Effects of Selected Variables on the Extractability of Oils from Coacervate Capsules

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The hypothesis that certain pharmaceuticals may be stabilized by coacervation led to the investigation of encapsulation requirements for pharmaceutical oils. Saponification values, acid values, and surfactant properties were imparted to light liquid petrolatum by the introduction of oils with high saponification values, organic acids, and surfactants. The effects of these substances on the degree of protection offered by coacervation were determined by submitting the coacervates to extraction with ethyl ether for periods up to 1 hour, then comparing the extracts spectrophotometrically to known absorption spectra. Results showed that saponification values had little effect on encapsulation and that acid values and surfactants decreased the degree of encapsulation.

THE TERM "coacervation" has recently been used to describe the salting out of a lyophilic solid into liquid droplets rather than solid aggregates (1). The term was introduced into colloidal chemistry by Kruyt and Bungernberg de Jong (2) to describe the flocculation or separation of liquids from solution, where at least one of the liquids contained a colloidal solute.

Coacervation has been subdivided into simple coacervation and complex coacervation. Briefly, simple coacervation usually deals with systems containing only one colloidal solute, while complex coacervation usually deals with systems containing more than one colloid (3).

Simple coacervation is a process involving the addition of a strongly hydrophilic substance to a solution of a colloid. This added substance causes two phases to be formed—one phase rich in colloidal droplets and the other poor in such droplets. This process is dependent primarily on the degree of hydration produced, and this is more difficult to control.

Complex coacervation, however, has been found to be primarily dependent on pH. It has been reported that in a gum arabic–gelatin system, complex coacervation into microcapsules occurred at pH values below the isoelectric point of the gelatin and would not occur above this pH regardless of how other factors were changed (4). The same was true of other systems containing two dispersed colloids of opposite electrical charge.

The optimum conditions for complex coacervation were achieved by adjusting the pH to a point at which equivalents of oppositely charged molecules of the two colloids were present, since the greatest number of salt bonds were formed at that point (4). In such a system, the pH must be adjusted so that the gelatin particles are positively charged (below the isoelectric point), since gum arabic particles are always negative in colloidal dispersions (5). Therefore, the principal condition necessary for successful complex coacervation is pH. Since this factor is more easily and precisely adjusted and controlled than the degree of hydration, it was decided that the process of complex coacervation would be more practical to the purposes of this study.

Green and Schleicher made practical use of the process of coacervation in various patents (6–10). Two of the patents (7, 10) indicated that various oils, some of which contained dissolved dyes, had been entrapped in gelatin-acacia microcapsules. No quantitative method to evaluate the strength of the coacervate or the degree of coacervation was given.

It appeared that encapsulation through coacervation has great potential application in pharmacy. Some properties that might be especially useful include (*a*) prevention of vaporization of volatile substances (*e.g.*, volatile oils), (*b*) protection of moisture-sensitive or light sensitive substances (*e.g.*, vitamins A and K), (*c*) separation of incompatible substances within a single system, and (*d*) dispersion of water insoluble substances in an aqueous medium more readily.

Before such applications could be explored, it was apparent that some methods should be available to predict necessary conditions to achieve good encapsulation. Since the properties of oils used in pharmacy differ considerably, it was decided to study the effects of varying saponification value, acid value, and surface activity to ascertain their effects, if any, on the encapsulation process. A second objective was to attempt to develop a method to determine the permeability of the coacervate shell.

EXPERIMENTAL

Preparation of Coacervates.—The method of Green and Schleicher (7, 10) for preparation of

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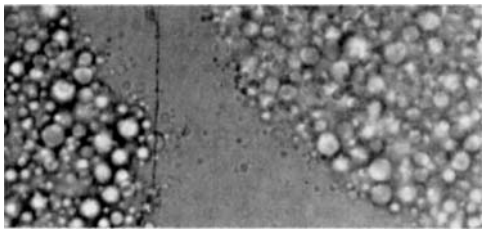

Fig. 1.—Photograph of the coacervation process at pH 4.5 and at 10°.

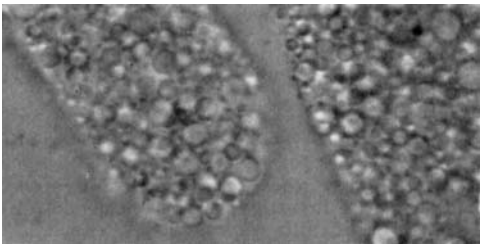

Fig. 2.—Photograph of the coacervation process after the addition of formaldehyde at pH 4.5 and at 10°.

