concentration of ~2% (w/w) for the mixing of isohydric, isogravimetric solutions coacervated to pH_c 3.5 (Fig. 2). The increasing efficiency of coacervation with increasing total colloid concentration up to a maximum of ~2.0% probably resulted from increased reactivity of the gelatin and pectin linear molecules as the forces of intramolecular aggregation limiting their movement were minimal. Above 2% (w/w) of total colloids, the decreasing efficiency of coacervation arises from an increasing salt or gegenion (*e.g.*, Na^+, Ca^{2+}, Cl^-) concentration which insulates the oppositely charged colloids, suppressing coacervation and increasing the mutual solubilities of the coacervate and equilibrium solution phases (14).

Figure 3 shows the relationship for the 2% colloid concentration between percent yield[6] and microglobule morphology as a function of pH_c

[6] Expressed as percent (w/w) of the total mass of pectin and gelatin in the coacervate system.

Table II—Effect of Glycerin on the Morphology and Powder Characteristics of Pectin–Gelatin Microglobules Recovered from Coacervate Systems Prepared from 2% (w/w) Pectin–Gelatin (33:67) at pH_m 10.0 and pH_c 4.5

Glycerin, % w/w[a]	Microglobule Characteristics: Morphology[b]	Microglobule Characteristics: Dimension, μm[c]	Recovered Product Characteristics[d]
0	S	11	C
13.5	S	14	C
25.7	S	12	C
31.9	S	13	C
49.1	E	22	F
56.1	E	20	F
72.4	E	21	F

[a] Percent by weight of coacervate system after achieving pH_c 4.5. [b] S—spheres; E—ellipsoids. [c] Maximum dimension of largest particle in field of view at 1000× magnification. [d] C, coarse powder; F, fine, light powder; both spontaneously revertible to individual units in aqueous suspension.

for pH_m values of 8.0, 9.0, and 10.0. Microglobule size increased as the pH_c decreased, until the maximum yield was reached, at which point the shape was transformed from spherical to ellipsoidal or amorphous.

The dependence of microglobule size on pH_c at fixed pH_m likely arises from the fact that as the pH_c is lowered, the relative ratio of pectin to gelatin increases in the coacervate. Furthermore, the more intense complex ionic relations of the pectin–gelatin *versus* the acacia–gelatin system would cause a greater coacervate reduction in the water content of the coacervate and a total coacervate colloid concentration >10%, a value reported for the gelatin–acacia system (15), thereby affecting droplet size and solubility[7].

As the system is adjusted to lower pH_c, the number of anionic charges on pectin decreases while the number of cationic charges increases on gelatin, resulting in more and more pectin being required for an equivalent union with gelatin. Therefore, at high pH_c values, the combination of both a high total colloid concentration and a low pectin–gelatin ratio in the coacervate resulted in stabilization of the coacervate drops as a result of a high viscosity and partial gelling of the microglobules precluding their coalescence. As the pH_c was lowered, the pectin–gelatin

