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* U. S. Department of Agriculture, Veterinary Toxicology and Entomological Research Laboratory, College Station, Tex.

† Department of Entomology, University of Georgia, Athens, GA 30601

# Microbiologic Determination of Drug Partitioning I: Gelatin–Acacia Complex Coacervate System

**SADEGH JAVIDAN, RIAZ-UL HAQUE*, and ROBERT G. MRTEK†**

**Abstract** □ An experimental method was developed for the determination of the extent of the partitioning of phenylmercuric nitrate in coacervate systems, as measured by the effect of the antimicrobial drug on the growth of *Escherichia coli*. Bacterial growth is utilized to investigate changes in the coacervate partition coefficient as a function of drug concentration. The method is sensitive for microgram quantities of this antimicrobial agent and is useful in drug partitioning at levels found in physiologic systems.

**Keyphrases** □ Drug partitioning—microbiological determination, gelatin–acacia coacervate system □ Phenylmercuric nitrate—microbiological determination of partitioning in coacervate systems □ Antimicrobial agents—determination of drug partitioning using coacervate systems □ Coacervates, complex—drug partitioning of antimicrobial agents □ Gelatin–acacia complex coacervate system—microbiological determination of drug partitioning

The term coacervation has been used to describe the salting out of certain types of lyophilic colloids into liquid droplets (1). Kruyt and Bundenberg de Jong (2) introduced the word in 1929 to characterize the flocculation or separation of liquids from solution when at least one separated phase contained a colloidal solute. The term has since been subdivided into simple coacervation and complex coacervation; complex coacervation is mainly dependent on the charges on the molecules, while simple coacervation results from phase separation due to the interaction of nonionized entities of macromolecules (1, 3). The mechanism for the coacervation process was studied by Kruyt (4), Bundenberg de Jong (5, 6), Voorn (7), Cohen and Vassiliades (8), Takruri (1), and others.

Coacervates generally contain a large amount of water; when these systems separate, the more dense coacervate settles to the bottom and contains about 99% of the colloidal solute. The upper layer is termed the "equilibrium liquid" or "dissolved coacervate layer" (2) and is in equilibrium with the coacervated phase. Water in the coacervate layer is structurally different from regular (liquid) water or from water in equilibrium with the coacervate phase. The state of aggregation of water molecules in coacervates was compared to the state of water in living systems. It was postulated (1) that the difference in aggregation states between water in coacervates and in the equilibrium layer is an important determinant in the distribution of electrolytes in these systems.

Takruri (1) studied the high uptake of organic molecules in complex coacervate systems and compared them to similar systems existing in nature. Several researchers (1, 9–12) suggested that these systems may serve as models for the study of absorption and distribution characteristics of drugs.

The purpose of this study was to utilize complex coacervates containing gelatin and acacia as a model system whereby drug partitioning of antimicrobial agents could be determined. Concentrations of phenylmercuric nitrate below the minimum inhibitory con-

Table I—Components Used to Study the Partitioning of Phenylmercuric Nitrate in Gelatin–Acacia Complex Coacervates

| Tube | A–G Stock Solution, ml. | 0.1 $N$ $HNO_3$, ml. | Milliliters Phenylmercuric Nitrate, 600 mcg./ml. | Sterile Distilled Water, ml. | Abbreviation of the System[a] | Coacervate, ml. |
|---|---|---|---|---|---|---|
| A | 4 | 1.5 | 0 | 24.5 | $C_0$ | 2 |
| B | 4 | 1.5 | $n$[b] | q.s. 30 ml. | $C_+$ | 2 |
| C | 4 | 0 | 0 | 26 | $N_0$ | 0 |
| D | 4 | 0 | $n$[b] | q.s. 30 ml. | $N_+$ | 0 |

[a] $C_0$ = coacervate system, no drug; $C_+$ = coacervate system with drug; $N_0$ = noncoacervate system, no drug; and $N_+$ = noncoacervate system with drug. [b] 0.2, 0.4, 0.8, 1.6, 3.2, 4.8, 6.4, or 12.8 ml. of phenylmercuric nitrate. (Only one concentration of drug was used in each experiment.)

centration (MIC) were used to measure the inhibitory effect on the growth of *Escherichia coli* by means of the Coulter counter. Garrett and Miller (13) applied microbial kinetics in the prediction and quantification of antimicrobial agents with the use of the Coulter counter.

