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Electron-microscopic examination of freeze-dried solutions of alkylpolyethylene oxides and other detergents.

by M. Kehren and M. Roesch.

Translated from: *Melliand Textilberichte*, 37: 680-685 (1956).

Introduction.

We fully realize that the work leading to the publication of this paper represents a new departure, and for this reason we do not consider our test results to be the final clarification of the complicated, exceedingly difficult problems involved, but we submit them for general discussion. It is possible that the questions related to our subject have been treated elsewhere, so that the further development of research is served by an exchange of ideas even when opinions diverge.

a) Basic considerations. The field of detergent colloids has always been marked by great interest in the peculiarities of concentrational variability in aqueous solution. The formation of micelles is involved here, occurring in active colloid electrolytes already in the range of greater dilutions in the form of a conversion from an ionogenic or molecular-disperse state to certain aggregational or orderly states; the latter probably owe their inception to the attractile forces between the hydrophobic fractions of the bipolar structure of the detergent molecule and the interrelation of the water molecules among themselves, which due to their dipolar character try to displace the hydrophobic molecular fractions from the solution with progressively rising concentrations.

According to existing concepts of the washing process, a certain amount of significance has been attached to micelle formation, since they may be considered first of all as "recruitment reservoirs" for the release of surfaceactive molecules to the limiting surfaces fiber/dirt, fiber/wash solution, and dirt/wash solution; in addition, they are called "concentration points" of electric charge due to accumulation of ions into electronegative force fields, which are stronger than that of the individual molecules. Finally, the micelles are credited with the capability of harboring dirt particles of both oily and solid nature and thus of offering a decisive contribution to the formation of stable dirt emulsions or suspensions, in contrast to the individual detergent molecule.

Finally, no ultimate decision is permissible regarding the direct need for micelles in the washing process. Preston's (1) descriptions seem to indicate that he attributes the washing action to the individual detergent ion. This concept certainly is not altogether correct. Preston says himself that c_k , the critical concentration of micelle formation, coincides with the critical concentration of the washing action. In the summary he states verbatim: "The colloid formation starts, and washing action and surface activity reach their maximum, at the concentration at which additional supplies of detergent either do not dissolve (at lower temperatures) or dissolve to form colloids (at higher temperatures), so that a further rise in the concentration of long-chain ions in the solution is impossible." This means that a real washing activity exists only in the range of micelle formation.

The methods for the demonstration of micelle formation are numerous and predominantly physical. Preston has shown schematically that the washing action, the osmotic pressure and high frequency conductivity reach their optimum in the range of the critical concentration, that the density curve and equivalent conductivity show a sharp bend at c_k , and that surface tension as well as limiting surface tension reach their minimum at c_k . Long before Preston, the same concept was treated in more detail by means of an identical sketch by Hess, Philippoff and Kiessig (2).

Moreover, the ultramicroscope permits observation of colloidal particles, though not in their true shape, but by inference from the light scattered due to their presence.

We were stimulated thereby to find a method that would make micelles or superior aggregates of active substances visible and possibly would permit fixation in the original state of solution. At first glance the electron microscope seems to be suited for this work due to its analytic powers in the range applicable to micelles, but certain difficulties are found upon closer scrutiny that oppose its use for the desired purpose. Thus, the examination of aqueous solutions is contra-indicated by the simple fact that the electron stream requires a high vacuum, in which the solvent would evaporate. This point alone apparently presents a basic, insurmountable obstacle to the extension of electron microscopy to the examination of aqueous solutions. The problem takes on a hopeless character when the solutions represent concentration-variable systems, subject to one or several changes in the solutional state in dependence on the concentration. Such systems exist in the case of active detergent colloids, and this is probably the reason why intensive attempts at electron-optical examination of such colloidal systems are lacking (with a few exceptions to be discussed later).

First, questions were raised regarding the fixation of solutional states of detergent colloids, with the stipulation that they were to be testable by electron-microscopic means. The ultramicroscopic investigation with its goal of fixation of colloidal micelles also had the purpose of establishing the three-dimensional structure of the colloidal form.