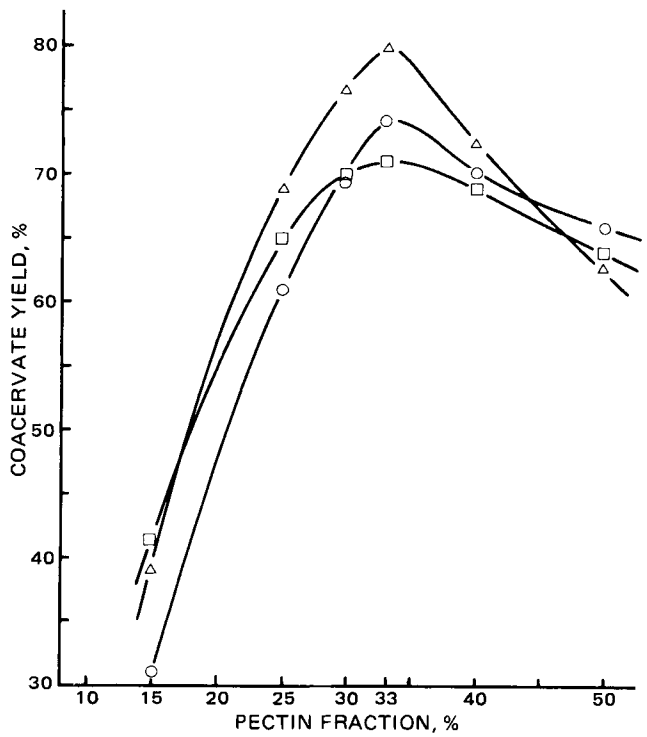


Figure 5—*Coacervate yield* versus *pectin–gelatin and pectin solutions adjusted to pH_m values of 8.0, 9.0, and 10.0 and coacervated to pH_c 3.5. Key: ○, pH_m = 8.0; □, pH_m = 9.0; △, pH_m = 10.0.*

[7] The lower the equivalent weight of anionic colloid the higher is its charge density and, thus, the stronger are its complex ionic relations with cationic gelatin. The equivalent weights of acacia and pectin (pectate) are 1200 and 200, respectively (1, 15).

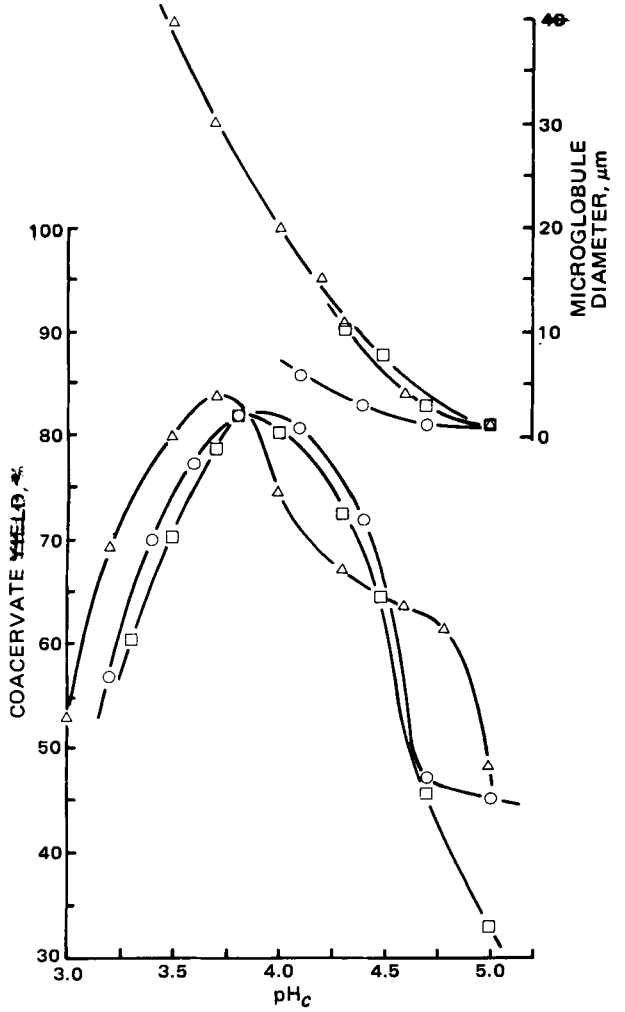


Figure 6—*Coacervate yield (left ordinate) and microglobule diameter (right ordinate)* versus *pH_c for coacervate systems prepared from a 33:67 gravimetric ratio of 2% (w/w) pectin and gelatin solutions, respectively, adjusted to pH_m values of 8.0, 9.0 and 10.0. Key: ○, pH_m = 8.0; □, pH_m = 9.0; △, pH_m = 10.0.*

