2. A preliminary investigation revealed that many different compounds could be used to coacervate gelatin solutions.

3. By varying the proportion of the components of a coacervate system, the amount of a coacervate phase and/or the composition of a coacervate could be controlled.

4. Almost any insoluble particle can be coated with coacervate drops.

5. Of all the variables investigated, it appeared that only those that affected interfacial tension had an effect on coacervate drop size.

6. Pharmaceuticals can be coated by coacervation, but they may intensify the degree of coacervation.

7. A process was originated for obtaining coacervate-coated pharmaceuticals as fine, dry powders.

8. The following pharmaceuticals were coated and recovered as fine, dry powders: riboflavin, cod liver oil, procaine penicillin G, carbon tetrachloride, charcoal, a micronized ion-exchange resin, castor oil, aspirin, and acetanilid.

9. Coacervation not only seems to be an effective method of coating pharmaceuticals but also a superior method of coating many drugs.

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Preparation of a Phase Diagram for Coacervation

By RUSSELL E. PHARES, JR., and G. J. SPERANDIO*

The objective of this study was to develop a method of preparing a phase diagram of coacervated systems using physical measurements instead of chemical analyses. The per cent weight-in-weight concentration of three-component solutions of micro and macromolecules was related to the refractive indexes and specific gravities of the solutions by an algebraic equation. The composition of both phases of several different coacervated systems was determined by using the derived equations. By making accurate quantitative dilutions, it was also possible to determine the composition of solutions the specific gravity of which did not remain linear because of a high concentration of electrolyte. This study indicates that the points needed for plotting a phase diagram (including tie lines) of three-component systems can quickly and accurately be determined by using measurements of specific gravities and refractive indexes.

MOST OF THE quantitative assay procedures currently available cannot be universally adapted to three-component systems without separating the components. The procedure of Leach and Lithgoe, as corrected by Williams (1, 2), for the determination of the percentage composition of a solution of water plus methanol plus ethanol could be very useful if it were not for the need of preparing extensive tables. If this technique, which uses specific gravity and refractive index, could be improved to eliminate the need of tables, it would be useful not only for the analysis of alcohol but also for obtaining the data necessary for plotting three-component phase diagrams. The role of phase diagrams in pharmacy will become increasingly important as coacervation, which was recently introduced as a pharmaceutical operation (3), is used as a means of obtaining many improved pharmaceuticals.

The purpose of this work was to develop a simple method of preparing a three-component phase diagram which would eliminate the need for complicated chemical assays of the ingredients. It was felt that the per cent weight-in-weight concentration of a solution of three components could be calculated directly from the experimentally determined specific gravity and refractive index of the solution without using tables.

THEORY

The weight-in-weight composition of an ideal three-component solution in which there is no interaction may be represented by

$$m + n + p = 1 \qquad \text{(Eq. 1)}$$

where m, n, and p represent the grams of solvent, ingredients 1 and 2, respectively, per gram of solution. Therefore

$$m = 1 - n - p \qquad \text{(Eq. 2)}$$

The refractive index of the solution can be written

$$\text{R.I. of solution} = \text{R.I. of solvent} + Cn + Dp$$

or

$$p = (\Delta\text{R.I.} - Cn)/D \qquad \text{(Eq. 3)}$$

where C and D are proportionality constants for ingredients 1 and 2, respectively. These constants must be determined experimentally on known samples.

The following equation, which is the third one needed to solve for the three unknowns, is true for dilute solutions and ideal solutions

$$\text{sp. gr. of solution} = \text{sp. gr. of solvent} + AN + BP \quad \text{(Eq. 4)}$$

A and B are proportionality constants for ingredients 1 and 2, respectively, while N and P are the grams of ingredients 1 and 2 per gram of solvent.

$$N = n/(1-n-p) \text{ and } P = p/(1-n-p) \quad \text{(Eq. 5)}$$

It follows from Eqs. 3, 4, and 5 that

$$n = \frac{(\Delta\text{sp. gr.})(C) - (\Delta\text{R.I.})(\Delta\text{sp. gr.} + A)}{(BC - AD) + (\Delta\text{sp. gr.})(C - D)} \quad \text{(Eq. 6)}$$

By using Eqs. 2, 3, and 6, one can find the relative weight-in-weight concentrations of the three components.

