

1. The "concentration" of the charges of both sign is relatively high in the molten salt, but relatively small in the coacervate (it contains $\pm 16\%$ colloids and 84% water, from which the concentration of the "gelatin arabinat" expressed in aequivalent p. L. can be roughly calculated to be only 0.07 N).

2. The electrical attraction in the coacervate is diminished considerably ($80\times$) because the macromolecular ions are inbedded in a medium (water) possessing a high dielectrical constant. This medium is the same as the equilibrium liquid (apart from some dissolved macromolecules in the latter), so that no contribution to the interfacial tension results from it.

Both factors would perhaps lead to even too small values for the interfacial tension of the coacervate, which may be due to the fact that we did not account for the very great difference in temperature.

Without doubt the two above points would not suffice for a quantitative theoretical treatment of the problem, and other factors (the attachment of the ionized group on flexible macromolecules) should also be taken into account.

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Biochemistry. — *Oleate systems containing potassium chloride in which the KCl concentration is still too low for coacervation. I. The viscosity-KCl curve.* By H. G. BUNGENBERG DE JONG and G. W. H. M. VAN ALPHEN.

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1. *Introduction. Technic of measurement.*

In previous research about coacervation of oleate sols by KCl, we have already mentioned that KCl concentrations still too low for coacervation, induce peculiar systems behaving as liquids of a high viscosity and showing elastic properties moreover ¹⁾).

In this and the next communication, we discuss in principle their viscous behaviour, in the third communication follows a discussion of the elastic behaviour.

This investigation, being only of an orientating nature, does not aim in the first place, at measuring the characteristics already mentioned with a high degree of accuracy.

The purpose is rather to become acquainted with the variables which we have to deal with and especially to learn also something about the influence of non-electrolytes (in particular here the influence of primary normal alcohols) exercising such an enormous great influence on oleate coacervates, as shown in previous research ¹⁾).

The expression $\frac{\eta_s - \eta_0}{\eta_0}$, in which we are interested in the first place at constant oleate concentrations, varies so enormously with increasing KCl concentrations that one has not enough of one and the same viscosimeter to record accurately the KCl curve but needs a whole set of them.

But in the circumstance that in these sols of high viscosity elastic properties are clearly present beyond a definite KCl concentration, the presented difficulties are not to overcome by increasing the accuracy of time measurement which may be obtained from the use of a set of mutually tested viscosimeters.

For our purposes we rather need to record with one viscosimeter the whole KCl curve, which necessitates the selection of a small value for the time of flow for water and KCl solutions resp.; at the same time we are limited to relatively small oleate concentrations, lest the times of flow become impractically great in adding KCl.

But small oleate concentrations produce a relative time of flow of the sol

¹⁾ H. G. BUNGENBERG DE JONG, H. L. BOOY and G. G. P. SAUBERT, *Protoplasma* 28, 543 (1937).

with regard to water at a KCl conc = 0 being hardly the experiment fault greater than 1, so that the expression $\frac{\eta_s - \eta_0}{\eta_0}$ at KCl = 0 is not measurable accurately.

Thus in the next we just express the results in relative times of flow $\frac{t_s}{t_0}$ in which we will use as t_0 either the time of flow for water or that one of the KCl solution being present in the oleate system.

The viscosimeter (fig. 1) is especially constructed with the intention to perform serial work.

It may be easily pushed up and down as a whole (pulley and counter weight) in the vertical, along the rails of a heavy frame. It is filled by

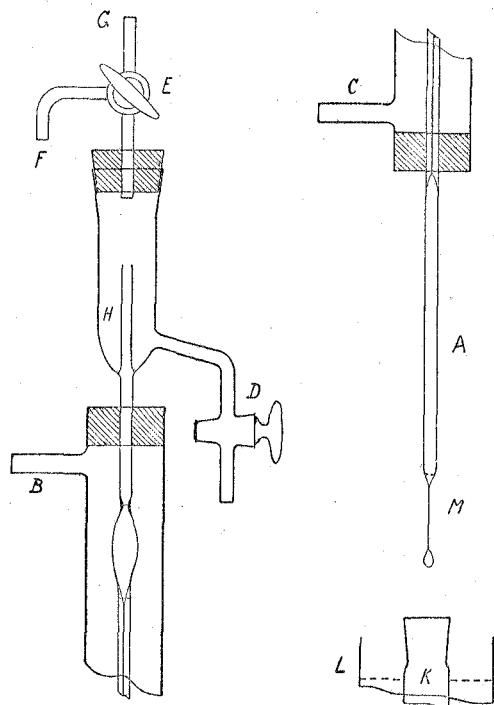


Fig. 1.

plunging of the lengthening-piece A into the system, which has to be measured and applying suction with closed tap D and correct position of the three-way tap E (F is connected with a tank filled with air of reduced pressure).

