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Phase Separation Induced in Gelatin-Base Coacervation Systems by Addition of Water-Soluble Nonionic Polymers I: Microencapsulation

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Abstract □ A microencapsulation procedure in which water-soluble nonionic polymers (especially, polyethylene oxide or polyethylene glycol) were added to gelatin-base coacervation systems is described. The advantages of this method are: (a) The addition of a small amount of polyethylene glycol (PEG) or polyethylene oxide (PEO) to a complex coacervation system (e.g., gelatin-acacia) allows microencapsulation to occur over an expanded pH region (pH 2-9 in gelatin-acacia). (b) These polymers induce phase separation in an aqueous solution of gelatin alone and enable the preparation of gelatin-coated microcapsules not only in the vicinity of the isoelectric point (pH 9.0), but over a wide pH range (pH 5.5-9.5). (c) Spherical single-seeded microcapsules can be obtained.

Keyphrases □ Microencapsulation—gelatin-base coacervation systems, water-soluble nonionic polymer □ Phase separation—coacervation induced by the addition of water-soluble nonionic polymer □ Coacervation—gelatin-acacia, polyethylene glycol, polyethylene oxide, phase separation, microencapsulation

Microencapsulation using coacervation of gelatin or gelatin-acacia is the most popular of the aqueous media procedures. The practicality of the gelatin-acacia complex coacervation method (1-3) is evidenced by products such as no-carbon paper and liquid crystal thermometers. Complex coacervation is the phase separation caused in a mixture of anionic and cationic polymers (e.g., acacia and gelatin) under re-

stricted pH conditions and colloid concentration. This limitation is the disadvantage of the conventional microencapsulation procedure by complex coacervation.

On the other hand, under certain conditions the addition of a water-miscible organic solvent (e.g., ethanol) or salt (e.g., sodium sulfate) to the aqueous solution of a polymer (e.g., gelatin) causes phase separation. This phenomenon has been used in microencapsulation as a simple coacervation method (4-8). The disadvantages of the simple coacervation method are the requirement of a large quantity of the organic solvent, the necessity of desalting, and the difficulty of setting the optimum conditions to obtain satisfactory microcapsules. Therefore, in this work, improvement of the conventional coacervating techniques was attempted.

EXPERIMENTAL SECTION

Materials—The gelatin used has the following specifications as provided by the manufacturer¹: bloom, 300; viscosity, 60.9 millipoises; pH 4.2; moisture, 9.9%; and isoelectric point, 9.0. The anionic polymers were acacia², carboxymethylcellulose (CMC)³, and ethylene-maleic anhydride copolymer

¹ Miyagi Kagaku Kogyo.

² Spray-dried powder; Gokyo Sangyo.

³ Serogen PR; Daiichi Kogyo Seiyaku.

Table I—Microencapsulation Procedure with the Addition of Water-Soluble Nonionic Polymers to Complex Coacervation Systems

Example	Core Substance		Anionic Polymer		Cationic Polymer		Nonionic Polymer		pH	Shape of Microcapsule ^a
	Species	Final Conc., % w/w	Species	Final Conc., % w/w	Species	Final Conc., % w/w	Species	Final Conc., % w/w		
1	Fluid paraffin	5.7	Acacia	2.0	Gelatin	2.0	PEO-1	0.85	7.7	H.S.
2	2,6-Dinitro- <i>N,N</i> -dipropyl-4-trifluoroaniline ^b	4.3	Acacia	2.0	Gelatin	2.0	PEG-6000	2.3	7.6	I.S.
3	Fluid paraffin	10.6	CMC	0.5	Gelatin	3.9	PEO-1	0.88	8.3	I.S.
4	Fluid paraffin	10.0	Acacia	2.2	Gelatin	2.2	PVA	1.0	7.6	R.S.
5	Fluid paraffin	10.0	Acacia	2.2	Gelatin	2.2	Dextran	1.5	7.5	R.S.
6	Fluid paraffin	10.0	Acacia	2.2	Gelatin	2.2	PVP	3.0	6.2	R.S.
7	Fluid paraffin	10.0	Acacia	2.2	Gelatin	2.2	Polysaccharide	2.0	7.3	R.S.
8	Fluid paraffin	6.7	EMA	0.23	Gelatin	2.5	PEG-6000	2.0	7.5	I.S.