Even in the negative case, inferences ought to be possible from a single projection, i.e. a smooth drop of lamellar or spherical micelle aggregates (see references in chapter b), onto the pictorial plane of the slide. In the most unfavorable event, fragments of the original structure would still be extant, depending on the technique of preparation. This alone would already represent a certain step forward.

b) Literature on the micelle formation in solutions of detergent substances.

This is not the place for an extensive perspective on the relevant literature from the past 40 years; it can at best serve as an introduction to the work to be discussed, in a demonstrative or registering manner.

McBain probably was the first to offer reasons for the micelle hypothesis. In order to explain the results of osmotic and conductivity measurements, he assumed the existence of different types of micelles. The results obtained by the classification of the components of a soapy solution according to the various molecular states over a great concentrational range were expressed by McBain by means of a so-called "condition diagram" of the soapy solution. He claims the existence of the dissociated single molecule, the undissociated single molecule, the ionic micelle and the neutral colloid, depending on the solution's concentration.

Hartley (3), on the other hand, defends the view that the assumption of a single type of micelle in spherical form suffices. The sphere's shell is occupied by ionized groups of surface-active molecules, the interior of the spherical micelles could contain paraffin chains (i.e. the hydrophobic molecular ends) in loose order. The opposing ions are partly held to the ionized spherical surface of the micelle, partly free in solution.

The work of Hess, Kiessig and Philippoff (4) as well as Stauff (5) indicated on the basis of numerous roentgenographic investigations the existence of the so-called Hess micelle or, according to Stauff (5), the large micelle with a lamellar structure. It cannot be identified with Hartley's spherical micelle (which belongs to the small micelles), and Philippoff (4) concluded from his measurements of viscosity that the micelles are not spherical, but consist of bimolecular lamellae in the form of hexagonal panes or blocks.

While roentgen interferences occur only when the presence of identity periods in (liquid) crystals is manifold, Harkins and his coworkers (6) found a roentgen maximum in soapy solutions that is claimed to correspond to the micellar interval M , i.e. the bimolecular molecular double layer in the micelle. According to the authors, M is quite independent from the concentration --- claimed also by Hartley for his spherical micelle --- and, in the opinion of these Americans, corresponds to the Hess micelle or Philippoff's model. However, Hartly (7) also explained this roentgen maximum in terms of his spherical micelle, if the latter shows a spherical structure with the greatest density hexagonally.

It cannot be decided yet, which of these micellar types is the correct one. Stauff (8), who has furnished a thorough perspective on the status of contemporary knowledge concerning marginal surface-active substances, indicates that Hartley's spherical model is better suited to the solubilization, for instance, of hydrocarbons in the micelles, whereas the enlargement of the lateral intervals of paraffin chains upon addition of electrolyte observed by Harkins et al. is more easily explained by the lamellary model.

The divergent opinions prevalent for a time among the individual research groups, concerning the form and size of micelles of detergent substances in aqueous solutions, may have had their origin in the insufficient consideration of concentrational conditions during the various measurements. McBain dealt primarily with soap, and his different associational and aggregational conditions were related to concentrations of at least 0.1 n. If the molecular weight of soap is assumed to be 250 on the average, McBain worked with solutions of 2.5 percent by weight and higher. However, numerous examinations of the changes in physical properties of detergent solutions indicate that c_k , the critical concentration of micelle formation, is located in the range of $m \cdot 10^{-2}$ (soap) to $m \cdot 10^{-4}$ (AeO substances), depending on the substance. Hartley had treated this concentrational range and above, i.e. the range of strongly diluted solutions, while Hess et al. used concentrations in their roentgen investigations that amounted to 20 to 30-fold magnitudes of c_k .

Based on measurements of scattered light in connection with a cation active substance, Debye and Anacker (9) observed the occurrence of rod-shaped particles, described by them as long cylinders.

Finally, Orthner (10) expounds a new concept of a spherical micelle composed of several bimolecular shells, which consolidates Hartley's concept with the principle of Hess, but fails to substantiate its structure in detail. Still, it is conceivable that the soap molecules have a quasi-parallel position in the spherical shell model, provided the radius of the micelle is sufficiently large in relation to the length of a single molecule or the thickness of a spherical shell; the layers would thereby assume a lamellar character and would produce corresponding roentgen interferences.

