UNIVERSITÉ DU QUÉBEC À RIMOUSKI

L'effet de la force ionique et du contenu en oxygène dissous sur l'agrégation et la dissolution de divers types de nanoparticules d'argent

Mémoire présenté

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«Ceux qui rêvent éveillés ont conscience de milles choses qui échappent à ceux qui ne rêvent qu'endormis.» *Edgar Allan Poe* extrait d'*Éléonora*

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RÉSUMÉ

L'utilisation accrue des nanoparticules d'argent (AgNP) pour leurs propriétés antibactériennes augmentera leurs rejets dans l'environnement aquatique, mais les effets et le comportement des AgNP dans l'environnement sont encore mal connus. La dissolution des AgNP en ions argent (Ag^+) est un danger potentiel pour beaucoup d'organismes marins. Il est donc important de comprendre le mécanisme de cette dissolution dans les eaux naturelles. Dans cette étude, la libération d'ions argent et l'agrégation de différents types de nanoparticules (nues, avec recouvrement de citrate ou de polyallylamine [PAAm]) ont été étudiées dans différents types d'eau pendant 16 jours. La concentration d'oxygène dissous des eaux était maintenue à saturation dans une série d'échantillons alors que des conditions hypoxiques (< 3 $mg \cdot L^{-1}$) ont été maintenues dans une seconde série. Une forte concentration de départ en ions argent et un phénomène de réadsorption ont été observés dans les eaux à faible force ionique pour les AgNP sans recouvrement et celles avec le PAAm. Les AgNP s'agglomèrent dans tous les types d'eau, sauf quelques exceptions. Une forte agglomération est observée en eau de mer, mais elle est accompagnée aussi d'une dissolution importante. La dissolution dans l'eau de mer a été plus importante dans l'ordre suivant : AgNP citrate > AgNP PAAm > AgNP nues. En général, la variation de l'oxygène dissous semble avoir peu ou pas d'effet sur la dissolution des AgNP, mais semble influencer l'agrégation. Quelques mécanismes sont présentés pour expliquer les résultats obtenus.

Mots clés : [Nanomatériaux; Nanoparticules; Argent; Dissolution; Agglomération; Oxygène; Citrate; Polyallylamine; nues; Eau naturelle; Eau de mer, Eau de rivière]

ABSTRACT

The massive use of silver nanoparticles (AgNP) for their antibacterial properties increases their potential release into the aquatic environment. The effect and behavior of AgNP in the environment are still poorly known. The dissolution of AgNP into free silver ions (Ag^+) is a potential risk for many aquatic organisms. It is thus important to understand the dissolution mechanism in natural waters. In this study, the formation of free silver ions and the aggregation of different types of AgNP (bare, citrate- or polyallylamine [PAAm]-coated) were observed in different types of water during 16 days. The concentration of dissolved oxygen was regulated at saturation in one series of samples whereas a hypoxic level (< 3 mg·L^{-1}) was maintained in another series. A high starting concentration of silver ions followed by a re-adsorption process was observed in water with low ionic strength for bare and PAAm-coated AgNP. AgNP aggregated in most types of water, except in some particular cases. A strong aggregation was observed in seawater, but an important dissolution was also observed. The dissolution in seawater followed the order: AgNP-citrate > AgNP-PAAm > bare AgNP. The variation of dissolved oxygen did not have a clear effect on dissolution, but had some effects on aggregation. A number of mechanisms are proposed to explain our results.

Keywords : [Nanomaterial; Nanoparticle; Silver; Dissolution; Aggregation; Oxygen; Citrate; Polyallylamine; Bare; Natural water Seawater, River water]

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INTRODUCTION GÉNÉRALE

Une nanoparticule est définie comme un objet dont l'une des dimensions est comprise entre 1 et 100 nm. Les nanomatériaux possèdent des propriétés mécaniques, catalytiques et optiques ainsi qu'une conductivité électrique qui résultent directement de leur taille nanométrique. Les nanoparticules peuvent être manufacturées, mais sont aussi présentes naturellement dans l'environnement (Klaine et al. 2008). Leurs formes et leurs tailles varient selon la méthode de synthèse. Il est possible de produire des nanomatériaux sphériques, cylindriques, triangulaires, étoilés, et autres et pouvant aller de quelques nm à une centaine de nm. Ces nanomatériaux peuvent être composés de carbone, de métaux comme l'or et l'argent, d'oxydes de métaux comme l'oxyde de titane, l'oxyde de zinc et l'oxyde de manganèse (Klaine et al. 2008). Les nanoparticules naturelles ont toujours été présentes dans l'air, l'eau et les sols, mais jamais formellement identifiées et des noms différents leur ont été donnés selon le compartiment environnemental où elles ont été trouvées. Dans l'air, les nanoparticules sont appelées « particules ultrafines » et dans l'eau et les sols, elles sont considérées comme des colloïdes (Klaine et al. 2008).

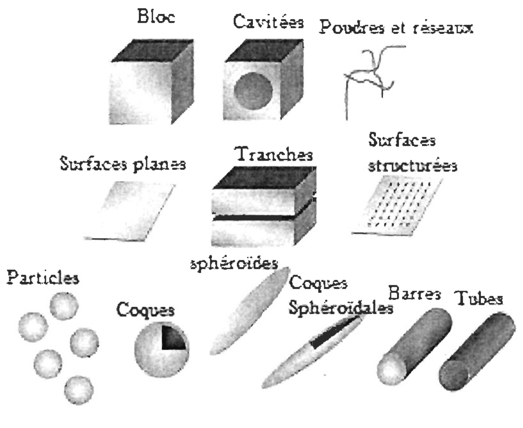


Figure 1 : Différentes formes de nanoparticules (Opensource Handbook of Nanoscience and Nanotechnology par Kristian Molhave).

Les propriétés antibactériennes de l'argent sont connues depuis l'Antiquité par les Romains, les Grecs, les Égyptiens et d'autres anciennes civilisations. Ils utilisaient des récipients en argent pour stocker les denrées périssables et utilisaient aussi ce métal pour désinfecter et soigner les blessures et brûlures. Des publications récentes montrent que l'argent est toxique pour certains organismes marins (Navarro et al. 2008), sous forme ionique ou nanoparticulaire. Les propriétés antibactériennes de l'argent ne sont cependant pas universelles car certaines communautés bactériennes ne sont pas affectées par la présence du métal (Panyala et al. 2008; Fabrega et al. 2009; Marambio-Jones and Hoek 2010; Fabrega et al. 2011; Doiron et al. 2012). L'argent se trouve naturellement dans les eaux, mais à des concentrations faibles. La combinaison des propriétés antibactériennes de l'argent et le développement de méthodes de synthèse à grande échelle des nanoparticules ont contribué à la naissance de l'industrie des nanoparticules d'argent (AgNP). Les nanoparticules d'argent ont vu leur utilisation dans l'industrie exploser au cours de la dernière décennie. Elles sont de plus en plus utilisées dans différents domaines, comme dans l'industrie des vêtements anti-odeurs, des produits cosmétiques et domestiques, des emballages alimentaires et d'autres produits vendus comme antibactériens (Doiron et al. 2012; Echegoyen et Nerin, 2013).

Si d'une part on observe une augmentation exponentielle de leur utilisation dans les produits de consommation, on peut supposer que d'autre part leur présence dans l'environnement augmente tout autant. Les industries peuvent déverser leurs produits ou déchet industriels dans les eaux usées et les stations d'épuration, mais aussi directement dans les rivières ou estuaires et le milieu marin avoisinant. Des études ont aussi montré que des AgNP sont relarguées à partir des produits domestiques pendant leur utilisation ou la lessive des vêtements. Les vêtements anti-odeurs contenant de nanoparticules d'argent perdent la plus grande partie de leur argent particulaire après 2 ou 3 lavages (Benn et Westerhoff, 2008). Une migration des AgNP contenues dans des emballages alimentaires a été démontrée comme possible par Echegoyen et Nerin (2013). La Figure 2 (Pan et Xing, 2010) représente les routes que peuvent emprunter les nanoparticules pour se retrouver dans l'environnement.

