

## Hybrid Organic-Inorganic Colloids with a Core-Corona Structure: A Transmission Electron Microscopy Investigation

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**Summary:** The present paper describes a simple route to prepare organic-inorganic colloids with a core-corona structure. Colloids are obtained by complexation of multivalent metal cations by anionic-neutral double hydrophilic block copolymers leading to hybrid polyion complex micelles. The hybrid colloids can then be mineralized in suspension by performing hydrolysis of metal cations within the micelles. The present study focuses on the structural characterization of the colloidal complexes and of the mineralized nanoparticles using a combination of transmission electron microscopy (TEM) techniques including conventional bright-field imaging (with and without negative staining) and cryo-TEM observations. It is shown that the different types of TEM observations are complementary and necessary to get a complete picture of the core-corona colloids.

**Keywords:** cryo-TEM; hybrid colloids; nanoparticles; polyion complex micelles; transmission electron microscopy

### Introduction

The preparation in aqueous suspension of highly stable nanoparticles of controlled properties is still a challenge. Most of the time, for various applications, the colloids characteristics that are simultaneously required include controlled size, shape, composition and structure. The present paper describes a preparation route in aqueous medium of organic-inorganic hybrid colloids that are obtained by using double hydrophilic block copolymers (DHBC)<sup>[1-4]</sup>. Such polymers have known a considerable development in the

recent years in the field of controlled inorganic synthesis<sup>[1,5,6]</sup>. The copolymers used in the present study contain an ionizable block and a neutral block. The two blocks present very different affinities for metal ions : the first block is a metal binding polymeric segment, while the second block is neutral and allows to stabilize particles in suspension. Hybrid polyion complex (PIC) micelles are formed by complexation of multivalent metal cations by the anionic block of the copolymer. The metal cationic entities can be either  $M^{n+}$  metal ions ( $n=2, 3, \dots$ ) such as  $Cu^{2+}$ ,  $Al^{3+}$ ,  $La^{3+}$  or polycations such as the Keggin tridecamer  $Al_{13}^{7+}$  or  $Al_{30}^{18+}$  or nanoparticles obtained by controlled partial prehydrolysis of metal ions<sup>[1-3]</sup>. The complex micelles can be transformed into polymer-stabilized metal hydroxide nanoparticles by performing hydrolysis and polycondensation of metal cations in the presence of the copolymers<sup>[1]</sup>. This mineralization step of the hybrid PIC micelles simply consists in adding a strong base to the micelle suspension.

The goal of this paper is to characterize the size and the structure of hybrid polyion complex micelles and of the colloidal metal hydroxide-based particles using a combination of different transmission electron microscopy (TEM) analyses. In order to get a description as complete as possible of the hybrid colloids, the use of different TEM techniques is needed: conventional bright field imaging, at room temperature, of air-dried droplets of suspension on carbon films, with or without additional negative staining, and cryo-TEM imaging of quench-frozen thin films. Moreover, TEM analyses are combined with characterization by dynamic light scattering (DLS) and small-angle neutron scattering (SANS) in order to complete the colloids description.

## Experimental

### *Materials*

#### *Metal cations and polymers*

Solutions of  $Al(NO_3)_3 \cdot 9H_2O$  (Aldrich) in water were used as  $Al^{3+}$  sources. Pure solutions of  $Al_{13}^{7+}$  clusters were prepared by controlled prehydrolysis of  $Al^{3+}$  solution. Hydrolysis of a 0.1 M  $Al^{3+}$  solution (100 ml) was performed by adding a 0.246 M solution of NaOH (100 ml), drop by drop, into the aluminum cation solution, under continuous stirring at  $90^\circ C$ <sup>[7]</sup>. The hydrolysis ratio reached equals 2.46 corresponding to  $Al_{13}$  species ; the  $Al_{13}$  solution looks clear and the pH equals 4.9. Ultrapure deionized water (MilliQ, Millipore, France) was used for all solution preparations. The block copolymers used in this study

are polyacrylic acid (PAA)-b-polyhydroxyethylacrylate (PHEA) or polyacrylic acid (PAA)-b-polyacrylamide (PAM), the chemical formulae are indicated in figure 1, and compositions and molecular weights  $M_w$  ( $\text{g}\cdot\text{mol}^{-1}$ ) in table 1. The following notations were used :  $\text{PAA}_{Mw1}\text{-b-PAM}_{Mw2}$  and  $\text{PAA}_{Mw1}\text{-b-PHEA}_{Mw2}$ . Block copolymers were provided by Rhodia, their synthesis was described earlier<sup>[8]</sup>. Before mixing the copolymer with solutions of metal cations, the pH of the copolymer solution was adjusted to 5.5.