In this investigation, an antimicrobial drug was incorporated into coacervating and noncoacervating systems. Partitioning between equilibrium and coacervate phases was allowed to occur in the system. After separation of the phases, an aliquot of equilibrium liquid was withdrawn and incubated with organisms. The amount of antimicrobial agent in the equilibrium layer was presumed to be directly related to the amount of drug that had partitioned into the coacervate phase. By comparing the inhibitory effect of this equilibrium liquid with an aliquot of noncoacervate systems, an estimate of the amount of drug incorporated into the coacervate phase may be offered.

## EXPERIMENTAL

**Organism and Reagents**—*E. coli* B/r (ATCC 23227) was used as the test organism throughout this investigation. This strain (UV and X-ray resistant) of *E. coli* is uniform in size, with the majority of a rapidly growing cell population distributed between 0.98 and 1.23 $\mu$ in diameter. Furthermore, this strain does not stick to the orifice of the Coulter counter aperture tube.

The lyophilized pellet was reconstituted, and organisms were transferred to an agar slant. The slant was incubated at 37° for 16 hr. to allow growth and then stored in the refrigerator at 5° for up to 2 weeks before subcultures were made.

Phenylmercuric nitrate[1] was the antimicrobial agent used in the partitioning studies. No. 1 granulated acacia USP[2] and Bacto gelatin[3] were used as colloidal solutes in the coacervate system. Nitric acid[4] and hydrochloric acid, analytical grade[5], were used to form coacervates. The 0.1 $N$ solutions of these acids were filtered through a 0.22-$\mu$ millipore filter[6] and stored in brown bottles. Formaldehyde solution USP[7] was used to arrest the growth of the organisms.

**Media**—Thirty-seven grams of the dried Difco E.C. medium was rehydrated in 1 l. of cold, sterile, distilled water and stirred at room temperature until a clear solution was achieved. This solution was filtered through 2- and 0.22-$\mu$ millipore filters to remove all cellular and particulate debris. Filtered media were distributed in batches of 18 ml. among 125-ml. conical flasks and autoclaved at 15 lb. pressure (121°) for 15 min.

**Preparation of Coacervate Systems**—*Stock Solution*—A modification of the method used by Takruri (1) was adopted. The concentration of gelatin was changed from 5 to 4.325% w/v. The procedure was followed by dissolving 24 g. acacia in about 150 ml. hot distilled water in a 250-ml. beaker. In another 250-ml. beaker, 17.3 g. gelatin was dispersed in 150 ml. hot distilled water. Stirring was maintained throughout the process until complete dissolution was achieved. The hot solutions were strained separately through gauze and combined in a large beaker.

The total volume was adjusted to 400 ml. by the addition of warm distilled water. The final concentrations of gelatin and acacia in this solution were 4.325 and 6% w/v, respectively. This solution, labeled as A–G stock solution, was distributed in 35-ml. batches among several 60-ml. bottles. After autoclaving for 15 min., the bottles of A–G stock solution were stored at 5° until used.

*Formation of Coacervate*—Prior to the formation of the coacervate systems, samples of A–G stock solution were removed from the refrigerator and allowed to come to room temperature. Then, under a bacteriological hood equipped with UV light, 2 ml. of the stock solution was pipeted into each of several 15-ml. sterilized, graduated, centrifuge glass tubes; 0.8 ml. of 0.1 $N$ HCl (or $HNO_3$) was added to the stock solution from a 1-ml. pipet, and the volume was adjusted to 15 ml. using sterile distilled water. The instantaneous appearance of cloudiness after adding the distilled water indicated the coacervation process. The final concentrations of acacia and gelatin in the coacervate system were 0.8 and 0.576% w/v, respectively. The tubes were capped and shaken vigorously by hand until complete mixing of the components was achieved. Then the tubes remained undisturbed in a cabinet at 37° for 24 hr. for complete separation of the layers in the coacervate system. In some experiments, when rapid separation of the coacervate layer from the equilibrium layer was desired, centrifugation at 1200×$g$ in a centrifuge[8] for 10 min. was performed instead. At the end of the 24-hr. standing time (or after centrifugation), the volume of coacervate at the bottom of the graduated centrifuge tube was measured visually.

