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Evaluation of Cryotechniques for TEM Observation of Sols - Application to Boehmite Sols Used in Catalysts Forming

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Résumé — Évaluation de cryotechniques pour l'observation de sols en MET - Application à des sols de boehmite utilisés au cours de la mise en forme de catalyseurs — L'utilisation de suspensions dans la fabrication des catalyseurs hétérogènes étant très prometteuse, il apparaît important de maîtriser cette étape au mieux. La taille des objets en suspension, l'état d'agrégation, l'homogénéité ou l'hétérogénéité (appelés texture ou structure dans cet article) sont autant de paramètres qu'il est parfois difficile de connaître par des techniques traditionnelles telles que la diffusion des rayons X ou la rhéologie. Les techniques cryogéniques utilisées conjointement avec la microscopie électronique par transmission (MET) peuvent alors être une alternative intéressante pour évaluer ces propriétés.

Trois méthodes de cryofixation ont été testées sur des suspensions de boehmite peu concentrées en solide du type de celles habituellement utilisées au cours de l'étape de dispersion du processus de mise en forme de supports d'alumine. Deux cryoprotecteurs ont également été testés. Nous avons montré que les méthodes de congélation par immersion dans le propane liquide (PL) et par projection (*slam freezing* - SF) nécessitent l'utilisation d'un cryoprotecteur, alors que la congélation "haute pression" (HP) permet d'éviter toute formation de cristaux de glace sans ajout de cryoprotecteur à la suspension initiale. Pour être efficace avec les méthodes PL et SF, le DMSO doit être ajouté en concentration importante (50 %). Dans ces conditions, le glycérol est plus intéressant car il permet de vitrifier l'eau à partir de 30 % d'ajout. La comparaison entre des préparations haute pression avec et sans ajout de glycérol permet de montrer qu'il existe très peu de différences de texture sur les répliques. Quelques arguments sont donnés pour expliquer les petites différences de densité d'objets observées, mais d'autres expériences sont en cours pour tenter de clarifier le rôle du glycérol.

Finalement, la cryomicroscopie apparaît comme une technique intéressante pour étudier la texture de suspensions telles que celles utilisées au cours de la peptisation ou bien d'un procédé par voie colloïdale.

Mots-clés : suspensions colloïdales aqueuses, technique de cryofixation, cryofracture, réplique, vitrification, cryoprotecteur, microscopie électronique par transmission, boehmite.

Abstract* — *Evaluation of Cryotechniques for TEM Observation of Sols - Application to Boehmite Sols Used in Catalysts Forming — *The use of suspensions in the heterogeneous catalysis field being a big issue, it appears important to be able to control this step as much as possible. Size of the objects in the suspension, aggregation state, homogeneity or heterogeneity (all called texture or structure here) are some important parameters which are sometimes difficult to evaluate with "traditional" methods such as X-ray scattering or rheology. The cryotechniques applied to transmission electron microscopy is then an interesting alternative.*

Three different methods of cryofixation have been tested on low solid content suspensions of boehmite commonly used during the dispersion step of alumina carrier forming. Two different cryoprotectants were tested as well. It is shown that liquid propane immersion cooling (PL) and slam freezing (SF) methods require the use of a cryoprotectant whereas high pressure technique (HP) avoids ice crystal formation without any addition of cryoprotectant in the suspension. It is shown also that DMSO is efficient with PL and SF techniques only when added at a high concentration (50%) to the suspension. Glycerol is preferred because water is vitrified since that 30% of glycerol is added. Comparisons of high pressure preparations with and without glycerol show very little differences of texture on the replica. Explanations of the origin of the small differences of density are tentatively given. More analyses are now under progress to clarify the role of glycerol.

Cryomicroscopy is conclude to be a very good technique when the texture of a suspension must be studied for example in the elaboration or in the improvement of a peptisation or a colloid formation process.

Keywords: aqueous colloidal suspensions, cryofixation techniques, freeze-fracture replication, vitrification, cryoprotectant, cryotransmission electron microscopy, boehmite.

INTRODUCTION

Highly hydrated systems, as for a final product or an intermediate step of processing, are of great interest in most of the industries (cosmetology, petroleum, etc.) as well as in more fundamental studies. Of course, this is especially true in life science where both composition and environment of cells are mainly liquid water. This is also true in material science, when dispersions of solids in liquid or more generally sol-gel processing are used to synthesize or form materials.

Before this kind of system can be used in an industrial process, properties and general conditions of stability of sols must be optimized. In this perspective, it is important to study the characteristics of the sols in order to insure that they fit with both the other steps of the process and the final properties of the product. Numerous characterization means are used during this perfecting operations. Apart from techniques using a separation of the solid from the liquid, a few techniques allow one to study sols as they are. For example, rheology, which gives precious information on flowing properties, and light or X-ray scattering can be used to study the structure of these so-called heterogeneous systems. However, these techniques suffer from two main disadvantages: first, the structure is not visualized directly and one needs to use a model to extract information on the texture. Second, models are often based on restrictive hypothesis about the monodispersed state of the sol, and detailed study of sols with a large object size distribution or with different kind of particles are very difficult. In order to overcome these difficulties, cryomicroscopies can be of a great help. As a matter of fact, it is thus possible to have a direct picture of the sol and the polydispersity is not a big issue. More, results from cryomicroscopy observation can be used for properties modelling.

In the petroleum heterogeneous catalysis field, sols and pastes are used at different steps of catalyst making. Forming of small alumina beads by an oil-drop process is one of the step making use of a sol-gel system. It is a crucial part of the processing since it will fix important properties of the catalyst carrier such as mechanical resistance, surface area and porosity. When one wants to understand and to improve these properties, it is necessary to find the evolution and the relations between properties of the initial powder, suspension characteristics and properties of the final product.