Ekwall (11) proceeds from the well-known fact that substances capable of forming micelles act like ordinary 1:1-value electrolytes in highly diluted solutions, and that radical changes take place in the solution's properties with increasing concentrations. He then comes to the conclusion that the assumption solely of a critical concentration, above which micelle formation commences, is inadequate for the explanation of the observed discontinuous changes of the saturation concentrations of added substances, dissociation constants, degree of hydrolysis, activity, the osmotic coefficients, the equivalent conductivity and the sudden appearance of roentgen interferences. Observations of cholates, laurates, caprates and

oleates have led to the recognition of three characteristic concentrations of varying distinctiveness, which lead to structural changes in the solutions, interpreted by Ekwall as the formation of double ions, the small micelles and the large micelles. The properties of the dissolved substances within the individual concentrational stages are highly constant.

In closing this chapter, the micellar models discussed here fleetingly shall be reproduced more clearly in Sketch I after a compilation by Kling (12), including the "spherical shell micelle" after Orthner (10).

c) Literature dealing with the electron microscopic investigation of soaps and soap-like products.

Electron-optical studies of the crystal structure of ordinary soaps and their concentrated solutions have already been pursued.

Thus, Marton, McBain and Vold (13) examined 5.6 % wt. alkaline solutions of sodium laurate gel and demonstrated that this substance consists of a number of fibrils formed by thin ribbons with a diameter approximating the multiples of two soap molecule lengths.

In addition, Hattiangdi and Swerdlow (14) found during ultramicroscopic studies of alkali soaps (pure substances) and their mixtures that soaps may be differentiated and characterized electron-optically according to the chain length of the hydrophobic molecular fraction and according to the soap cation, similarly to trade products of unknown composition. Still, judging from the published illustrations, the criticism of Kling and Mahl (15) is justified when they point out that basically no positive differences are recognizable between the tested soaps.

In the United States, dispersions of lithium soaps in oils have recently acquired increasing significance as lubricants, since they are dispersive in the various mineral oils, petroleum and synthetic oils, in contrast to most other soaps. Moreover, lithium soap oil dispersions are said to produce lubricants with better resistance against water; they have higher melting points than the greases made with calcium or aluminum soaps, making them suitable for aircraft engine greases, etc. Hotton and Birdsall (16) therefore studied these lithium soaps in oily dispersion under the electron microscope and found at 8,000-fold magnification that the lithium salts of saturated fatty acids form long "micelles," whereas the corresponding salts of unsaturated fatty acids produce round, cruder aggregates.

Chwalow (17) recently studied soaps of Na-butyrate to Na-stearate dried from 0.02 % aqueous solutions and discovered that they consisted primarily of ribbon-like fibers which in the case of high-molecular soaps are arranged in rings. The maximal width of the fibers narrows with increasing molecular weight of the soaps; he bases his findings on fairly extensive theoretical considerations about the cohesion of the fatty acid chains. He resorts to the findings of Marton, McBain and Vold for the confirmation of the correlation of the densities of bimolecular lamellae, but he does not state expressly that these authors studied a sodium laurate gel from a solution with a considerably higher percentage.

Kling and Mahl (12) were probably the first to make an electron microscopic picture of a synthetic detergent prepared from a vacuum-dried solution, sodium-dodecyl sulfate, (in addition to the fibrillar network of soap gel mentioned above), in the hope that the preparatory technique used would somehow preserve the original micelles as structural elements and possibly would make them visible. In contrast to the fibrillar network of the soap, the fatty alcoholic sulfate predominantly reveals

superimposed layers of platelets; however, they also noted that structures other than those indicated could occur in detergents, apparently in co-existence according to the preparatory conditions (during the drying of the solution). The latter result may be confirmed on the basis of personal tests with air-dried solutions of soaps, anion active synthetic detergents as well as non-ionogenic alkylpolyethylene oxides, carried out comparatively with solutions prepared by freeze drying; test results will be published elsewhere.

If Ekwall's (11) discoveries are considered, namely, that different α may appear in the solutions of soap-like substances, all of which ought to cause a change in the solution's structure, then the assumption that residues of micelles can be found in air-dried solutions, presumably does not quite conform to the realities.