Noncoacervate systems were prepared by the same procedure used for forming coacervates except the acid was eliminated.

**Effect of Heat of Sterilization on Coacervation**—To determine optimum conditions for the preparation of large volume coacervate phases, the effect of heat of sterilization on the formation of coacervates was determined by autoclaving the various components at 121° for 15 min. The final volume of the coacervate layer was measured after 10 min. of centrifugation at 1200×$g$. For this purpose, several different coacervate systems were used. Gelatin and acacia were separately autoclaved, sterilized in combination, or not sterilized. Additionally, studies were made on systems that had been sterilized by autoclaving after the coacervation process was completed. For all of these experiments, 0.8 ml. 0.1 $N$ HCl was used to adjust the pH for coacervation, and 2 ml. of A–G stock solution was used. The total volume of each system was 15 ml.

**Effect of Different Acids on Coacervation**—Two milliliters of A–G stock solution was placed in each of four 15-ml. centrifuge tubes. Then 0.8 ml. 0.1 $N$ HCl, $HNO_3$, $H_2SO_4$, or $CH_3COOH$ was added to each tube. The volume was adjusted to 15 ml. by the addition of distilled water. The tubes were capped and shaken vigorously by hand until all components were mixed. Then after centrifugation at 1200×$g$ for 10 min., the volume of the coacervate layer was recorded and the pH of the equilibrium layer was measured with a pH meter[9]. The effect of different acids on coacervation was also determined in the presence of 6 ml. stock solution of phenylmercuric nitrate in each tube.

**Effect of Drug Concentration on Coacervation**—A stock solution of phenylmercuric nitrate (100 ml.) was prepared by dissolving

[1] Eastman grade, Eastman Organic Chemicals.
[2] Penick Co.
[3] Difco Laboratories, Detroit, Mich.
[4] Baker analyzed reagent.
[5] E. I. duPont de Nemours & Co.
[6] Millipore Corp.
[7] J. T. Baker Chemical Co.
[8] International, model HN.
[9] Sargent model DR.

Table II—Effect of Heat of Sterilization[a] on the Volume of Gelatin–Acacia Complex Coacervate

| Coacervate System[b] | Volume of Coacervate, ml. |
|---|---|
| Gelatin and acacia separately sterilized | 1.0 |
| Sterilized A–G stock solution | 1.0 |
| Nonsterilized A–G stock solution | 1.3 |
| Coacervate formed from A–G stock solution by addition of acid and then sterilized | 0 |

[a] 121° for 15 min. [b] Total volume of the system is 15 ml.

60 mg. of the drug in 100 ml. sterile distilled water at room temperature with the aid of a magnetic stirrer. The solution was filtered once through a 0.22-$\mu$ millipore filter and stored at room temperature in a brown bottle until used. This stock solution contained 600 mcg./ml. phenylmercuric nitrate and was prepared fresh every 3 weeks.

Into eight 45-ml. graduated centrifuge tubes were placed 0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 ml. of phenylmercuric nitrate stock solution, respectively. To each tube were added 4 ml. of A–G stock solution and 1.5 ml. 0.1 $N$ $HNO_3$. Hydrochloric acid could not be used in these experiments, because it reacted with phenylmercuric nitrate and formed a white precipitate of phenylmercuric chloride which is less soluble in water. The volume of each tube was adjusted to 30 ml. by addition of sterile distilled water. After the tubes were capped and the caps were secured to the neck of the centrifuge tubes with rubber bands, they were shaken horizontally in a 5-gal. reciprocating water bath shaker[10] at 37° for 6 hr.

Then the capped tubes were stored undisturbed in a cabinet at 37° for 24 hr. The volume of the coacervate layer was recorded at the end of this period. The sequence of the addition of different components such as drug, acid, A–G stock solution, and water or the duration of the shaking period was altered to study any possible effect of those factors on the final volume of coacervate. The shaking period was tested for 0.5, 1, 2, 3, 4, 5, and 6 hr.

**Partitioning of Phenylmercuric Nitrate in Gelatin–Acacia Complex Coacervate**—In each experiment, four 45-ml. centrifuge tubes were identified as A, B, C, and D. Tubes A and B were used to form coacervating systems; in Tubes C and D, noncoacervating systems were prepared. The amounts of different components pipeted in each tube are shown in Table I. From the tests run in the previous section, the specific sequence of the addition was determined to be immaterial.