In this particular case, a powder of boehmite (AlOOH, nH$_2$O), which is the Al_2O_3 precursor, is dispersed in an acid solution. The sol (or paste) produced must have specific properties: rheology properties must be compatible with next steps of processing and "textural" properties have to fit with final expected properties of the beads. It is known that most of the objects present in this suspension will still be in the dry shaped alumina carrier. Thus homogeneity or heterogeneity of this aqueous dispersion as well as size and type of objects are very important characteristics that have to be controlled. Cryotechniques with scanning electronic microscopy have already been applied on a high-boehmite-concentration water system forming a paste (Rosenberg *et al.*, 1995). In this work, authors have sucessfuly characterized aggregates (size and distribution) and their interconnections with a sluch nitrogen cryofixation. This was possible because of the high concentration of boehmite. However, when solid concentration is low (water content up to 80% wt) and when size of objects to be observed is small, other cryofixation techniques have to be used and transmission electron microscopy (TEM) is then the only means that can provide the adapted spatial resolution to these materials. Note that in other cases of heterogeneous catalysis preparation, like the use of a colloidal method for metal particles synthesis, the characteristic size of objects is also of the order of a few nanometers and TEM can be of a great interest.

However, cryotechniques for transmission electron microscopy require a specific specimen preparation that have to be adapted to the sample itself and to the problems to be solved. In this article, three techniques often used by the material scientist are described and they are applied to a boehmite suspension. An overview of most of the preparation techniques can be found in the book of Echlin (Echlin, 1992).

The first step of a cryopreparation is a fast freezing of the sample, (so called cryofixation or cryoimmobilization). The aim of this freezing is to fix the structure of the sample without any modification compared to its room temperature texture properties. This is only achieved by vitrification of the water (for aqueous samples). Indeed, crystallization and ice crystal growth change drastically the structure of the suspension. In order to perform vitrification of the whole suspension, freezing rate of at least $10 \cdot 10^3$ K·s^{-1} are needed (Moor, 1971) in most of the cases.

If cryoprotectants have been added to the initial sample, one can expect to avoid water crystallization with lower freezing rates. However, this procedure may alter the sample structure.

In the second step two techniques may be applied: the frozen sample is transferred into the microscope and examined in its “frozen-hydrated” state (direct imaging), or a replica is prepared from the fractured surface of the sample (freeze fracture technique).

Direct imaging needs a cryo-sample holder, which may be cooled either by liquid nitrogen or liquid helium. In addition, it is necessary to use a transfer device to bring the frozen-sample from the low-temperature preparation equipment to the microscope cold stage. Furthermore, this technique can be used only for thin films either directly obtained by liquid propane immersion of a thin sample deposited on a microscope grid, or after an ultramicrotomy cut of a frozen-sample. In both cases, preparation of the frozen sample thin enough to be directly observed in a TEM, is not an easy task and more, imaging require a specific cryo-sample holder. That may be why a lot of laboratories are not equipped for this technique and prefer to use an alternative technique of TEM observation.

The freeze fracture technique consists of four essential steps: cryofixation of the sample suspension, fracture of the cryofixed sample, heavy metal replication of the fractured surface, cleaning of the replica. Following the cleaning step, the replica is suitable for examination in classical conditions at room temperature in any transmission electron microscope without any specific sample-holder.

In this paper, the results of TEM observation of alumina sols by the freeze fracture and replication techniques are presented. We have evaluated the efficiency of three different cryofixation techniques on suspensions with high water content (up to 85% wt). First, we studied the possibility of characterizing our samples as they are, without any addition of cryoprotectant. However, it is well known that a low solid content suspension can be a real challenge to cryofixe properly, i.e. without formation of ice crystal. Thus, in a second time, we tested the efficiency of two different cryoprotectants on the suspensions.

1 MATERIALS AND METHODS

1.1 Sample Preparation

Boehmite powder was supplied by *Condea Petrochemie Gesellshaft*. This is a microcrystalline boehmite of high purity and high surface area (♠ 300 $m^2 \cdot g^{-1}$) generated as a side product in the manufacture of straight-chain alcohols. The formula of the sample ($AlOOH\ nH_2O$; n ♠ 0.45), as determined from thermogravimetric measurements, indicated a water excess from the well crystallized boehmite form (AlOOH), principally due to water physically adsorbed at the crystallite surface (Baker and Pearson, 1974).

1.1.1 Un-Pretreated Sol

Boehmite sols were prepared by peptizing the initial powder with a water-nitric acid solution ($[HNO_3] = 7.5 \cdot 10^{-2}$ mol·l^{-1}). The resulting suspension contains ♠ 15% wt of solid and appeared slightly heterogeneous due to a non-peptized powder fraction.

1.1.2 Pretreated Sol

Our systems being highly hydrated, there may be a possibility that none of the cryofixation techniques work properly. In order to provide for this contingency, two different cryoprotectants have been tested on our boehmite suspensions.

Cryoprotectants are chemical agents that were originally employed in biology to protect cells from damages during slow equilibrium cooling procedures used for the cryo-preservation of tissues. However, these chemical agents are also effective in preventing ice crystal damage in structural studies when used in conjunction with high cooling rates.

Cryoprotectants are thought to interact with water (presumably by means of their hydrogen bonding potential) perturbing the solution and suppressing the anomalous structuring of water. This activity results either in the formation of many small ice crystals rather than a few large crystals, or in a complete vitrification. In addition, they increase viscosity of the medium, which decreases molecular mobility and slows down the rate of ice crystal growth (Franks, 1983; Echlin, 1992).

In this study, two cryoprotectants among the more frequently used have been tested: glycerol and DMSO (dimethyl sulphoxide), in standard concentration: 30% wt (as suggested by Moor, 1971).

Pure (un-pretreated) suspensions as well as pretreated suspensions have always a white diffuse colour. This colour depicts the presence of aggregates of several tens nanometers.

1.2 Cryofixation Techniques

The goal of this step is to quench rapidly enough so that after cryofixation the structure is the same as it was at the initial temperature. For this purpose, solid as well as liquid cryogens can be used. A suitable cryogen should have a low melting point and a high boiling point; a high thermal conductivity and thermal capacity; and a high density and a low viscosity at its melting point. Furthermore, it should be safe, inexpensive and readily available.

In spite of its low cost, nonflammability, and readily availability, liquid nitrogen at its equilibrium boiling point (77 K) is not a good primary cryogen. Indeed, liquid nitrogen suffers from the disadvantages of the Leidenfrost phenomenon: a thin boiling film surrounds immediately any warm object plunged in liquid nitrogen, creating an insulating layer of nitrogen gas. This highly reduces the cooling rate of the specimen. Furthermore, the low thermal conductivity of liquid as well as slush nitrogen does not provide to these materials a sufficient cooling capacity. However, liquid nitrogen can be used as a primary cryogen in processes for which fast cooling rates are not critical. This includes high pressure cooling process and processes using chemically cryo-protected samples.