EXPERIMENTAL

The experimental work was divided into two parts. Part I entailed verification of Eqs. 3 and 4 and the determination of constants A, B, C, and D for sodium sulfate, gelatin, and water. Part II was the preparation of a phase diagram of sodium sulfate, gelatin, and water, using Eqs. 2, 3, and 6. This coacervate system was selected so that the resulting phase diagram could be compared with the one presented by Bungenberg de Jong (4).

Part I.—The refractive indexes and specific gravities of several sodium sulfate solutions and gelatin solutions of known concentrations were plotted against concentration on rectangular coordinate paper (Tables I–IV and Figs. 1, 2, and 3). As can be seen from the graphs, a straight-line relationship exists between refractive index and concentration of both sodium sulfate and gelatin solutions; a straight-line relationship also exists between specific gravity and concentration of gelatin solutions. It will be noticed, however, that a linear relationship exists between specific gravity and con-

TABLE I.—COMPOSITION OF GELATIN SOLUTIONS FOR DETERMINATION OF CONSTANTS A AND C

Sample	Gelatin, Gm.	Water, Gm.
1	5.00	99.25
2	7.54	98.76
3	9.99	99.25
4	15.00	99.25
5	15.02	99.23

TABLE II.—SPECIFIC GRAVITIES AND REFRACTIVE INDEXES OF GELATIN SOLUTIONS FOR DETERMINATION OF CONSTANTS A AND C

Sample	Sp. Gr.	N^a	R. I.	n^b
1	1.01438	0.0504	1.339443	0.0479
2	1.02026	0.0764	1.343206	0.0710
3	1.02611	0.1007	1.346748	0.0915
4	1.03887	0.1511	1.353621	0.1312
5	1.03993	0.1514	1.353682	0.1315

[a] N = Gm. of gelatin per Gm. of water. [b] n = Gm. of gelatin per Gm. of solution.

TABLE III.—COMPOSITION OF SODIUM SULFATE SOLUTIONS FOR DETERMINATION OF CONSTANTS B AND D

Sample	Sodium Sulfate, Gm.	Water, Gm.
1	2.01	99.46
2	4.01	99.56
3	5.00	99.25
4	6.00	99.54
5	7.99	99.51
6	9.96	99.51
7	9.99	99.25
8	10.01	99.25
9	10.03	99.27
10	14.54	99.25
11	25.11	99.24

TABLE IV.—SPECIFIC GRAVITIES AND REFRACTIVE INDEXES OF SODIUM SULFATE SOLUTIONS FOR DETERMINATION OF CONSTANTS B AND D

Sample	Sp. Gr.	P^a	R. I.	p^b
1	1.01785	0.0202	...	...
2	1.03443	0.0403	...	...
3	1.04323	0.0504	1.338463	0.0480
4	1.05092	0.0603	...	...
5	1.06706	0.0804	...	...
6	1.08311	0.1001	...	...
7	1.08375	0.1006	...	...
8	1.08332	0.1008	1.344637	0.0916
9	1.08378	0.1010	1.344730	0.0917
10	1.12218	0.1465	1.350125	0.1278
11	1.19135	0.2530	1.359930	0.2019

[a] P = Gm. of sodium sulfate per Gm. of water. [b] p = Gm. of sodium sulfate per Gm. of solution.

centration of sodium sulfate solutions only at concentrations less than 10 Gm. of sodium sulfate per 100 ml. of water.

The refractive indexes of all samples in this work were determined at 37° using an immersion refractometer. All specific gravities were determined at 37° using a 25-ml. pycnometer weighed on a Mettler type TB5 analytical balance. When the constants
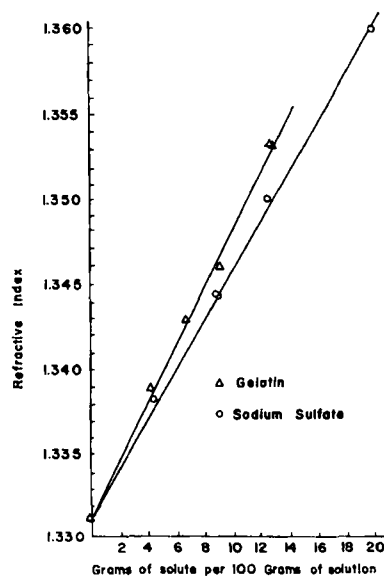