The four tubes were glass stoppered. The stopper was attached to the neck of the tube with rubber bands and placed horizontally in a water bath shaker for 6 hr. as previously described. During this period, the partitioning of drug was completed. Afterward the tubes were removed and placed undisturbed at 37° for 24 hr.

**Growth Conditions**—A separate slant of *E. coli* B/r was used for each experiment. Material was scraped off and inoculated into a 125-ml. flask containing 18 ml. E. C. medium. The culture was allowed to grow without agitation at 37° for 16 hr. From this culture, a loopful was inoculated into 18 ml. of fresh broth in a 125-ml. loosely capped flask. The new culture broth was allowed to grow for 6 hr. in the water bath shaker (37°). At the end of 6 hr., 0.1 ml. of the growing culture was removed and the total number of organisms was determined using a Coulter counter[11]. This procedure required approximately 7–10 min. The growing culture was appropriately diluted with fresh E. C. medium to contain $10^3$ organisms/ml. of the culture.

One milliliter of this diluted inoculum was added to each of 12 replicate flasks containing 18 ml. of fresh broth. The cultures were placed in the water bath shaker (37°) for 90 min. before incorporating the drug samples. At the end of this period, 1 ml. of noncoacervate system (Tubes C and D, Table I) or 1 ml. of the upper equilibrium phase of the coacervate system (Tubes A and B, Table I) was added to each of the three replicate cultures. The flasks containing the drugs were permitted to grow an additional 4.5 hr. on the shaker.

[10] Eberbach Corp., Ann Arbor, Mich.
[11] Model B, Coulter Electronic Co., Hialeah, Fla.

Table III—Effect of Different Acids on the Volume of Gelatin–Acacia Complex Coacervate in the Presence or Absence of Phenylmercuric Nitrate

| 0.8 ml. 0.1 $N$ Acid | In Presence of Phenylmercuric Nitrate | | In Absence of Phenylmercuric Nitrate | |
|---|---|---|---|---|
| | pH | Coacervate, ml. | pH | Coacervate, ml. |
| HCl | — | —[a] | 3.67 | 1.0 |
| $HNO_3$ | 3.65 | 1.0 | 3.70 | 1.0 |
| $H_2SO_4$ | 3.80 | 0.9 | 3.81 | 0.8 |
| $CH_3COOH$ | 4.20 | 0.5 | 4.25 | 0.4 |

[a] Insoluble phenylmercuric chloride precipitated.

**Viable Count**—At the end of 4.5 hr. of incubation, 0.5 ml. of each culture was withdrawn into sterilized flasks and appropriately diluted with fresh medium so that 60–100 colonies/plate would result. One milliliter of this diluted solution was pipeted onto each of three replicate agar plates. The plates were incubated at 37° for 48 hr. before counts were made. The colonies were counted with a colony counter[12].

**Total Count Method**—After withdrawing samples for viable counts, one drop of formaldehyde solution was added to each of the 12 flasks to kill the organisms. Then the content of each flask was diluted to 100 ml. with filtered, sterile, normal saline. One-tenth milliliter was diluted to 100 ml. (1000×) using Isoton[13] so that the total organism count would not exceed the coincidence limit at the following setting on the Coulter counter (model B): $A = I = 1/2$, gain = 88, $T_L = 5.2$, $T_U = \infty$, $k = 0.185$, $t = 5$ sec., and tube orifice = 30 $\mu$. This setting registers as counts all organisms above 0.78 $\mu$ in diameter without significant interference from the background noise. The mean of four total counts was obtained for each sample.

To relate the total number of organisms in each flask to the number of organisms inoculated (here $10^3$), two parameters are defined: "reproductive coefficient" (R.C.) and "reproductive index" (R.I.):

$$\text{R.C.} = \frac{\text{total number of organisms in each flask}}{\text{total number of organisms inoculated}} \quad \text{(Eq. 1)}$$

$$\text{R.I.} = \frac{\text{R.C. in presence of drug}}{\text{R.C. in absence of drug}} \times 100 \quad \text{(Eq. 2)}$$

**Coincidence of Total and Viable Counts of Organisms**—Into nine 15-ml. centrifuge tubes were placed 0, 0.8, 1.6, 2.4, 3.2, 4.0, 4.8, 5.6, and 6.4 ml. phenylmercuric nitrate stock solution (600 mcg./ml.), respectively. Then, 2 ml. A–G stock solution was added to each tube and the volume was adjusted to 15 ml. by addition of sterile distilled water. The tubes were capped, shaken by hand, and then stored at 37° for 24 hr. Since no acid was added to these tubes, no coacervation occurred.