Liquid nitrogen is more frequently used as a primary cryogen to cool secondary cryogens such as some organic gases. Indeed, liquid propane or ethane, because of their low melting point, high boiling point and high thermal conductivity (respectively 0.22 and 0.24; vs 0.13 $J \cdot m^{-1} s^{-1} K^{-1}$ for liquid nitrogen), give the highest cooling rates. A review of the literature suggests that ethane and propane are the most efficient (and used) coolants for immersion cooling (Silvester *et al.*, 1982; Ryan and Purse, 1987; Echlin, 1992).

Solid coolants are normally blocks of very pure metals like copper or silver with a highly polished, contamination-free surface, which are cooled either by liquid or gaseous nitrogen at 77 K or helium at 4 K. This solid cryogens form the basis of the so-called impact cooling devices (slam-freezing for example). Indeed, the very low temperature and high thermal conductivity (11 300 $J \cdot m^{-1} s^{-1} K^{-1}$ at 4 K for copper) of these materials makes them excellent cryogens (Bald, 1983).

In the following, details will be given on the three techniques that have been tested on our low content solid suspensions, namely the liquid propane immersion cooling (using a liquid cryogen), the slam freezing (using a solid cryogen) and the high pressure cooling (using a liquid cryogen and a high pressure).

1.2.1 Liquid Propane Immersion Cooling (Liquid Cryogen)

This technique consists in plunging the sample into a suitable organic cryogen such as liquid propane or ethane, as fast as possible. It is important that the sample and its holder are as small as possible and that the specimen support is of low mass and high thermal diffusivity. However, it is not sufficient just to immerse the sample rapidly below the surface; it must also continue at the same velocity well into the cryogen in order to maximize forced convective cooling, which is the most efficient process of heat transfer (Ryan and Purse, 1985; Ryan *et al.*, 1990). It is sometimes necessary to use some sort of spring-loaded mechanical device to give higher injection velocities (up to 2-4 $m \cdot s^{-1}$).

In our experiments, a small droplet of suspension is deposited on a flat copper device. Copper is used because of its good thermal conductivity. The cryofixation of the sample is obtained by plunging it rapidly by hand, into liquid propane, cooled by liquid nitrogen (77 K) (Fig. 1) (Aggerbeck and Gulik, 1986; Robbe *et al.*, 1998).

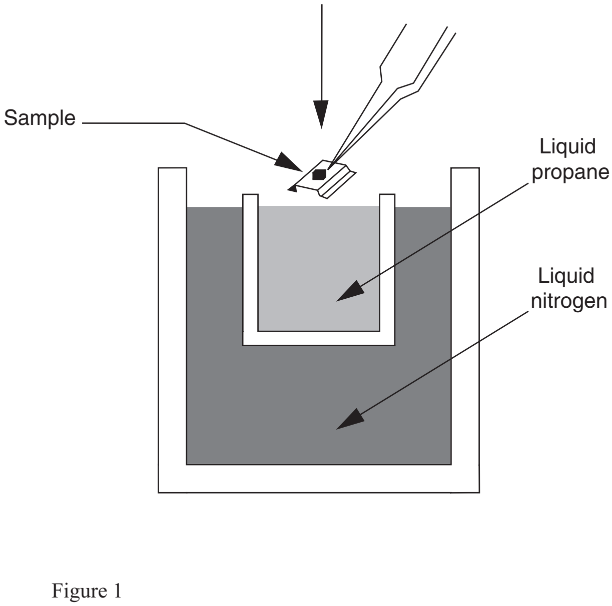

Figure 1

Schematic representation of the liquid propane cooling technique (modified and redrawn from Aggerbeck and Gulik, 1986).

Ability and experiences allow one to rapidly see if the cryofixation has some chance to be successful. On our sample, a white film on the sample holder indicates the formation of ice crystals due to a too slow quenching.

1.2.2 Slam Freezing (Solid Cryogen)

In this method, the material to be cooled is pressed rapidly against the highly polished surface of a metal block maintained at 15-25 K, by means of liquid helium (Fig. 2). The main advantage of this method is that although metals such as copper or silver have a thermal capacity about the same as organic liquids, they have higher thermal conductivity. Consequently the same amount of heat is transferred 10 000 times faster through copper than through propane (Echlin, 1992). However, the metal surface must be scrupulously clean as contaminants such as water vapour will substantially lower the surface thermal conductivity. This cleanliness is achieved by continually flushing the metal surface with dry helium gas. The purity of the metal is also important, since even the smallest trace (♠ 200 ppm) of some element will reduce the conductivity of pure (at 99.999%) copper by 10-15% (Escaig, 1982; Echlin, 1992).

The success of the cooling depends, to some extent, on the speed at which the sample makes contact with the mirror surface and the thrust developed by the system following the initial contact. Best results have been obtained at initial speeds of 5 $m{\cdot}s^{-1}$. At faster speeds there may be a deformation or a total destruction of the sample (Echlin, 1992).

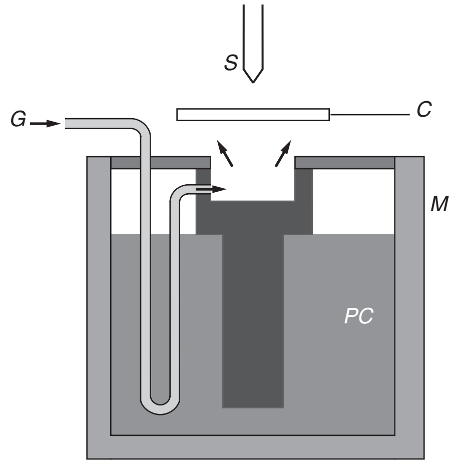

Figure 2

Slam cooling apparatus. The metal block (in black) is cooled in a Dewar (*M*) by the primary cryogen (*PC*), liquid helium in our case. The highly polished surface of the metal block is continually flushed with pre-cooled dry He (*G*) to prevent surface frosting. The cover (*C*) is removed just before the sample (*S*) is rapidly pressed against the cold metal surface (from Echlin, 1992).

