

Chemistry. — *Soap coacervates with special properties, hitherto only known in coacervates of phosphatides. I Oleate coacervates at a pH above 12.* I. By H. G. BUNGENBERG DE JONG and L. J. DE HEER.

(Communicated at the meeting of September 24, 1949.)

1. *Introduction.*

In KRUYT's Colloid Science Vol. II one of us (B. de J.) has given a survey of the peculiar phenomena observed with drops of phosphatide coacervates ¹⁾.

These phenomena, viz. a) abnormal behaviour of the vacuoles, b) abnormal behaviour of the drops in a d.c. field, c) occurrence of multiple coacervate drops, had since their discovery ²⁾ not been observed in coacervates of other colloids, neither in those of macromolecular colloids (e.g. gelatin) nor in those of association colloids (e.g. soaps). This isolated position of phosphatide coacervates has now come to an end as we have learned to realize coacervates of soaps showing quite the same phenomena.

In the following this new type of soap coacervates will be called *P-coacervates* (coacervates with phosphatide-like properties), to distinguish them from the "ordinary" soap coacervates (lacking these properties), which will be called *O-coacervates*.

2. *Morphology of drops of O- and P-coacervates of oleates, including their behaviour in a d.c. field.*

For the present investigation, which deals only with oleate coacervates, we started from "Sodium Oleate, Neutral, Powder" from BAKER, which preparation, though not representing really pure Na-oleate, was the only one accessible to us in the after wartime ³⁾. As its impurities — mainly the soaps of the C₁₆ and C₁₈ saturated fatty acids — may show P-coacervation also under similar conditions (though not at numerically the same), this admixture will not radically change the results which may be expected from chemically pure oleate.

¹⁾ H. R. KRUYT, Colloid Science Vol. II, Elsevier Publishing Company, Inc. New York, Amsterdam, London, Brussels 1949. Compare Chapter XI, § 6.

²⁾ H. G. BUNGENBERG DE JONG and R. F. WESTERKAMP. Biochem. Z. **248** 335 (1932).

³⁾ The first indications for the occurrence of P-coacervates were found during the war starting from Na oldanicum med. pur. pulv. MERCK. As it was impossible to buy this preparation anew, we applied to the ROCKEFELLER Foundation, which kindly put at our disposal a large quantity of the preparation mentioned in the text in order to enable us to continue our researches on oleate systems. We are glad to express our great thanks for his help.

As we know already that oleates behave with regard to O-coacervation in an uncomplicated way only, if the pH is high enough (e.g. at pH 12 and higher) to exclude all hydrolysis ⁴⁾, it seemed advisable to start our study on P-coacervation under the same precaution. As far as we know at present P-coacervation of oleates can be obtained only by the combined action of a salt (e.g. KCl, K₂CO₃) and a typical polar organic non-electrolyte of not too small molecular weight (e.g. isoamylalcohol, n.hexanol, n.heptanol, cyclohexanol, terpineol, benzylalcohol, phenylaethylalcohol). For the present investigation our choice fell on the combination KCl + isoamylalcohol, (CH₃)₂CH-CH₂-CH₂OH, a purissimum preparation of MERCK being used for all experiments.

If we now prepare a dilute oleate solution (e.g. 2 %) containing 0.1 N. KOH (to ensure a pH above 12) and add 10 ml 2N.KCL to 10 ml of it, we obtain after rigorously shaking a homogeneous and (apart from entrapped air bubbles) a clear solution which is very highly viscous and which shows marked elastic properties as well. Starting from this one phase system we can readily obtain O-coacervation and P-coacervation by adding different amounts of isoamylalcohol and rigorously shaking (for the experimental equipment actually used see section 4). A few small drops will suffice already to start O. coacervation. The total system now consists of two phases: the O coacervate and the equilibrium liquid. If we inspect the system under the microscope (for technique see section 4) we will see the coacervate in the form of perfectly round drops ⁵⁾.

When two of such drops meet, they readily fuse. If vacuoles are present in the coacervate drops, their behaviour is quite normal (when two vacuoles meet, they readily fuse; a vacuole meeting the innerside of the drop boundary breaks though and the drop boundary rounds off). Also in a d.c. field we do not perceive the peculiar behaviour of the vacuoles which we will meet with the P-coacervate. The main effect is electrophoresis of the drops, which show a negative charge at their surface.