After 24 hr., 1 ml. from each tube was pipeted into each of three replicate 19-ml. cultures, growing in 125-ml. flasks inoculated 90 min. prior to the incorporation of drug. After 4.5 hr., viable and total counts were made as described previously.

## RESULTS AND DISCUSSION

The effect of the heat of sterilization on the volume of coacervate layer is shown in Table II. The conditions of sterilization reduced the volume of coacervation when compared to the nonsterile system. The presence of acid in the system totally destroyed the coacervate. This was probably due to the hydrolysis of the colloidal solutes composing the coacervate layer in the presence of acid.

The effect of different acids on the volume of coacervate is shown in Table III. This table indicates that the effect of the drug on the pH and, consequently, on the volume of coacervate is negligible. Hydrochloric acid could not be used in the presence of phenylmercuric nitrate because these two components reacted and formed the highly insoluble phenylmercuric chloride which precipitated at the bottom of the tubes.

[12] Cenco, Central Scientific Co.
[13] Coulter Electronic Co., Hialeah, Fla.

**Table IV**—Coacervation Volume in Gelatin–Acacia System Due to Fermentation of Glucose by *E. coli*

| Glucose in System, % | Volume of Coacervate Formed, ml.—Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0.1 | 0.1 | 0.12 | 0.14 | 0.15 | 0.2 | 0.3 | 0.3 |
| 2 | 0.1 | 0.2 | 0.2 | 0.4 | 0.5 | 0.7 | 0.75 | 0.8 | 0.82 | 0.85 |
| 3 | 0.5 | 0.8 | 1.0 | 1.1 | 1.3 | 1.5 | 1.6 | 1.75 | 1.9 | 1.9 |
| 4 | 1.0 | 1.2 | 1.5 | 1.75 | 1.85 | 2.0 | 2.1 | 2.2 | 2.5 | 2.5 |
| 5 | 1.1 | 1.5 | 1.6 | 1.8 | 2.0 | 2.2 | 2.4 | 2.4 | 2.4 | 2.4 |

The importance of pH in biological systems using coacervates can be illustrated by the continuously changing volume of the coacervate phase when *E. coli* is incubated in the system. Over a 10-day period, the acidic metabolites produced from glucose fermentation by *E. coli* sufficiently reduced the pH of the system to produce a measurable increase in the coacervation phase (Table IV).

When different amounts of phenylmercuric nitrate were added to coacervating systems, no difference in the volume of coacervate occurred (Table I). The ratio of the volume of coacervate to the volume of A–G stock solution was consistently 1:2. In forming coacervates, the sequence of the addition of different components, as well as the length of the shaking period (for partitioning of the drug in coacervate), had no effect on the volume of the coacervate layer. These results suggest that the presence of partitioning of phenylmercuric nitrate in the experimental system had no measurable effect on the volume of the coacervate phase.

Since pH is a critical factor in the determination of the total volume of the coacervate phase, the absence of a volume effect attributable to the addition of phenylmercuric nitrate provides a rapid means of visually determining that the drug did not alter the pH of the system.

The partitioning studies of phenylmercuric nitrate in coacervate systems are based on the assumption that if phenylmercuric nitrate partitions uniformly, then the concentration of drug in the equilibrium layer of a coacervate system must be the same as in the noncoacervate system. Consequently, the same aliquots of each system should inhibit the growth of *E. coli* to the same extent. From Fig. 1 and Table V, it is evident that the distribution of phenylmercuric nitrate in the coacervate system is not uniform. This is apparent