In our case, sample suspension was placed on a gold-copper cup (made by *Bal-Tec*) with a centred shaft of 1 mm diameter. The preparation was placed on a sample holder and transferred to the quick-freezing apparatus. Cooling was obtained by projection of the sample against a highly cleaned copper block (made by Reichert and Jung) cooled by liquid helium (4 K) and maintained under vacuum (Lechaire *et al.*, 1994).

1.2.3 High Pressure Cooling (Liquid Cryogen + High Pressure)

An examination of the phase diagram of water shows that vitreous water should form when liquid water is rapidly cooled under high pressure. As water freezes it increases in volume, and high pressures will hinder such expansion and, in turn, hinder crystallization. This effect is manifest in a decrease in the freezing point and a reduction in the ice germs nucleation rates and the ice crystal growth rate. At a pressure of 2045 bar the freezing point of water is lowered to 251 K and the temperature of homogeneous nucleation reduced to 181 K (Studer *et al.*, 1989). Consequently, less heat is produced by crystallization and less heat has to be extracted per unit time by cooling (Moor, 1987). Because the procedure takes advantage of a reduced critical cooling rate, high pressure cooling should not really be considered as a rapid cooling procedure. This means that adequate cryofixation can be achieved with reduced cooling rates.

For our experiments, we used a high pressure cooling device HPM 010 that is available from Balzers Union. The simple diagram in Figure 3 shows the principal features of this equipment. A more in-depth description of the apparatus and its use is given by Moor (1987). The samples are subjected to pressures of about 2100 bar by the application of a high pressure jet of warm liquid propanol a few milliseconds before the sample is cooled by a jet of liquid nitrogen.

Samples may be cooled rapidly by a number of different ways using liquid or solid cryogens. Although most of the methods give cooling rates of about $10{\cdot}10^3$ Ks^{-1}, there is wide variation of the characteristics of the cryofixation methods, and no single method gives good results with all samples.

Immersion cooling (by hand or using a mechanical injector) gives acceptable results for small samples (< 10 mm^3), or very thin films. Besides, this method is simple to use and the equipment inexpensive to construct or purchase. Impact cooling (slam-freezing) is better suited for the surface regions of samples and for thin, flat specimens and suspensions. Good preservation of inorganic aqueous gels can be routinely achieved by slam freezing (Favard *et al.*, 1989, 1992). However, the small depth of vitrification that can be obtained and the compressive stress applied to the sample during the cooling process (which can induce deformation of the microstructure) represent important limitations (Leforestier *et al.*, 1996).

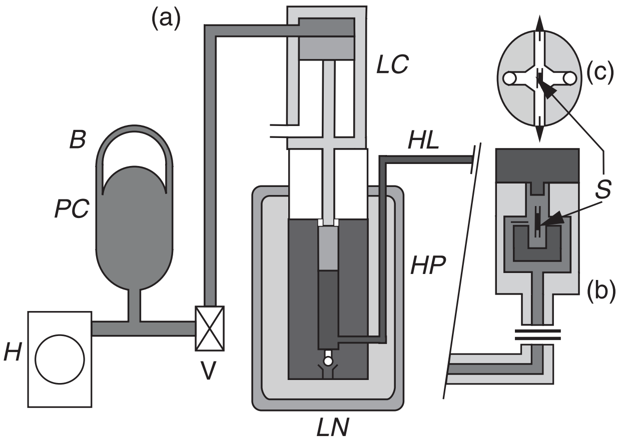

Figure 3

High pressure cooling apparatus. (a): a hydraulic pump (*H*) forces oil into a pressure container (*PC*) containing a rubber balloon (*B*). The pressurized oil is released by opening the main valve (*V*), which forces the piston down in the low-pressure cylinder (*LC*). The lower part of the piston is in the high pressure cylinder (*HP*) immersed in a liquid nitrogen dewar (*LN*). This movement compresses liquid nitrogen, which is driven through the insulated high pressure line (*HL*) into the sample chamber shown in more detail in (b). The metal foil sandwiched sample (*S*) is momentarily bathed in pressurized iso-propyl alcohol before the two jets of pressurized liquid nitrogen are directed onto the opposite sides of the specimen shown in more detail in (c). This delay cooling by about 15 ms and allows the sample to be exposed simultaneously to high pressure and low temperature (from Dahl and Staehelin, 1989).

High pressure cooling is able to overcome the most severe limitation of the slam freezing technique; namely it is possible to significantly increase the vitrification depth. However, decompression effect may occur during post-cooling procedures and it seems more difficult to obtain large replicas for example. Furthermore, high pressure equipment is considerably more expensive than the others.

Usually, one considers the cryofixation efficiency to be respectively higher for the high pressure than for the slam freezing and than for the hand liquid propane immersion.

After the cryofixation has been performed successively, it is important to keep the sample under the devitrification temperature (T_v ♠ 130 K) in order to avoid any phase and any texture modification (Dubochet *et al.,* 1982). In our case, after freezing samples are stored in a vessel containing liquid nitrogen.

1.3 Replica Preparation

Following these three cryofixation techniques, we have been using exactly the same method to produce replica that can be observed at room temperature.

1.3.1 Fracture of the Cryofixed Sample

The frozen sample is mounted on a cold table (77 K) still under liquid nitrogen. Then the table is inserted into the freeze fracture equipment (Balzers BAF400 or BAF400T) and kept at 123 K. The fracturing is performed, when a vacuum of 10^{-7} Torr is reached, by a single edge scalpel blade pre-cooled to 123 K (Fig. 4). A fracture plane is established through the cryofixed sample suspension. Part of the small particles present in the initial suspension and "confined" in the cryofixed sample are removed with the upper part and the others remain in the lower part of the specimen (Fig. 4). Thus, the fracture surface of the sample exhibits holes representing particles which have been removed with the upper half of the specimen and emerging particles which stayed in the lower part.