Dans le milieu marin, la capture des AgNP par les organismes peut se faire de différentes façons, en particulier par ingestion, que la particule soit individuelle et isolée ou sous la forme d'un auto-agrégat de particules. Les nanoparticules peuvent aussi avoir un accès direct aux organes en traversant les branchies, les organes olfactifs ou les parois corporelles de l'organisme du fait de leur taille nanométrique (Moore, 2006). Si les AgNP sont ingérées ou incorporées par l'organisme, la surface des nanoparticules peut alors générer des ROS (dérivés réactifs de l'oxygène) en s'oxydant. Les ROS peuvent endommager les cellules et aussi dégrader l'ADN (Moore, 2006; Klaine et al. 2008). La présence d'AgNP dans l'environnement représente donc un risque certain pour la santé des organismes vivants puisque la forme nanoparticulaire tout autant que la forme dissoute présentent des effets toxiques reconnus.

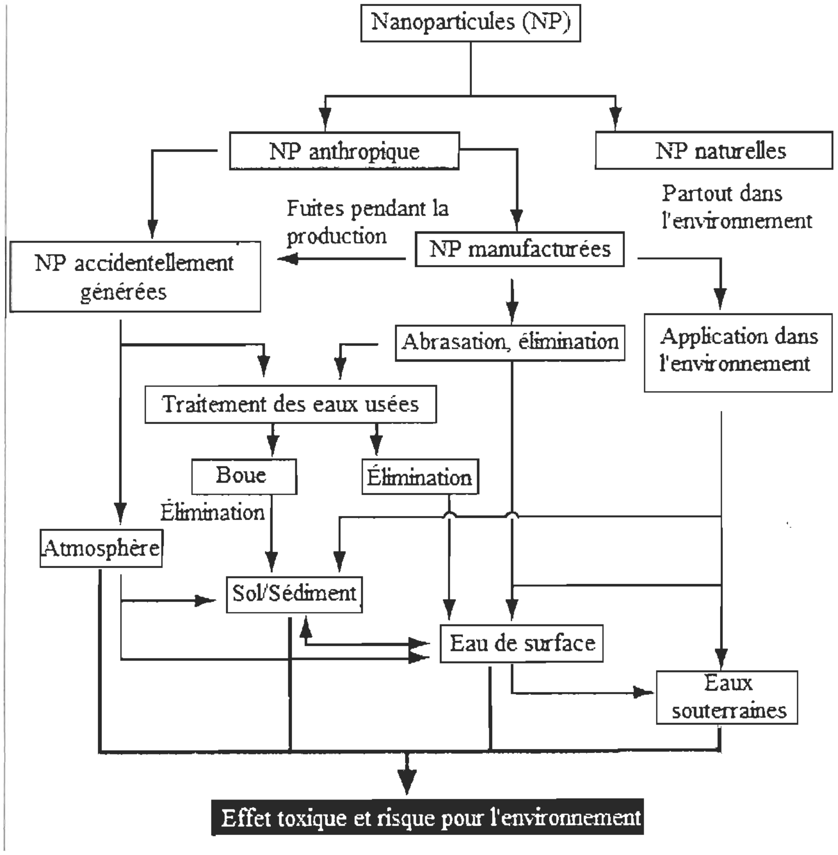


Figure 2 : Parcours des AgNP dans l'environnement (traduit de Pan et Xing, 2010)

Les nanoparticules dans l'eau sont sujettes à l'agrégation et à la dissolution. Plusieurs études ont montré que l'agrégation des nanoparticules est influencée par la force ionique du milieu. Donc, les nanoparticules formeront des agrégats plus gros et plus rapidement dans l'eau de mer que dans de l'eau de rivière. L'équation [1] représente la dissolution de AgNP dans de l'eau contenant de l'oxygène dissous. La dissolution procède par une oxydation de la surface des nanoparticules pour libérer les ions d'argent. Sans oxygène, les nanoparticules d'argent ne devraient pas se dissoudre, comme l'ont observé Liu et Hurt (2010).

Eq.[1] $$2AgNP_{(s)} + O_{2(aq)} + 4\ H^{+}_{(aq)} \leftrightarrow 2Ag^{+}_{(aq)} + 2\ H_2O_{(l)}$$

Dans un environnement oxygéné, les nanoparticules devraient se dissoudre et ne pas persister. L'impact de la variation de la concentration en oxygène dissous sur la dissolution n'a pas encore été étudié. Liu et Hurt (2010) ont montré en laboratoire que dans un milieu anoxique, les nanoparticules d'argent ne se dissolvent pas. Après une étude sur la dissolution des nanoparticules en eau déionisée pendant 125 jours, Kittler et al. (2010) ont constaté que les nanoparticules ne se dissolvaient pas entièrement, ce qui implique une réaction très lente. De plus, l'oxydation des nanoparticules d'argent en milieu oxygéné produit des peroxydes intermédiaires qui sont plus réactifs que l'oxygène moléculaire et accélèrent la dissolution (Liu et Hurt, 2010).

Les AgNP dans les eaux naturelles

La composition chimique des eaux varie fortement selon que l'on étudie le milieu lacustre ou le milieu marin. Les différences des propriétés physico-chimiques entre les deux milieux sont importantes et le comportement des nanoparticules sera différent.

1) Comportement des AgNP dans l'eau déionisée

Dans l'eau déionisée, les interactions des nanoparticules entre elles et avec le milieu sont limitées puisque cette eau est presque entièrement dépourvue d'ions et aussi de matière organique. Les nanoparticules d'argent ne devraient pas s'agglomérer, mais on devrait observer une dissolution causée par la présence de l'O_2 dissous qui oxydera les nanoparticules d'argent en surface comme montrée par l'équation 1.

2) Comportement des AgNP dans les eaux des stations d'épuration

L'efficacité de l'enlèvement des nanoparticules part les systèmes d'épuration des eaux usées est controversée (Kaegi et al. 2013). Une forte stabilité de la suspension des AgNP recouvertes par une substance organique n'implique pas une plus grande mobilité des AgNP dans l'environnement (Kaegi et al. 2013). Les résultats de Kiser et al. (2010) suggèrent que les nanoparticules avec un recouvrement peuvent passer à travers un système d'épuration plus facilement que des nanoparticules non recouvertes. Lors de la simulation d'un réacteur séquentiel discontinu pour le traitement des eaux usées, Hou et al. (2012) ont observé un retrait quasi-total des nanoparticules recouvertes de citrate (AgNP-citrate). Pourtant, une autre étude similaire n'avait montré qu'un faible taux d'enlèvement des nanoparticules lors du traitement primaire des systèmes d'épuration (Jarvie et al. 2009). Le comportement des nanoparticules dans les systèmes d'épuration n'est pas encore bien connu, bien que des transformations chimiques et physiques surviennent certainement dans les eaux usées, puisque celles-ci contiennent des fortes concentrations de sulfures et de thiols (Nielsen et al. 2008). À cause de la forte dilution du système d'épuration et de la précipitation des biosolides, les nanoparticules sont plus susceptibles d'entrer en collision avec des molécules autres que des nanoparticules et donc, une hétéro-agrégation et/ou une déposition peuvent déterminer le comportement et le devenir des nanoparticules dans un système d'épuration (Kaegi et al. 2013).

3) Comportement des AgNP dans les eaux douces

Dans les eaux douces, le comportement des nanoparticules d'argent est plus complexe, car il sera grandement influencé par la composition de l'eau et sa concentration en matière organique. Plusieurs études ont été consacrées à la dissolution et l'agrégation des AgNP dans les eaux douces (Prathna et al. 2011 ; Levard et al. 2012 ; Li and Lenhart, 2012 ; Dobias et al. 2013). Puisque la composition chimique et les propriétés physiques des systèmes étudiés ainsi que le type et la taille de nanoparticules utilisées dans ces études sont différents, elles ne peuvent pas être comparées directement, mais, en général, une agrégation et une dissolution des nanoparticules ont été observées.