If we gradually add now more and more isoamylalcohol, the total oleate system in the tube changes its character (for details of the various successive states of the oleate system see section 4) and when a fairly large number of drops is added, it becomes homogeneous and clear again. The obtained one phase system now needs only one or two drops of isoamylalcohol to start coacervation afresh, this time the P coacervate separating. Microscopical inspection at once reveals an unexpected morphological richness:

- 1) Many coacervate drops are not perfectly round, but have the shape of spheres

⁴⁾ At lower pH (e.g. at 10 already) complications occur (the oleic acid formed by hydrolysis causing "auto sensibilisation") and below pH 8.70 coacervation is no longer possible at all by KCl.

⁵⁾ The interfacial tension coacervate/equilibrium liquid being very small, the drops are very easily deformed by convection currents and the rounding off (after these currents have come to a standstill) is a relatively slow process.

from which one or more segments are cut away. Cf. microphotograph *) *A* and fig. 1 *A* and *B*. Also smaller defects in the ideal spherical form may be observed (fig. 1 *C*).

2) It may happen that a number of drops drift along in a very conspicuous way: they are at short distances and have each (apart from the two outer ones) two plane faces which stand perpendicular to the direction in which the drops drift along (fig. 1 *D*). As the whole train of drops behaves as a mechanical unit the individual drops must be united by invisible films attached to the circular edges of the plane or slightly curved faces.

3) It has been observed that two drops of the shape of Fig. 1 *A*, formed an analogous mechanical unit, now of two members only when they happened to come with their plane faces up to a short distance from another ⁶⁾ (fig. 1 *E* → *F*).

This observation makes it clear that the shape of Fig. 1 *A* must be caused by a closed film protruding in the equilibrium liquid and attached to the circular edge of the plane face as depicted in Fig. 1 *H*.

The film in question is invisible as a rule but with special efforts it was vaguely seen a few times and had indeed the shape of Fig. 1 *H* (dotted).

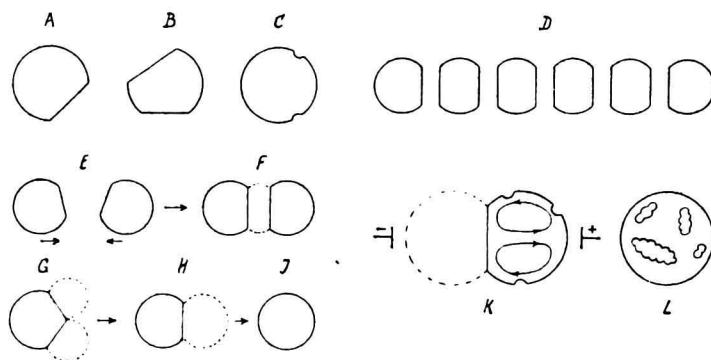


Fig. 1.

4) It may happen that a coacervate drop of the type of Fig. 1 *B* has its two plane faces situated in such a way that they seem to touch at one point. It then often occurs that the drop suddenly changes in shape into a drop of the type of Fig. 1 *A*. The two films of adjacent vesicles have then made real contact and have fused to a single one (see Fig. 1 *G* → *H*). This sideways fusion is in principle the same phenomenon as occurs in the formation of drop chains (see above 2 and 3).

5) The presence of these films follows further from the observation that a mechanical disturbance outside the coacervate drop at a small distance from the plane face may result in a rounding-off of the coacervate drop (Fig. 1 *H* → *I*). The protruding film which was not visible, was evidently destroyed then.

It is important that as a consequence of this destruction no other particulars can be seen at the place where the protruding vesicle was attached. It means that the liquid enclosed in this vesicle is wholly miscible with the surrounding equilibrium liquid. So the film in question has the property to be stable notwithstanding the fact that it divides two wholly miscible (and as we shall later see: identical) liquids.

6) Apart from electrophoresis (the direction of which indicates that the drops are

*) The microphotographs have been taken by Mr W. W. H. WEYZEN, to whom we express our sincere thanks.

⁶⁾ If drops having the shape of Fig. 1 *A* drift up against the glasswall and so come close to one another they may also unite into chains. In this case often networks of chains are formed, some drops having three faces functioning as branching points of the network.

negatively charged) we see in a d.c. field a number of phenomena inside the drop, some details of which are schematically given in Fig. 1 *K*. They are quite the same as those which are characteristic of negatively charged phosphatide coacervate drops, (which is a variant of the phenomena common to all negatively charged complex coacervate drops ⁷⁾).

Without entering here upon a discussion why small vacuoles are formed and why streaming inside the drops arises, we discuss here only the fate of those small vacuoles which happen to come into contact with the inside of the drop surface.