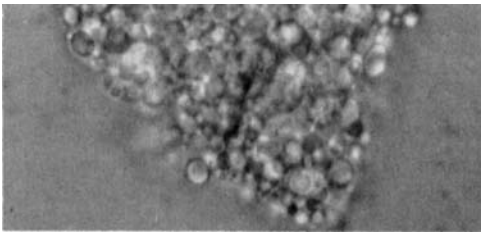

Fig. 3.—Photograph of the coacervation process at pH 9.

complex coacervates was modified. The primary coacervating solutions were made by dissolving 3.0 Gm. of acacia and 3.0 Gm. of gelatin separately in 100 ml. of purified water at 55°. The acacia used was powdered acacia U.S.P. and the gelatin used was 300 bloom, pigskin gelatin[1] with an isoelectric point at pH 8.0.

A 20% sodium hydroxide solution was used to adjust the pH of the solutions to 6.5. An emulsion was formed by adding 12 ml. of the selected oil to the acacia solution and passing the mixture through a hand homogenizer. The gelatin solution was then added with gentle stirring. At no time was the temperature of the mixture allowed to fall below 50°.

The pH of the mixture was adjusted gradually to 4.5 by the dropwise addition of diluted hydrochloric acid U.S.P. while stirring. At this pH the gelatin particles were positively charged and were attracted to the negatively charged acacia particles. The combined droplets are assumed to have coalesced about the oil droplets. In any event, the oil droplets were isolated from the rest of the system.

Then 10 ml. of formaldehyde solution U.S.P. was added, and the mixture was cooled to 10° in an ice bath. The pH was adjusted to 9.0 with 20% sodium hydroxide solution. The addition of formaldehyde denatured the gelatin-acacia complex and entrapped the oil more permanently. Chilling and adjusting the pH merely enhanced this effect.

The coacervation mixture was diluted to 400 ml. and filtered. After filtration, the wet coacervate was wrapped in blotting paper to remove some of the excess moisture. The resulting soft rubbery mass was passed through a 20-mesh screen and dried at 50° for 24 hours. The coacervate was then coarsely granular.

Method to Evaluate Permeability of Coacervate Shell.—The method used to evaluate the strength of the shell was based on the ability of the shell to prevent or allow extraction of the entrapped oil by ethyl ether (analytical reagent). To measure the degree of extraction, the oils were colored with dye[2] which was present in the oils in the same ratio both before and after extraction. The oils were then encapsulated through coacervation and dried by the method described.

Colored oils were scanned for wavelength of maximum absorption using a Bausch and Lomb spectronic 505 recording spectrophotometer. A broad peak with a maximum at 500 mμ was seen. Beer's law plots were obtained using a Bausch and Lomb spectronic 20 at this wavelength. This facilitated assay of the ether extracts of the various coacervates containing the colored oils.

Choice of Oils for Coacervation Properties Study.—To provide a series of oils with a controlled range of saponification values, light liquid petrolatum N.F., which has no saponification value, was mixed with coconut oil[3] which had a saponification of 250 but no acid value.

A controlled acid value series was prepared by mixing oleic acid U.S.P., which had an acid value of 190 and no saponification value, with light liquid petrolatum N.F. Series using lauric acid and benzoic acid were also prepared.

The effect of surfactants on the coacervation was studied by preparing a series using trioleate 85[4] as an additive to the mineral oil before coacervation was carried out. Sodium oleate, polysorbate 20,[5] and sodium acetate were added to the aqueous phase in separate qualitative studies to see if they exerted an effect on the encapsulation process.

Microphotography of the Coacervation Process.—Photomicrographs of the process were taken at various steps in the procedure. Figure 1 shows the agglomeration of oil droplets that occurred when the pH was lowered to 4.5 and the temperature reduced to 10°. At this stage in the process a matrix apparently had not completely formed. Figure 2 was photographed after the addition of the formaldehyde solution with the pH still at 4.5. The outline of the oil droplets is clearly delineated and, in some areas, shows what appears to be a matrix.

Figure 3 was taken after the pH was raised to 9.0. Although the coacervate consisted of a

[1] Obtained from the American Agricultural Chemical Co., Detroit, Mich.

[2] Sudan III. Marketed by General Dyestuff Corp., New York, N. Y.

[3] Marketed as Coconut Oil 76° by Welch, Holme, and Clark Co., New York, N. Y.

