It is to be remarked that this equation is identical with the equation derived by SEZAWA, if we put the function f, occurring in his notation, equal to our function k_1^2/ζ.

In the following special cases the reduction of equation (1) will be obvious:

1. If

$$N = \frac{p\,d}{\mathfrak{B}_2} = 0,$$

in other words if the depth of the layer is infinitely small in comparison with the transversal wave-length $\left(=\frac{2\pi\mathfrak{B}_2}{p}\right)$, the equation can be reduced to

$$4\sqrt{(1-\zeta)(1-\nu_1\zeta)} - (2-\zeta)^2 = 0,$$

the RAYLEIGH equation of medium 1. ($\varphi = tgh\,\alpha = tgh\,\beta = 0$.)

2. If $N = \sim$ then $\varphi = tgh\,\alpha = tgh\,\beta = 1$; equation (1) becomes

$$\{4\sqrt{(1-\omega\zeta)(1-\gamma\zeta)} - (2-\omega\zeta)^2\}\{P - Q_2 + S - Q_1 - R_1 - R_2\} = 0:$$

the RAYLEIGH equation of medium 2 and the STONELEY equation.

3. If $\frac{\varrho_2}{\varrho_1} = \frac{\mu_2}{\mu_1} = 0, \qquad \omega = \frac{\varrho_2\mu_1}{\varrho_1\mu_2}$ remaining finite

we get

$$(2-\eta)^4\,tgh\,\alpha\,tgh\,\beta - 8(2-\eta)^2\sqrt{(1-\eta)(1-\nu_2\eta)}.\,\varphi + 16(1-\eta)(1-\nu_2\eta)\,tgh\,\alpha\,tgh\,\beta = 0$$

where

$$\eta = \omega\zeta,\ \alpha = N\sqrt{\frac{1-\nu_2\eta}{\eta}} \text{ and } \beta = N\sqrt{\frac{1-\eta}{\eta}}.$$

It is evident that in this case ($\mu_1 = \varrho_1 = 0$) the subjacent medium does not exist; this equation must therefore relate to the vibrations of an isolated layer. We shall investigate in the next paragraph the problem of the wave-systems occurring in an elastic plate, as it has an important bearing on the general equation (1).

(To be continued.)

Biochemistry. — *Coexisting complex coacervates.* By H. G. BUNGENBERG DE JONG and E. G. HOSKAM. (Communicated by Prof. H. R. KRUYT.)

(Communicated at the meeting of February 28, 1942.)

1. *Introduction.*

We have previously described how two coexisting complex coacervates are formed in mixtures of gelatine, gum arabic and Na-Nucleinate sols in certain mixing proportions with sufficient pH reduction [1]. The results were set out in a ternary diagram which showed that the area of mixing proportions in which there are two coacervates is roughly between the mixing proportions located on the sides of the triangle in which the reversal of charge lies in the two systems gelatine + gum arabic and gelatine + Na-Nucleinate.

The problem of the significance of the charge for the formation of coexisting coacervates is again discussed in the following pages. We were especially interested in the course of the lines connecting the coexisting coacervates in the area of the two coexisting coacervates.

2. *Material and technique.*

In the previous investigation we made use of unpurified colloid preparations, but for this investigation we used them purified, viz. isoelectric gelatine, Na-Arabinate and Na-Yeast nucleinate, the preparation of which has been described elsewhere [2]. In the following pages we refer to these preparations as G (gelatine), A (Na-Arabinatie), and N (Na-Nucleinate).

Of these 3 preparations we prepared stock sols by dissolving 5 g. air-dry samples in 250 g. dist. water. These stock sols were preserved in the refrigerator for future use.

In the previous investigation the pH reduction was caused by diluted acetic acid, in the present investigation acetate buffers were used. As neutral salts, however neutralize the complex coacervation it is recommendable to keep the Na-Acetate final concentration in this buffer rather low, for which we chose 10 m aeq. p. L. To 10 cc stock sol or mixture of stock sols we always added 5 cc buffer, the composition of which was as follows: 30 cc Na-Acetate 0.1 N + 50 cc acetic acid 1 N, dist. water being added until 100 cc. For the three stock sols separately (H electrode at 40°) the pH was then: G = 3.65, A = 3.57; and Na = 3.76. So the three buffered sols are not exactly, but approximately isohydric, which is not to be wondered at, as only a comparatively slight Na-Acetate concentration was admissible, so that better buffering was not to be expected with the comparatively great colloid concentrations. In the area of mixing proportions, in which 2 coexisting coacervates are formed (extending between ca. 50 % A + 50 % G with pH = 3.61 and between ca. 30 % N + 70 % G with pH = 3.68) the pH does not vary quite 0.1 pH.