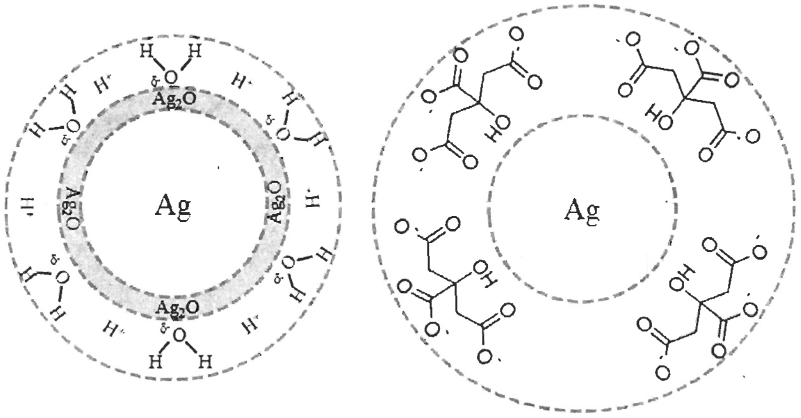
4) Comportement des AgNP dans l'eau de mer

Plusieurs études ont montré l'effet des chlorures sur les nanoparticules d'argent. Dans les eaux riches en chlorures, comme l'eau de mer, l'interaction de l'argent avec les ions chlorures vient compliquer le devenir des nanoparticules. L'argent dissous peut former des chlorures d'argent AgCl insolubles, mais avec des concentrations en chlorures plus importantes, on observe aussi la formation de complexes anioniques $AgCl_x^{(x-1)-}$ tel illustré dans l'équation 2 (Levard et al. 2012). Comme le composé AgCl est insoluble dans l'eau douce, l'argent dissous provenant d'une nanoparticule et qui est lié au chlorure n'est plus disponible, cependant les ions $AgCl_2^-$, $AgCl_3^{2-}$ et $AgCl_4^{3-}$ demeurent en solution et peuvent donc interagir avec les différentes composantes du milieu marin, incluant les AgNP présentes. Levard et al. (2013) ont d'ailleurs montré que la vitesse de dissolution des nanoparticules était liée à la concentration des ions chlorures dans l'eau. Plus la concentration des chlorures était grande, plus la dissolution des nanoparticules était rapide. Ces résultats indiquent que les nanoparticules d'argent ne devraient pas persister en milieu marin. Il y a peu d'études pour confirmer ou infirmer ces résultats sur l'agrégation et la dissolution des nanoparticules d'argent dans le milieu marin.

Eq [2] $$\mathbf{Ag_{(aq)} + Cl_{(aq)} \leftrightarrow AgCl_{(s)} \leftrightarrow AgCl_2{}^{(-)}{}_{(aq)} \leftrightarrow AgCl_3{}^{(2-)}{}_{(aq)} \leftrightarrow AgCl_4{}^{(3-)}{}_{(aq)}}$$

Recouvrement des NP et effet sur le devenir

Pour améliorer la stabilité des nanoparticules d'argent dans l'eau, un enrobage de molécule organique peut être appliqué sur les nanoparticules d'argent. La molécule la plus couramment utilisée est l'ion citrate, mais d'autres types de recouvrement existent comme le polyvinylpyrrolidone (PVP) et le polyallylamine (PAAm). En fonction du recouvrement, les nanoparticules ont des comportements différents quant à la résistance à l'agrégation ou à la dissolution. Une AgNP sans recouvrement est jusqu'à un certain point électrostatiquement stabilisée contre l'agglomération à cause de sa charge de surface négative (Levard et al. 2012). Cette charge vient de la présence de groupements négatifs, -hydroxo (OH^-) et –oxo se trouvant dans le milieu. Ces charges négatives limitent l'agglomération des nanoparticules car deux particules ayant la même charge se repoussent. Enrober de citrate la même nanoparticule augmentera le nombre de groupements –hydroxo et –oxo à sa surface et devrait réduire l'agrégation des nanoparticules dans l'eau ayant une faible force ionique (Levard et al. 2012). Donc, dans l'eau déionisée, les nanoparticules nues et celles recouvertes de citrate devraient rester stables et ne pas s'agglomérer (Figure 3). Pour produire des nanoparticules stables et recouvertes d'un polymère, le polymère doit porter des groupements thiols ou amines (Sardar et al. 2007). Les nanoparticules d'argent recouvertes de PVP ou de PAAm devraient donc rester stables.



AgNP nue AgNP citrate

Figure 3 : Exemples de la couche de surface des AgNP nues et recouverte de citrate

Comme il a été indiqué plus hauts les AgNP ne devraient pas persister dans un milieu marin. La Figure 4 montre qu'une fois dans le milieu les AgNP seront transportées et diluées vers l'océan et contamineront les organismes pélagiques. Le changement de température, de force ionique et la présence de matière organique avec la profondeur induiront une agglomération des AgNP qui précipiteront au fond de l'océan et pourront contaminer le sédiment et le benthos. Les nanoparticules peuvent aussi s'accumuler ou former des agrégats aux différentes interfaces. Après une accumulation à l'interface, il y aura une floculation des AgNP vers le fond de l'océan.

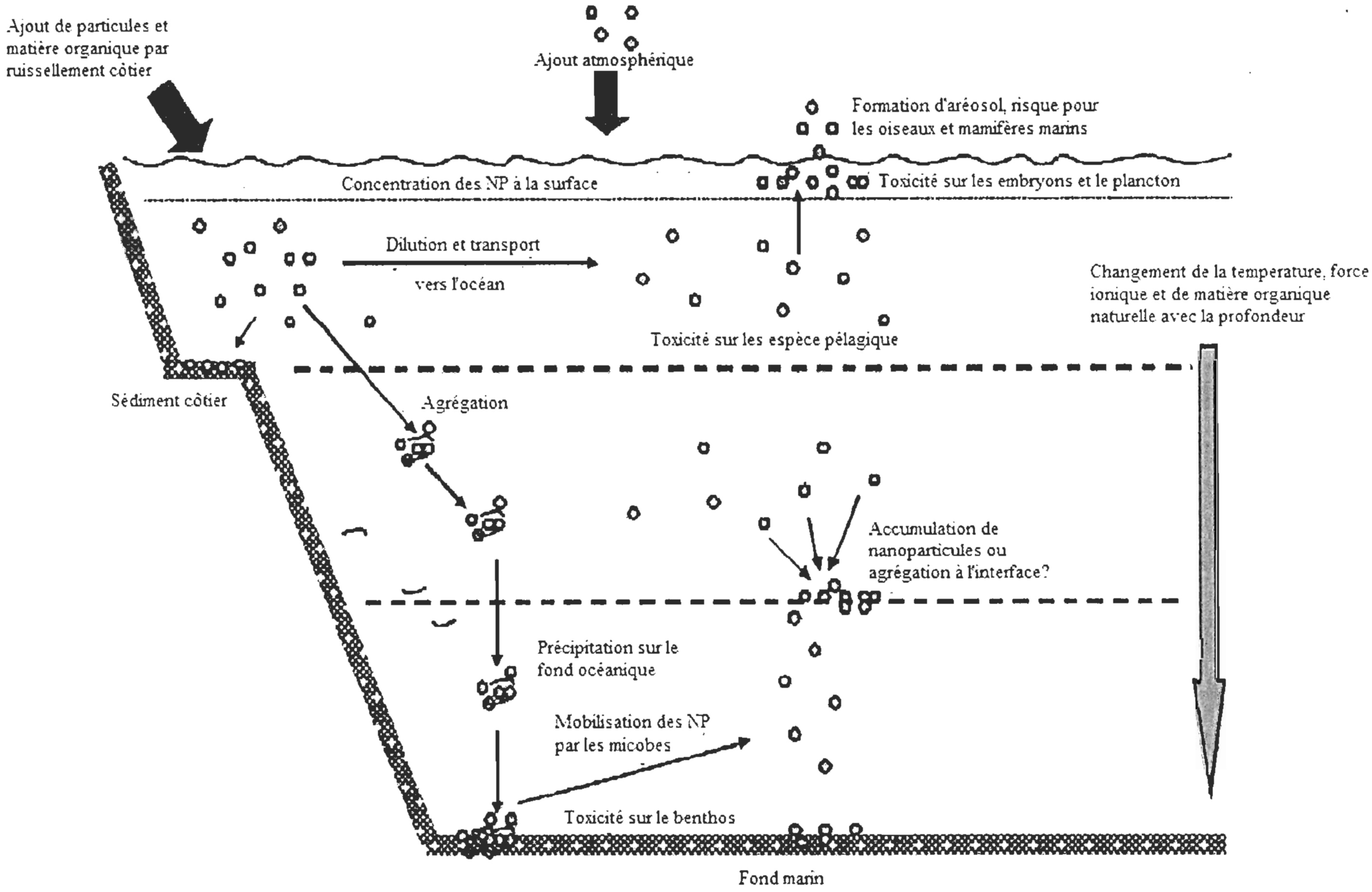


Figure 4 : Comportement des nanoparticules d'argent dans l'eau de mer (traduit de Klaine et al. 2008)

Les objectifs de cette étude sont d'observer et comparer le comportement d'agrégation et de dissolution de trois types de nanoparticules (sans recouvrement, recouvertes de citrate et recouvertes de PAAm) en fonction de plusieurs paramètres qui seront étudiés individuellement ou ensemble. Les paramètres observés sont : la force ionique du milieu, la concentration en oxygène dissous et le type de recouvrement des AgNP. À la suite de ces multiples observations en fonction du temps, notre objectif est de discuter le mécanisme général du comportement des AgNp en fonction des paramètres étudiés.