^a H.S. = spherical, single seed; I.S. = spindle, single seed; R.S. = irregular shape, single seed. ^b Trifluralin.

Table II—Microencapsulation Procedure with the Addition of Nonionic Surfactants to Aqueous Solution Containing Gelatin and Core Substances

Example	Core Substance		Final Conc. of Gelatin, % w/w	Nonionic Polymer		pH	Shape of Microcapsule ^a
	Species	Final Conc., % w/w		Species	Final Conc., % w/w		
9	2,6-Dinitro- <i>N,N</i> -dipropyl-4-trifluoroaniline ^b	4.8	2.8	PEG-6000	3.0	9.0	I.S.
10	Fluid paraffin with pigment	12.0	2.8	PEO-1	1.2	8.6	H.S.
11	Fluid paraffin with pigment	5.7	2.0	PEO-18	0.39	9.0	H.S.
12	Chlorpromazine (dissolved in sesame oil)	0.5 (5.2)	2.0	PEO-1	1.5	9.0	I.S. H.S.

^a H.S. = spherical, single seed; I.S. = spindle, single seed. ^b Trifluralin.

(EMA)⁴. Nonionic polymers used were polyethylene glycol (PEG)⁵, polyethylene oxide (PEO)⁶, polysaccharide⁷, polyvinylpyrrolidone⁸, dextran⁹, and polyvinyl alcohol¹⁰. All the polymers were used as supplied by the manufacturers.

Both PEG and PEO have the same general structural formula: HO—(CH₂CH₂O)_nH. The former is usually used for polymers having molecular weights < ~20,000 and the latter for those with molecular weights greater than tens of thousands.

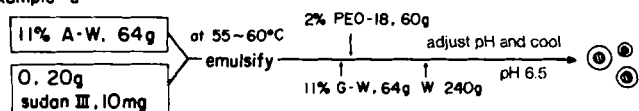
Microencapsulation Procedures—The microencapsulation methods are illustrated in Fig. 1. Examples a and b demonstrate the procedure for the complex coacervation system using PEO-18 and for the aqueous solution of gelatin using PEG-6000, respectively. In these example, high concentrations (11%) of acacia and gelatin were used as starting concentrations according to the method of Green and Schleicher (9, 10), although low concentrations [e.g., 3% (11)] can also be used. First, the core substances were emulsified

above 40°C with a homogenizer¹¹ in aqueous solutions containing acacia (Example a) or gelatin (Example b) in 100-mL beakers. After emulsification, the colloidal dispersions were added to 500-mL beakers in which three-blade metal propellers with 2.5-cm blades were fixed 1.5 cm from the bottom, and water, and aqueous solution of gelatin (Example a), and nonionic polymers were successively added with stirring until the final concentrations of gelatin and acacia were adjusted to 1.5–3.0%. In this step, the nonionic polymers may be added in a neat state (powder or flakes) instead of as an aqueous solution.

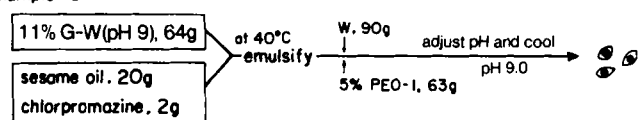
After adjusting the pH with 20 and 1% NaOH solutions at 40°C, the mixtures were cooled gradually to 20°C with continuous stirring at 400–900 rpm and then cooled abruptly to ≤10°C. The mixtures were subjected to hardening treatment for 14 h after a dropwise addition of diluted glutaraldehyde (8%, 10 mL) and/or formaldehyde (7.4%, 10 mL)¹². The products

Microencapsulation Procedure

Example a



Example b



O: Fluid paraffin. A: Acacia. G: Gelatin. W: water

Figure 1—Microencapsulation procedures by the addition of water-soluble nonionic polymers to a complex coacervation system (Example a) and the simple aqueous system of gelatin (Example b).

