

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

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5. *Free and bound isoamylalcohol at the limit of the P-coacervation.*

In this section we intend to compare at a number of constant KCl concentrations the location of the boundary between the regions *e* and *f* (fig. 5) at three or more oleate concentrations. The experimental method followed in section 4, which would do for an approximate location of the boundaries in fig. 5, is not accurate enough for our present aims and is therefore replaced by a macroscopical method now, working in a thermostat at 20°. This method consists in measuring the relative volume of the coacervate at a number of isoamylalcohol additions very close to the above boundary between *e* and *f*. We shall describe this method more detailed with the help of the series of measurements at the constant KCl concentration of 0.475 N. We started from a stock oleate solution of the composition 29.95 g Sodium oleate of BAKER + 675 ml distilled water + 75 ml KOH 2 N. In the thermostat we placed three groups of glass-stoppered cylindrical measuring flasks of 50 ml capacity. In the first group we pipetted 15 ml oleate solution + 5 ml 1.9 N KCl, in the second 10 ml oleate solution + 5 ml H₂O + 5 ml 1.9 N KCl and in the third 5 ml oleate solution + 10 ml H₂O + 5 ml 1.9 N KCl.

In this way we obtained three series of mixtures in which the oleate concentration varies in the ratio of 3 : 2 : 1. We then added to each tube a different number of isoamylalcohol drops (with the apparatus of fig. 4) which lay in the neighbourhood of the coacervation limits of the three oleate concentrations at the given KCl concentration (roughly determined beforehand). After being mixed thoroughly the mixtures were left till the following day. The height of the column of coacervate was read then and expressed in per cent of the total volume in the tube (see Table II). If we plot these values as a function of the number of drops (fig. 6A) we can read in this graph the number of drops needed to reach the same coacervate volume (e.g. 60 %, 80 % and — by extrapolation — 100 %, the latter representing the coacervation limit). We now plot these numbers (also recorded in table II) as a function of the oleate concentration, cf. fig. 6B) in which the oleate concentrations are arbitrarily indicated by 1, 2 and 3).

The graph thus obtained shows that *the amount of isoamylalcohol needed to reach the same coacervate volume (60 %, 80 % or 100 %) is a linear function of the oleate concentration.*

This can be explained by assuming that the isoamylalcohol is present partly as free molecules dissolved in the KCl solution, and partly bound

in some way or other to the oleate (e.g. taken up in oleate "micelles"). The concentration of free isoamylalcohol can then be calculated from the

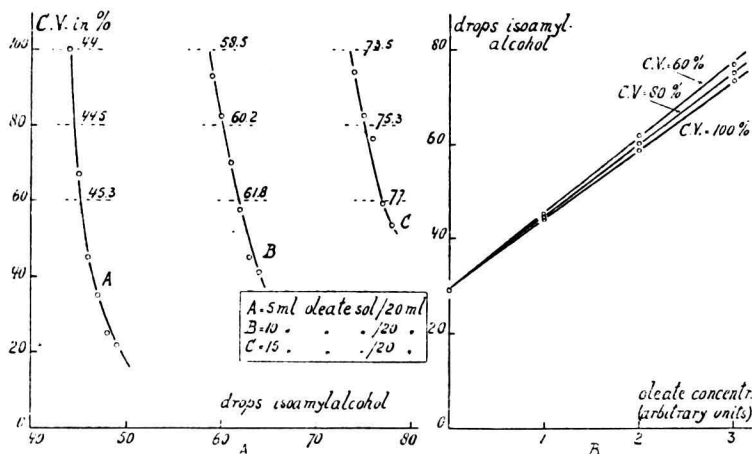


Fig. 6.

TABLE II.

Volume (in per cent) of the P-coacervate at three different oleate concentrations (KCl concentration = 0.475 N and temperature = 20°) as a function of the number of drops of isoamylalcohol added (1 drop = 12.4 mg).