Les eaux par leurs compositions variées vont influencer différemment le comportement des AgNP, dans l'eau déionisée n'ayant rien pour interagir les AgNP ne devraient pas se dissoudre rapidement, tandis que plus l'eau contient d'ions plus le comportement des AgNP risque de changer. Dans l'eau salée qui contient beaucoup de chlorures, les nanoparticules sans recouvrement devraient se dissoudre rapidement par comparairon avec des AgNP recouvertes d'un polymère. Est-ce que les AgNP restent sous forme nanoparticulaire dans de l'eau douce ou estuarienne ? Ou vont-elles se dissoudre ? L'oxygène aussi est important à prendre en compte puisque l'équation de dissolution des AgNP consomme de l'O_2. Si le milieu est pauvre en oxygène (hypoxie) est- ce que cela va ralentir le processus de dissolution ou plutôt le favoriser ?

Méthode expérimentale

1) Observation de l'agglomération des AgNP

Les méthodes pour observer l'agrégation des nanoparticules sont nombreuses. Beaucoup d'études utilisent un couplage de données obtenues en microscopie électronique à balayage (MEB) et en transmission (MET) avec une moyenne des tailles d'un échantillon analysé par diffraction de la lumière (DLS) au Zetasizer®. Les eaux contenant des hautes concentrations en sels, comme l'eau de mer, posent un problème particulier pour ces méthodes, car le MET observe un échantillon qui a été séché et donc des cristaux de sels se forment et apparaissent sous le microscope. La distinction entre un agrégat de nanoparticules et des cristaux de sel devient très difficile. Pour la méthode DLS, les échantillons d'AgNP dans des eaux naturelles deviennent vite hautement polydispersés, ce qui induit un biais lors des analyses. Donc, l'observation d'agrégats de nanoparticules dans l'eau naturelle est assez complexe. De plus, la mesure par DLS a besoin d'une concentration minimale de nanoparticules d'argent dans l'échantillon afin de fournir une moyenne de la taille statistiquement fiable.

2) Quantification de la dissolution des AgNP

Pour la dissolution, un spectromètre de masse à plasma couplé par induction (ICP-MS) a été utilisé pour analyser et quantifier les valeurs en argent dissous du surnageant après ultracentrifugation ou dialyse des échantillons. En connaissant la quantité d'argent nanoparticulaire qui a été injectée dans le milieu étudié et sachant que la solution ne contenait pas (voir très peu) d'argent dissous, l'augmentation de l'argent dissous indique donc une dissolution en fonction du temps. L'inconvénient de l'utilisation de l'ICP-MS est que les échantillons contenant de l'eau de mer doivent être dilués le plus possible pour éviter des interférences et ne pas encrasser l'appareil après la digestion acide. Il est donc difficile de travailler avec des concentrations très faibles qui reflèteraient les concentrations en AgNP normalement présentes dans l'environnement. Les concentrations utilisées dans cette étude sont de 3625 $\mu g.L^{-1}$ pour les AgNP nues, 265 $\mu g.L^{-1}$ pour les AgNP-citrates et 2550 $\mu g.L^{-1}$ pour les AgNP-PAAm.

Dans cette étude, trois types d'AgNP ont été étudiées : nanoparticules «nues» (sans recouvrement), nanoparticules recouvertes de citrate et nanoparticules recouvertes de polyallylamine (PAAm) (Figure 5). Les comportements d'agrégation et de dissolution ont été observés et mesurés pendant une période de 16 jours pour quatre types d'eau différents : 1) l'eau déionisée ; 2) l'eau de rivière, 3) l'eau de mer diluée et 4) l'eau de mer sans modification. Le dernier paramètre à être pris en compte a été la concentration en oxygène dissous. Les bouteilles contenant l'eau et les nanoparticules ont été bullées à l'air ou à l'azote afin de les saturer en oxygène dissous ou en réduire la concentration selon les différents types d'eau.

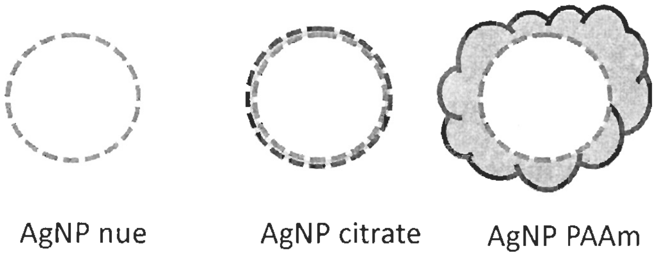


Figure 5 : Représentation des différents types d'AgNP utilisés dans ce mémoire.

Des essais préliminaires ont servi à déterminer le pH des différents types d'eau, des suspensions de nanoparticules et du mélange des deux. Une autre série d'essais a permis de déterminer les concentrations en oxygène dissous obtenues après le bullage des bouteilles avec de l'air ou de l'azote.

CHAPITRE 1

L'effet de la variation de la force ionique et du contenu en oxygène dissous sur l'agrégation et la dissolution de divers types de nanoparticules d'argent.

1.1 RÉSUMÉ ET ABSTRACT

Résumé:

Parmi les nanomatériaux sur le marché, les nanoparticules d'argent (AgNP) sont utilisées de plus en plus pour leurs propriétés antibactériennes dans les vêtements, les emballages alimentaires, les produits cosmétiques, l'électronique et autres. La libération des AgNP durant l'utilisation quotidienne ou le lavage de ces produits introduit des AgNP dans les eaux usées, les eaux douces et les eaux de mer. Peu d'éléments sont connus sur la dissolution des AgNP dans l'environnement et les études qui s'y consacrent se limitent généralement aux eaux douces. Cette étude observe l'agrégation et la dissolution des AgNP possédant différents types de recouvrements : nues, recouvertes de citrate et recouvertes de poly(allyl)amine (PAAm), dans différents types d'eaux (eau déionisée, eau de rivière et eau de mer) et avec ou sans aération pour obtenir une saturation en oxygène ou une valeur similaire à des conditions hypoxiques (< 3 mg·L^{-1}). Les résultats montrent que l'agrégation se produit dans tous les types d'eaux, particulièrement dans l'eau de mer pour tous les types de nanoparticules. La dissolution diffère selon le type de nanoparticules et le type d'eau; l'oxygénation joue un rôle dans certains cas précis. La dissolution dans l'eau de mer se produit plus rapidement pour les nanoparticules recouvertes de citrate que pour celles recouvertes de PAAm. Dans le cas des nanoparticules sans recouvrement, la dissolution atteint un plateau en concentration d'ion argent libre après quelques jours d'exposition.

Mots clés : [Nanoparticule; Argent; Dissolution; Agglomération; Oxygène; Citrate; Polyallylamine; Sans recouvrement; Eau naturelle; Eau de mer, Eau de rivière]

Abstract:

Among nanomaterials on the market, silver nanoparticles (AgNP) are increasingly being used as an antimicrobial agent in clothing, food storage containers, pharmaceuticals, cosmetics, electronics, and optical devices. The release of AgNP from these products during their use, particularly during washing, introduces AgNP into wastewaters, fresh, estuarine and seawater. Little is yet known about the dissolution of AgNP in the aquatic environment and most studies to observe dissolution of AgNP have been conducted in freshwater. This research pays attention to the aggregation and dissolution of AgNP with different coating types: bare, coated with citrate and coated with poly(allyl)amine (PAAm), in different types of water (deionized, fresh and saline waters), and with or without aeration to obtain dissolved oxygen saturation or depletion near the hypoxic level ($< 3\ \mathrm{mg{\cdot}L^{-1}}$). The results show that aggregation occurred in all types of water, particularly in seawater with all types of coating. The dissolution of AgNP differed with coating and type of water; oxygen levels also played a role in some rare cases. Dissolution in seawater happened faster for citrate-coated AgNP than PAAm-coated ones and seems to achieve a plateau after a few days for uncoated AgNP. The dissolution is faster with oxygen saturation for uncoated and PAAm-coated AgNP, and faster at low oxygen levels for citrate-coated AgNP.