It is constructed in a glass-container with side tubes B and C through which thermostat water is pumped. The temperature ($25.0^\circ \pm 0.05^\circ$) of this water may be controlled by a thermometer in the container.

The systems which have to be measured are prepared at room temperature in a tube of Jenaglass (K), with ground tight fitting stopper and are shaken vigorously by hand (at least 1 minute).

After that, the tube is placed in a vessel (L) situated below the end of the viscosimeter through which is also running thermostat water. After 6 minutes the oleate system has the same temperature as the vessel.

The glass-stopper is removed and the viscosimeter pushed downwards; the end is now plunged deep in the oleate system and the mixture is sucked upwards as described above, whereby it flows over in H. In this way the rests of the previous contents are washed away.

When nearly everything has been sucked upwards, the three-way tap E is placed in such a position that a connection with the air outside is obtained via G, and the viscosimeter is emptying itself. Immediately there after one pushes the viscosimeter upwards and the liquid of the viscosimeter runs out freely in the air.

With a stop-watch one measures the time the liquid needs to pass the marks on the top and the lower end of the bulb.

The mixture is now sucked upwards again still a number of times (3 to 4) and measured, and the average time of flow is computed.

The width of the tube joined to the end of the capillary is chosen in such a manner that the share of friction existing herein may be disregarded with respect to the friction in the capillary (length 24 cm, diameter 0.85 mm) and thus the temperature of the soap system passing this tube does not matter. To prevent the ascension of airbubbles, the end of the plunging tube had to be narrowed to ± 1 mm.

If the oleate system has a very viscous and gelatinous character after preparation and shaking the mixture is placed first in the thermostat of 25° to allow the airbubbles to ascend. Very small enclosed airbubbles do not influence the time of flow.

The viscosimeter is always used for the measurement of series mixtures, the order of measurement running in small steps from low to high values of the concerning variable (e.g. increasing KCl conc.). In this way a non-ideal removal of the rest of the preceding mixture can hardly influence the time of flow of the next mixture.

The free outlet into the air, though disputable from point of view of a theoretically correct technic of measurement, appeared to be of great value, as it was now possible to observe beyond a definite KCl conc., the formation of shorter or longer threads (M) instead of separate drops. Then the system becomes "thread-pulling".

2. Viscosity-KCl-curve.

In preliminary investigations about oleatecoacervates always Na-oleate of MERCK was used and coacervation was accomplished by adding a concentrated KCl solution.

The concentration of K-ions is large when coacervations starts with regard to the Na-ions originating from the Na-oleate solution, but it seemed more perfect to us if the system contained just K-ions.

Thus at first we made K-oleate stock solutions, by dissolving oleic acid purissimum of KAHLBAUM in a surplus of KOH.

The surplus of KOH was used to prevent "sensibilisation" by oleic acid hydrolytically liberated²⁾.

With this oleate sols completely different viscosity-curves were obtained as similarly composed oleate sols from Na-oleate of MERCK.

Further investigation into this difference excluded immediately the small percentage of Na-ions in these latter sols as the cause.

Indeed, by the liberation of oleic acid from Na-oleate and preparation of K-oleate sols from this oleic acid in exactly the same manner as K-oleate sols were being prepared from oleic acid purissimum "KAHLBAUM", the same typical difference still existed (see 4).

Finally it was found that recrystallisation of Na-oleate prepared from oleic acid "KAHLBAUM"³⁾ does profoundly alter the shape of the KCl-curve hitherto found starting from oleic acid.

The recrystallised sample now shows the characteristic shape of Na-oleate (MERCK).

After these experiences we limited ourselves to Na-oleate MERCK, as oleic acid purissimum KAHLBAUM evidently contained a contamination, greatly influencing the shape of the KCl-curve.