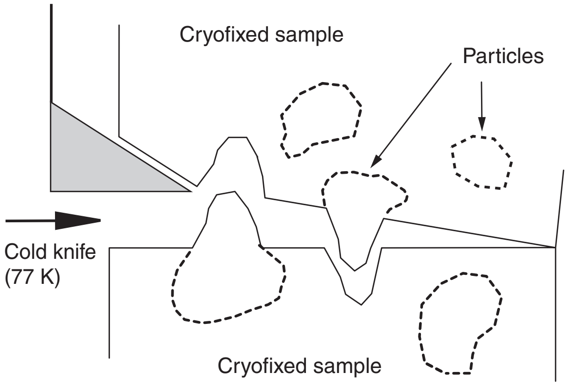

Figure 4

Sketch of the fracture showing particles on both side of the sample. Note that temperature has to remain lower than the devitrification temperature.

1.3.2 Replication of the Fracture-Exposed Surface

The replication of the surface exposed by cryofracture is a two part-process. In the first stage, a thin layer (2 nm) of a heavy metal such as platinum is evaporated onto the specimen from an oblique angle (at 30 or 45° in our case) to provide contrast enhancement of the topographic features of the fracture surface (Fig. 5 (a)). The shadow coating will not be continuous over the whole sample, so it is necessary to hold all these patches of material together by a second, somewhat thicker continuous layer (approximately 20 nm) of an electron transparent material such as carbon or silica (carbon in our case). In addition, as the platinum deposit is too fragile to be manipulated or observed in the electron microscope, the carbon layer allows to strengthen the replica (Fig. 5 (b)).

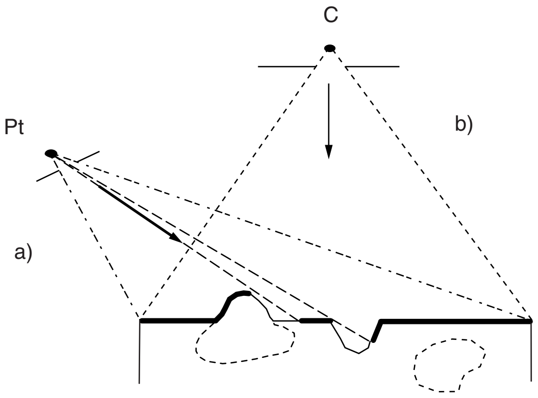

Figure 5

Sketch of the replication technique showing the platinium (Pt) shadowing that will create the contrast (a) and the carbon (C) evaporating that will strengthen the replica (b).

These two layers form the replica; the carbon layer faithfully replicates the features of the fracture surface, while the heavy metal provides the necessary contrast for visualizing the replica in the transmission electron microscope. The film thickness determination (platinum and carbon) is controled by a quartz crystal thickness gauge.

1.3.3 Cleaning of the Replica

After replication of the fractured sample, the replica is washed. Thus we eliminate the sample by solubilization or digestion with an appropriate solvent system such as distilled water followed by sulfochromic acid. After elimination of the sample, the replica (Fig. 6) is washed with distilled water and deposited on an electron microscope grid.

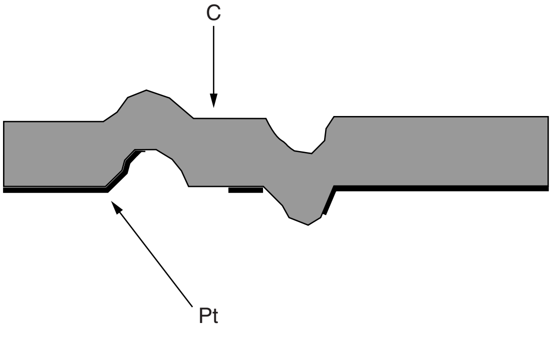

Figure 6

Replica of the cryofractured sample.

1.4 Observation in Transmission Electron Microscopes

Transmission electron microscopy (TEM) measurements were performed on three different TEM depending on the laboratory equipment: a Jeol 100 sx top entry type working at 100 kV *(Parfums Christian Dior SA*); a Jeol 2010 high resolution working at 200 kV *(Institut français du pétrole*) and a Zeiss 912 (equipped with an omega filter) working at 120 kV *(Université Pierre et Marie Curie*). Although all these microscopes have very different performances and are usually used for different applications, results obtained on our samples can be directly compared; point resolutions of the microscopes are not a big issue for this study. Resolution or observation of very small features would be much more limited by the replica itself and its quality. Image contrast is lower with the Jeol 2010 working at 200 kV but platinum being a high Z-element, there is no difficulty at all to see very small features. The Zeiss TEM is equipped with an energy omega filter which enhances the contrast by removing all the inelastic contribution to the image.

2 RESULTS AND DISCUSSION

First, we compare the cryofixation efficiency of the three techniques applied to the (un-pretreated) initial suspension.

In all the cases, small traces are seen on the replicas. These traces correspond to emergent objects or holes that were on the cryofixed surface of the sample. The maximum size of these traces is estimated to be around 12 nm. This measurement squares very well with the size of the elementary boehmite particles in the initial powder. Thus, we conclude that these traces represent individual particles of boehmite. Nevertheless, the spatial repartition of these particles can be very different from one cryopreparation to an other. In some cases, it is clear that the initial sample has been modified.

Both propane immersion by hand and slam freezing are not suitable for the vitrification of the sample. Indeed, although the low-viscosity initial suspension is optically isotropic, observations of the replica show the formation of a slit-shaped-like structure (Fig. 7). The solid particles have obviously segregated around ice crystals in the process of ice-growing. This rearrangement induces the lost of all structural information about the initial suspension. One can note that ice crystals seem to be bigger in the immersion preparation than in the slam preparation (from 400-900 nm up for the immersion, against 300-400 nm for the slam freezing). If the ice crystal growth is considered to be the limiting factor, this could confirm the trend of rating slam freezing as a more efficient cryofixation method than the immersion. In contrast, and as we have already suggested, high pressure method allows an adequate cryofixation. Indeed, the absence of segregation pattern is a generally accepted indication of good cryoimmobilization. No ice crystals are formed and the cryofracture shows an homogeneous dispersion of the particles (Fig. 8). In some areas, one can see that an important number of prints are very close from each other. Sometimes, these prints seem to be linked. We suggest that these specific arrangements of primary prints represent the traces of non-peptized particle clusters present in the initial suspension. Dimension of these specific areas evaluated on the images represents the minimum size of the aggregates. This minimum size ranges approximately from 80 to 175 nm for the un-pretreated suspension.