Keywords : [Nanoparticle; Silver; Dissolution; Aggregation; Aggregation; Oxygen; Citrate; Polyallylamine; Bare; Natural water; Seawater, River water]

1.2 EFFECT OF THE VARIATION OF IONIC STRENGTH AND DISSOLVED OXYGEN CONTENT ON AGGREGATION AND DISSOLUTION OF DIFFERENT TYPES OF SILVER NANOPARTICLES.

Introduction

The use of nanomaterials has increased considerably in the past decade as they are widely used in many different industrial fields such as microelectronics, micro-machineries, optics and composite materials. In the particular case of silver nanoproducts, nanoparticles (AgNP) can be found in cosmetics, clothing, toothpaste, food storage products and in coatings on medical instruments (Wijnhoven et al. 2009, Echegoyen and Nerin, 2013). Silver is mostly used for its antibacterial properties which have been known since ancient time (Huang et al. 2011; Luoma 2008). Recent publications have revealed that silver is toxic for some aquatic organisms in both ionic and nanoparticulate forms as well as harmful for natural bacterial communities in aquatic environments (Panyala et al. 2008; Fabrega et al. 2009; Marambio-Jones and Hoek 2010; Fabrega et al. 2011; Doiron et al. 2012). Navarro et al. (2008) were first to conclude that nanoparticles are a source of free silver ions and these ions contributed directly and mainly to its toxicity to cell cultures of *Chlamydomonas reinhartii*. However, Fabrega et al. (2009) showed that some toxic effects observed for silver nanoparticles cannot be explained only by free silver ions. In experiments on *Pseudomonas fluorescens*, they showed that nanoparticles could have their own effects on bacterial cells.

Natural nanoparticles have always been present in air, water and soil but not clearly identified due to a lack of appropriate instruments and they were named differently depending on the environmental compartment in which they were present. In the air they were referred as ultrafine particles and in water and soil they were considered as colloids (Klaine et al. 2008). The recent intensive use of AgNP has likely increased their presence in the environment and most of the studies on their environmental impacts are limited to natural freshwater (Klaine et al. 2008). It is now recognized that AgNP are released from the products during normal use or washing (Benn and Westerhoff, 2008; Geranio et al. 2009, Echegoyen and Nerin, 2013) and can be found in the aquatic environment, although the analysis of nanoparticles dispersed in environmental samples is still a major analytical challenge (Hull et al. 2012; Gray et al. 2013). As AgNP are released into wastewater after the washing of clothes, they will eventually be present in river water and seawater. Nanoparticles can contaminate soils and be transported to aquatic system by wind and rainwater runoff. Also many industrial discharges could lead nanomaterials directly to estuarine and marine environments (Klaine et al. 2008).

Little is yet known about the behaviour of AgNP in marine ecosystems. Aggregation and dissolution processes must be characterized and quantified since they dictate the long term fate of AgNP and their toxicity (Levard et al. 2012). The changes in the chemical and physical properties of water produced by mixing natural freshwater with marine water, as observed in an estuary are complex and have to be studied in presence of nanomaterials. Properties such as alkalinity, ionic strength, as well as nature and concentration of natural organic matter vary greatly in the estuarine mixing process. The high ionic strength of seawater compared with freshwater will tend to cause aggregation of nanoparticles (Klaine et al. 2008). The aggregation and sedimentation could lead to a deposition of nanoparticles in the sediment (Klaine et al. 2008).

Oxidation of silver nanoparticles in natural waters or in a biological fluid is a surface process requiring the presence of dissolved molecular oxygen and protons with the formation of a water molecule (Eq.1). Thermodynamically, only dissolved oxygen is the only species that can oxidize metallic silver in water (Liu and Hurt, 2010).

Eq. [1] $$2Ag_{(s)} + ½O_{2(aq)} + 2H^{+}_{(aq)} \rightarrow 2Ag^{+}_{(aq)} + H_2O_{(l)}$$

Also depending on their synthesis, nanoparticles can have different shapes and sizes which regulate their physical and chemical properties. A coating agent can also be added on the surface of most metal nanoparticles including AgNP. The most common carboxylic acid used as a capping and reducing agent is citrate, although polymers like polyvinylpyrrolidone (PVP), polyallylamine (PAAm) and some polysaccharides can also be used as capping agents (Levard et al. 2012). The coating can enhance the stability of nanoparticles in water (Li and Lenhart, 2012) but properties of the AgNP can be changed along with their shape and their coating agent in waters with different chemical compositions. It should also be noted that citrate coated AgNP behaviour is thought to be highly affected by changes in pH, ionic strength and organic matter content (Ho et al. 2010).

Because of the high ionic strength of saline waters, researchers have suggested that nanoparticles dispersed in seawater should rapidly aggregate and be found in the sediment biofilms (Klaine et al. 2008). Although a fast aggregation of nanoparticles is expected in seawater, a fast dissolution has also been proposed by some authors (Liu and Hurt, 2010) in such a way that nanoparticles might not contaminate the marine sediment because silver nanoparticles will not be persistent in environmental compartments containing dissolved oxygen. However, other authors observed only a partial dissolution of AgNP, and even after 125 days, silver nanoparticles were still present in the suspension (Kittler et al. 2010). It was concluded that aggregation process should slow down dissolution of nanoparticles by a reduction of the chemically active surface of nanoparticles.

The objective of this study is to improve our understanding of the fate of silver nanoparticles in natural waters in comparison with pure water. The aggregation and dissolution of AgNP (bare, citrate and polyallylamine coated) with different coatings have been studied for 16 days in water of different chemical compositions: deionized water (DW), river water (RW) and seawater (SW) effects of high and low dissolved O_2 concentration were also studied.

2. Material and Methods

2.1 Materials

Silver nitrate powder ($AgNO_3$, >99%), maltose powder (90-95%) and polyallylamine (PAAm) as a viscous liquid were purchased from Sigma Aldrich®. Trisodium citrate powder (>99%) was purchased from SAFC and sodium hydroxide pellets from Anachemia. Ammonium hydroxide (29.2%), nitric acid and hydrogen peroxide solutions were trace metal grade and purchased from Sigma Aldrich. The deionized water (18 MΩ·m resistivity) used in this experiment was provided by a deionized water system Nanopure Infinity^{TM} ultrapure water system from Barnstead. All labware and glassware used in this study were washed using 10% nitric acid and rinsed with deionized water before use.

2.2. Synthesis of silver nanoparticles

Three types of nanoparticles were prepared in our laboratory and used for this study: bare silver nanoparticles (bare-AgNP), citrate-coated silver nanoparticles (citrate-AgNP) and PAAm-coated Ag nanoparticles (PAAm-AgNP).

Suspension of bare- and citrate-AgNP were synthesised following the method of Li and Lenhart (2012) with slight modifications to obtain nanoparticles with a diameter around 40 nm. PAAm-AgNP were synthesised following the method of Sardar et al. (2007) with slight modifications.

In the case of bare-AgNP, in a 50-mL beaker, 2 mL of 0.01 $mol \cdot L^{-1}$ silver nitrate ($AgNO_3$) were poured with 10 mL of 5.84 $mmol \cdot L^{-1}$ ammonium hydroxide, then 250 μL of 0.1 $mol \cdot L^{-1}$ sodium hydroxide and 8 mL of 0.025 $mol \cdot L^{-1}$ D-maltose were added. The solution turned yellow-brown quickly and was stirred with a magnetic bar for 15 min. The suspension was dialysed with 10 kDa molecular weight cut off regenerated cellulose membranes from Thermo Scientific against deionized water over a 24-h period. The dialyzed water was changed at least 4 times every 2 h to remove free silver ions and maltose. Bare-AgNP were stored in the dark at 7 °C for a maximum of one day before use.

Immediatly after dialysis, a sub-sample was analysed for total silver. Another one was ultracentrifuged (50,000 g for 20 min) and the supernatant was recovered and analysed for free silver ions. The bare-AgNP suspension had a total concentration of 438 mg·L^{-1} with 0.26 % free silver ions (1.69 mg·L^{-1})

To prepare citrate-AgNP, 1 mM of trisodium citrate was added to a dialysed bare-AgNP suspension and shaken for 24 h. The suspension was dialysed as described above with a 10 kDa membrane against deionized water for 24 h. Citrate-AgNP suspensions were stored in dark at 7°C for a maximum of 24 h before use. Again, one sub-sample was used for total silver analysis and an other one was ultracentrifuged (50,000g for 20 min) and supernatant analysed for free silver ions. The citrate-AgNP solution had a concentration of 31 mg·L^{-1} with 0.32% free silver ions (0.1 mg·L^{-1}).