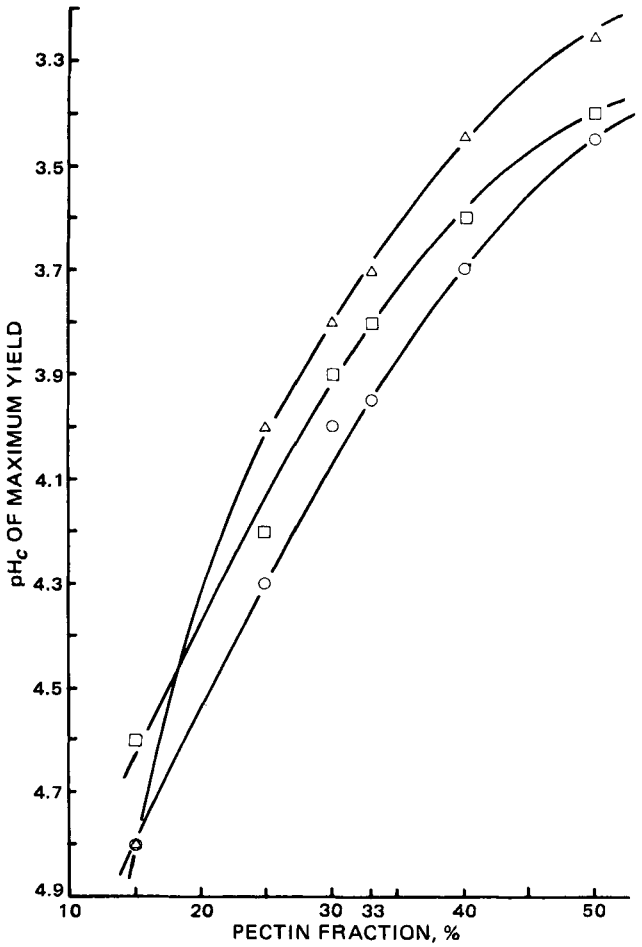


Figure 7—*pH_c of maximum yield* versus *pectin weight fraction of total colloid in coacervates prepared from 2% (w/w) gelatin and pectin solutions adjusted to pH_m values of 8.0, 9.0, and 10.0. Key: ○, pH_m = 8.0; □, pH_m = 9.0; △, pH_m = 10.0.*

ratio in the coacervate increased allowing microglobule growth to proceed further before an equilibrium sphere occurred at a higher equilibrium size.

Microglobule size increases with decreasing pH_c and with increasing pectin–gelatin ratio in the coacervate, probably as a result of increasing coacervate viscosity until the stabilizing forces become too weak to prevent deformation of the microglobules by the stirring forces. This results in ellipsoidal particles which cannot readily return to the spherical state because of their very viscous and gel-like state and finally amorphous particles as a result of agglomeration and partial coalescence of ellipsoidal particles. The microglobules are further stabilized from coalescing into a single phase by the anionic charge they acquire as they form above the optimum pH_c. As for the gelatin–acacia system (16), as the PH_c is lowered, the net charge on the microglobules remains negative because of the predominance of dissociated carboxylate groups on pectin and gelatin as opposed to cationic amino groups on gelatin.

The pronounced effect of pH_m on microglobule morphology is probably related to the pectin–gelatin ratio in the coacervate and the gegenion concentration. It has been reported that pectin molecules in solution exist in a high state of aggregation in solutions >0.1%, as a result of suppressed ionization of carboxyl groups (17). It was found through titration and pH *versus* viscosity studies of 2% solutions at 45° that some carboxyl groups are not readily ionizable until pH 7.5, above which such solutions show a sharp decrease in viscosity, which can be attributed to increased repulsive forces tending to further lower the degree of aggregation. Therefore at pH_m 8.0 in 2% (w/w) solutions, the reactivity of pectin with gelatin is limited by the higher degree in intramolecular aggregation as compared to that at higher pH_m, *i.e.*, 9.0 or 10.0. Subsequently, this results in a lower pectin–gelatin ratio at pH_c. As the pH_m is raised >8, deaggregation of pectin increases, permitting more pectin molecules to react with gelatin molecules and microglobules and higher yields.

Figure 4 indicates that as the solution concentration increases, the pH_c of maximum coacervation decreases; an observation also made previously (14) for gelatin–acacia coacervates. Again, as the concentration of solutions increases, so does that of the accompanying gegenions which suppress coacervation. Hence, pH_c must be lowered to increase the cationic charge on gelatin, while causing a smaller decrease in the anionic charges on both pectin and gelatin.

As expected, there was a linear relationship between the volume of 0.5 *N* HCl added and pH_c, verifying that the complex ionic relations between pectin and gelatin were stoichiometric and rapid (9, 18).