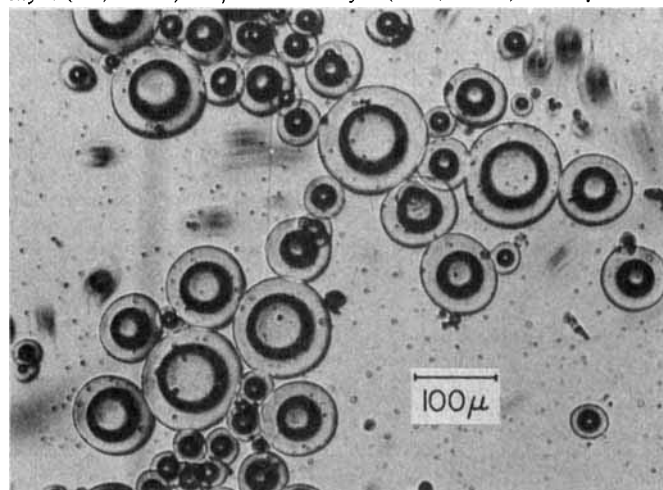


Figure 2—Photomicrograph of spherical single-seed microcapsules obtained from the dispersion system of gelatin-acacia and liquid paraffin by the addition of PEO-18 at pH 6.5.

⁴ EMA-31; Monsanto Chemical Co.
⁵ PEG-6000, PEG-20000; Nippon Oils & Fats Co., Ltd.
⁶ PEO-1, PEO-18; Seitetsu Kagaku Kogyo.
⁷ Pullulan PF-10, mol. wt. 100,000–300,000; Sumitomo Chemical Co., Ltd.
⁸ Luviscol K-30 (mol. wt. 40,000); Badische Anilin and Soda Fabrik A.G.
⁹ Mol. wt. 100,000–200,000; Wako Pure Chemical Industries, Ltd.
¹⁰ Gohsenol GM; Nippon Gosei Kagaku Kogyo.

¹¹ Silverson Machines Ltd.
¹² It is described in the patent (Japan 52-38097) that the combined use of glutaraldehyde and formaldehyde prevents yellowing. Formaldehyde is preferable to glutaraldehyde for hardening of microcapsules in a neutral pH region, because the latter has some tendency to cause aggregation of microcapsules in this pH region.

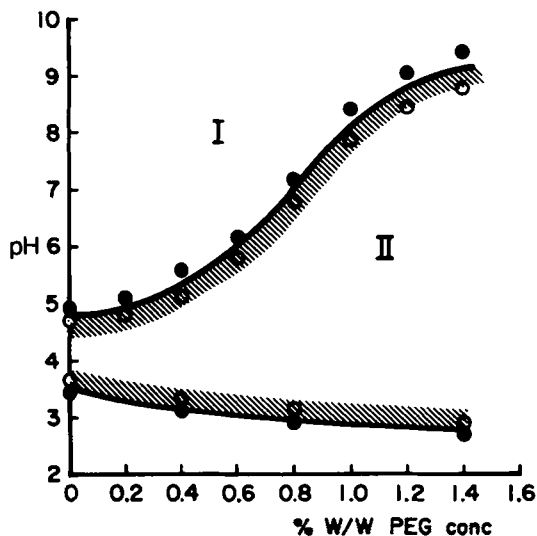


Figure 3—Phase diagram of the acacia-gelatin (2.2% w/w each) complex coacervation system containing PEG-20,000. The coacervating region is represented by the shaded area (II); I represents a region of one-phase colloidal solution. Key: (●) no coacervation; (○) formation of coacervate.

were then removed by filtration and washed. Microcapsules in the form of a free-flowing powder can be obtained according to the method of Madan *et al.* (6) or Palmieri (12) by the use of colloidal silica. The unhardened microcapsules can also be recovered as discrete free-flowing particles according to the aforementioned methods.