These vacuoles are unable to make their exit, but stick in the surface of the drop as protruding vesicles. The vesicle wall, however, is once more very thin and, as rule, not visible. Therefore, what is actually observed is only an indentation in the drop boundary, wholly comparable to Fig. 1 *C*. These indentations are transported by the moving surface of the drop (drops may creep in a d.c. field on a glass surface) towards one side (cathode side) of the drop and gradually form there by sideway fusing (see 4) the well known picture of Fig. 1 *A*, with only this difference that all coacervate drops now have one large plane face perpendicular to the electrical field on the same side of the drops apart from a number of small indentations (on their way of being transported) on other places. The same occurs if a d.c. field is applied on coacervate drops of the type Fig. 1 *A* and *B*. The plane faces pointing to chance directions all move to the cathode side of the coacervate drops, but now the large attached vesicles are transported and not the small indentations. It therefore becomes very probable that the peculiar forms described, which are spontaneously present without the aid of a d.c. field, all originated from "vacuoles" originally imprisoned in the coacervate.

7) In harmony herewith is the fact, that by careful addition of isoamylalcohol to the KCl containing oleate system coacervate drops may be obtained which contain vacuoles.

The rounding off of the finely divided coacervate (as long as convection currents are present in the total system, the coacervate is deformed to liquid threads, c.f. notes) is a slow process here and one may observe that actually equilibrium liquid gets entrapped as vacuoles in the coacervate. Evidently in the neighbourhood of the coacervation limit the entrapped vacuoles have not much chance in this case to come into contact with the inner surface of the drop-wall. Yet some have succeeded, the drops also showing plane faces here and there.

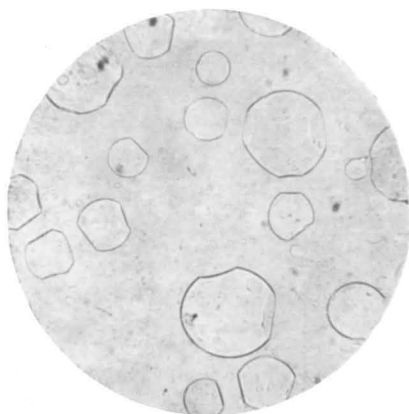
If another one or two drops of isoamylalcohol are added the coacervate drops do no longer contain vacuoles, and have now the typical shapes of fig. 1 *A* and 1 *B*. The factor which slowed down the meeting of vacuoles and inner surface of the drop, has evidently been removed.

8) Above (7) we have referred to vacuoles present in the coacervate drops obtained in the neighbourhood of the coacervation limit, but we did not yet describe the peculiar morphology in question. They are united in groups which because of their shape might be called "*articulated vacuoles*". In reality these units are chains of vacuoles, in which the individual members are separated by invisible films (or by films that are visible only with great difficulty) at the positions of the articulations, fig. 1 *L*. Here once more an extremely thin film separates two miscible, nay identical liquids (the vacuole contents being equilibrium liquid).

The articulated vacuoles are most characteristic if we have obtained the P-coacervation with the smallest possible number of added drops of isoamylalcohol thus having just crossed the coacervation limit. The volume of the coacervate in this case being large in proportion to the volume of the equilibrium liquid, the latter is present in the form of articulated vacuoles embedded in a continuous layer of coacervate. The shape of the articulated vacuoles is still more characteristic if benzylalcohol is used in stead of isoamylalcohol. Cf. microphotograph *B*.

⁷⁾ See H. R. KRUYT's Colloid Science II, loc. cit., p. 345, 452 and 479.

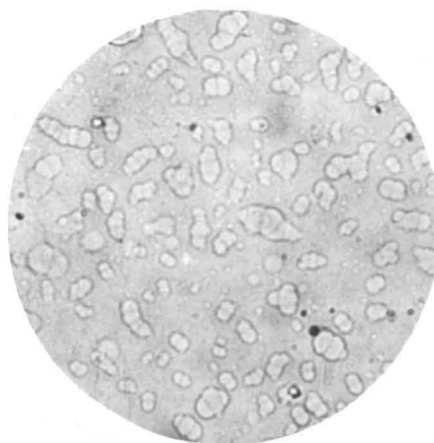
H. G. BUNGENBERG DE JONG and L. J. DE HEER: *Soap coacervates with special properties, hitherto only known in coacervates of phosphatides. I Oleate coacervates at a pH above 12.*



A (35 ×)

P-coacervate drops with superficially attached vesicles. The protruding vesicle wall is not visible.