With such a preparation, it is thus possible to image the structure of the suspension. For example, beside the presence (and the size) of aggregates that were already expected because of the white diffuse colour of the suspension, it is seen that numerous elementary boehmite particles have been isolated during the peptisation process. This is very important to know this kind of "pitch of the reaction" because the type of object in the suspension defines the texture of the alumina carrier. In this particular case, this confirms the partial peptization of the boehmite with the acid concentration used here. More acid would push the reaction forward and more individual particles would be obtained. However, with other materials (other boehmites or colloidal preparations), one could also wonder if these two different behaviours would be due to two types of particles (intrinsic limitation) or more to an other type of "chemical" limitation like a limit of dissolution, complexion, etc.

In order to test the efficiency of the two chosen cryoprotectants (glycerol and DMSO) only cryofixations by liquid propane by hand immersion and slam freezing were performed. Indeed, as we have already seen, in the case of high pressure method, which is the most efficient, suspensions do not need any pretreatment. When pretreated, suspensions contains 30% wt of cryoprotectant.

The DMSO-suspension cryofixed by liquid propane by hand immersion gives results similar to the initial un-pretreated

Figure 7

Liquid propane immersion cryofixation.

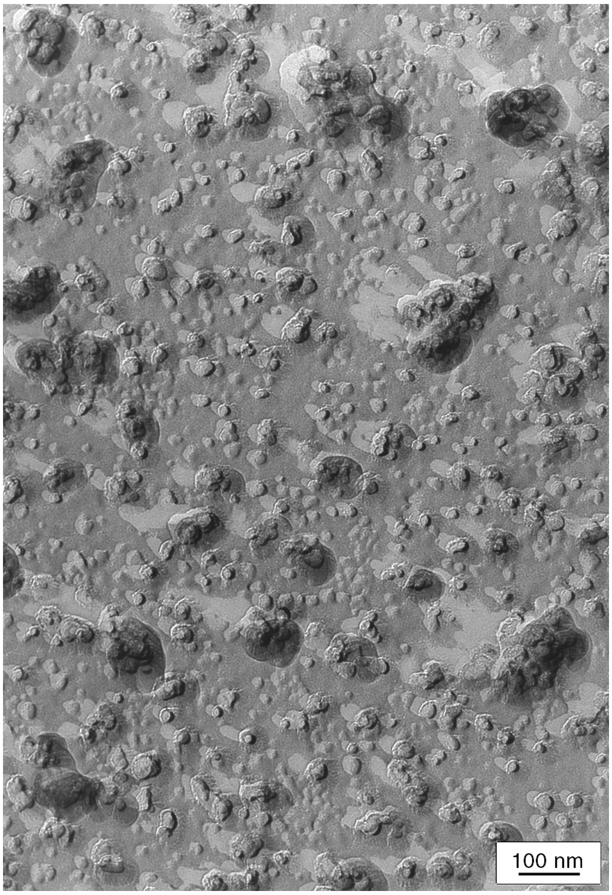

Figure 8

High pressure cryofixation.

one: water crystallisation is observed. The solid particles segregate around ice crystals (ranging from approximately 300 to 550 nm) and again the replica shows the formation of a slit-shaped-like structure. Once more, there is no information available about the real texture on these cryopreparations. The slam freezing technique gives heterogeneous cryofixation: some areas seem to be comparatively well vitrified whereas others show the classical segregation pattern due to ice crystal formation. In the latter situation ice crystals are smaller (from 50 to 100 nm) than these formed for the un-pretreated suspension.

We suggest that DMSO don't act as an efficient, cryoprotectant in our case. Indeed, different cryofractures on 30% wt DMSO-water system (without solid phase) show an heterogeneous fractured surface with probably little ice-crystals. In addition, experiments with various DMSO concentrations (10 to 50% wt) on DMSO-water systems, show that a minimum amount of 50% wt of DMSO is necessary to perform correct cryofixations without ice crystallization. However such a quantity of DMSO added to the suspension may involve a complete destabilization of the initial equilibrium state of the system. Thus, it does not seem reasonable to use this procedure to characterize the texture of highly hydrated samples like the boehmite suspensions considered here.

The first tests with glycerol were performed by hand immersion in liquid propane. On these samples large domains were investigated on the replicas and crystallization of water remained absent. This means that an addition of 30% wt of glycerol is sufficient to avoid ice crystallization. The replicas show an homogeneous dispersion of the particles which sizes correspond to the range observed in the powder (Fig. 9), and, like in the case of the high pressure method, some areas present higher trace concentration. As we mentioned above, we speculate that these specific arrangements of prints represent the traces of aggregates.

It can be noticed that the density of objects (both elementary particles and aggregates) is slightly lower and the aggregate size is smaller than for the high pressure preparation. Thus, even if 30% of glycerol allow to obtain a good cryofixation, one can wonder if the glycerol has some effects on the studied suspension structure. Indeed, the dielectric constant of glycerol is 42.5 against 80 for water. Replacing 30% wt of the acid-water solvent by glycerol is then reducing the dielectric constant (ε) of the suspension medium. This is equivalent to a decrease of the Debye length (κ^{-1} with $\varepsilon^{1/2}$) which is a characteristic of the repulsive interaction between particles (Derjaguin-Landau-Verwey-Overbeek, DLVO theory). Such a decrease of κ^{-1} , which is equivalent to an increase of the ionic strength, is known to get particles closer to each others until they aggregate. In spite of the expected decreasing Debye length due to the addition of glycerol and the white diffuse colour of the suspension, only very few and very small aggregates are seen on the liquid propane replica. The glycerol has either no visible effect on the sample or either some small effects on the suspension structure (since it seems to reduce a little the size and the density of aggregates that were expected) that are not described by this theory. There may be some specific adsorption of glycerol on surfaces of boehmite particles inducing some sterique interaction for example that are not considered in the DLVO theory. A more general point of view of all possible cryoprotector effects is given by Franks (1977).