As example of the characteristic viscosity KCl-curve we show fig. 2, in which the mixtures contain a final concentration of 0.454 % Na-oleate, always a constant quantity of 0.0454 N KOH²⁾, and a variable KCl concentration.

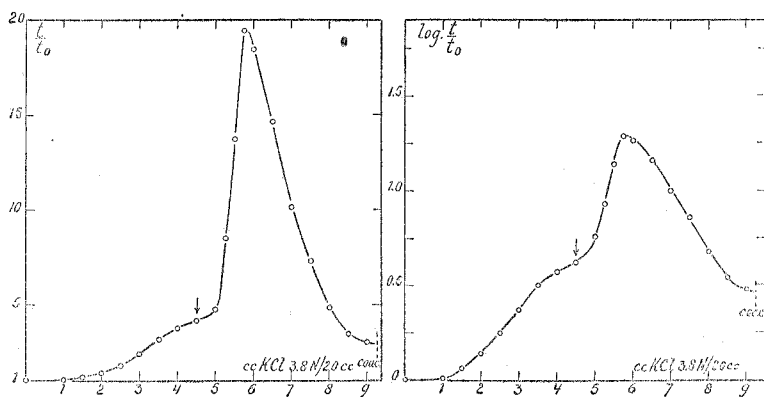


Fig. 2.

²⁾ Always a small constant KOH concentration is added. This serves to prevent complications resulting from the hydrolysis of the oleate. Free oleic acid actually acts as every non-electrolyte with long carbon chain very strong "condensing" on the oleate system. Thus we have to take care for a sufficient alkaline reaction to exclude this complication. Compare about this auto-sensibilisation, which was noticed for the first time in the study of oleate-coacervates, H. G. BUNGENBERG DE JONG, H. L. BOOY and G. G. P. SAUBERT, *Protoplasma* 29, 536 (1938).

³⁾ Oleic acid KAHLBAUM + the required quantity NaOH for neutralisation is dissolved by heating in alcohol and filtrated, after cooling the Na-oleate crystallises.

The mixtures were prepared from 5 cc of a 4 × concentrated Na-oleate sol [40 gr. Na-oleate + 2000 cc H₂O, to which is added after dissolving 200 cc KOH 2N] + a cc KCl 3,8 N + (15—a) cc dest. water. See Table I.

TABLE I.

a (cc KCl 3.8 N per 20 cc)	t sec	t KCl solution	t/t ₀ KCl	log. t/t ₀	Observations about threads
0	9.0	8.9	1.01	0.00	pulls no threads
1	9.2	8.9	1.03	0.01	"
1.5	10.2	8.85	1.15	0.06	"
2	12.2	8.8	1.39	0.14	"
2.5	15.6	8.75	1.78	0.25	"
3	20.4	8.7	3.38	0.37	"
3.5	27.2	8.67	3.14	0.50	"
4	32.2	8.63	3.73	0.57	"
4.5	35.8	8.6	4.16	0.62	threads long and thin.
5	49.0	8.58	5.71	0.76	} gradual transition to short and thick threads
5.25	72.8	8.56	8.50	0.93	
5.5	117.0	8.54	13.70	1.14	
5.75	165.6	8.52	19.44	1.29	
6	156.8	8.5	18.45	1.27	
6.5	124.	8.475	14.63	1.16	} gradual transition to long and thin threads
7	85.4	8.45	10.11	1.00	
7.5	61.4	8.425	7.29	0.86	
8	40.2	8.4	4.79	0.68	
8.5	28.6	8.36	3.43	0.54	
9	25.2	8.33	3.03	0.48	} coacervated
9.5	coacervated	—	—	—	

From the table it becomes clear (column 2) that the time of flow at KCl = 0 within the experimental faults, corresponds with that of water, but soon increases considerably, passes through a maximum and decreases strongly again before the KCl concentration is attained, where coacervation occurs.

We want to imply however these times of flow at those of similarly composed KCl solutions containing a constant quantity of 0.0454 N KOH. The times of flow of some of similar KCl mixtures were determined and the others of column 3 interpolated. Column 4 gives the relative times of flow with regard to the corresponding KCl solutions.

These values thus represent approximately⁴⁾ the relative viscosity of the oleate systems showing an enormous influence of KCl.