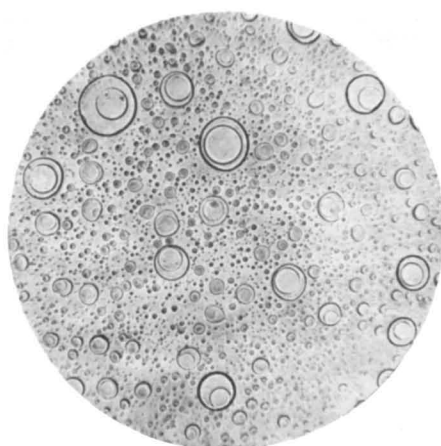
(KCl containing oleate + isoamylalcohol)



B (106 ×)

Articulated vacuoles embedded in a continuous coacervate layer. The dark lines at the articulations are only special light effects, as the septa separating the individual members in a chain of vacuoles are not or hardly visible.

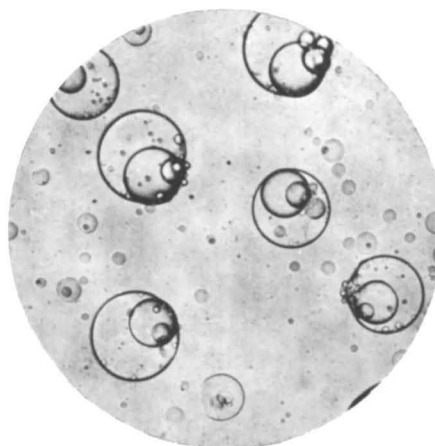
(KCl containing oleate + benzylalcohol)



C (105 ×)

Double drops, consisting of a coacervate shell and a central drop of the isoamylalcohol rich phase.

(KCl containing oleate + isoamylalcohol)



D (60 ×)

Triple drops, consisting of two coacervate shells and a central drop of the isoamylalcohol rich phase.

(KCl containing oleate + benzylalcohol after warming to 36°)

All the above very highly remarkable morphological particularities of these oleate coacervates, were observed on phosphatide coacervates many years ago already and we think that for the explanation of the nature of the peculiar films which play a prominent part in them, essentially the same line of reasoning can be followed as BUNGENBERG DE JONG and BONNER⁸⁾ gave in the case of phosphatide coacervates.

We must start then from the contrast between ready fusibility of coacervate drops with each other on contact and the formation of stable films on contact of vacuoles with each other (above under 8) or of a vacuole with the inside of the drop surface. From this it follows already that the boundary coacervate/equilibrium liquid has different properties on the two sides. If, indeed, two such boundaries come into contact with one another while their surfaces are directed towards the equilibrium liquid, coalescence takes place (two coacervate drops meeting). On the other hand a stable film is formed when these boundaries encounter one another with their surfaces directed towards the coacervate side (two vacuoles meeting in the interior of the coacervate; vacuole and the inside of the surface of the drop meeting).

If we assume that there is a film of orientated oleate molecules at the coacervate/equilibrium liquid boundary, then it is understandable without any further argument that the two sides of this film bear very different characters. This renders it probable that the very peculiar films which may separate two identical liquids are bimolecular. In section 5 we will return to the question with which side the monolayers lay together in this bimolecular film.

3. *Multiple coacervate drops.*

The analogy between phosphatide coacervates and P-coacervates of oleates goes still further, as also in the latter case formation of "multiple" drops may be observed.

Double drops are formed from the P-coacervates for instance when we gradually add more isoamylalcohol to the coacervated system (see microphotograph *D*). If still more is added, the outer shell, consisting of the P-coacervate diminishes in amount and finally disappears entirely, thus leaving the inner drops alone (fig. 2 *A*, $a \rightarrow b \rightarrow c \rightarrow d$).

The latter are rich in isoamylalcohol and dissolved oleate, but show no peculiar phenomena at all and cannot be regarded as coacervate drops.

The case described is a simple one: the formation of the double drops is evidently connected with a transgression of the solubility of isoamylalcohol in the total oleate system (being composed of: solubility in the $KCl + H_2O$ medium and the solubilizing capacity of the oleate, cf. section 5). The inner drops, however, are not indifferent, but absorb a certain amount of oleate.

⁸⁾ H. G. BUNGENBERG DE JONG and J. BONNER, *Protoplasma* **24**, 198 (1935) and a shorter summary in *Proc. Kon. Akad. v. Wetensch., Amsterdam*, **38**, 797 (1935).

and when more isoamylalcohol is added the oleate is displaced almost completely from the sphere of the aqueous medium (P-coacervate and equilibrium liquid) towards that of the medium rich in isoamylalcohol.