Effect of Colloid Ratio—On the basis of results obtained in studies of the optimum total colloid concentration to maximize microglobule yield, 2% (w/w) was used in evaluating the effect of varying pectin–gelatin ratios of 15:85, 25:75, 33:67, 40:60, and 50:50. Figure 5 indicates that the maximum yield was obtained at a colloid ratio of pectin–gelatin (33:67)

Table III—Flocculation Rates by Four Alcohols and Acetone of Coacervate Microglobules Prepared from 2% (w/w) Pectin–Gelatin (33:67) at pH_m 10.0 and pH_c 4.5

Flocculating Agent	Relative Flocculation Rate[a]	Product Characteristics[b]
1-Propanol	5	C, D
Isopropyl alcohol	4	C, D
Ethanol	3	G, N
Methanol	2	F, N
Acetone	1	F, N

[a] Rate increases from 5 to 1. [b] C, coarse powder; D, spontaneously dispersible to individual spheres in water; G, granular powder; N, not dispersible as individual spheres in water; F, fused filter cake mass.

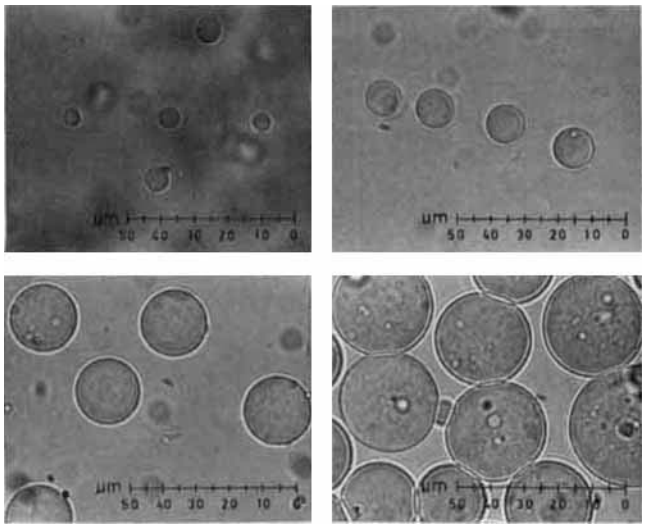


Figure 8—*Pectin-gelatin microglobules having nominal diameters of 5, 10, 20, and 30 μm.*

for the mixing of isohydric solutions coacervated to a pH_c of 3.5. The percent yields of microglobules ranged from ~70–80% of total colloids available, indicating a high reactivity in the complex coacervate system.

The fact that at a 15% pectin fraction the maximum yield was higher than expected is misleading because the microglobules or amorphous particles obtained were highly vacuolated, containing uncoacervated colloid-rich liquid, referred to as hollow spheres (19).

Figure 6 demonstrates the relationship for 2% (w/w) solutions between percent yield, microglobule diameter, and pH_c for pectin–gelatin colloid ratios of 33:67 at pH_m values of 8.0, 9.0, and 10.0. The microglobule diameter increased by ~3.3 μm/0.1 pH unit decrement, *i.e.*, from 20–40 μm as pH_c was lowered from 4.0 to 3.6 at pH_m 10.0. Microglobule diameter increased proportional to increasing pH_m and decreasing pH_c probably for the same reasons previously discussed. At $pH < pH_c$ of maximum yield, the globules became elliptical and amorphous.

Figure 7 shows that as the pectin fraction increased in coacervate systems of 2% (w/w) colloid concentration, the pH_c of maximum coacervation decreased from pH_m 8.0, 9.0, and 10.0.

Figure 8 shows typical microglobules having nominal diameters of 5, 10, 20, and 30 μm demonstrating control of microglobule size and the ability of spontaneously reverting to a polydisperse system like that of the gelatin–acacia microglobules in a figure from a study published previously (9).

Effect of Additives on Recovery—The effect of glycerin added to coacervate systems at 45° after achieving pH_c is shown in Table II. With the addition of ≥49% (w/w) glycerin, ellipsoidal particles were obtained, a possible result of partial dehydration of the pectin and gelatin molecules in the coacervate and of increased intramolecular association, resulting from the decreasing dielectric constant of the system. A similar result was found with gelatin–acacia coacervates where from 23 to 41% (v/v) of glycerin yielded nonvacuolated, spherical microglobules, but ≥44% (v/v) glycerin resulted in vacuolated ellipsoids (20). In the pectin–gelatin system, glycerin improved the fineness of the dry recovered powder, but had no apparent effect on the dispersibility of the microglobules in water.