While at KCl = 0, the relative viscosity within the experimental error is equal to water, it increases greatly and attains a value of more than 19 at the maximum of the curve.

⁴⁾ In fact the values t/t₀ are still troubled by an error, as the used test liquids do not possess the low σ of the soap systems. As this low σ is constant (see note 5), we have to deal here with a systematic error, but at the given dimensions of the viscosimeter (diameter measuring bulb and hydrostatic pressure) this error is practically to neglect.

The left ascending branch of the curve is not continuous but there appears to be an inflexion point. At this point or a somewhat higher KCl concentration (at $a = 4.5$) appears for the first time the phenomenon that a loosening drop from the point of the viscosimeter pulls a thread.

This thread is still here long and thin, but gets shorter and thicker at higher KCl concentrations, to get longer and thinner again at still higher concentrations.

When a drop is losing from the bearing thread one may frequently observe that it was elastically stretched, it withdraws itself to the point of the viscosimeter.

Thus there must take place peculiar structure alterations in the oleate solution and we cannot leave the impression that KCl causes an appearance of elastic properties about which more in the third communication⁵⁾.

To get a surveying diagram we rather choose in the following, the logarithmus of the relative time of flow as ordinate (column 5). In this manner the arised curve (fig. 2 B) shows a shape which is nearly the same as that of fig. 2A. Here again we meet an inflexion point in the left ascending branch.

The fact, that the relative viscosity strongly decreases again ($19 \rightarrow 3$) when approaching the coacervation limit (KCl concentration of $a = 9.25$) is highly important for the theory of the coacervation of oleates (association colloid).

In this respect there is an similarity with the behaviour of macromolecular sols.

Here a strong decrease in the relative viscosity does always occur when approaching the coacervation limit.

We may not attach to much value to this similarity however, as this strong decrease of the viscosity is missing at very small oleate concentrations, while nevertheless coacervation occurs.

Compare fig. 3 giving the results ($\log t$) for different but for every curve constant oleate concentrations (the final concentration in % i.e. gram per 100 cc, are added to every curve), while the final concentration of KOH was always the same (0.0454 N).

It appears from fig. 3 that at increasing oleate concentrations, the KCl concentration whereat the threads are pulled for the first time gets smaller and further that the simple shape of the curve becomes more complicated (the curves at the two highest oleate concentrations were not further examined than indicated).

The viscosity maximum also moves to smaller KCl concentrations.

⁵⁾ Measurements with the tensionmeter of DU NOUY have shown, that the surface tension of the oleate systems, though considerably lower than those of water (± 28 dynes) remains practically constant in the whole KCl concentration trajectory. The appearing phenomenon of pulling threads at the inflexion point of the viscosity KCl curve thus has no bearing on an alteration of surface tension, but must be due to alterations in the internal structure of the three dimensional oleate system.

On the other hand the coacervation limit does not, or hardly move with increasing KCl concentrations.

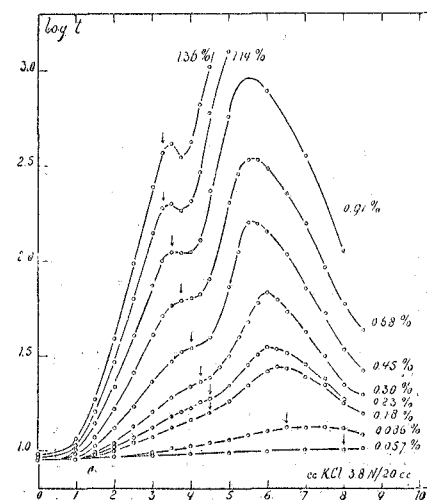


Fig. 3.

This limit was situated always very near to $a = 9$ cc KCl 3.8 N (per 20 cc), also for the highest used oleate concentrations.

3. Influence of the temperature.

Finally we observed, that the temperature also has a strong influence. One compares fig. 4, in which t_0 in the expression $\log t/t_0$ presents the time of flow of the corresponding KCl—KOH solution at the indicated temperatures.