In harmony with this explanation is, that having arrived at the state *d* of fig. 2 *A*, we may transgress the whole series of morphological states in a

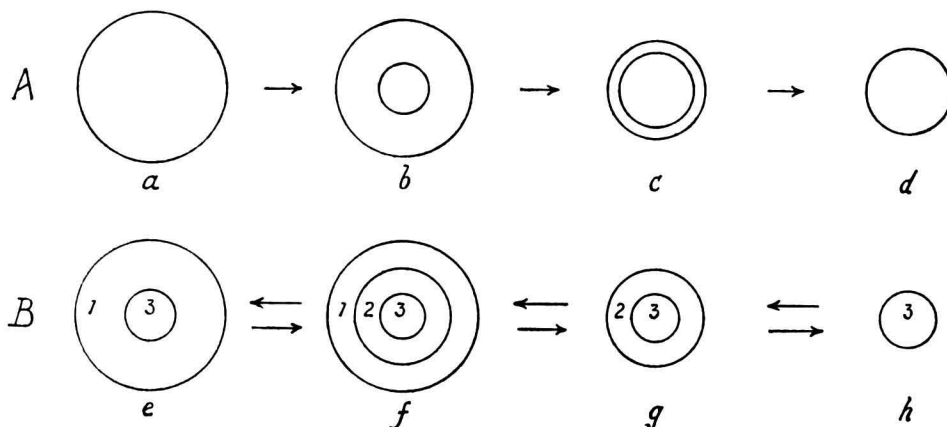


Fig. 2.

reverse direction (so $d \rightarrow c \rightarrow b \rightarrow a$) by now gradually adding 1 N. KCl solution, which is approximately the electrolyte-water medium. Double drops may also be obtained by heating a system containing homogeneous P-coacervate drops ($a \rightarrow b$) while by cooling down the initial state is restored again ($b \rightarrow a$).

This may in principle be explained in the same way, as the solubility of isoamylalcohol in water is reduced on increasing the temperature.

A more complicated case is met with, if one uses benzylalcohol instead of isoamylalcohol to produce P-coacervation of the oleate. At room temperature additional benzylalcohol produces once more double drops. If we heat, however, this system under the microscope (e.g. with an electrical hair dryer, or more conveniently by applying an a.c. field⁹⁾) a new liquid shell is produced at the boundary of the inner drops¹⁰⁾. The triple drops thus formed (fig. 2 *B*, $e \rightarrow f$) are composed of a central drop (3) mainly consisting of benzylalcohol, and two shells (1) and (2) (see microphotograph *D*) which both may be considered as oleate coacervates. At further increase of the temperature the outer shell (1) first disappears, and finally the shell left (2) and the drop rich in benzylalcohol (3) remains ($f \rightarrow g \rightarrow h$).

By subsequent cooling we return to the original state of double drops in a reverse direction ($h \rightarrow g \rightarrow f \rightarrow e$).

⁹⁾ Apart from the heat it produces the a.c. field, has no influence on the morphology of the coacervate drops.

¹⁰⁾ Details as to the consecutive morphological changes (comprising also vacuolation processes) actually taking place when going from $E \rightarrow F$ in fig. 2, are omitted here. We hope to return to this interesting case of dynamic colloid morphology in a later communication.

We will not try to explain this more complicated case of benzylalcohol in details, we have recorded it here merely to emphasize once more on the phosphatide like character of the oleate coacervates obtained by adding so much of an appropriate polar non electrolyte that we are in the neighbourhood of its solubility. For also in phosphatide coacervates we may obtain double and triple coacervate drops¹¹⁾ by transgression of the solubility.

4. *Location of the regions in a KCl-isoamylalcohol diagram in which O- and P-coacervates are present.*

We shall have to judge microscopically the nature of the oleate system in a large number of combinations of KCl and isoamylalcohol concentrations. This can be simplified by using a method, the essential point of which consists in the microscopical inspection of the oleate system contained in a closed vessel and observing this through the glasswall. For this purpose we use a bent pyrex glasstube, containing the oleate system and being closed by a rubber stopper, which tube can be placed upon the stage of an inverted microscope as indicated in fig. 3.