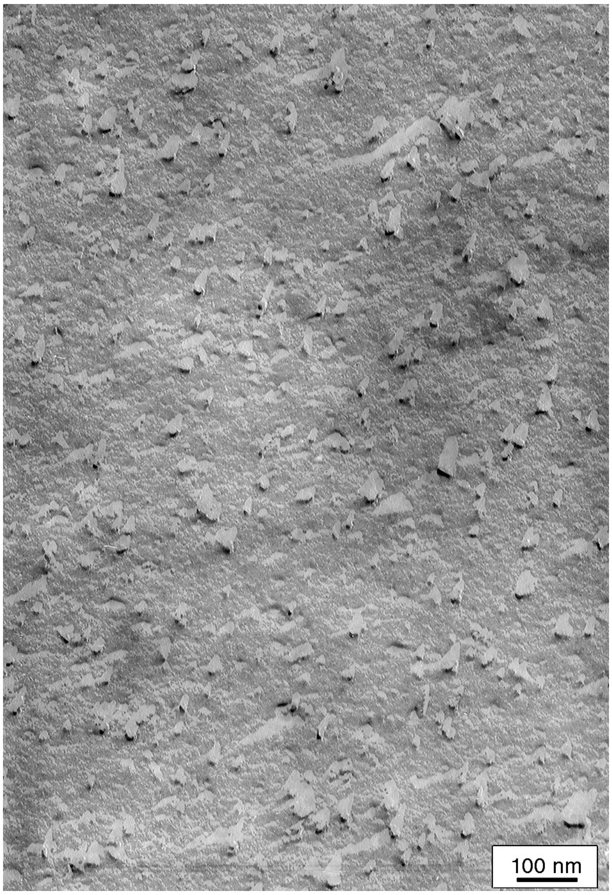

Figure 9

Glycerol-suspension cryofixed by liquid propane immersion.

In order to identify the possible glycerol effect on our systems, we have performed comparative tests on the initial (un-pretreated) suspension and pretreated glycerol-suspension. For this purpose, we have used high pressure technique, which is the only cryofixation method allowing the vitrification of the pure suspension.

Even if it is clear that such an addition of glycerol affects somehow the chemistry of the medium, one wants to know if there is a subsequent modification of the texture of the system and if the cryo-TEM method adopted here can depict them. If no modification is observed, meaning that the glycerol does not change the system or that the characterization technique is not sensitive enough to depict any evolution, then the glycerol can be used to prepare the samples and high pressure technique is not required for our suspensions.

Observation of the glycerol-suspension replicas shows that there is no ice crystals, and once again the spatial distribution of particles is relatively homogeneous (Fig. 10).

Like the case of the un-pretreated suspension, some areas show some higher concentration of traces. As we mentioned above, we speculate that these specific arrangements of prints represent the traces of aggregates. In general, the two preparations (with and without glycerol) are similar to each other.

However, the replica of the pre-treated glycerol-suspension, seems to be slightly different from the un-pretreated one. The binary thresholds of these two representative pictures allow to enhance these differences (Fig. 11 and Fig. 12).

After thresholding, the two preparations are slightly different (even if spatial distribution and size of the elementary objects are still similar). The general density of objects is lower and the areas corresponding to aggregates are smaller for the pre-treated suspension than for the un-pretreated (pure) sample. Indeed, the minimum aggregate size ranges approximately from 50 to 130 nm for the pre-treated glycerol-suspension, against 80 and 175 nm for un-pretreated suspension.

As we mentioned above, we may speculate that these differences are, in a first approximation, only due to the physico-chemical effect of glycerol on the equilibrium state of the initial suspension. However, once more, relative characteristics of replicas are opposite to those predicted by the DLVO theory: density and aggregate size are smaller for the glycerol pretreated sample. Even if other effects of glycerol may be considered, an other explanation can be raised.

Contrary to the direct modification of the initial suspension texture, there may be a modification of the suspension

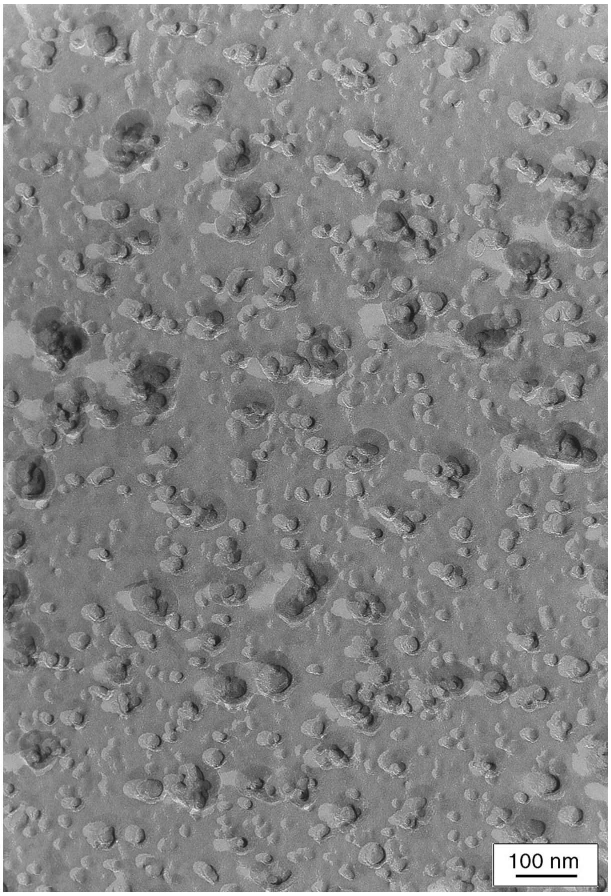

Figure 10

Glycerol-suspension cryofixed by high pressure.