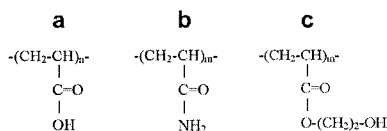


Figure 1. Chemical structures of the hydrophilic blocks in DHBCs : a) poly(acrylic acid) PAA, b) poly(acrylamide) PAM, c) poly(hydroxyethylacrylate) PHEA.

Table 1. Characteristics of PAA-b-PHEA and PAA-b-PAM block copolymers (number-average degree of polymerization (N) and molecular weight ( $M_w$ )).

Sample	$N_{\text{PAA}}$	$N_{\text{PHEA}}$	$N_{\text{PAM}}$	$M_w$ total
$\text{PAA}_{1900}\text{-b-PHEA}_{8200}$	26	79		10100
$\text{PAA}_{2800}\text{-b-PHEA}_{11100}$	39	107		13900
$\text{PAA}_{3000}\text{-b-PAM}_{10000}$	42		140	13000
$\text{PAA}_{3000}\text{-b-PAM}_{30000}$	42		417	33000
$\text{PAA}_{5000}\text{-b-PAM}_{30000}$	69		417	35000

### Hybrid PIC Micelles

The hybrid nanoaggregates were prepared by adding copolymer solutions (5 wt.%) into metal cations containing solutions, under fast stirring at room temperature. Nanoaggregates were characterized by the metal complexing degree noted R which is the molar ratio between the acrylate functions and the metal cations. Metal concentrations in the final suspensions vary between  $5.10^{-3}$  and  $10^{-1}$  M. Typically, for a  $\text{Al}_{13}$  /  $\text{PAA}_{1900}\text{-b-PHEA}_{8200}$  ( $R=1$ ) micelle suspension : In 800  $\mu\text{l}$  of an  $\text{Al}_{13}$  solution ( $[\text{Al}]=5.10^{-2}\text{M}$ ), 731 mg of a copolymer solution (2.094 wt.%) is added. Then, milliQ water is added for completion to a total 4g sample. The mixture is stirred for 2 hours

### Polymer-Stabilized Inorganic Colloids

Mineralization of the complex micelles in order to form metal hydroxide particles is performed by adding sodium hydroxide to the micelle suspension, at room temperature. The total hydrolysis degree of Al ions equals 3. Typically, the procedure for mineralization of an

Al<sub>13</sub> / PAA<sub>1900</sub>-b-PHEA<sub>8200</sub> micelle suspension (R=1) is the following : In 2 ml of the previously described micelle suspension, 65µl of NaOH (2.5.10<sup>-1</sup>M) are added in order to complete Al hydrolysis up to a final OH/Al ratio of 3. The solid content in hybrid particles in the present suspension is 0.45 wt.%. The particle suspensions can be dried and redispersed at the desired concentration. Suspensions are studied by TEM and diffusion techniques at least one day after the metal ion hydrolysis step.

#### *Transmission electron microscopy*

TEM specimens were prepared by depositing a drop of colloidal suspension onto glow-discharged carbon-coated copper grids. After 1 min, the liquid in excess was blotted with filter paper and the remaining film allowed to dry. Negatively stained samples were also prepared by depositing a drop of 2 wt.% uranyl acetate prior to sample drying. TEM images of dried samples were recorded at room temperature with a Jeol 1200 EXII microscope operated at 80 kV. According to a method described elsewhere<sup>[9-12]</sup>, cryo-TEM samples were prepared by quench-freezing thin films of the aqueous colloidal suspensions into liquid ethane cooled down to -171 °C ; colloidal particles are then embedded in a film of vitreous ice. The specimen were mounted onto a Gatan 626 cryo-holder, transferred into the microscope and observed at low temperature (-180 °C), using a Philips CM200 'Cryo' microscope operated at 80 kV.