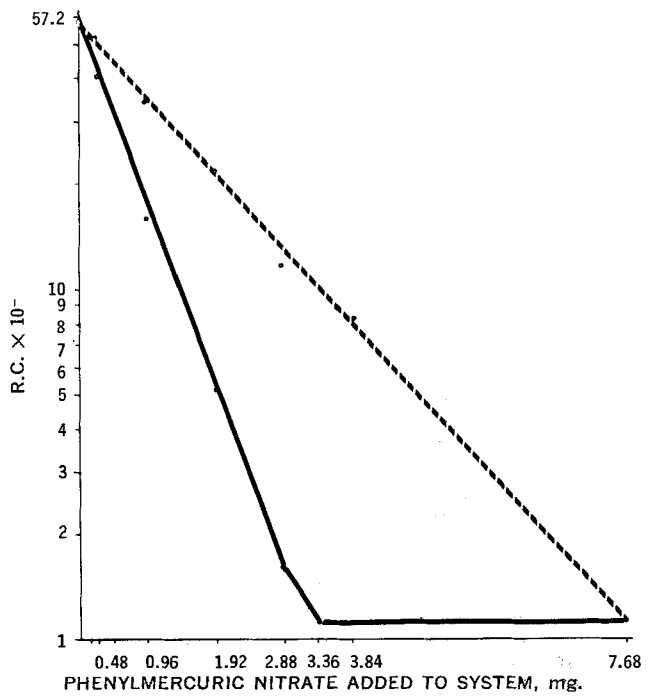


**Figure 1**—*Effect of concentration of phenylmercuric nitrate on R.C. of* E. coli *B/r. Key: - - -, coacervate system; and ——, noncoacervate system.*

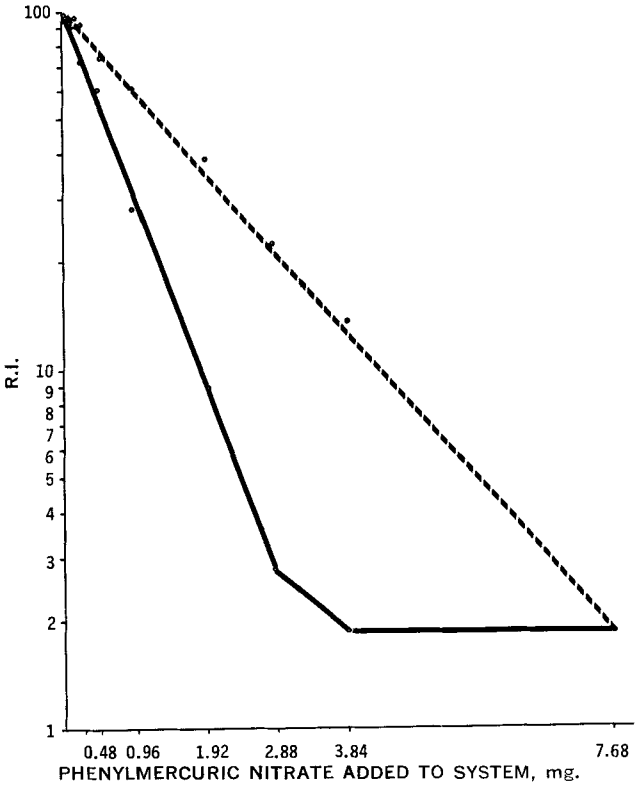


**Figure 2**—*Effect of concentration of phenylmercuric nitrate on R.I. of* E. coli *B/r. Key: - - -, coacervate system; and ——, noncoacervate system.*

when Columns 4 and 5 in Table V are compared. The difference in the slopes of the lines in Fig. 1 is related to the partitioning of phenylmercuric nitrate in the coacervate layer and the equilibrium liquid.

Aliquots taken from tubes $N_0$ or $C_0$ (see Table I for abbreviations) resulted in maximum microbial growth (R.I. = 100), as shown in Fig. 2. The gradual increase of drug decreased R.C. and R.I. until these parameters remained constant with increasing amounts of drug, when complete bacterial growth inhibition occurred. In the absence of drug, R.C. for the noncoacervate system was slightly larger than the corresponding value for its equilibrium layer in the coacervate system (Table V). This difference is attributed to the presence of larger amounts of acacia in the noncoacervate aliquot than in the aliquot of equilibrium layer. When R.C.'s were converted into an R.I., this difference was eliminated since the cell growth at each concentration of drug was divided by the cellular growth at zero drug concentration in the same system (Fig. 2). The calculation of R.I. uses $N_0$ and $C_0$ as growth standards in the presence of drug. In Table V, constant R.C. values of under 16 at high phenylmercuric nitrate concentration may be interpreted as the R.C. for the 90 min.