The results of formaldehyde treatment of the microglobules showed that at least 10 ml of 37% HCHO, preferably 15 ml, per 40-g batch prepared from 2% (w/w) pectin–gelatin solutions (33:67) was required to obtain microglobules in dry powdered form spontaneously revertible to a dispersed suspension in water. This result was comparable to that for gelatin–acacia microglobules (9). A minimum formaldehyde treatment time of 9 hr was required to obtain redispersible microglobules prepared by the previously mentioned conditions.

Glycerin, propylene glycol, and polyethylene glycol were equally satisfactory as redispersing media for the formaldehyde treated, unflocculated, sedimented microglobules. Water and 50% (v/v) isopropyl alcohol yielded a finer dry powder, but the rate of filtration of the centrifuged sediments was unacceptably lengthened.

The flocculation rates of the microglobules, by 35-ml portions of four aliphatic alcohols and acetone added to the centrifuged sediments of microglobules (~3–4 ml, Fig. 1) resuspended in 5 ml of glycerin after preparation from 2% (w/w) pectin and gelatin solutions according to previously specified conditions, are described in Table III. Flocculation by only 1-propanol and isopropyl alcohol elicited microglobules spontaneously dispersible in aqueous suspension, whereas ethanol, methanol, and acetone resulted in filter cakes which were not revertible to suspensions of individual microglobules in water. Although these results corroborate those for gelatin–acacia (9) and gelatin (21) coacervate products, there is no readily discernible relationship, *e.g.*, with dielectric constant, ε[8]. The greater dehydration of pectin and some remaining hydrophilic groups on gelatin[9] by ethanol, methanol, and acetone, resulting from their stronger hydrogen bonding affinity for water, could explain the apparent intramolecular–intermolecular colloid aggregation and the failure of the microglobules to disperse as individual units upon suspension in water.

CONCLUSIONS

Based on these studies, it was possible to recover microglobules of controlled and uniform diameter from complex coacervates of pectin–gelatin by regulating the concentrations of pectin and gelatin solutions, pH_m, pH_c, and the pectin–gelatin ratio as well as certain additives. The spherical microglobules so obtained are spontaneously revertible to a dispersed system upon suspension of the powdered products in aqueous solutions.

REFERENCES

(1) H. G. B. de Jong, in "Colloid Science," vol. 2, H. R. Kruyt, Ed., Elsevier, New York, N.Y., 1949, pp. 186–193, 243–276, 338–450.

(2) A. B. Dhruv, T. E. Needham, Jr., and L. A. Luzzi, *Can. J. Pharm. Sci.*, **10**, 33 (1975).

(3) M. Glicksman and R. E. Sand, in "Industrial Gums," 2nd ed., R. L. Whistler, Ed., Academic, New York, N.Y., 1973, pp. 234–238.

(4) L. A. Luzzi and R. J. Gerraughty, *J. Pharm. Sci.*, **53**, 429 (1964).

(5) *Ibid.*, **56**, 634 (1967).

(6) *Ibid.*, **56**, 1174 (1967).

(7) S. Javidan, R. Hague, and R. G. Mrtek, *J. Pharm. Sci.*, **60**, 1825 (1971).

(8) P. L. Madan, L. A. Luzzi, and J. C. Price, *ibid.*, **61**, 1586 (1972).

(9) D. W. Newton, J. N. McMullen, and C. H. Becker, *ibid.*, **66**, 1327 (1977).

(10) H. Takenaka, Y. Kawashima, and S. Y. Lin, *ibid.*, **69**, 513 (1980).

(11) Ciba Ltd., French pat. 1,462,506 (1966); through *Chem. Abstr.*, **67**, 67587g (1967).

(12) A. P. Sal'chinkin and F. L. Movshovich, *Colloid J. (USSR)*, **6**, 15 (1940); through *Chem. Abstr.*, **36**, 29119 (1941).

(13) H. Deuel, J. Solms, and A. Denzler, *Mitt. Lebensm. Hyg.*, **45**, 73 (1954); through *Chem. Abstr.*, **48**, 9578b (1954).