For PAAm-AgNP, a volume of 190 mL of deionized water was poured in a 500-mL two-neck round-bottom flask with glass beads. A volume of 5 mL of 200 mg·mL^{-1} silver nitrate and 5 ml of 280 µl·mL^{-1} polyallylamine were added to the flask. The solution was brought to slow ebullition for 60 min. The solution was cooled down and ultrafiltered through Ultracel® 30 KDa (ultrafiltration Disc regenerated cellulose from Millipore®) several times to remove unreacted silver ions. The synthesis was performed in dark. PAAm-AgNP suspensions were stored in dark at 7°C for a maximum of 24 h before use. After ultrafiltration, a sample was analysed for total silver and a sub-sample was ultrafiltered, filtrate was sampled and analysed for free silver ion concentration. PAAm-AgNP suspension had a total silver concentration of 306 mg·L^{-1} with 2.6% free silver ions.

2.3 Experimental setting

The dissolution and aggregation of different types of nanoparticles were observed over a period of 16 days in different types of water and with different concentrations of dissolved oxygen. Modified brown bottles 125 mL (HDPE, Nalgene) were used for the experiment with 2 syringe needlés, one located on the shoulder of the bottle and reaching near the bottom for bubbling air or nitrogen and the other one through the screw cap of the bottle for adding reactants or removing samples. The bottles were filled with 119 mL of one of the 4 different types of water: deionized water (DW), river water (RW), deionized water + 0.5% of seawater (hereafter DSW) or seawater (SW).

Then, some bottles were bubbled with air to obtain a saturated concentration of dissolved oxygen, for 15 min before the experiment and for 5 min after each sampling to maintain oxygen saturation during the experiment. Similarly, in other bottles nitrogen gas was bubbled instead of air to reduce the O_2 level in water samples to reproduce a hypoxic situation. Dissolved O_2 concentration was measured with an oxygen probe YSI 550A from YSI Inc. Bottles were kept in the dark at 7°C. A total of 24 conditions were studied: 4 different water compositions x 3 different types of nanoparticles x 2 different oxygen contents. Each condition was tested in duplicate.

Sampling, injection and bubbling were performed via the needles in a way that the water in bottles had no contact with the atmosphere during the experiment period.

At the starting point of the experiment, 1 mL of one of the three types of nanoparticles (bare-, citrate- or PAAm-AgNP) was injected in the bottles. Concentrations of nanoparticles in bottles were of 3,625 $\mu g \cdot L^{-1}$ for bare-AgNP, 265 $\mu g \cdot L^{-1}$ for citrate-AgNP, and 2,550 $\mu g \cdot L^{-1}$ for PAAm-AgNP. The starting concentrations are different due to an analytical error after dilution of the stock suspension. The stock suspension was analysed by ICP-MS and then a dilution was performed just before starting the experiment, butthe analysis of the diluted stock did not match the concentration desired. The sampling started immediately after the injected of AgNP suspension (t=0) and was repeated after days 1, 2, 3, 4, 6, 8, 10, 13, and 16. Before each sampling, the bottle was vigorously shaken. A volume of 5 mL was sampled from each bottle at each time using a 5-mL syringe. One mL was transferred in a disposable cuvette to determine the size distribution of the nanoparticles using DLS analysis and the rest of the sample was ultracentrifuged at 50,000g for 20 min with an Optima max XP-Ultracentrifuge (Beckman Coulter) for bare- and citrate-AgNP. For PAAm-AgNP separation, ultracentrifugation was not efficient enough and ultrafiltration was performed with a 10 KDa copolymer styrene/butadiene using a centrifugal filter device (Amicon Ultra-4, Millipore); samples were centrifuged at 7,150 rpm for 15 min with a Centrifuge 5430R from Eppendorf. After the separation was completed, 2 mL of the filtrate were sampled; 1 mL was added to 2 mL nitric acid in cryovials in duplicate and stored at 7°C for further digestion (Section 2.4.3) and ICP-MS analysis. The same protocol was used for all types of AgNP.

A second series of experiments was performed to observe short term aggregation of different AgNP over a 6-h period in DW and SW with a saturated concentration of dissolved oxygen using the same conditions as described above. The sampling rate was every 15 min, and only the size distribution of AgNP was determined.

2.4. Characterization

2.4.1. Characterization of natural water sample

The conductivity and pH were measured with a conductivity probe, EC215 Conductivity Meter Hanna instrument, and a pH probe, pH213 Microprocessor pHmeter Hanna instrument, respectively (Table 1). River water (RW) was sampled from Neigette River (QC), a small river mainly draining a forested area with no anthropogenic inputs. Samples for DSW (deionized water + 0.5% of seawater) were obtained by mixing 118.4 mL of deionized water with 0.59 mL of seawater. Seawater samples (SW) were collected from the biological station of ISMER/UQAR, located along the St Lawrence Estuary near Rimouski (QC). All water samples were filtered through 0.2 μm nylon sterile filters (Fisher Scientific) before use in experiments. Major ions of the different types of water were analysed by ICP-MS (Table 1)

	DW	Neigette RW	DSW	SW
Calcium ($mg \cdot L^{-1}$)	< dl	41.0	22.9	458
Magnesium ($mg \cdot L^{-1}$)	< dl	8.47	58.9	1180
Sodium ($mg \cdot L^{-1}$)	< dl	9.46	456	9110
Potassium ($mg \cdot L^{-1}$)	< dl	3.72	15.7	314
Chloride ($mg \cdot L^{-1}$)	< dl	4.50	775	15500
Argent ($\mu g \cdot L^{-1}$)	< dl	0.54	0.01	0.26
pH	5.10 – 5.15	8.02	7.81	7.61
Conductivity($\mu S \cdot cm$)	0.6 – 1.0	255	280	44 000

Table 1 : Major ions and silver concentrations, pH and conductivity of deionized, river and sea water. (< dl, below detection limit)

2.4.2. Diameter and size distribution of nanoparticles

The hydrodynamic diameter and the distribution size of the three AgNP types synthesized were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern instrument UK). The measurement temperature was 7°C, and the scattering angle was 173°. To determine a mean size distribution, six measurements of six successive runs of 15 s each were performed. Disposable cuvettes, pre-washed with 10% hydrochloric acid and rinsed with deionized water and ethanol (100%) were used. Zetasizer measurements can give the mean hydrodynamic diameter from the intensity, volume, or number of particles, in this study all hydrodynamic diameters are from volume calculations. If the hydrodynamic diameter increases greatly it can be assumed that nanoparticles are forming aggregates.

Transmission electron microscopy (TEM, LVEM5 Delong Instrument®) was also used to observe the size, shape and aggregation of AgNP. One drop of a nanoparticles suspension was diluted in an appropriate volume of ethanol (100%) and one drop of the diluted sample was placed on a holey carbon film coated on 400 mesh copper grid from TED PELLA INC.

Table 2 presents the mean distribution size of the different nanoparticle suspensions just before injection into the experimental bottles.

2.4.3. Silver analysis

The total silver content and total ionic silver concentration of AgNP stock suspensions were determined using inductively coupled plasma mass spectrometry (ICP-MS). Before ICP-MS analysis, 2 mL concentrated nitric acid and 0.5 mL hydrogen peroxide were added to samples and, without sealing the cap, samples were put under the hood for 45 min. Digestion was completed in a water bath (65°C) for 45 min. After digestion, 1 mL of digested sample was mixed with 5 mL of DI water, stored at 7 °C and analysed within 2 days.

Silver nanoparticles	Hydrodynamic diameter by DLS (nm)	Diameter by TEM (nm)
Bare-AgNP	41 ± 2 (SD) (n = 6)	33.1 ± 4.9 (SD) (n=43)
Citrate coated-AgNP	46 ± 9 (SD) (n = 6)	35.8 ± 10.2 (SD) (n=63)
PAAm coated-AgNP	337 ± 9 (SD) (n = 6)	19.2 ± 4.9 (SD) (n=87)

Table 2 : Mean diameter of silver nanoparticles using Zetasizer (DLS) (n= number of measures obtained by DLS) and transmission electron microscopy (TEM) (n= number of nanoparticles measured).