Fig. 1.—Concentration *vs.* refractive index of gelatin and sodium sulfate solutions.
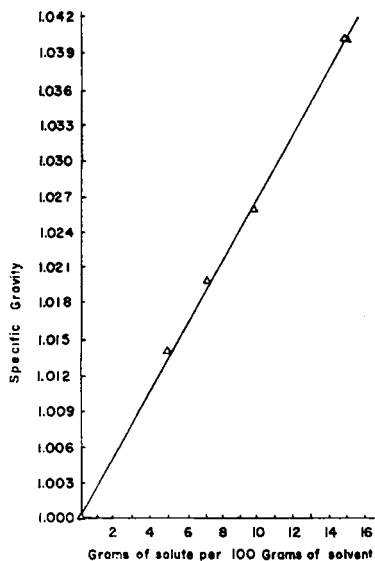
Fig. 2.—Concentration *vs.* specific gravity of gelatin solutions.

Fig. 3.—Concentration *vs.* specific gravity of sodium sulfate solutions.

A, B, C, and *D* (which are the slopes of the lines on Figs. 1, 2, and 3) were statistically calculated along with their 99% confidence interval estimates, the following values were obtained: $A = 0.250058 \pm 0.0298$ (gelatin), $B = 0.820425 \pm 0\ 0128$ (sodium sulfate), $C = 0.169258 \pm 0.0046$ (gelatin), and $D = 0.141455 \pm 0.0024$ (sodium sulfate).

Equations 3 and 6 were verified by using them to predict the concentrations of several dilute solutions, all of which contained known amounts of gelatin, sodium sulfate, and water (the concentrations of gelatin and sodium sulfate were never more than 10% w/w). In all samples tested, the true concentration and the calculated concentration based on Eqs. 3 and 6, using the experimentally determined refractive index and specific gravity of each solution, were within 0.5% of one another.

Part II.—Five samples containing known amounts of water, sodium sulfate, and gelatin (Table V) were prepared and stored at 37° for 5 days. Sample 1 was the only system that did not coacervate. After 5 days, the two phases of the coacervated samples were separated by centrifuging at 5000 r.p.m. for

10 minutes. Since both the equilibrium phase and the coacervate phase of each system was a clear solution, the weight-in-weight composition of each phase was determined in the following manner, using the previously verified equations. From each coacervate phase, a sample weighing approximately 15 Gm. was accurately weighed and then diluted with approximately 100 Gm. of distilled water; the weight of the water was then accurately determined. The specific gravities and refractive indexes of these dilutions were measured. From these measurements, the compositions of the undiluted coacervate phases were calculated using Eqs. 2, 3, and 6 (Table VI). It was necessary to dilute the coacervate phases because they were too viscous to pour into standard pycnometers and because they had specific gravities too high to be linearly related to possible sodium sulfate concentrations.

The composition of each equilibrium phase was calculated using Eqs. 7 and 8. Equation 7 is the equation of the tie line which connects the composition of the total system with the composition of the coacervate phase and the composition of the equilibrium phase. The equation of each tie line

TABLE V.—COMPOSITION OF COACERVATE SAMPLES USED FOR PREPARING PHASE DIAGRAM

Sample	Gelatin, Gm.	% w/w	Sodium Sulfate, Gm.	% w/w	Water, Gm.	% w/w
1	5.88	5.00	11.76	10.0	99.25	85.0
2	5.11	5.00	13.09	11.0	99.27	84.0
3	6.02	5.00	14.46	12.0	99.21	83.0
4	6.10	5.00	15.85	13.0	99.25	82.0
5	6.17	5.00	17.28	14.0	99.24	81.0

TABLE VI.—PERCENTAGE COMPOSITION OF EACH PHASE OF THE COACERVATED SYSTEMS[a]

Sample	Coacervate Phases, Compn.			Equilibrium Phases, Compn.		
	Gelatin[b]	Sodium Sulfate[b]	Water[b]	Gelatin[b]	Sodium Sulfate[b]	Water[b]
1	Did Not Coacervate					
2	17.00	9.50	73.50	3.00	11.25	85.75
3	22.00	9.50	68.50	2.00	12.25	85.75
4	25.00	9.50	65.50	1.50	13.50	85.00
5	27.00	9.50	63.50	1.00	15.00	84.00