(14) H. G. B. de Jong, in "Colloid Science," vol. 2, H. R. Kruyt, Ed., Elsevier, New York, N.Y., 1949, pp. 340–370.

(15) *Ibid.*, pp. 340–345, 362, 364, 368–370.

(16) *Ibid.*, pp. 322–326.

(17) H. S. Owens, H. Lotzkar, R. C. Merrill, and M. Peterson, *J. Am. Chem. Soc.*, **66**, 1178 (1944).

(18) H. Terayama, *J. Polym. Sci.*, **8**, 243 (1952).

(19) H. G. B. de Jong, in "Colloid Science," vol. 2, H. R. Kruyt, Ed., Elsevier, New York, N.Y., 1949, pp. 454–466.

(20) D. W. Newton, Ph.D. dissertation, University of Florida, Gainesville, Fla., 1976.

(21) J. R. Nixon, S. A. H. Khalil, and J. E. Carless, *J. Pharm. Pharmacol.*, **20**, 528 (1968).

(22) "Handbook of Chemistry and Physics," 56th ed., C. R. Weast, Ed., CRC, Cleveland, Ohio, 1975, p. E-56.

[8] The decreasing order of ε at 25° for the solvents is: methanol, 32.6; ethanol, 24.3; acetone, 20.7; 1-propanol, 20.1; isopropyl alcohol, 18.3 (22).

[9] Formaldehyde treatment renders the gelatin component partially insoluble by condensing with nonprotonated RNH_2, R'NHR, and RCONH groups, *i.e.*: $RNH_2 + R'NHR \xrightarrow{HCHO} RNHCH_2R'NHR + H_2O$, resulting in a more hydrophobic (less hydrogen bonding) product (23).

(23) H. L. Fraenkel-Conat, M. Cooper, and H. S. Olcott, *J. Am. Chem. Soc.*, **67**, 950 (1945).

ACKNOWLEDGMENTS

Abstracted in part from a dissertation presented by J. N. McMullen to the Graduate Council of the University of Florida in partial fulfillment of the Doctor of Philosophy degree requirements.

The authors thank Mrs. Ginette Simard and Mrs. Donna Earnshaw for their assistance in preparing this manuscript.

Pharmacokinetic Profile of Progabide, a New γ-Aminobutyric Acid-Mimetic Drug, in Rhesus Monkey

IKUO JOHNO, B. TERI LUDWICK, and RENÉ H. LEVY ˣ

Received March 30, 1981, from the *Departments of Pharmaceutics and Neurological Surgery, Schools of Pharmacy and Medicine, University of Washington, Seattle, WA. 98195.* Accepted for publication September 10, 1981.

Abstract □ The pharmacokinetic profile of progabide was investigated in five chronically catheterized male rhesus monkeys. The experimental design included single-dose intravenous bolus and oral administration at two dose levels (50 and 100 mg) and zero-order intravenous infusion for 7 days. Plasma samples were analyzed by electron-capture–GLC. Protein binding of the drug was determined by equilibrium dialysis (4°). A one-compartment open model with monoexponential decay was proposed to describe the pharmacokinetics. The mean parameters (±*SEM*) of the 50- and 100-mg iv bolus were: total body clearance, 2.09 (±0.15) and 1.53 (±0.18) liter/hr/kg; half-life, 0.656 (±0.054) and 0.789 (±0.079) hr; distribution volume, 1.97 (±0.08) and 1.79 (±0.21) liter/kg. Progabide was highly bound to plasma proteins and also to erythrocytes. The drug was rapidly absorbed (T_{max} < 1 hr at both doses). The mean bioavailability was attributed principally to a first-pass effect. In the constant rate infusion study, systemic clearance was larger than that of the single dose studies.