15 ml oleate/20 ml		10 ml oleate/20 ml		5 ml oleate/20 ml	
drops isoamylalcohol per 20 ml system	coacervate volume per cent	drops isoamylalcohol per 20 ml system	coacervate volume per cent	drops isoamylalcohol per 20 ml system	coacervate volume per cent
69	(100)	59	93.0	44	(100)
74	94.0	60	82.5	45	67.0
75	82.5	61	70.0	46	45.0
76	76.5	62	57.5	47	35.0
77	59	63	45.0	48	25.0
78	53.5	64	41.5	49	23.5

from the above figures follow graphically:

77	60	61.8	60	45.3	60
75.3	80	60.2	80	44.5	80
73.5	100	58.5	100	44	100
	(coacervation limit)		(coacervation limit)		(coacervation limit)

distance between the origin and the intersection point of the straight line with the ordinate axis (29 drops per 20 ml; weight of 10 drops = 0.124 g; concentration therefore = 1.80 g per 100 ml). The slope of the straight line, on the other hand, makes it possible to calculate the ratio bound isoamylalcohol/oleate. For the coacervation limit (coacervate volume

exactly 100 %) 73.5 drops/20 ml are needed for the mixture containing 15 ml oleate solution, and 44 drops/20 ml for the mixture containing 5 ml oleate solution. The oleate in 10 ml stock solution (0.383 g = 1.262 millimol) binds therefore $73.5 - 44 = 29.5$ drops = 4.152 millimol isoamylalcohol. This gives at the coacervation limit the ratio $4.152/1.262 = 3.29$. Similarly we find at the coacervate volumes 80 % and 60 % the ratios 3.44 and 3.56.

As we have described the series at 0.475 N KCl more in details, it may suffice to give the results at other KCl concentration in a concise form. It has been invariably found that the quantity of amylalcohol just needed to start P-coacervation is a linear function of the oleate concentration. Cf. fig. 7, which is analogous to fig. 6 B, but in which we have only given the

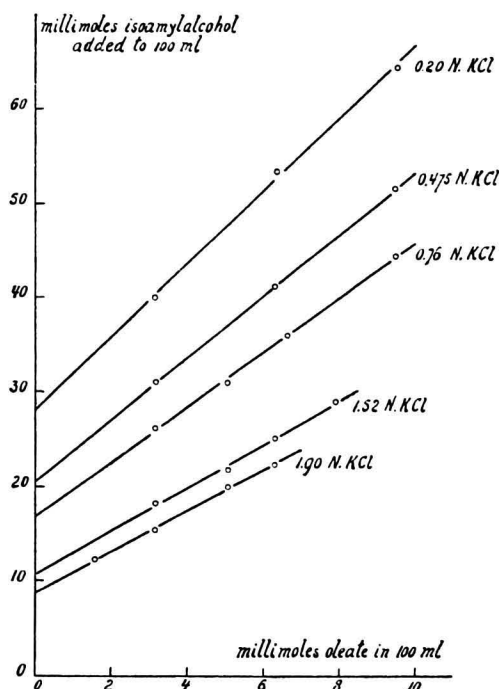


Fig. 7.

results for the coacervation limit (coacervate volume exactly 100 %) and in which the quantity of isoamylalcohol added is expressed in millimol per 100 ml; similarly the oleate concentration in millimol per 100 ml. The best fitting linear regression lines have been calculated by adopting a statistical method, from which we obtain for the concentration of free isoamylalcohol and the ratios of bound isoamylalcohol/oleate, the values recorded in the columns 2, 3 and 4 of the next survey.

In fig. 8 A we have plotted the figures of column 3 as a function of the KCl concentration (white dots). On the ordinate axis we have further indicated, with a black dot, the solubility (2.90) of isoamylalcohol in water

at 20°, expressed in g added to 100 ml water ¹²). If we connect the black point on the ordinate axis with the first white dot (at 0.2 N KCl) a line is

KCl conc. N.	Conc. free isoamylalc.		bound isoamylalcohol oleate (in molecular ratio)
	g added to 100 ml	millimol added to 100 ml.	
0.20	2.48	28.2	3.86
0.475	1.80	20.6	3.29
0.76	1.47	16.8	2.90
1.52	0.94	10.7	2.31
1.90	0.77	8.7	2.19

obtained (dotted) which practically coincides with what would appear as the probable course of the curve through the white dots if this curve is extrapolated to a KCl concentration zero. This suggests that the curve of fig. 8 A represents the solubility of isoamylalcohol at 20° as a function of the KCl concentration (in the absence of oleate) ¹³).