3. *The coacervation areas.*

First we investigated the coacervation in the binary combinations gelatine + Na-

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1) H. G. BUNGENBERG DE JONG and A. DE HAAN, Biochem. Z. **263**, 33 (1933).

2) Isoelectric gelatine, prepared from gelatine F00 extra of the "Lijm- en Gelatinefabriek 'Delft'" at Delft. Method of preparation see Koll. Beihefte, **43**, 256 (1936).

Na-Arabinate prepared from gomme Senegal pepite boule blanche I of ALLAND et ROBERT, Paris (preparation see Kolloid Beihefte **47**, 254 (1938)).

Na-Nucleinate prepared from N.-Nucleinate of E. MERCK (preparation see Kolloid Beihefte **47**, 254 (1938)).

Arabinate, resp. gelatine + Na-Nucleinate at 40° C. Therefore the following series of mixtures were prepared in sedimentation tubes:

a cc A + (10—a) cc G + 5 cc buffer (I)

resp.

a cc N + (10—a) cc G + 5 cc buffer (II)

In which G, A and N stand for the stock sols mentioned in 2 (5 G air dry colloid + 250 cc dist. water). The sedimentation tubes were left in the thermostat at 40° till the following morning, when the coacervate volumes were read in 0.1 cc, namely:

Combination G + A		Combination G + N	
a	vol.	a	vol.
2	0.2	1.5	0.2
3	3.4	2	2.4
4	6.8	2.5	3.9
4.5	8.3	3.5	3.9
5	9.1	4.5	3.8
5.5	9.2	5	3.4
6	8.6	6	3.0
7	4.8	8	1.1
8	0.1	9	0.2

Graphically we found that for series I the coacervation takes place between 19 % and 81 % A, for series II between 9 % N and 93 % N.

Electrophoretic measurements at 40° gave the following points of charge reversal for these series:

48 % A for series I and 27 % N for series II.

Subsequently larger quantities of these 48 % A resp. 27 % N mixtures were made and with these a series of mixtures of the following composition:

a cc (48 % A) + (10—a) cc (27 % N) + 5 cc buffer (III)

was prepared in sedimentation tubes and the coacervate layers were noted down after ca. 40 hours in the thermostat.

The results are pictured in Fig. 1.

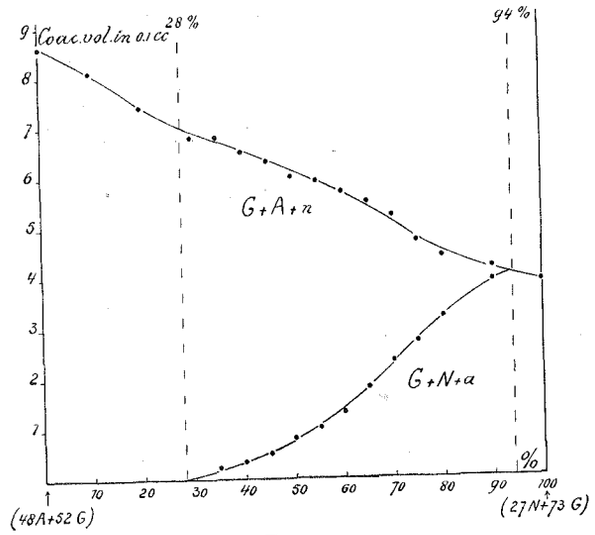

Fig. 1.

From this it is seen that in a certain section of mixing proportions (expressed in % of the N + G system this section extends from 28 % to 94 %), two coexisting coacervates are formed. The one with the greatest specific gravity and the highest nucleinate percentage is indicated in the figure as G + N + a. To the right of the dotted line it passes without any interruption into the G + N coacervate. The complex coacervate of less specific gravity and high arabinate percentage is indicated in the figure as G + A + n, to the left of the dotted line it passes without interruption into the G + A coacervate.

In the following survey the boundaries are indicated of the area in which coexisting coacervates occur, expressed in % of the system indicated as second system. The series III, IV, V, VI and VII were obtained from determinations of the coacervate volume curves (analogous to fig. 1). In the case of VIII and IX we followed a different method, in which a number (climbing up with 1 % of the mixing proportion in the critical area) of mixtures was prepared and microscopically investigated. In order accurately to determine the boundary it is necessary to keep the mixtures belonging to the critical area in the thermostat at 40° for at least one hour.