## **Results and discussion**

### **Colloids of hybrid PIC micelles**

As it was shown earlier, the present block copolymers, PAA-b-PHEA and PAA-b-PAM, in solution at pH 5.5 behave as soluble polymers in water<sup>[4]</sup>. No polymer association, which could be due to H bonding, is observed at that pH.

By mixing DHBCs with metal cationic entities, the spontaneous formation of colloidal objects is observed<sup>[11,13]</sup>. Depending on the polymer molecular weight, suspensions appear clear or turbid; the colloids are very stable in any case. Measurement of the hydrodynamic sizes of the colloids by DLS shows that the objects are 3 to 4 times larger than the initial polymer coils in solution<sup>[4]</sup>.

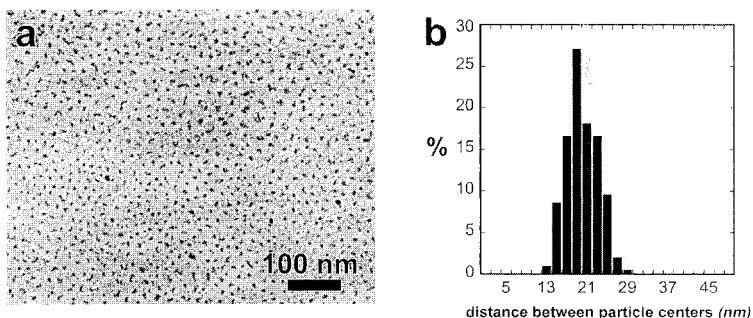


Figure 2. a) Cryo-TEM image of hybrid micelles of  $\text{Al}_{13}^{7+}$  and  $\text{PAA}_{2800}\text{-b-PHEA}_{11100}$ ; b) histogram indicating the mean distance between 200 particles embedded in vitreous ice.

As presented in a previous paper <sup>[1]</sup>, a direct observation of the colloids by TEM at cryogenic temperature could be obtained by quench-freezing liquid films of suspensions and then observing the sample at cryogenic temperature. The advantages of cryo-TEM techniques for direct imaging of colloids and polymer solutions has already been emphasized in the case of amphiphilic block copolymer assemblies <sup>[14]</sup>. This analysis allows to observe colloids as they are in suspension, avoiding any structural change eventually caused by a drying step. The cryo-TEM image shown on figure 2 represents colloids obtained by mixing  $\text{Al}_{13}^{7+}$  and  $\text{PAA}_{2800}\text{-b-PHEA}_{11100}$ . Well dispersed objects of isotropic shape are observed. The TEM image reveals the inorganic-rich part of the colloids which is denser in electrons. It shows that mixing anionic-neutral DHBCs with  $\text{Al}_{13}^{7+}$  leads to a controlled nanophase separation: complexation of metal cationic entities by the anionic block of the DHBCs leads to segregation of inorganic entities in nanodomains. In figure 2, the mean TEM size of the objects equals 9 nm is much smaller than the hydrodynamic size  $D_h$  obtained by DLS (*number-average*  $D_h=30$  nm, *intensity-average*  $D_h=47$  nm). This observation confirms that the TEM image only reveals the inorganic-rich core of the colloids. It is interesting to note on figure 2 that the distribution of distances between centers of inorganic-rich particle cores is very narrow (see the distribution on figure 2 b). That suggests that particles must be in close contact, forming a film where polymeric coronas touch each other. The value of the mean distance measured between core centers equals  $20 \pm 3$  nm, suggesting that particle coronas may interpenetrate.

In order to visualize the whole colloidal aggregates, TEM images of colloids stained with a uranyl solution were recorded. They could then be directly compared to images obtained on

the dried samples without staining. These experiments were done by using a larger copolymer having a bigger neutral block in order to make the observation of the stained colloids easier. As larger and asymmetric PAA-b-PHEA polymers were not available, we chose to work with a PAA-b-PAM type polymer, it exhibits properties of solubility in water similar to those of PAA-b-PHEA polymers. Figure 3 shows a TEM image of a dried sample of micellar aggregates of  $\text{AlI}_3^{7+}$  and  $\text{PAA}_{3000}$ -b- $\text{PAM}_{30000}$  which were stained with  $\text{UO}_2^{2+}$ .