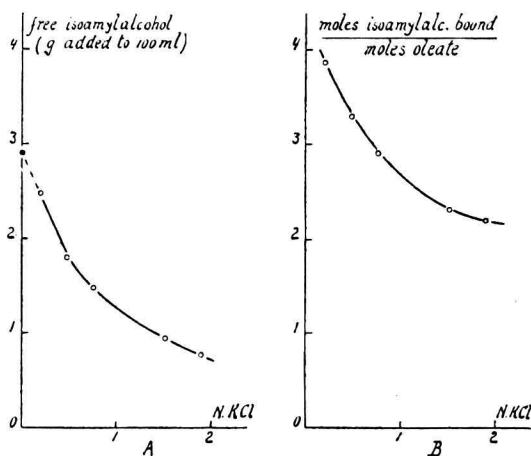


Fig. 8.

This then would mean consequently that P-coacervation sets in when the free isoamylalcohol standing in equilibrium with the amount of isoamylalcohol bound to the oleate, has reached its maximum value in the medium bathing the soap "micelles".

Now turning to the bound isoamylalcohol it self, cf. fig. 8 B, we observe

¹²) Calculated from the value 2.82 g per 100 g solution at 20°, quoted in Seidell, Solubilities of inorganic and organic compounds, 2e Edition, New York 1928, see p. 1053.

¹³) Unfortunately no data are known to us in the literature concerning this solubility of isoamylalcohol in KCl solutions at 20°, to adstruck this assumption. Not in contradiction with it is the fact that we have actually observed that KCl diminished this solubility considerably.

that it is appreciable, and further that its molecular ratio depends on the KCl concentration. Yet we must lay stress on the fact that the P-coacervate is not at all a phase rich in isoamylalcohol, but one very rich in water.

When we consider e.g. a 1 % oleate system in which a P-coacervate has been obtained with a volume of 50 % or 25 % of the total system, its oleate contents cannot be larger than 2 % or 4 %. When we take the highest ratio of the survey (3.86) these coacervates would contain approximately 2.2 % or 4.4 % isoamylalcohol bound to the oleate only. The rest of the coacervate (93.8 or 91.6 %) must necessarily be constituted of the components of the equilibrium liquid (KCl solution saturated with isoamylalcohol). *The coacervate therefore mainly consists of a solution of KCl in water.*

This turns up a question, which we did not yet consider in section 2, viz. how the monolayer of oleate molecules present at the boundary P-coacervate/equilibrium liquid is oriented. We cannot yet give a definite answer to this problem, but we will warn for the too rash conclusion that these oriented oleate molecules "of course" have their polar heads directed towards the equilibrium liquid and their hydrocarbon chains towards the coacervate. For as we saw above there is not a very great difference at all in the constitutions of coacervate and equilibrium liquid (both being constituted mainly of a KCl solution in water). Therefore we may not consider the oleate coacervate as a much less polar liquid than the equilibrium liquid. It might even be considered as more polar than the other, taking into consideration that the concentration of the very polar oleate ions is many times higher in the coacervate than in the equilibrium liquid. This then would lead to the reverse position of the oleate molecules in the monolayer and as a consequence in the bimolecular membranes ¹⁴). Further experiments, however, are needed to settle this problem.

Another question upon which the experiments of this section may shed some — though not a sufficient — light, is the rôle of isoamylalcohol in this kind of coacervation. As we have found that an appreciable amount of it is bound to the oleate at the appearance of the P-coacervate, it seems likely that the inner structure of the soap micelles is affected by the isoamylalcohol molecules taken up.

Assuming here with BOOIJ ¹⁵) that the micelles present in the O-coacervate are plates consisting of a double film of oriented oleate ions, the said affection might partially consist in a cleavage of the plates. In this way associations of oleate ions would be formed which have different properties at both sides and therefore could assemble at the interface P-coacervate/equilibrium liquid to form the postulated monomolecular layer.