**Table V**—Microbial Growth of *E. coli* B/r in the Presence of Phenylmercuric Nitrate in Gelatin–Acacia Complex Coacervate and Noncoacervate Systems

| Milligrams Drug Added to 30-ml. Systems (1) | R.C. Noncoacervate System (2) | R.C. Coacervate System (3) | R.I. Noncoacervate System (4) | R.I. Coacervate System (5) |
|---|---|---|---|---|
| 0 | 572 | 558 | 100 | 100 |
| 0.120 | 520 | 531 | 90.91 | 95.16 |
| 0.24 | 410 | 519 | 71.68 | 93.01 |
| 0.48 | 342 | 416 | 59.79 | 74.55 |
| 0.96 | 161 | 340 | 28.15 | 60.93 |
| 1.92 | 52 | 215 | 9.09 | 38.53 |
| 2.88 | 16 | 125 | 2.79 | 22.40 |
| 3.84 | 11 | 80 | 1.92 | 14.33 |
| 7.68 | 11 | 11 | 1.92 | 1.97 |

**Table VI**—Comparison of Total and Viable Counts of *E. coli* Growth in Cultures Treated with Phenylmercuric Nitrate

| Phenylmercuric Nitrate Added to System, mg. (1) | Phenylmercuric Nitrate Concentration, mcg./ml. In Noncoacervate System (2) | Phenylmercuric Nitrate Concentration, mcg./ml. In Culture Media (3) | R.C. Total Count (4) | R.C. Colony Count (5) | R.I. Total Count (6) | R.I. Colony Count (7) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 580 | 569 | 100 | 100 |
| 0.48 | 32 | 1.6 | 342 | 338 | 58.96 | 59.40 |
| 0.96 | 64 | 3.2 | 161 | 156 | 27.76 | 27.41 |
| 1.44 | 96 | 4.8 | 100 | 93 | 17.24 | 16.34 |
| 1.92 | 128 | 6.4 | 52 | 49 | 8.96 | 8.61 |
| 2.40 | 160 | 8.0 | 28 | 25 | 4.83 | 4.39 |
| 2.88 | 192 | 9.6 | 16 | 6 | 2.76 | 1.05 |
| 3.36 | 224 | 11.2 | 11 | 0 | 1.90 | 0 |

of growth prior to the addition of large amounts of phenylmercuric nitrate.

Coincidence in total (Coulter count) and viable (colony count) numbers of *E. coli*/ml. in drug-free and phenylmercuric nitrate-treated cultures is evident for all concentrations of drug up to 10 mcg./ml. (Table VI). Viable counts at the end of 90 min. confirmed these figures. As can be seen from Table VI, Column 7, at concentrations in excess of 160 mcg./ml. phenylmercuric nitrate, the requirements for the calculated parameters to be valid were not met. The R.I. is totally based on live, albeit partially inhibited, cells. At concentrations above 160 mcg./ml. phenylmercuric nitrate, there were no viable cells as determined by colony counts of virtually zero.

Additional studies are now in progress to quantify the partitioning of various drugs and to determine the effect of total drug concentration in the system on the apparent partition coefficient by applying this method.

## SUMMARY AND CONCLUSIONS


1. Gelatin–acacia complex coacervate was utilized as a model to study the partitioning of phenylmercuric nitrate.

2. It was shown that phenylmercuric nitrate did not affect the coacervation process; therefore, during its partitioning, the volume of the coacervate layer was constant.

3. To apply microbial growth to the investigation of drug distribution in the coacervate system, two parameters were defined:

$$\text{reproductive coefficient (R.C.)} = \frac{\text{total number of organisms in each flask}}{\text{total number of organisms inoculated}}$$

$$\text{reproductive index (R.I.)} = \frac{\text{R.C. in presence of drug}}{\text{R.C. in absence of drug}} \times 100$$

4. R.C. and R.I. provide an estimation of the partitioning characteristic of the drug in a coacervate system.

5. This method is valid at R.I. values higher than 25 when applied for phenylmercuric nitrate.


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* Department of Microbiology, College of Medicine, University of Illinois at the Medical Center, Chicago, IL 60612

† To whom requests for reprints should be directed.