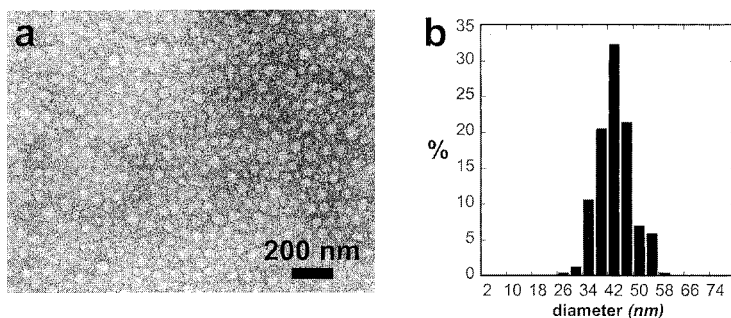


Figure 3. a) TEM image of a dried sample of micellar aggregates of  $\text{AlI}_3^{7+}$  and  $\text{PAA}_{3000}$ -b- $\text{PAM}_{30000}$  negatively stained with  $\text{UO}_2^{2+}$ , at room temperature ; b) size-distribution histogram calculated from 450 particles on a negatively stained specimen.

The objects appear now lighter than the dark  $\text{UO}_2^{2+}$  rich background ; they are quite regular in size (figure 3b) and their shape seems isotropic. The mean diameter measured from TEM images of the dried and stained micelles equals  $42 \pm 5$  nm (figure 3b), it is very close to the hydrodynamic diameter (*intensity*-average  $D_h=48$  nm) of the colloids. TEM pictures a dried suspension of those micelles were difficult to obtain due the combination of the two following facts : a very small size and an insufficient contrast of the PAA-Al complex core with the grid support. The pH was then increased up to 7 in order to make the inorganic-rich core denser due to polycondensation of Al cations. The pH increase leads to a concentration of metal ions rich in electrons, which seems to confer the micelle core a sufficient density and maybe rigidity ; this allows a better observation thanks to a higher contrast with the carbon grid support. A room temperature TEM image on figure 4 shows the inorganic-rich cores of the dried colloids which have a mean size of about 7 nm.

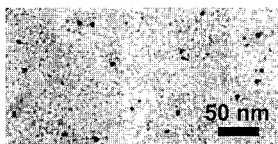


Figure 4. TEM image of unstained micellar aggregates of  $\text{Al}_{13}^{7+}$  and  $\text{PAA}_{3000}\text{-b-PAM}_{30000}$  (after a pH increase to 7).

The use of different TEM techniques combined with DLS measurements allows to propose a description of the nanostructure of the colloids. As proposed in figure 5, complexation of metal cationic entities by oppositely charged DHBCs leads to the formation of colloids constituted of a small core made of inorganic species and collapsed polyacrylate blocks and a diffuse corona of neutral PHEA or PAM blocks. This was confirmed by small angle neutron scattering studies (SANS) <sup>[4]</sup> which support a core-corona architecture of the colloids. SANS results show that the interface between the core and the corona is well defined and sharp ( $I$  varies as  $q^4$ ) and that the corona is made of polymer chains in a good solvent ( $I$  varies as  $q^{-1.7}$  at high  $q$  values). Direct imaging and indirect structure characterization techniques such as DLS and SANS are clearly complementary to study organic-inorganic core-corona systems. Other authors have reported on the advantages of combining DLS and cryo-TEM to investigate polymer-based suspensions <sup>[14]</sup>.

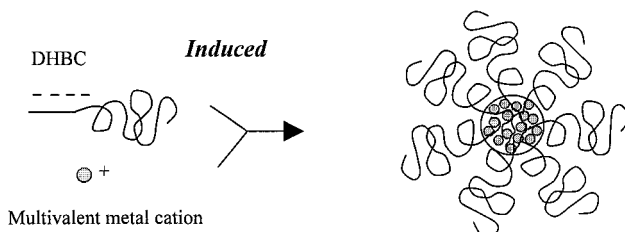


Figure 5. Schematic representation of the hybrid polyion complex micelles.