The destruction of the micelles once begun may proceed still farther by

¹⁴) Compare the discussion of the analogous question in the case of phosphatide coacervates in KRUYT's Colloid Science, Vol. II, Ch. XI, small print on p. 482.

¹⁵) H. L. BOOIJ in KRUYT's Colloid Science II, loc. cit. See Chapter XIV, § 5.

further addition of isoamylalcohol for instance into wholly free oleate ions. The fact that all morphological peculiarities become less pronounced if after just passing the coacervation limit, more isoamylalcohol is added, gives support to the above idea, that it are really intermediate cleavage products of the plate micelles and not wholly free oleate ions, which play the prominent part in the described phenomena.

6. *Other examples of P-coacervation of soaps.*

The above experiments all refer to oleate at a $\text{pH} > 12$ and KCl as salt. P-coacervation of oleate may be obtained at conditions which do no longer allow for O-coacervation: 1) at much lower pH (e.g. at pH 7.3, using a phosphate buffer), 2) when using LiCl or NaCl instead of KCl at room-temperature.

A qualitative investigation has further been made (still using isoamylalcohol and as a rule KCl) to see if P-coacervation is possible with other soaps. We could realize this with myristate, palmitate and stearate, further with "T-pol", a technical mixture of sulfate soaps (in this case MgCl_2 was used as salt) and with cetyltrimethylammouniumbromide (technical preparation). Thus the carbon chain may be altered, the COO' group may be replaced by another of the same electrical sign or by one of the reverse sign, without loss of the possibility of P-coacervation.

As phosphatides may be considered as amphoionic "soaps", we may generalize by saying that P-coacervation is possible with the three types of soaps: anionic, cationic and amphoionic.

7. *Summary.*

1) There are two kinds of oleate coacervates:

- a) Coacervates showing a morphological behaviour hitherto known in phosphatide coacervates only and therefore called here *P-coacervates* (P stands for "phosphatide like").
- b) Coacervates lacking this morphological behaviour and therefore called here *O-coacervates* (O stands for "ordinary").

2) The present communication dealing with P-coacervates of oleate obtained by the joint action of isoamylalcohol and KCl, first gives a description and microphotographs concerning the special morphology of the P-coacervate drops. The phenomena observed may be brought under three headings: a) abnormal behaviour of the vacuoles meeting one another or meeting the inner surface of the drop boundary, b) abnormal behaviour in a d.c. field, c) formation of multiple drops.

3) In the phenomena sub 2) under a) and b) the formation of very thin (invisible or visible only with extreme difficulty) membranes play the main rôle. They are only formed if two boundaries of coacervate/equilibrium liquid meet with their sides directed towards the coacervate. This

suggests the presence of a layer of oriented oleate molecules at the coacervate boundary, from which follows that the above mentioned thin membranes, having the property to separate two indetical liquids mainly consisting of water (+ KCl), must be bimolecular films.

4) When adding isoamylalcohol dilute oleate systems containing KCl may pass through eight successive states, each characterised by the number and kinds of the phases present. In two of these states the O-coacervate is present as one of the phases, in two others the P-coacervate. A KCl-isoamylalcohol diagram is given for 1 % oleate systems at approximately 16°, in which these eight states are represented as regions. The diagram shows that O-coacervates, can already be obtained with KCl only, that P-coacervates, however, can only be obtained by the joint action of KCl and isoamylalcohol.

5) The amount of isoamylalcohol just needed to start P-coacervation at a given KCl concentration is a linear function of the oleate concentration. From this we calculated the concentration of isoamylalcohol which is present free in solution and the molecular ratio bound isoamylalcohol/oleate.

6) The results obtained sub. 5) were used for a preliminary discussion of two problems: a) why the P-coacervate boundary is the seat of an oriented layer of oleate molecules, b) the direction of the molecules in this layer.

7) P-coacervation is possible with the three types of "soaps": anionic, cationic and amphoteric soaps.

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