Phase Diagram—The nonionic polymers were added to the aqueous gelatin-acacia (2.2% each) solution or the aqueous gelatin (2.2%) solution without core materials under stirring at 40°C, and the formation of coacervates at various pH values was observed with a microscope at room temperature (~23°C). The dependence of the phase diagram on the concentrations of gelatin and acacia was also observed for PEO-1 and PEG-20,000.

RESULTS AND DISCUSSION

Microencapsulation—The various types of microcapsules formed in gelatin-base systems using water-soluble nonionic polymers are listed in Tables I and II. Examples of complex coacervation systems are shown in Table I, while examples of simple gelatin coacervation systems are shown in Table II.

In Examples 3 and 8, carboxymethylcellulose (CMC) and ethylene-maleic anhydride copolymer (EMA) were used instead of acacia as the anionic polymer, respectively. Examples 4-7 are of microencapsulation using polyvinyl

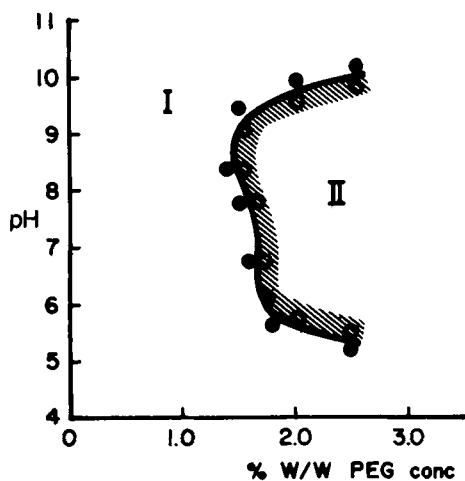


Figure 4—Phase diagram of a simple coacervation of gelatin (2.2% w/w) with PEG-20,000. The coacervation region is represented by the shaded area (II); I represents a region of one-phase colloidal solution. Key: (●) no coacervation; (○) formation of coacervate.

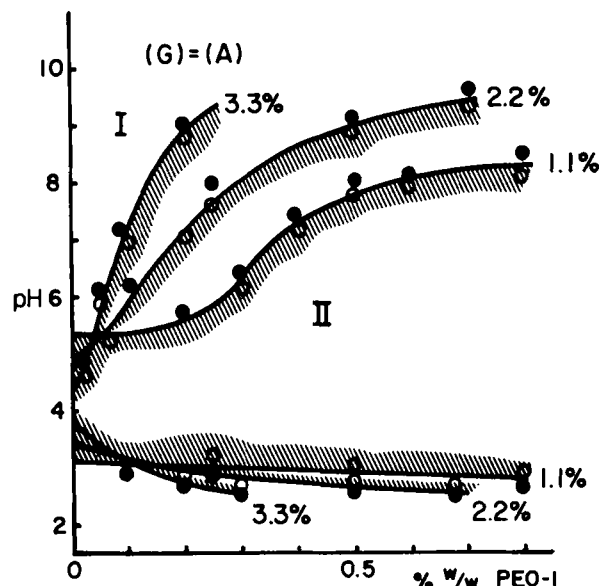


Figure 5—Phase diagrams of acacia-gelatin complex coacervation systems containing PEO-1. The effect of colloidal concentrations of acacia and gelatin (1.1, 2.2, and 3.3% w/w) on the phase diagram was observed. The coacervating regions are represented by shaded areas (II); I represents the regions of one-phase colloidal solution. Key: (●) no coacervation; (○) formation of coacervate.