Mixing series No.	Composition of the two systems combined		Mixing section in which coexisting coacervates occur, expressed in % of the 2nd system [1]
	1st System	2nd System	
III	48 A + 52 G	27 N + 73 G	$28^{(a)} - 94^{(n)}$
IV	30 A + 70 G	85 N + 15 A	$11.5^{(a)} - 26.5^{(n)}$
V	75 A + 25 G	25 N + 75 G	$22^{(a)} - 96^{(n)}$
VI	15 N + 85 A	17 N + 83 G	$48^{(a)} - 93^{(n)}$
VII	10 N + 90 A	10 N + 90 G	$35^{(n)} - 75^{(a)}$
VIII	100 G	30 N + 70 A	$35^{(n)} - 58^{(a)}$
IX	100 G	60 N + 40 A	$26^{(a)} - 43^{(?)}$

With the assistance of these data we have drawn in Fig. 2 the closed curve within which coexisting coacervates occur.

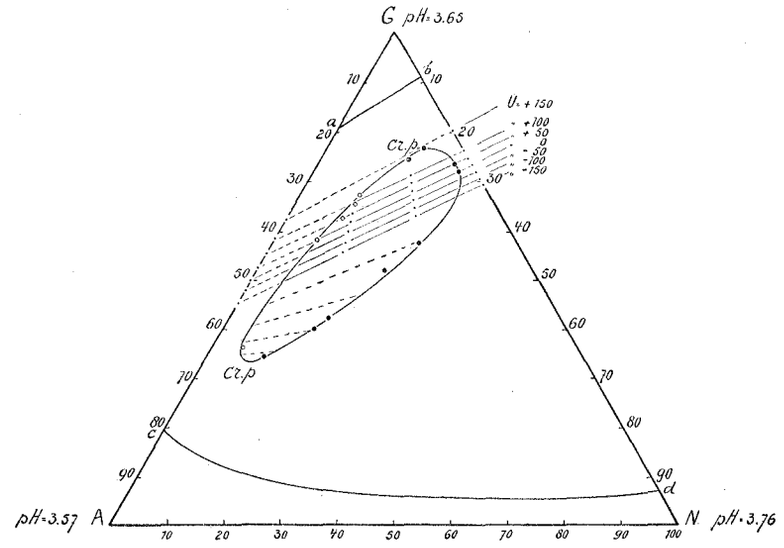

Fig. 2.

[1] The significance of (a) and (n) is explained in § 5.

Moreover curves ab and cd have been drawn which indicate the boundaries of the coacervation. So coacervation does not take place in area Gab, nor in area AcdN.

In area abdc coacervation does take place, one coacervate occurring outside the closed curve (containing the three colloids), two coexisting coacervates within the closed curve, of which the one has a high A and a low N percentage, the other a high N and a low A percentage.

The results described thus far agree very well with the results obtained previously with unpurified colloids and without buffers.

4. *Electrophoretic measurements.*

We have pointed out in our former publication that the location of the area of the coexisting coacervates extends approximately between the points of charge reversal of the combinations G + A resp., G + N on the sides of the triangle, but accurate measurements were not made at the time.

Now, however correct measurements have been made in a microscopic electrophoresis cuvette at 40°, in which, after a short time of centrifuging of the coacervated system, we suspended a little quartz powder in the equilibrium liquid and measured the electrophoresis velocity of the quartz particles. These measurements were made of four mixing series [1]).

The following survey gives the two systems, combined each times and the mixing proportions with which a certain electrophoresis velocity is attained.

Composition of the two systems combined		Mixing proportions in % of the second system, in which the electrophoresis velocity (U) indicated is reached [2])						
1st System	1nd System	+ 150	+ 100	+ 50	0	− 50	− 100	− 150
100 G	100 A	38	42	45	48	50.5	52.5	55
100 G	70 A + 30 N		34.9	36.8	38.5	40.4	42.7	—
100 G	40 A + 60 N		29	30.5	32.2	34	35.7	37.3
100 G	100 N	20	24.1	25.7	27.1	28.5	29.8	31.1

The results have been drawn in Fig. 2. The points belonging to the reverse of charge (U = o) show that the uncharged systems within the plane of the triangle lie practically on the straight line connecting the points of charge reversal of the two sides of the triangle G A and G N. The line divides the plane of the triangle into two parts, in an upper half until vertex G, in which the systems are positive, and in a lower half in which the systems are negative.