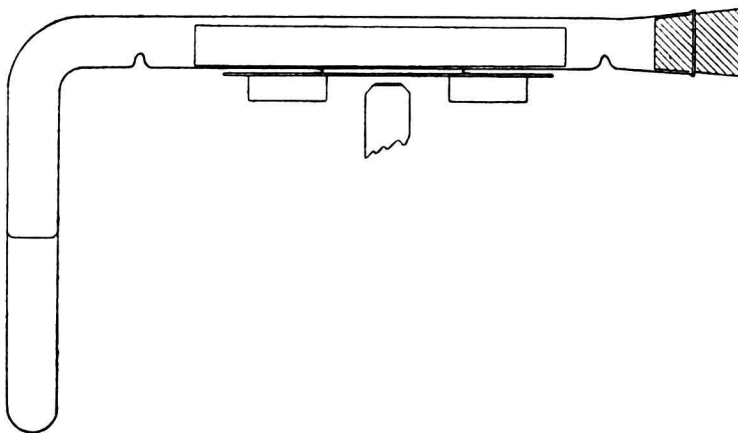


Fig. 3.

If an objective of small magnifying power is used and the horizontal limb lies in drop of water on a large cover-glass or on thin sheet of mica, one can obtain already a reasonable microscopic image of the oleate system close to the wall of the tube.

It is still inconvenient that the layer of the oleate system may be too thick, so that the image is too dark; while this layer may also continue to move for a very long time, which makes microscopic inspection either impossible or very difficult. Both inconveniences are satisfactorily removed by the presence of a cylindrical tube, open at both ends, in the horizontal limb. The oleate system in the capillary space between the bent tube and the smaller one inside the former can very satisfactorily be observed. Indeed, all morphological details recorded in the section 2 and 3 (apart from those in a d.c. field) can and actually have been investigated by using this method.

¹¹⁾ Reviewed in KRUYT's Colloid Science II, loc. cit, Chapter XI, § 6.

The possibility of examining microscopically the state of the oleate system in the closed tube has the advantage that for the objects of the present section we need not prepare separately a great many oleate systems containing different KCl and different isoamylalcohol concentrations. Instead of doing so we prepare an oleate KCl mixture in the bent tube to which we successively add small amounts, of isoamylalcohol and after rigorous shaking (causing the contents to run 50 or more times from one limb to the other) we can observe the state of the oleate system after each addition.

For the purpose of adding known amounts of isoamylalcohol we used the dripping pipette shown in fig. 4. The apparatus consists of three parts, a 1 cc micropipette *A*

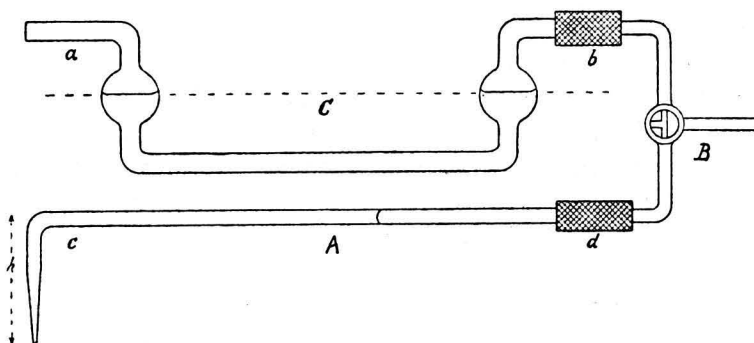


Fig. 4.

the lower part having been bent rectangularly, a centre part *B* with three way stopcock and an upper part *C* which serves as a flow resistance, and consists of two bulbs connected by a relatively wide capillary. The three parts are connected by short pieces of rubber vacuum tube, which gives flexibility at *b* and *d*. The apparatus is supported at *a*, *b*, *c* and *d* in such a way that the "horizontal" limb of *A* is actually in a horizontal position and that the levels of the very viscous liquid (paraffine oil or castor oil) are in one horizontal plane in the bulbs of *C*.

Drops of constant weight may be expected if the pipette (with stopcock turned as in fig. 4) is slowly dripping at a constant rate. This constant rate is obtained here by separating the two factors involved. The hydrostatic pressure *h* is independent of the degree of filling of micropipette *A* and the same practically also holds for the total viscous resistance (the resistance of the two liquids in series being practically that of the castor oil in the capillary connecting the two bulbs). By weighing 10 drops at different degrees of filling of the micropipette, we checked that the weight of the drops was practically constant (1 drop of isoamylalcohol = 12.4 mg). For filling the micropipette the three way stopcock is turned 90° anticlockwise and slight suction is applied at the horizontal tube of *B*. Then the stopcock is turned as in the figure and the first few drops which are delivered are disregarded. After use the stopcock is turned 90° clockwise, which allows the transported castor oil to flow back. This may be too slow a process and it may be necessary to readjust part *C* after some time (by changing the position of the support at *a*) in order to ensure that the levels in the bulbs remain in one horizontal plane.