alcohol (PVA) dextran, polyvinylpyrrolidone (PVP), and polysaccharide as the nonionic polymers, respectively. PEO and PEG were better than these nonionic polymers, which gave coacervation systems of high viscosity in the cooling process and made it difficult to prepare good microcapsules. On the other hand, the results of experiments on the homologous series of PEO and PEG indicated that the minimum concentrations of the molecules required to obtain microcapsules became lower with increasing molecular weight, although the system containing the larger molecule was more viscous. In the conventional method, spindle-shaped microcapsules are usually formed under the conditions used for obtaining single-seed microcapsules; in the present method, however, spherical shapes were obtained in many cases using PEO with a higher molecular weight than PEO-1. Although the viscosities were not measured, the systems in which the spherical shapes were obtained were, in general, more viscous in appearance. Therefore, the viscosity of the medium is considered an important factor in forming spherical shapes, but extended polymer chains with high molecular weight included in the medium might prevent the deformation (from sphere to spindle) of the microcapsule induced by the stress of distortion. On the other hand, stirring speed is also an important factor, and a speed of 400-900 rpm was necessary to overcome the retardation or to stop the flow of the dispersion in the vicinity of gelation temperature.

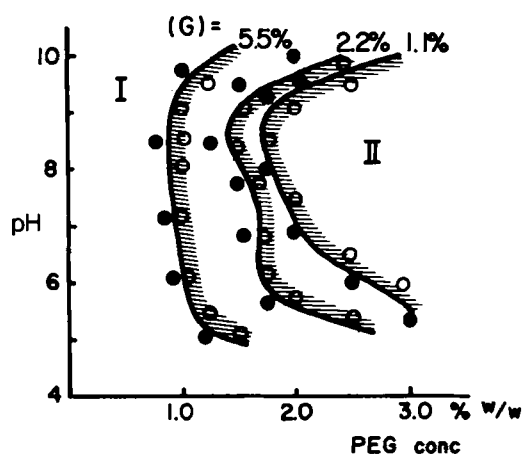


Figure 6—Phase separations induced in the simple coacervation systems containing different amounts of gelatin (1.1, 2.2, and 5.5% w/w) by the addition of PEG-20,000. The coacervating regions are represented by shaded areas (II); I represents the regions of one-phase colloidal solution. Key: (●) no coacervation; (○) formation of coacervate.