4. Influence of auto-sensibilisation and of contaminations.

In the note on page 852 we mentioned already that a small addition of a constant KOH concentration aimed at preventing the auto-sensibilisation. Different bottles of Na-oleate MERCK supply solutions in dest. water

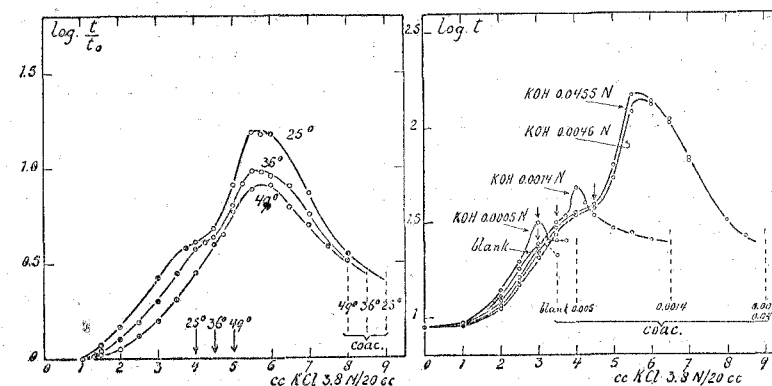


Fig. 4.

Fig. 5.

which may originate mutually very different viscosity KCl curves caused by their variable degree of auto-sensibilisation.

It may be recommended to open the bottles of Na-oleate powder just before use.

In opened situation the auto-sensibilisation may increase by absorbing CO_2 from the air.

Fig. 5 shows the viscosity KCl curves for a given oleate sample, which had been opened for a longer time and to the solutions of which no (blank), or increasing amounts of KOH were added (the final concentrations are added to the curves).

Apparently the oleate dissolved in dest. water produces strongly sensibilised oleate sols.

Here is nothing left of the characteristic appearance of the viscosity KCl curve in fig. 2 B.

The viscosity may still increase by adding KCl and the system may be just pulling threads (at 3 cc KCl 3.8 N in 20 cc final volume), but at a little higher concentration coacervation occurs already (dotted curve).

Already at small KOH concentrations the coacervation limit moves to higher KCl concentrations and gradually the shape of the curve develops itself into the typical one.

This is reached already at 0.0046 N, which curve hardly differs from that at 0.0455 N.

This latter KOH addition, which we always used previously and which will also steadily be maintained throughout the following communication, gives 10 times as large a security against the occurrence of auto-sensibilisation effects.

These effects we previously ascribed to non-ionised oleic acid, of which may be expected that it will act in principle in the same way as every other polar organic non-electrolyte with sufficient long carbon chain.

The series of curves of fig. 5 are indeed very much alike to the curve-series such as we are to meet in the next communication (see fig. 1 and 2) about the influence of methyl-hexyl-keton or N. octanol resp.

In 2. we mentioned already that oleate sols prepared from oleic acid (KAHLBAUM) produce deviating viscosity KCl curves.

The difference with Na-oleate MERCK consisted of a much lower viscosity maximum, situated at much smaller KCl concentrations, a much lower coacervation limit, further the lack of an inflexion point in the left ascending curve branch i.e. phenomenons as occur in fig. 5 at a too small KOH concentration.

However sufficient KOH was present to exclude auto-sensibilisation. As after re-crystallisation of Na-oleate prepared from this oleic acid, a viscosity KCl curve was produced with all characteristic features of the Na-oleate MERCK, we must conclude that oleic acid KAHLBAUM contained a sensibilising non-electrolyte.

For the experiments in this and the next communication we always used therefore Na-oleate MERCK.

Finally it must be mentioned, that this preparation also cannot be considered to be free from sensibilising contaminations, which latter must be always present in small but various amounts. Indeed, oleate sols prepared from different unopened bottles produce viscosity-KCl curves and coacervation limits, which do not entirely cover each other.

Thus it will be advisable to use one and the same oleate stock solution in examining the influence of one variable.

Summary.

1. The viscosity of non sensibilised oleate sols (0.3 % and higher) increases through KCl to a maximum and afterwards decreases considerably again before the concentration has been reached where coacervation occurs.
2. The left ascending curve branch contains an inflexion point. Here the system starts to show "thread-pulling". This property at first strongly increases, but only to decrease strongly again when approaching the coacervation limit.
3. The typical shape of the viscosity-KCl-curve becomes less pronounced with increasing temperature. It disappears at extremely low oleate concentrations although in both instances coacervation occurs in a normal manner.
4. The influence of auto-sensibilisation and of contaminations, in the oleate system on the shape of the viscosity-KCl-curve has been discussed.