[4] Marketed as Arlacel 85 by the Atlas Powder Co., Wilmington, Del.

[5] Marketed as Tween 20 by the Atlas Powder Co., Wilmington, Del.

TABLE I.—COCONUT OIL SERIES

Coconut Oil, %	Sapon. Value	Oil Extracted in 60 Min., ml.	Oil Extracted as % Total Wt. of Sample
1	2.5	0.006	0.45
2	5.0	0.005	0.40
3	7.5	0.005	0.40
4	10.0	0.006	0.45
5	12.5	0.005	0.40

TABLE II.—OLEIC ACID SERIES

Acid in Oil, %	Acid Value of Oil	Oil Extracted in 60 Min., ml.	Oil Extracted as Total Wt. of Sample, %
0	0	0.012	1.02
1	2.15	0.012	1.02
2	4.22	0.061	5.18
3	6.08	0.091	7.70
4	8.24	0.248	21.08
5	10.47	0.320	27.20

rubbery mass at this point, it was not apparent in the photograph.

RESULTS

Effects of Saponification Value.—The series of oils with saponification values ranging from 2.5 to 12.5 were studied after coacervation. Table I shows that the permeability of the shell was constant since the amount of oil extracted by ether in 1 hour was about the same throughout the series.

Effects of Acid Value.—Results of the coacervation and extraction of the oleic acid series are shown in Table II. The amount of oil extracted increased directly as the acid value increased. Oil samples having higher acid values gave results that were somewhat erratic and difficult to measure quantitatively.

A series using lauric acid followed a similar pattern. However, lauric acid was soluble in mineral oil only to the extent of 2%, thereby limiting the usefulness of this series.

Benzoic acid was used as an additive, since it contributed acid value without surfactant properties (both the oleic acid and lauric acid exhibit some surfactant properties). However, the benzoic acid lowered the pH to 4.0 very abruptly, and the condition of gradual approach to a pH of 4.5 could not be achieved. No acid could be found which was soluble in mineral oil, insoluble in water, and which had no surfactant properties.

Effect of Surfactants.—Since this study involved a water soluble phase and an oil soluble phase it was desirable to include surfactants in first one phase and then the other.

Trioleate 85, an oil soluble surfactant, was used as an additive. The results shown in Table III indicate that this surfactant interfered with the encapsulation process, although the results were somewhat more erratic than those in the coconut oil or oleic acid series.

Polysorbate 20, a water soluble emulsifier, was added to the aqueous phase (acacia solution) prior to coacervation. No quantitative data were obtained, since none of the colored oil was retained by the coacervate after dilution and filtration.

TABLE III.—TRIOLEATE-85 SERIES

Trioleate in Oil, %	Acid Value	Oil Extracted in 60 Min., ml.	Oil Extracted as Total of Sample, %
0	0	0.012	1.02
1	0.14	0.02	1.70
2	0.24	0.14	11.45
3	0.33	0.10	8.50
4	0.55	0.35	30.09
5	0.78	0.13	11.05

Sodium oleate was also tried; the results were the same and indicated that surfactants in the aqueous phase seemed to prevent proper encapsulation.

DISCUSSION

Encapsulation of oils occurred when colloidal droplets of oppositely charged colloids agglomerated about the oil droplets to form a shell which probably consists of several layers of coacervate droplets. Denaturization of the colloidal film or shell gave it a degree of permanence.

Surfactants exhibited interference with the encapsulation process and the strength of the protective shell. It is theoretically possible this effect was because surfactants may compete with the gelatin-acacia complex at the oil-water interface If this reasoning is acceptable, then the shell would not be continuous and leakage could occur through the incomplete shell. It would then follow that the more surfactant present, the greater the number of spaces possible in the shell.

The interference of the oleic acid in the coacervation process might have been due to the surfactant action of this fatty acid, or it might have been due to the acidity. In the saponification value series the esters added did not exhibit surfactant properties, nor were the conditions severe enough to cause hydrolysis to the more surfactant fatty acids.

SUMMARY

A spectrophotometric method was found to evaluate the permeability of coacervate shells.

Oleic acid and lauric acid interfere with encapsulation as the concentration of these acids in the oil was increased.

Surfactants incorporated in either the oil phase or water phase interfered greatly with the encapsulation process, and a possible reason for this behavior is discussed.

Increasing the saponification value of the light mineral oil with coconut oil showed no appreciable effect on the permeability of the coacervate shell.

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