Kling and Mahl (15) recently published what is probably the best illustrated investigation of Na-soaps. They examined air-dried aqueous and alcoholic solutions of the soap in relation to the concentration and obtained lamelliform crystals, different for each homologue, from diluted aqueous solutions; at higher concentrations they invariably found tendencies to ligamentous fibrils, partly with additional deposits.

Gels only yielded fibrils that appeared to have considerable morphological variations. The structures are changeable also in relation to pH. Their criticism of Hattiangdi and Swerdlow's findings were confirmed by their own studies. Only amylalcoholic soap gels yielded certain differences between the various entities, which were insufficient, however, for analytical purposes. This result was also confirmed by personal tests.

d) Literature on preparatory techniques for electron microscopic investigations of solutions, other than air drying.

It was pointed out at the close of chapter a) that the preparative fixation of micelles from aqueous solutions must try to preserve the three-dimensional structure of the colloidal system.

Unfortunately, attempts to preserve the three-dimensional structure of objects for the purpose of electron-optical studies have been pursued almost exclusively in the medical-biological field; the various preparatory methods are reported predominantly by Americans.

Thus, for instance, Anderson (18) has developed the "critical point technique," based on the circumstance that a liquid heated to its critical temperature under pressure, is converted directly to the gaseous state. The object is placed in a shell filled with liquid CO_2 at 25°C . Heat is applied past the critical temperature (31°C) to 35°C . The object is now situated in compressed, gaseous CO_2 , which is slowly evacuated through a valve. This method is said to yield objects in an excellent state of preservation, but must be eliminated from our considerations, since it

uses organic solvents in the preparation of the objects and resorts to carbon dioxide as the final fluid prior to study, and therefore cannot be compared to aqueous solutions.

In 1946, Wyckoff (19) described a method of freeze-drying electron-microscopic preparations, in which relatively large frozen drops of liquid are exposed to a vacuum on a pre-cooled metal block in the metal vaporization device, until the solvent is sublimated and the block with the object has warmed to room temperature. The samples are then subjected to vaporization and examined in the usual manner.

The value of this freeze-drying method is questioned by Williams (20), since in his opinion the object carrier has inadequate contact with the cooled block to insure sufficient thermal exchange; furthermore, the drops are claimed to be so large that they cannot be cooled intensely enough. He recently developed a new freeze drying method that sprays minute liquid drops on a metal surface inside an intensely cooled glass tube (liquid air), causing them to solidify in about 10^{-5} sec. Sublimation of the solvent in vacuo, warming to above room temperature to prevent condensation of atmospheric moisture seem to have been solved elegantly. Williams has also improved the spray method described by him and Backus in a separate paper (21), and has modified it so that the solution drops solidify in flight before they hit the metal support. Williams himself lists as a disadvantage of the latter method the fact that the rate of freezing is lower here than in spraying the liquid onto the cooled metal surface. Upon relatively low velocity spraying the drops receive an electric charge which they impart to the metal carrier upon contact, by which the drops are subjected to an electrostatic recoil. Here a practical placement of the object material on the object carrier is accomplished by a metallic spray device connected with the object carrier (i.e. the metal block) by a metallic conductor. This "grounding" is not required, however, when high velocity spraying is employed; but the latter may be used only in connection with objects that are not destroyed by the hurling effect.

Although it is extraordinarily elegant in the technique of freeze drying, Williams' method has the disadvantage that the objects must be transferred after freeze drying from the metal carrier to the slide proper. This manipulation must be considered a questionable operation when applied to detergent micelles.

Williams has used the freeze drying method developed by him with excellent results, e.g. for electron-optical determination of the structure of Na-desoxyribonucleate with a 0.01 % solution of this substance (22); in addition, for fixing the spatial organization of red blood corpuscles (20), as well as in the study of bacteriophages (23). The eminently successful pictures shown by Williams in comparison to pictures of identical air-dried objects attest to the favorable preservation of the spatial structure by the employment of the freeze drying method.

In contrast, Bretschneider and Eibero (24), in their thorough, comparative studies of electron microscopic cell analysis after freeze drying, make the disappointing discovery that crystalline ice leads to fragmentation of the cellular plasma, the nucleus, and to the displacement of certain fine structures, thus failing to insure the proper position of all fine structures. They negate, on the basis of their work, Mayall's (25) assumption that rapid freezing to -180°C produces amorphous and not crystalline ice.