This line of reverse of charge intersects the area of the coexisting coacervates, giving a confirmation of what we have said before: The mixability of complex coacervate G + N and G + A is especially slight with the optimal mixing proportions of G and N, resp. of G and A, i.e. there where the compensation of opposed charges is optimal.

From the fact that the line of reverse of charge intersects the area of the coexisting coacervates asymmetrically, into a smaller positive and a larger negative part it would seem that the mutual mixability of the negative G + A and G + N coacervates is smaller than that of the positive coacervates. We cannot as yet account for this fact. In the following section we shall discuss the significance of lines of constant electrophoresis velocity.

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[1]) In this place we thank Dr. H. L. BOOY for his assistance in performing the measurements.

[2]) The electrophoresis velocity is expressed here in arbitrarily chosen units. For details regarding method of the measurements see H. G. BUNGENBERG DE JONG and P. H. THEUNISSEN, Recueil des Trav. Chim. d. Pays Bas, **54**, 460 (1935).

5. *What is the colloid composition of the coexisting complex coacervates?*

This question might of course be answered at once by analyzing the coexisting coacervates. The difficulty arises, however, that although microscopically coexisting coacervates can clearly be distinguished [1]) macroscopically the coalescence to separate layers is generally far from easy, with some mixing proportions for instance, the coacervate of high N percentage persists in a division into small drops in the coacervate of high A percentage. Centrifuging is often not sufficient. For the present, therefore, we have to be satisfied with an indirect answer to the question asked.

As described in previous publications the coacervate drops of high A percentage take up those of high N percentage, so that microscopically composite drops are observed. In the electric field these composite drops behave differently according as the mixture

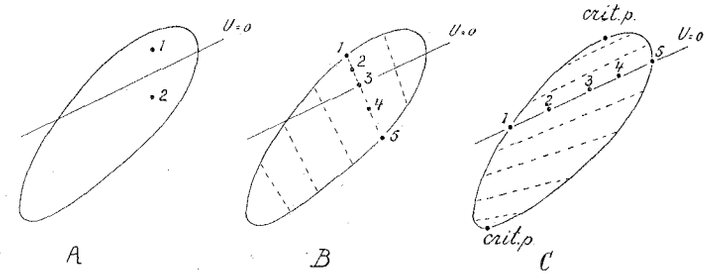

Fig. 3.

is chosen in the positive part (Fig. 3a, point 1) or in the negative one (Fig. 3a, point 2) of the area of the coexisting coacervates.

When the composite drop is positive (which appears from the cataphoretic direction of the composite drops) the drop of high Na percentage enclosed within the drop of high A percentage also moves into the direction in which the drop of high A percentages electrophoretizes. With negative composite drops the reverse takes place. From this one would be inclined to conclude that the two coexisting coacervates always have the same charge sign. From this it would again follow that the line of reverse of charge in the triangle connects two coexisting coacervates. But this reasoning is inadmissible, as the direction of movement of the enclosed coacervate drop is no indication of its charge sign. For any enclosure (vacuole, carbon particle, oil drop) moves in this way in a coacervate drop. Yet the theory that the line of reverse of charge connects two coexisting coacervates is plausible.

Suppose the connecting lines of the coexisting coacervates have a different course, for instance the one in Fig. 3b.

Then mixtures of the colloid compositions 2, 3 and 4 break up into coexisting coacervates of colloid compositions 1 and 5. One of these two is then the enveloping coacervate of the composite drops, and must therefore always give the same charge sign to these drops. But this is contradictory to our experience, for the composite drops formed from 3 are uncharged, from 2 they are positive and from 4 they are negative (see previous section).

In the same way any other direction of the connecting lines is contradictory to our experience, unless the line of reverse of charge itself is a connecting line of coexisting coacervates (Fig. 3c). What has been said of the line of reverse of charge also applies practically to the other lines of constant electrophoresis velocity, whose course follows from the data of the table in § 4. Therefore they are drawn in full in Fig. 2 for so

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[1]) Dyes stain the coacervate of high N percentage many times more intensively than the coacervate of high A percentage and thus the former can at once be recognized microscopically.

far as their course falls within the area of the coexisting coacervates. This indicates that they may be considered as approximately connecting lines of coexisting coacervates.

Two lines with constant U (electrophoresis velocity) will touch the closed curve of the two coexisting coacervates at the place of the two critical points.

While the location of the point of contact nearest to vertex G is practically known, the other one is not known on account of the absence of electrophoresis measurements. But from the coacervate volume curves of Fig. 3 we can obtain a control concerning the correctness of the location of the critical point mentioned first and indications concerning the location of the second critical point.