Using the above experimental equipment we investigated at room-temperature ($\pm 16^\circ$) the state of the oleate system (oleate concentration constant) at varying additions of isoamylalcohol for eight different KCl

concentrations. Starting from a stock solution of Na oleate, (4 g oleate + 90 ml H₂O + 10 ml 2 N. KOH) and a stock solution of 3.8 N. KCl, we introduce 5 ml oleate stock solution + a ml KCl 3.8 N. + (15—a) ml distilled water into the bent tube and investigate the nature of the system after adding ever more drops of isoamylalcohol. During the mixing after each addition care should be taken that the system is exposed to the warmth of the hands as less as possible. The results are recorded in Table I, which

TABEL I.

Number of drops of isoamylalcohol (1 drop = 12.4 mg) added to 20 ml 0.96% oleate system containing KCl at which one of the successive states a—h (see text) is present at 16°.

C _{KCl}	a	b	c	d	e	f	g	h	log. C _{KCl}
0.19						61—68	70—78	80	0.28—1
0.28						57—62	63—69	70	0.45—1
0.47					46—47	48—54	55—59	60	0.67—1
0.56	0—16	(18—21)		(22—41)	42—43	46—50	53—55	57	0.76—1
0.76	0—11	12	13	16—37	39—40	42—48	49—52	53	0.88—1
1.14	0—2	3—4	5	6—31	32	34—39	40—		0.06
1.90	—	0—1		2—21	22	23—29	30—31	32	0.28
2.85	—	—	—	0—16	17	18—22	23—25	26	0.45

gives the number of drops at which a certain morphological state of the oleate system was observed.

These states, which we shall indicate, by a, b, c and so on are eight in number, two of which (a and e) are one phase systems, four (b, d, f, h) two phase systems and again two (c and g) three phase systems.

The kinds of phases in the successive states a—h are as follows:

a) *liquid*, representing a homogeneous solution of all components. This solution may often be very viscous and may exhibit marked elastic properties.

b) *liquid + liquid*, one of which contains practically all the oleate present and is therefore indicated "oleate coacervate", while the other liquid contains oleate only in very slight concentration and is therefore called "equilibrium liquid". In the terminology adopted in the preceding section the coacervate in question is the "ordinary oleate coacervate".

c) *liquid + liquid + "semiliquid"*. The first two liquids are of the same kind as in b (equilibrium liquid + O-coacervate). The actual nature of the third phase cannot be identified with the above microscopic equipment. We know from former investigations however that this is a birefringent phase which has the nature of a fluid. Its viscosity must however be very high and we have therefore used the term "semiliquid". As a consequence of its high viscosity this phase appears as small granula, which often stick together to form small aggregates such as floccules, lumps, etc. The amount of this semiliquid phase increases at the cost of the O-coacervate by a further addition of isoamylalcohol.

d) *liquid + "semiliquid"*, these phases are already present in c minus the O-coacervate. On further increase of the isoamylalcohol concentration the turbidity of the total system may increase at first, but as a rule it decreases on further addition, as the amount of the "semiliquid" phase decreases.

e) *liquid*, the "semiliquid" phase, present in c) and d) now being dissolved.

f) *liquid + liquid*, being equilibrium liquid and an oleate coacervate, this time the *P-oleate coacervate*, the morphology of which has been investigated in section 2.

g) *liquid + liquid + liquid*, apart from the two liquids present in f) a third liquid, mainly consisting of isoamylalcohol, appears now. Drops of the P-coacervate take up drops of the latter phase to form the "double drops", which were studied in section 3. On further addition of isoamylalcohol the P-coacervate diminishes in volume.

h) *liquid + liquid*, one mainly consisting of a KCl solution in water the other which has practically taken up the oleate present in the system, consisting chiefly of isoamylalcohol.

In fig. 5 A these eight successive states of the oleate system have been pictured in a KCl-isoamylalcohol diagram by the regions a—h, the limiting curves of which can be drawn approximately from the data of Table I. For this purpose we took on every horizontal row the means of the last

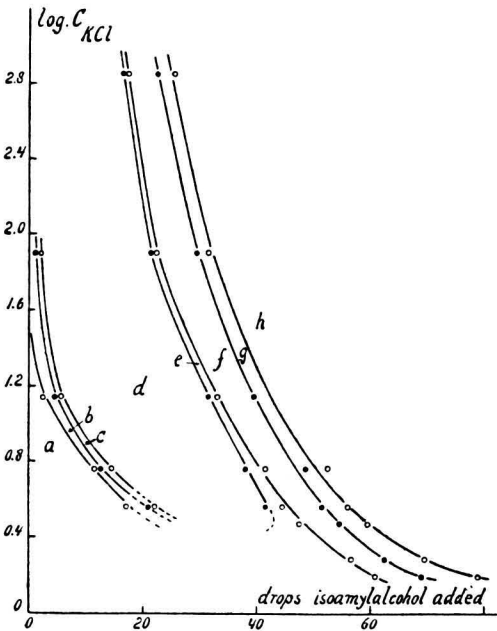


Fig. 5A.