Studer (26), on the other hand, defends the view that changes in the structure of biological material may be avoided during freeze drying. Spherical casein particles of milk, for instance, are preserved. In the case of inorganic colloids, however, he concludes that strong aggregates develop in systems with isolated individual particles (e.g. arsenic sulfide) during the sublimation of ice. Again, it is claimed that the original spongy structure of gels may be maintained (lime paste). Vanadinepentoxide sol with its thread-like particles offers a transition between sol and gel, depending on the concentration.

c) Description of the apparatus for freeze drying for the present study and the conditions of testing.

After a study of the existing papers, an attempt was made initially to consolidate the freeze drying methods of Wyckoff and Williams in one apparatus. Sketch 2 depicts this initial freeze drying device. A glass bell d is attached hermetically by means of a threaded ring c and rubber washer e to a hollow, cylindrical, nickel-plated copper stand a with dish b, having a depression for the placement of electron microscopic object diaphragms (and the thermometer bulb). The glass bell has a ground opening at its apex for the thermometer f, which penetrates into a bore hole in the center of the copper mount a filled with vacuum grease, for reasons of better contact with the stand. The glass bell d also has a lateral tube attachment with about 10 mm clearance, with glass stopcock g and a ground ball-and-socket joint h leading to cooling trap i.

The procedure used with this apparatus is as follows: The substance solutions produced in the measuring flask with twice distilled water in concentrations of 10^{-2} , 10^{-3} or 10^{-4} molar were allowed to stand overnight and used the next day. A sample was taken from each solution with glass tubes extended to fine capillaries, a drop of solution was formed on the tip of the capillary by gentle blowing and carefully deposited on the object diaphragm. Next, the diaphragms equipped with the solution were placed on the copper mount, precooled with liquid air, the glass bell was set in place and fastened with the threaded ring, the thermometer was inserted, the cooling trap was connected (the latter also dipped in liquid air), while the coolant was now removed from the copper stand, and evacuation by means of an oil pump commenced simultaneously. As soon as the ice was sublimated and the temperature had risen to above 0°C , the copper stand was heated with warm water until the temperature at the copper dish indicated about $6-8^{\circ}$ above room temperature. Stopcock g was now closed, the whole apparatus disconnected from the cooling trap and air was carefully admitted in the immediate proximity of the microscope, the glass bell was removed and the diaphragms were immediately placed into the exsiccator or fed into the microscope.

The freeze drying device described here shows certain disadvantages. Depending on the object diaphragm's contact with the cooled copper stand, the solutions froze after about $3/4$ to $1\frac{1}{2}$ seconds, visible to the naked eye, possibly an excessively slow rate of freezing.

During the cooling of the copper mount and also between the placement of the object diaphragms and the setting of the glass bell, condensed atmospheric moisture was deposited both on the dish and on the diaphragms containing the solutions, so that relatively large amounts of ice had to be drawn off by the pump. A blank test with an object diaphragm carrying only atmospheric moisture showed, however, that this diaphragm was electron-optically empty after freeze drying.

In addition, the vacuum-tight connection of the glass bell with the copper dish presented some difficulties, since the rubber washer had completely lost its elasticity at the cooling temperature of -80 to -100°C . Nevertheless, a few test series succeeded with this device, before construction of a second apparatus was started, shown schematically in Sketch 3. It represents a modification of Williams' devices and shall also be described briefly.

A glass tube a contains a copper carrier b, connected to the former by high vacuum grease for reasons of greater thermal conductivity; it is equipped with a wire c for withdrawal and insertion. The glass tube has a ground lip at its upper end, on which the tube cover with a corresponding fit d is placed. The tube cover is equipped with symmetrically arranged ground sleeves f, through which microburettes n are admitted for the placement of solutional drops onto the object diaphragms located on the copper carrier b. A fifth ground sleeve h in the center of the

tube cover serves for the insertion of the cold thermometer i, the end of which fits into the bore hole g of the copper carrier. Here, again, thermal transfer is established with high vacuum grease placed in the bore hole. The glass tube has, in its upper portion, a lateral tube connection with glass stopcock k and ball-and-socket joint l for the connection to the cooling trap m, through which the device is connected to the rotating oil pump and the Hg diffusion pump; evacuation was in two stages and high vacuum was produced.