The software used for analysis was ChemStation v.3.04, and the ICP was an Agilent ICP-MS 7500C. The element observed was isotope ^{107}Ag. One measurement was a mean of five repetitions; the acquisition time was 0.38 sec for a total time of 1.9 sec for one measurement. The calibration was made from silver standard in 2% HNO_3 (1.000 $mg{\cdot}L^{-1}$) purchased from Certiprep®. The calibration curve had 9 points from 0.75 to 200 $\mu g{\cdot}L^{-1}$ (ppb) with a blank (0 $\mu g{\cdot}L^{-1}$ or ppb). The detection limit is 15 $ng{\cdot}L^{-1}$ and the quantification limit is 120 $ng{\cdot}L^{-1}$

2.4.4. Statistics

All errors are expressed as standard deviation (SD). Differences in conditions were tested with repeated measure ANOVA with 3 factors: nanoparticle types, water types and dissolved oxygen concentrations. Normality of the residues and variance were not reached. Data were transformed but normality was not attained. Results were similar to untransformed data so the database was not modified. All statistical analyses were computed using JMP 10 software.

3. Results

3.1 pH, dissolved oxygen and initial AgNP concentration

The pH was monitored in experimental bottles for one hour following the addition of various nanoparticles species (Table 3). Results indicated that adding nanoparticles in water did not influence the pH, variations not being significantly different between nanoparticle types for the same water composition, except for PAAm-AgNP where pH increased from 5.10 to 6.50 in DW, where the pH was always low and variable due to the very low concentration of buffer ions.

After 1 hour	Without particles	Bare-AgNP	Citrate-AgNP	PAAm-AgNP
DW	5.10-5.20	5.28-5.45	5.27-5.32	6.50
RW	8.02	8.01	8.06	8.05
SW	7.61	7.70	7.59	7.68

Table 3 : pH values of water samples after 1 hour with and without nanoparticles. DW: deionized water; RW: River water; SW: Seawater)

Concentrations of dissolved O_2 were determined for different types of water after bubbling with air or nitrogen (Table 4). Water samples with low ionic strength (DW, RW and DSW) showed saturation levels in oxygen of about 12.7 ± 0.3 $mg \cdot L^{-1}$ whereas SW showed a lower value around 10.8 $mg \cdot L^{-1}$. Nitrogen bubbling did not remove all dissolved oxygen and residual O_2 was around 2.8 ± 0.3 $mg \cdot L^{-1}$for low ionic strength waters and 1.77 $mg \cdot L^{-1}$ for seawater. Taking saturation levels as 100% for each water sample, it is observed that N_2 bubbling reduced O_2 content below 25% saturation and the level decreased slightly with increasing ionic strength.

(mg-L-1)	DW	RW	DSW	SW
Bubbled with air	12.73 ± 0.64	13.00 ± 0.56	12.43 ± 1.00	10.79 ± 0.36
Bubbled with nitrogen (% saturation)	3.08 ± 1.18 (24.2%)	2.80 ± 1.17 (21.5%)	2.58 ± 0.93 (20.8%)	1.77 ± 0.56 (16.4%)

Table 4 : Mean concentrations of dissolved oxygen in mg-L^{-1} for deionized water, river water, diluted seawater and seawater after bubbling with air and nitrogen.

For this experiment, different concentrations of nanoparticles were used. Concentrations of AgNP solutions were analysed just before the beginning of the experiment. Table 5 summarizes the AgNP concentrations and dissolved ionic silver added in bottles. Using the mean diameter of nanoparticles as determined by TEM, the number of nanoparticles was estimated for each type of particles assuming a spherical shape and the absence of aggregation before the beginning of the experiment. The total surface of AgNP was also calculated. Citrate-AgNP was about 10 times less concentrated than the other ones.

Parameters	Bare-AgNP	Citrate-AgNP	PAAm-AgNP
AgNP concentration in bottle (μg·L^{-1})	3650	265	2550
Free silver ions concentration (μg·L^{-1})	10	0.8	65
Estimated number of AgNP (TEM)	2.14E+10	1.22E+09	7.63E+10
Total surface estimated (m^2)	7.33E-05	4.89E-06	8.82E-05

Table 5 : Concentration of AgNP and free silver ions added in one bottle and the number of particles calculated from the concentration and mean size of AgNP.

3.2. Aggregation over 6 hours

In a preliminary experiment, the aggregation of the three types of nanoparticles was studied in DW and SW during 6 h under O_2 saturated condition only (Figure 6). Most nanoparticles aggregated in DW and SW, but at different rates and reached different hydrodynamic diameters (sizes) according to the nature of AgNP used.

Bare-AgNP were faster to aggregate and in a more homogeneous manner. The mean size of particles after 30 min was of 170 nm and reached 302 nm after 1 h, then the size of nanoparticles increased at a slower rate, as some AgNP kept growing with time, but a large portion of particles were still around 300-400 nm in diameter.

The citrate coating protected AgNP from aggregation in DW with a mean diameter ranging between 45 and 160 nm over 6 hours. Similarly, aggregation of citrate-coated AgNP in SW was much less intense than with bare-AgNP, with a size ranging between 45 nm and about 400 nm at maximum. After 30 min, the mean size was around 59 nm and around 127 nm after 2 h.

PAAm-AgNP are of a very particular nature and cannot be directly compared to bare- and citrate-AgNP. As PAAm is the reducing agent in the synthesis of these AgNP, it is understood that the polymer is integrated within the nanoparticles giving the aspect of a hairy ball where the organic coating cannot be displaced or removed from the surface (Sardar et al. 2007). Results in Figures 6C and 6F clearly show that DLS cannot appropriately determine the size of these nanoparticles. Results are highly variable and do not show any trend in DW although in SW, a general increase of the size around 600 nm is visible with a large variation between 400 and 1200 nm.