Keyphrases □ Progabide—pharmacokinetics in rhesus monkeys, plasma bioavailability □ Bioavailability—pharmacokinetics of progabide in plasma of rhesus monkeys □ Pharmacokinetics—bioavailability of progabide in plasma of rhesus monkeys □ Electron-capture–GLC—determination of progabide in rhesus monkey blood plasma, bioavailability, pharmacokinetics

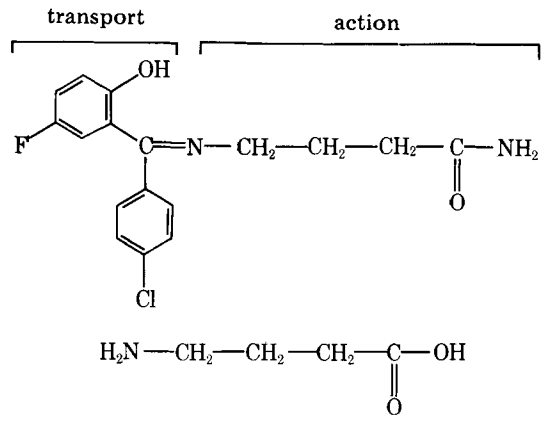
Progabide[1] is a new γ-aminobutyric acid-mimetic drug with a broad spectrum of anticonvulsant activity (1–6). Progabide was designed to be a carrier of γ-aminobutyramide into the central nervous system where it would be broken down and release γ-aminobutyramide.

The present study was undertaken to characterize the pharmacokinetic behavior of progabide in the rhesus monkey in view of its efficacy testing in the chronic epileptic monkey model.

EXPERIMENTAL

Five healthy male rhesus monkeys (*Macaca mulatta*) (2.7–3.8 kg) were used in this study. The monkeys were maintained on fresh fruits and monkey chow and chaired for the duration of the study. Each monkey had two chronic catheters, one for drug administration (femoral vein) and one for blood sampling (jugular vein). The experimental design included single-dose and chronic administration studies. In the single-dose studies, each monkey received 50 or 100 mg of the drug intravenously and orally in a randomized fashion. The drug was dissolved in 100% polyethylene glycol 400[2] at concentrations of 25 and 50 mg/ml. Two milliliters of this solution was injected over 5 min through the femoral vein or administered by intranasal gastric intubation. Fourteen (50-mg dose) or 15 (100-mg dose) blood samples were collected over a 5–6-hr period. Ethylenediaminetetraacetic acid tripotassium salt was used as an anticoagulant, and the blood samples were stored in ice. They were centrifuged at 4° and the separated plasma was frozen immediately. The samples were kept frozen until assayed. Some blood samples were also frozen to determine the blood level of the drug and the total blood clearance in each monkey.

I



I

The chronic administration studies consisted of constant rate intravenous infusion for 7 days. The drug was dissolved in 80% polyethylene glycol 400 at a concentration of about 3 mg/ml (1.91–3.84 mg/ml), and the infusion rate was 1 ml/hr. Blood samples were collected during the accession to steady state (0–6 hr), on a daily basis during steady state, and during the post steady-state decay phase.

Protein binding of progabide was measured by equilibrium dialysis at 4° to minimize the degradation of drug in plasma. A volume of 0.7 ml of pooled monkey plasma, and an equal volume of isotonic phosphate buffer system (pH 7.4), were used. The dialyzing system consisting of plexiglass cells was rotated at a rate of 10 rev/min for 12 hr. The dialysis was carried out with spiked plasma samples with concentrations ranging from 0.4 to 34.6 μg/ml (determined postdialysis). The drug on both sides of the membrane was analyzed by electron-capture–GLC.

Progabide concentrations were determined by the method of Gillet and Dring[3] using a GLC equipped with electron-capture detector[4]. The column consisted of 3% OV-17 on Chromosorb W-HP (column length was 184.3 cm and 2-mm i.d.). Temperatures of detector, injection port, and oven were 300, 250, and 240°, respectively. An analog of progabide, [α-(chloro-4′-phenyl)chloro -5- hydroxy-2-benzylideneamino]-4-butyramide, was used as the internal standard.

Areas under intravenous and oral curves were calculated by the trapezoidal method (with extrapolation to infinite time). Systemic clearance was determined by dose–area ratio. Oral bioavailability was calculated by ratio of oral and intravenous areas.

[1] Synthélabo-L.E.R.S., Paris, France.
[2] J. T. Baker Chemical Co., Phillipsburg, NJ 08865.
[3] Unpublished method, 1978.
[4] Model 5710A, Hewlett-Packard, Palo Alto, CA 94304.

0022-3549/82/0600-0633$01.00/0