In addition to the possibility of placing drops of solution on the object diaphragms, the alternative was explored of spraying the solution in finely disseminated form with a glass sprayer o, the construction of which is shown in Sketch 3. It was activated with a manual bellows, 5-6 thrusts were given during each spraying. It is obvious that not all droplets impinged directly on the diaphragm or the copper carrier. The result was an atmosphere of atomized droplets, which partially filled the tube and only gradually settled on the copper carrier or the walls of the tube. This produces the disadvantage that the object diaphragms do not only contain instantaneously frozen droplets, but also those in various stages of solidification, which indicates that microscopy would also deal with different structures from slowly crystallizing substance to rapidly frozen drops.

Williams (20) has specified an approximate relation for the determination of the rate of freezing:

$$Q/t = \frac{CA \Delta T}{d}$$

in which C is the thermal conductivity of water (0.0013 at 0°C), A the drop surface, d the drop diameter and ΔT the temperature difference between the uppermost and lowest drop zone.

The drop volume of $2 \cdot 10^{-2}$ ml for the drying of drops from a micro-burette yields a freezing time of about $3 \cdot 10^{-1}$ sec, assuming a spherical shape of the drop and $T = 150^\circ\text{C}$ (copper carrier -135°C , atmosphere -15°C); thus it is only 2 or 3 times as long as that obtained by placement freezing in the first freezing device. The advantage here consists in the circumstance that the drop was able to extend over the entire diaphragm and thus made direct contact with the copper carrier, leading to a freezing time of about 10^{-1} sec in the case of drop freezing.

In spray-drying with the glass sprayer o, the drop residues on the diaphragms were estimated under the microscope to have an average diameter of 30 microns (10 to 50 microns). This droplet diameter, included in the above calculation, yielded a freezing rate of about 10^{-3} sec. Although the diaphragms were in contact with the copper carrier via a very thin coat of high vacuum grease, it is likely that the drops impinged on the

poorly conducting carrier foils, which necessitates the deduction of one decimal power for reasons of safety, leaving a freezing rate of 10^{-2} sec for spray-drying.

Thus, three methods were used in freeze drying with two devices, yielding the following crudely calculated freezing rates:

- a) "Placement drying," about 1 sec.
- b) "Drop drying," about 10^{-1} sec.
- c) "Spray drying," about 10^{-2} sec.

The procedure for b) and c) with the second device is best described by means of the following divided steps:

1. Insertion of the copper carrier with object diaphragms in the glass tube;
2. slight heating of the tube's bottom by submersion in warm water for the purpose of softening the high vacuum grease and obtaining a seal between the copper carrier and the glass tube;
3. placement of the cover with inserted thermometer and ground stoppers (initially in place of the burettes or the sprayer);
4. cooling of the glass tube's bottom by submersion in liquid air up to the upper margin of the copper carrier; cooling progresses to about -140°C ;
5. transfer of liquid air cooling from the tube to the cooling trap;
6. exchange of the ground stoppers in the cover for microburettes and immediate release of a solutional drop, followed by immediate removal of the burettes and closure of the tube cover by means of ground stoppers;
7. open stopcock between tube and cooling trap and evacuate the apparatus to 10^{-4} Torr (diff. pump). Evacuate until the temperature in the glass tube has risen to room temperature;
8. heat the tube in vacuo up to slightly above room temperature by insertion in warm water, to prevent the settlement of atmospheric moisture on the objects during subsequent admission of air;
9. separation of the tube from the cooling trap with closed glass stopcock. Now admit air carefully, open the apparatus and remove the copper carrier.

Vacuum and sublimation of the ice were controlled with a high frequency vacuum tester; it was better than 10^{-3} Torr (no luminous phenomenon). At the start of sublimation, normally observed at about -40°C , a green fluorescence appeared, becoming strong at the apex of ice pumping (10^{-2} Torr). As soon as all of the ice had been removed, the vacuum again became better than 10^{-3} Torr, and fluorescence disappeared.

Some moisture settled on the copper carrier due to the opening of the ground sleeves for the placement of microburettes or the glass sprayer, and the air initially present in the apparatus, but this was considerably less than that incurred in the placement method with the first device.