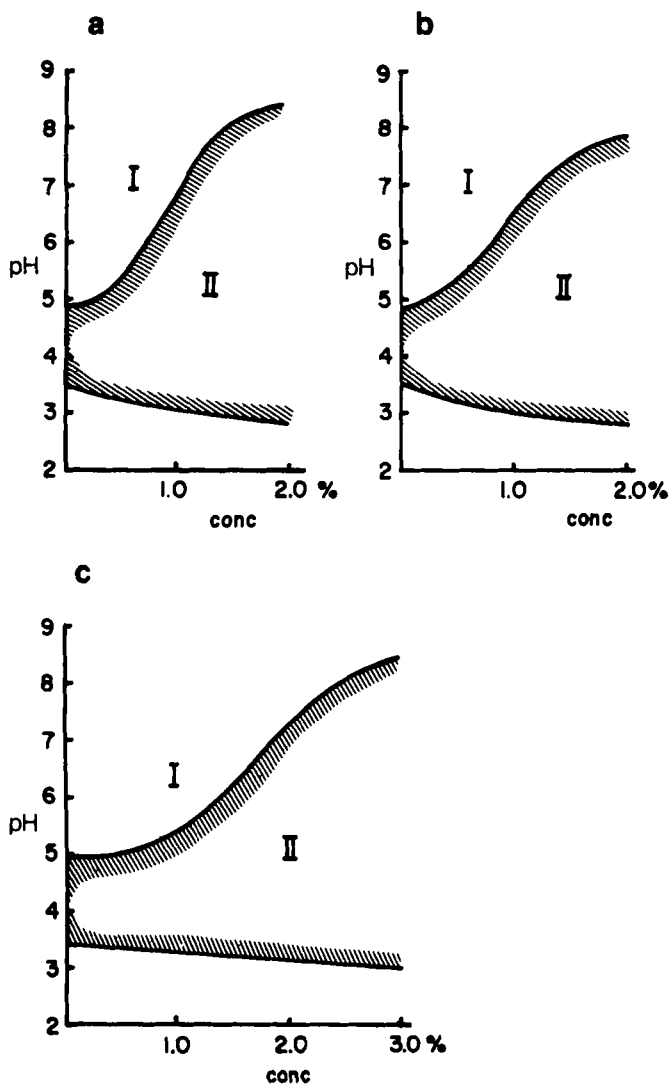


Figure 7—Phase diagrams of acacia-gelatin (2.2% w/w each) complex coacervation systems with various water-soluble nonionic polymers. The coacervation regions are represented by the shaded areas (II); I represents the regions of one-phase colloidal solution. Key: (a) dextran; (b) polysaccharide; (c) PVP.

Figure 2 shows a photomicrograph of spherical single-seed microcapsules obtained from the dispersion systems of gelatin-acacia (1.6% each) and liquid paraffin (4.7%) by the addition of PEO-18 (0.3%) at pH 6.5. The product was recovered as free-flowing powder (diameter, 30–100 μm) after filtration without hardening treatment, according to the method of Madan *et al.* (6).

Phase Diagram—The coacervation areas are represented by shading (indicated by the symbol II) in the phase diagrams. The areas represented by the symbol I indicate one-phase colloidal dispersions.

Figure 3 shows the result of the gelatin-acacia complex coacervation systems and Fig. 4 the results of the aqueous solution of gelatin alone. Figure 3 indicates that the intercept of the shaded area to the ordinate is in the pH range of conventional complex coacervation and that the addition of a small amount of PEG-20,000 to the gelatin-acacia coacervation system causes phase separation in a remarkably expanded pH range. Figure 4 shows that in the aqueous solution containing gelatin alone, phase separation does not take place at every pH value without the addition of foreign molecules, but with the addition of PEG-20,000, the pH range is expanded.

Figure 5 shows that the pH range of complex coacervation without the addition of foreign molecules (conventional method) decreases with increasing colloidal concentration, but that the addition of PEO-1 expands the effective pH range of phase separation with increasing colloidal concentrations of gelatin and acacia. The minimum concentration of PEG-20,000 required to cause the phase separation in the aqueous solutions of gelatin alone becomes lower with increasing gelatin concentration, as shown in Fig. 6.

The results of other water-soluble nonionic polymers (polysaccharide, dextran, and PVP) can be seen in Fig. 7. Although all of these nonionic polymers have molecular weights greater than that of PEG-20,000, they are not more effective.

Coacervation from ethylcellulose-cyclohexane solution is widely used in the microencapsulation of water-soluble drugs, using a combination of coacervation-inducing agents (*e.g.*, polyethylene and polyisobutylene) (13, 14). However, the role and effect of the coacervation-inducing agents in the organic solvent systems differ from those of the water-soluble nonionic polymers in the aqueous coacervation systems described in the present paper.

In the coacervation system of ethylcellulose-cyclohexane, the coacervation-inducing agents effectively cause phase separation and prevent aggregation of microcapsules, but the microcapsules can also be prepared without these agents. In the aqueous solution of gelatin, on the other hand, phase separation does not occur without the addition of the water-soluble nonionic polymers. In the acacia-gelatin complex coacervation system, the nonionic polymers can cause phase separation even at pH values at which separation does not occur using the conventional method. The phenomenon of inducing coacervation in these two solvent systems might be caused (possibly common to both systems) by the contributions of the added polymers to the thermodynamic properties of mixing (ΔH_{mix} and ΔS_{mix}).

CONCLUSIONS

The addition of a small amount of high molecular weight PEO or PEG to a gelatin-acacia coacervation system enables coacervation over an expanded pH range (pH 2–9). These polymers also induce phase separation in the aqueous solution of gelatin alone over a wide range (pH 5.5–9.5). The microencapsulation procedure using this phenomenon is useful for the microencapsulation of materials which are labile or water-soluble in the acidic pH region but stable and insoluble in the neutral pH region.

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