[a] The per cent compositions are given to the nearest 0.25%. [b] The values represent the per cent weight-in-weight concentration of each ingredient.
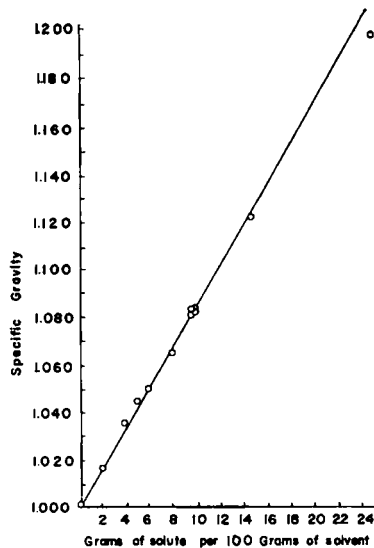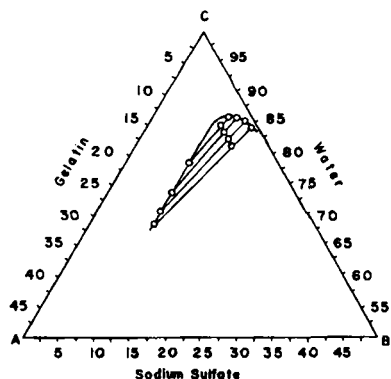
Fig. 4.—Phase diagram of gelatin, water, and sodium sulfate.

$p = \alpha + \delta n$, was determined as shown below by using the total known composition of each coacervated system and the calculated composition of the corresponding coacervate phase. $(p_C = \alpha + \delta n_C) - (p_T = \alpha + \delta n_T)$ can be rearranged to give

$$\frac{p_C - p_T}{n_C - n_T} = \delta$$

and therefore

$$\alpha = p_T - \delta n_T$$

$$p_E = \alpha + \delta n_E \qquad \text{(Eq. 7)}$$

The subscripts C, T, and E represent coacervate phase, total coacervate system, and equilibrium phase, respectively. By combining Eqs. 3 and 7

$$n_E = \frac{\Delta RI - \alpha_D}{\delta D + C} \qquad \text{(Eq. 8)}$$

The compositions of the equilibrium phases are presented in Table VI. The data in Table VI are plotted in Fig. 4.

SUMMARY AND CONCLUSIONS

1. The objective of this work was to develop a rapid method of determining the weight-in-weight concentration of a three-component solution using the experimentally determined specific gravity and refractive index of the solution.

2. Formulas were derived for calculating weight-in-weight concentrations without the need of tables.

3. The formulas are applicable to all three-component solutions in which there is no interaction of the ingredients.

4. In solutions in which there is interaction of the ingredients, such as aqueous solutions of ethanol, or methanol, or sodium sulfate, the weight-in-weight concentration can be determined by using a "diluting technique" which would minimize the effects of interaction.

5. The assay procedure is satisfactory for obtaining the data necessary for plotting three-component phase diagrams.

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Use of 3-Azabicyclo[3.2.2.]nonane in the Mannich Reaction III

γ-Amino Tertiary Alcohols

By C. DEWITT BLANTON, JR., and W. LEWIS NOBLES

Syntheses of a group of γ-amino tertiary alcohols by application of the Grignard reaction to the corresponding Mannich bases are described. These alcohols are to be screened for possible pharmacological activity.

IN OUR EARLIER publications (1, 2), a number of substituted β-amino ketones and γ-amino secondary alcohols employing 3-azabicyclo[3.2.2]-nonane as the amine moiety were synthesized for pharmacological screening. Denton and his associates (3) have previously established that certain structural modification of β-amino ketones

had an effect on physiological activity. The authors wish to report upon the extension of our earlier studies to include the preparation of γ-amino tertiary alcohols.

The general structure of the γ-amino tertiary alcohols prepared by Denton and co-workers (4–10), as well as those presented here, are represented as

$$R-\underset{\underset{R'}{|}}{\overset{\overset{OH}{|}}{C}}-(CH_2)_x\text{-}Am$$
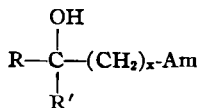
Of the tertiary amino carbinols which have been reported in the literature, many have shown