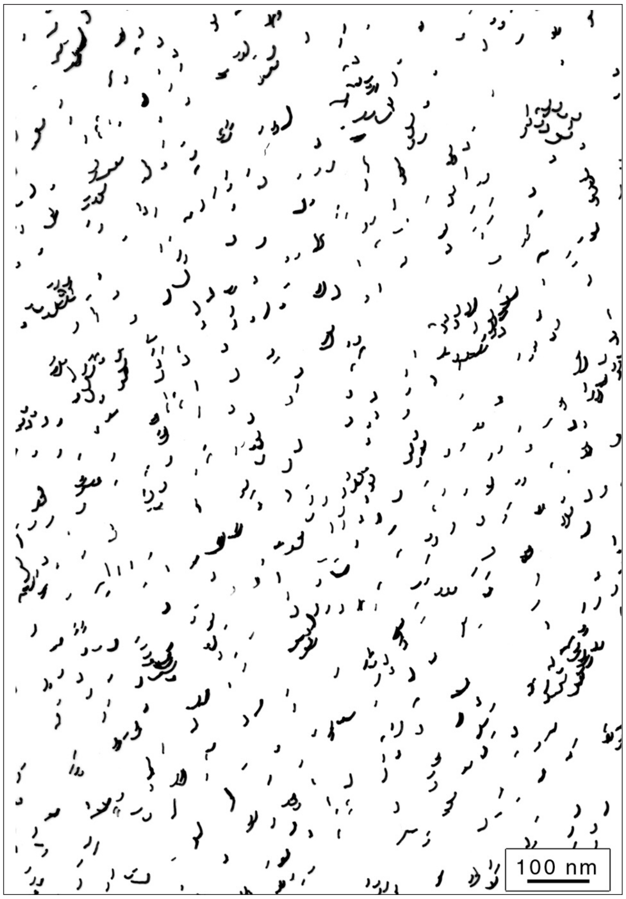

Figure 11

Binary threshold of un-pretreated boehmite suspension.

behaviour when it is cryofixed. For example, it has been shown that density of amorphous ice obtained under high pressure was increased by 10% relative to that formed at ambient pressure (Richter, 1994). Furthermore, molecular dynamics simulations of the water phase diagram (Tanaka, 1996) confirmed these results and showed the existence of two distinct amorphous phases: a high-density amorphous state at high pressure and a low-density amorphous phase at atmospheric pressure. Thus, images of high pressure un-pretreated samples may be slightly altered images of the real structure of the suspensions. The global contraction of the system (due to the increase of the density of water during the cooling) may move objects closer to each other. This would increase the density of objects and could slightly increase the number and the size of the aggregates as well. As it is well known that cryoprotectants change the properties of the suspensions when they are cooled rapidly, the addition of glycerol may limit this contraction phenomena (for example by lowering the molecules mobility in the suspension). Thus, one could imagine to depict smaller aggregates in the sample pretreated with glycerol. In this assumption, addition of glycerol is not considered to modify the structure of the initial suspension; only the cryofixation technique would be responsible of the differences observed on the replicas.

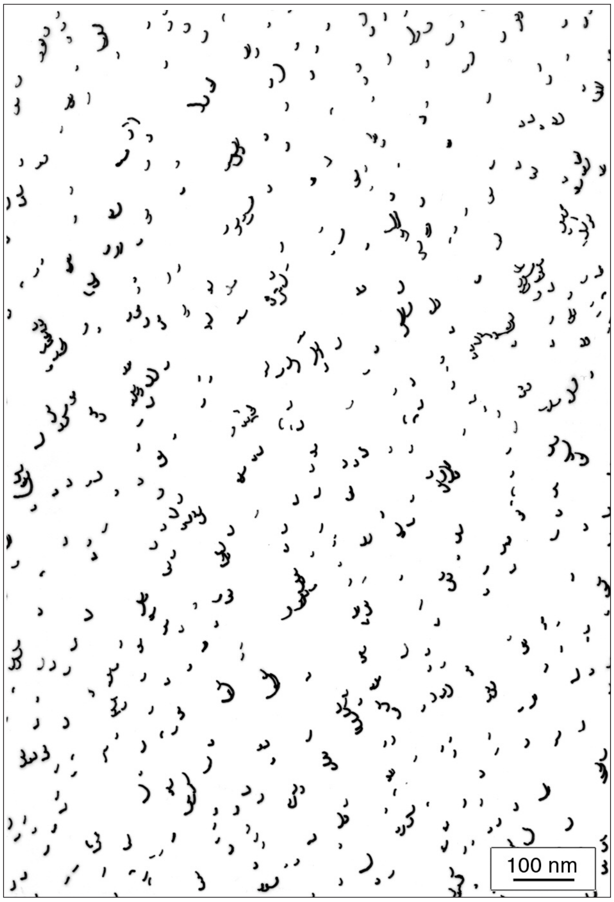

Figure 12

Binary threshold of glycerol-suspension.

In order to confirm either of the two possibilities, repeatability tests of cryo techniques and small angle X-ray scattering analysis of pure and pre-treated suspensions could be carried out. Note that there are small differences in terms of object density and aggregates size between pretreated samples cryofixed in liquid propane or by means of the high pressure apparatus. This may be because glycerol affects both the structure of the suspension and its behaviour during high pressure cooling.

CONCLUSION

Among all the different specific techniques of cryomicroscopy, three methods of cryofixation followed by a cryofracture and a replication have been tested on a (high water content) boehmite acid suspension:

- the liquid propane immersion cooling (by hand);
- the slam freezing;
- the high pressure cooling.

With no cryoprotectant added to the low solid content suspensions, only the sophisticated high pressure techniques give good cryofixed samples (i.e. with no ice crystal) allowing a texture study. In order to avoid the crystallization of water during the cryofixation step when the other methods are used, a cryoprotectant must be added to the suspension. We have shown that DMSO must be added to 50% to be efficient whereas 30% of glycerol (as it is generally used) is enough to vitrify water. Such a high quantity of DMSO is presumably thought to destabilize the initial suspension. Thus only glycerol has been concluded to be a “good” cryoprotectant for our suspensions. Comparisons of high pressure cryofixed samples show that the addition of glycerol does not change much the TEM characteristics of the replicas. Slight differences in terms of density and aggregates size may be due to either a modification of the suspension itself or to a modification of the behaviour of the modified suspension during cryofixation. Work is still under progress to clarify this point. In addition, observations by direct imaging of the freezed object, should be performed to complete this evaluation of cryomicroscopy of aqueous suspensions.

First images of our boehmite suspensions show that peptization produces individual boehmite particles evenly distributed. In addition, with the physico-chemical conditions used here, it was seen that the peptization was not total and aggregates could be measured. In the mean time, we can follow the evolution of the suspensions with different mixture parameters and try to find the best conditions for specific applications. This method, and the direct cryo-TEM, may be of great help to identify, for example, non-peptizable objects in initial powders in order to modify synthesis parameters of boehmite if necessary. These techniques will be used shortly to characterize colloidal suspensions of metal particles as well.

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