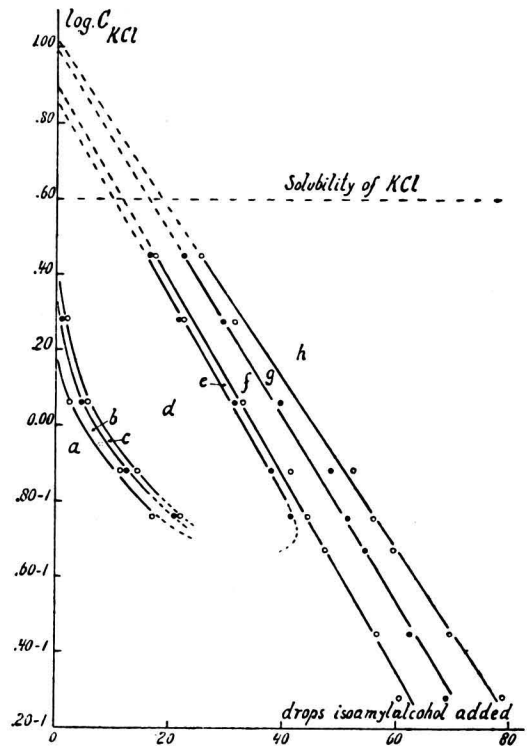


Fig. 5B.

figure in each column and the first in the next column to its right (thus e.g. the first row of the table gives as means 69 and 79).

The mean drop number values thus obtained have been plotted (alternatively as white and black dots) as abscissae against the corresponding KCl concentrations as ordinates. The position of the points is somewhat irregular so that simple curves cannot be drawn through all corresponding black and white points. Still we do not doubt that the limiting curves have the simple shape as given in fig. 5 A, the irregular position of the points being ascribed to the insufficient constancy of the room temperature.

We can read from this fig. 5 A how we can prepare O-coacervates

(present in the regions *b* and *c*) and P-coacervates (present in the regions *f* and *g*). First of all we must emphasize that in either case it is imperative that the *KCl concentration should not be too low*, for if isoamylalcohol is added to the oleate system in the absence of KCl, the states *b*, *c*, *d*, *f* and *g* cannot be realized. A considerable amount of amyralcohol can be dissolved in this case before we have reached its solubility, after which we suddenly enter region *h* (drops rich in isoamylalcohol appear).

We have not investigated how the regions *b*, *c*, *d*, *f* and *g* terminate downwards, but know for certain that those of *b*, *c*, and *d* disappear already below 0.5 N KCl, whereas the regions *f*, *g*, could still be obtained at 0.19 N KCl. It is therefore probable that in the lower part of the figure the regions *a* and *e* communicate.

We can further read from this figure that *O-coacervates* (present in the regions *b* and *c*) can be realized already with KCl only, the regions *a*, *b*, *c*, *d* touching the ordinate axis. *P-coacervates* (present in the regions *f* and *g*) can only be obtained by the joint action of KCl and isoamylalcohol. This conclusion however, seems not quite safe, as in our experiments the highest KCl concentration was 2.85 N, where as the solubility of KCl at room temperature amounts to approximately 4 N. If we re-draft fig. 5 A, using the logarithms of the KCl concentrations as ordinates, we get fig. 5 B, in which the curves limiting the regions *e*, *f* and *g* are practically straight lines now. Assuming for a moment that these straight lines might be extrapolated until they intersect the ordinate axis it would follow that P-coacervates could be obtained with KCl only. The minimum KCl concentration required (limiting line between *e* and *f*) would then amount to approximately $\log. C_{\text{KCl}} = 0.90$, that is about 8 N. KCl. As at room temperature the solubility of KCl amounts to approximately 4 N only, (indicated in fig. 5 B by a dotted line at $\log. C_{\text{KCl}} = 0.60$) these high concentrations cannot be realized.

That P-coacervation cannot really be reached with KCl only has been confirmed by adding isoamylalcohol to an oleate system which by the addition of solid KCl was saturated with KCl.

In the next section we will further investigate the rôle of isoamylalcohol in the production of P-coacervation.

(To be continued.)