As an example we take the mixing series pictured in Fig. 1. Here from left to right on entering the area of the coexisting coacervates we note the presence of the coacervate layer of high A-percentage and we see that the layer of high N-percentage increases from zero upward. On leaving the area of the coexisting coacervates on the other hand we see that the coacervate layer of high N-percentage is present and that the layer of high A-percentage decreases to zero. For this reason we have added the letters (*n*) or (*a*) to the mixing percentages in the survey table of § 3, in order to indicate what coacervate is present in abundance on passing the boundary. At the critical points mentioned the curve branch of the coacervates of high A-percentage must pass into that of the coacervates of high N-percentage. From Fig.. 4 in which we have indicated the coacervates of high A-percentage by open circles, those of high N-percentage by black dots (see survey Table 3), we see that the critical point on the side of vertex G of the triangle, as indicated by the course of the lines with U-constant, lies indeed between the series of the white points (left) and of the black points (right). Reversely the place of the other critical point is indicated by the space between the white and black dots on the other side of the area of the coexisting coacervates. Dotted lines within the area of the coexisting coacervates indicate the probable course of the connecting lines of the coexisting coacervates near this critical point.

Summary.

1. The occurrence of coexisting coacervates in mixtures of purified gelatine, Na-arabinate and Na-Yeast nucleinate was investigated in the presence of diluted buffers at pH ca. 3,7 and the results were put out in ternary diagrams.

2. The results agree very well with the results previously obtained with unpurified colloid preparations. The investigation was extended with electrophoretic measurements and with the measurement of coacervate volumes.

3. Thus the probable direction of the connecting lines of coexisting complex coacervates in the ternary diagram could be determined.

Leiden, Laboratory for Medical Chemistry.

Biochemistry. — *Behaviour of microscopic bodies consisting of biocolloid systems and suspended in an aequeous medium.* VI. A. *Auxiliary apparatus for studying the morphological changes of coacervate drops.* B. *Preparation and behaviour of composite drops consisting of coexisting complex coacervates.* By H. G. BUNGENBERG DE JONG. (Communicated by Prof. H. R. KRUYT.)

(Communicated at the meeting of February 28, 1942.)

1. When studying coacervate drops we often felt the need of an apparatus in which a coacervated system could be kept in stock in which on the one hand the coacervate drops could coalesce to larger ones, while on the other hand these larger drops remain suspended in their medium for some length of time.

A solution of these requirements was formerly found in an apparatus we called by the name of "Kreisröhre"[1]). This apparatus consists of a circular tube connected by spokes to a central axis and which rotates slowly. The coacervated system only partly fills the tube, so that when rotating it is always flowing. Although this apparatus has proved very useful it also has certain disadvantages, it is namely impossible during rotation to take small samples from it in order to check any changes in the coacervate drops from moment to moment, nor is it possible to add substances to study their effect on the coacervate drops.

The apparatus pictured in Fig. 1 removes these difficulties. Here the coacervated

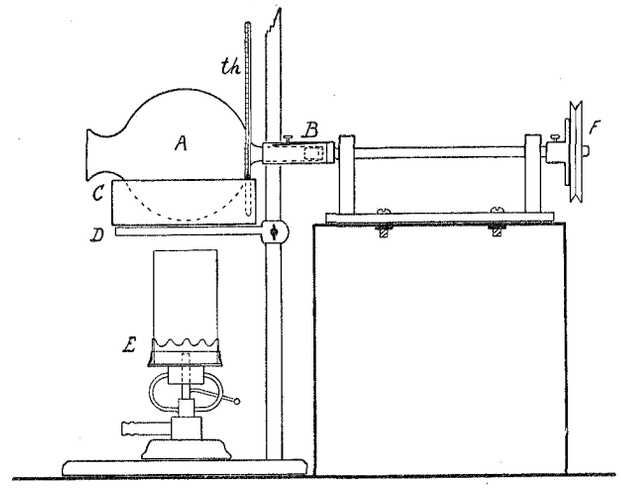

Fig. 1.

system is placed in a glass sphere (A), which turns horizontally round its axis. At the back this sphere is narrowed to a tube which is closed with a thin rubber stop and which fits into a copper case (B) of the horizontal axis, into which it is fixed by means of a screw.

In front there is a bell-shaped opening, through which the apparatus may be filled; during the rotation substances may be added and with a pipette or glass rod a sample may be taken from it for examination under the microscope. The sphere (A) is submerged

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1) H. G. BUNGENBERG DE JONG and O. BANK, Protoplasma **33**, 322 (1939).