### Hybrid colloids obtained by suspension mineralization

The organic-inorganic polyion complex micelles were then used as precursors for inorganic polycondensation reactions in suspension. Sodium hydroxide was added for hydrolysis and condensation of metal cations in order to form metal hydroxide-based particles. Depending on the metal complexation degree,  $R$ , expressed as the number of acrylate functions per metal atom, particles resulting from mineralization present different

sizes<sup>[1]</sup>. It was observed that particle sizes increase as R decreases, and the minimum particle size is fixed by the size of the micellar precursor. As briefly explained in reference 1, the micellar aggregates are the true precursors of the metal hydroxide particles. The micelles are reservoirs of inorganic ions. For high R values, they act as closed nanoreactors leading to mineralization confined within the core of the micelle, whereas for low R values, metal hydrolysis leads to metal hydroxide particle growth and the final size of the particle is a function of the copolymer amount. Figure 6 shows TEM images at room temperature of dried particles obtained for two different values of R for system  $\text{Al}_{13}^{7+}$  and  $\text{PAA}_{1900}\text{-b-PHEA}_{8200}$ . For R=1 (figure 6a), the precursor micelle and corresponding particle have similar structure and size (Dh=41 nm), while for R=0.6 (figure 6b), bigger metal hydroxide-based particles are formed (Dh=153 nm). On figure 6b, one can guess the presence of the polymeric corona around the mineral particle. As the PHEA blocks are water-soluble, the corona appears as a very fuzzy shape.

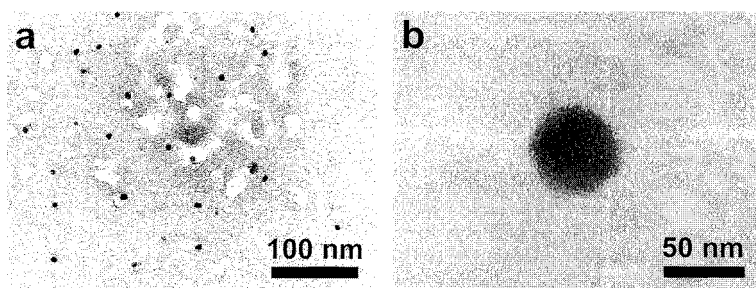


Figure 6. TEM images at room temperature of particles obtained by inorganic condensation in suspension of micelles of  $\text{Al}_{13}^{7+}$  and  $\text{PAA}_{1900}\text{-PHEA}_{8200}$ , for R=1 (a) and R=0.6 (b).

In order to better observe the polymer chains in the corona, a bad solvent of the corona blocks was added to the particle suspension. Figure 7 shows the case of particles obtained by hydrolysis of Al ions in a micelle formed from  $\text{Al}_{13}^{7+}$  and a polymer of higher molecular weight ( $\text{PAA}_{5000}\text{-b-PAM}_{30000}$ ), with R=1, in a 1:1 (v/v) mixture of dioxane and water. By adding dioxane to the aqueous particle suspension, particles acquire a core-shell structure : the collapse of the PAM polymer blocks, insoluble in dioxane, leads to the formation of a dense shell which seems to perfectly protect the Al hydroxide particle. The interface between PAM blocks and the solvent appears now as a sharp interface.



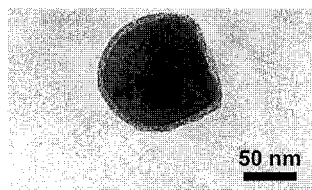


Figure 7. TEM image at room temperature of a PAA<sub>5000</sub>-b-PAM<sub>30000</sub>/ Al hydroxide particle obtained with R=1, in a 1:1 (v/v) mixture of dioxane and water.

The presence of the polymeric corona attached to the mineral core confers a very high colloidal stability to the aqueous particle suspension. The polymer-protected particles are highly stable with dilution and with an increase in ionic strength (up to 2.8M).

## Conclusion

This paper presents a route to prepare highly stable hybrid inorganic-polymeric complex colloids in aqueous medium. They are obtained by complexation between multivalent metal cations and oppositely charged DHBCs and can be mineralized in suspension to give rise to polymer-metal hydroxide particles. A combination of different TEM analyses allowed to obtain a complete picture of the hybrid core-corona nanoparticles. Performing TEM experiments at room temperature on dried films without and with staining together with observations at cryogenic temperatures of the quenched suspensions allowed to distinguish the inorganic-rich dense core and the soluble polymeric corona. Finally, the use of solvent mixtures is very convenient to reveal water-soluble polymer coronas and colloids architecture.

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