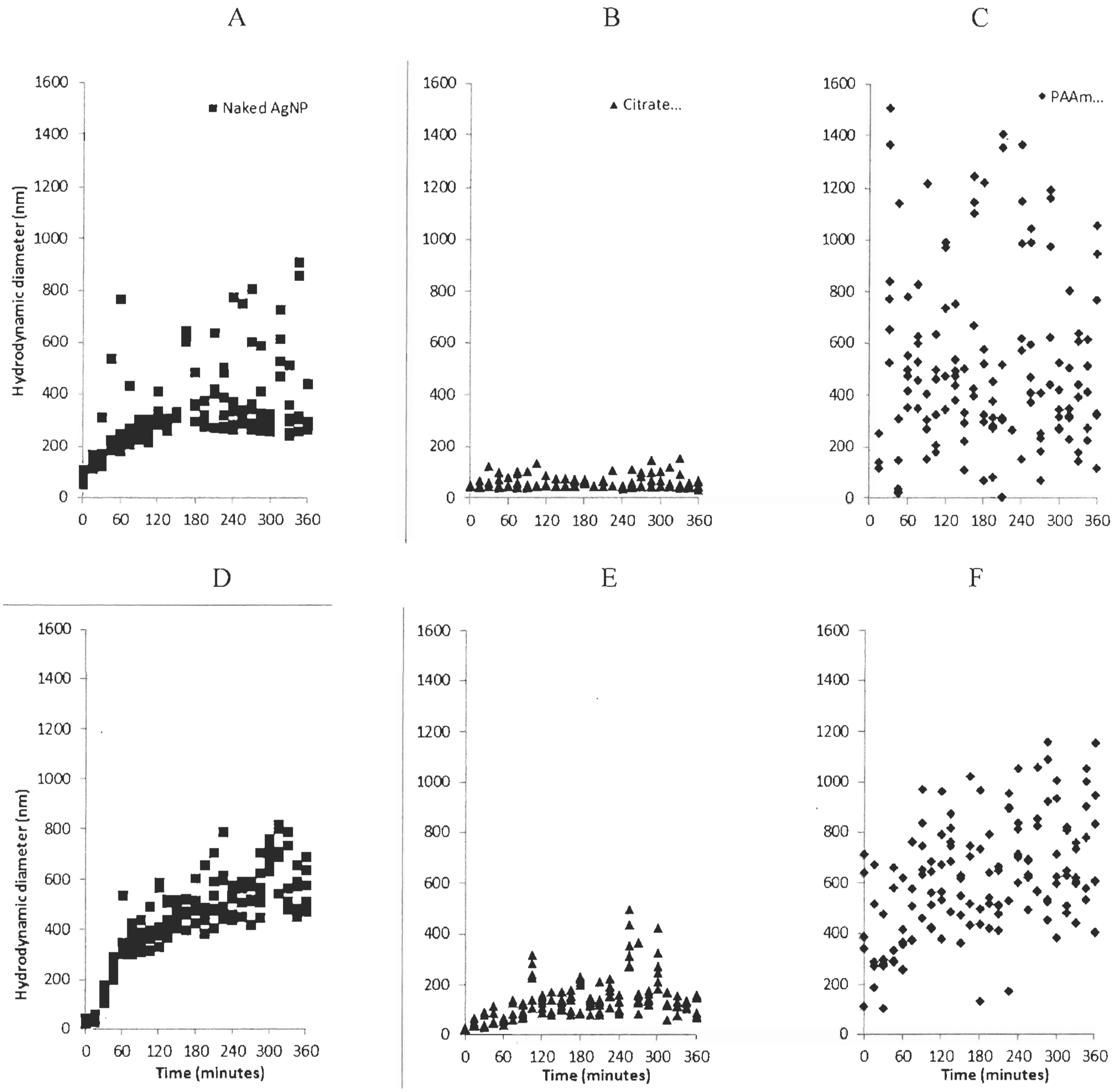


Figure 6 : Changes in hydrodynamic diameter (nm) over 6 hours for (A) Bare-AgNP in deionized water (B) Citrate-AgNP in deionized water (C) PAAm-AgNP in deionized water (D) naked AgNP in seawater (E) Citrate-AgNP in seawater and (F) PAAm-AgNP in seawater.

3.3. Aggregation over 16 days

3.3.1. Bare-AgNP

The change in hydrodynamic diameter of bare-AgNP over 16 days for the four types of water is shown in Figure 7. In DW with saturated oxygen (Figure 7A), an aggregation started at day 2 which increased the mean size of the nanoparticles up to 600 nm in day 6 and tended to slowly decrease to 500 nm at day 16. In strong contrast, bare-AgNP dispersed in low dissolved oxygen DW did not aggregate, and the mean size was observed around 40 nm for the 16 days of the experiment showing an apparent highly significant effect of dissolved O_2 in this particular case (Table 7). An artefact due to a too low concentration of AgNP during DLS analysis cannot be excluded.

When bare-AgNP were dispersed in RW (Figure 7B), aggregation started early for both conditions with a size around 70 nm at day 1. The maximum size of the aggregates was 300 nm at day 6 and do not seemed increase thereafter. No significant differences existed between waters with high and low contents in oxygen (Table 7). Aggregation in DSW (Figure 7C) with low dissolved oxygen concentration showed similarities with aggregation in DW, as it started with no aggregation then, a quick increase in size was observed.

In SW (Figure 7D), aggregation of nanoparticles was very fast. A few minutes after the addition of nanoparticles, a mean size of 200 nm was observed with DLS. The size of particles kept increasing in following days to reach a maximum of 820 nm at day 10. After day 10, the mean size of particles slowly decreased to 600 nm at day 16 in both oxygen saturated and hypoxic conditions. The two conditions evolved similarly with no significant difference between samples.

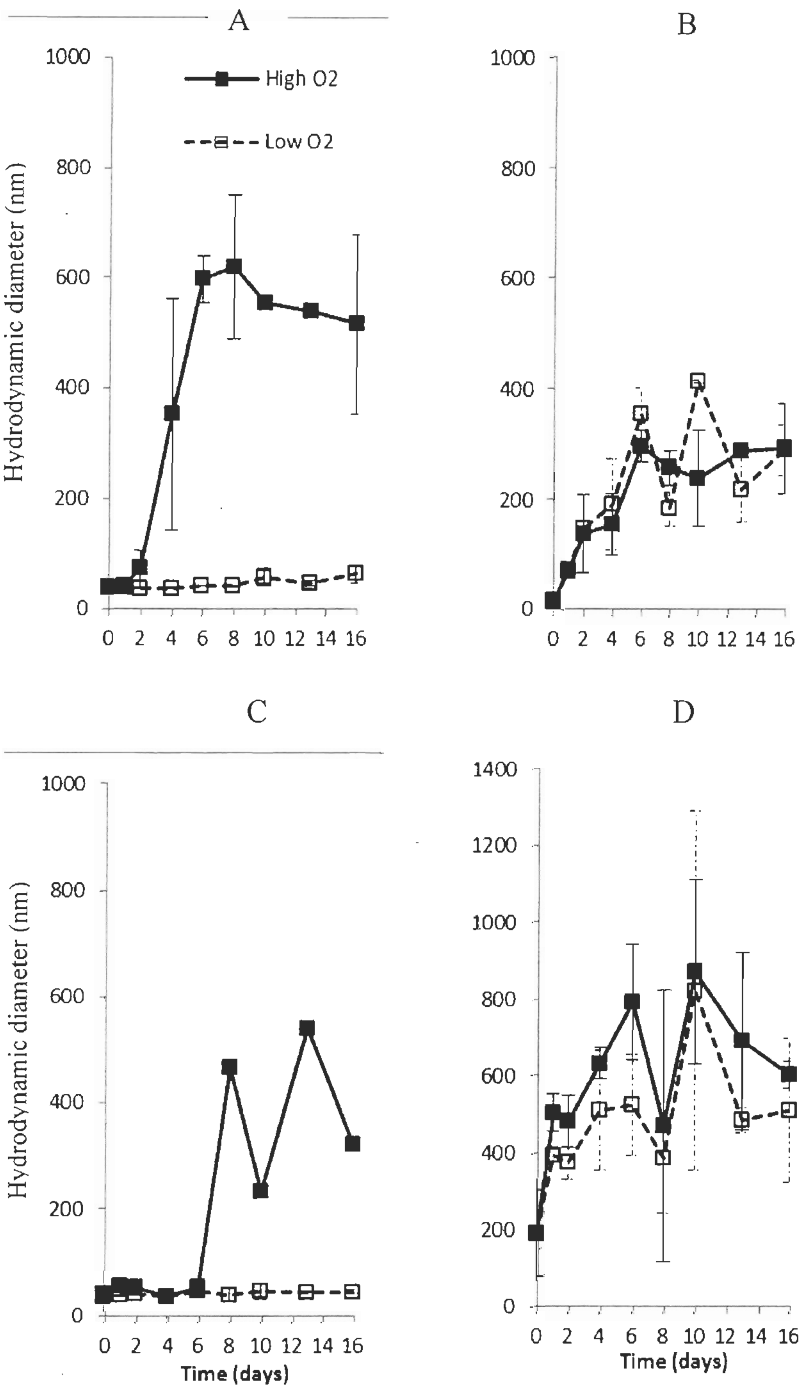


Figure 7 : Changes in hydrodynamic diameter (nm) for Bare AgNP over 16 days (A) in deionized water (B) in river water (C) in diluted seawater and (D) in seawater.

3.3.2. Citrate-AgNP

The change in hydrodynamic diameter of citrate-AgNP is illustrated in Figure 8. The water composition and dissolved gas conditions clearly induced a different evolution of the aggregation.

Aggregation reached 200 nm after 24 h, followed by a plateau for DW (Figure 8A) with high dissolved oxygen level. In low dissolved O_2 in DW, the mean size reached 150 nm after 24 h, but a slow decrease of the size was then observed to reach the lowest value of 120 nm at day 16.

The size of citrate-AgNP in RW (Figure 8B) reached a plateau near 100 nm for both conditions after one day. The two conditions evolved similarly, except on day 8 where a peak of the mean size of particles was around 250 nm for the condition with low dissolved oxygen. The average size values of nanoparticles from day 2 to day 16 were 90 ± 15 nm and 141 ± 56 nm for high and low dissolved O_2, respectively.

Aggregation barely occurred in DSW (Figure 8C) with low dissolved oxygen concentration, as the maximum mean size of aggregates was on day 8 around 88 ± 3 nm. At the end of the experiment, the mean size was 48 ± 2 nm, close to its initial value. Only one of the two replicates showed a high aggregation in high O_2 (illustrated here) with a fast increase of the size in the first 4 days followed by a decrease in size. Aggregation was not detected in the other replicate, which may be due to a too low concentration of particles to be detected by DLS.

A fast aggregation of citrate-AgNP in seawater (Figure 8D) occurred for both O_2 conditions as the size reached 500 nm in first 24 h and stabilized near 600-700 nm over the last 6 days. Aggregation initially seemed to evolve faster in a low concentration of dissolved oxygen but at day 16 the two conditions had the same mean size.

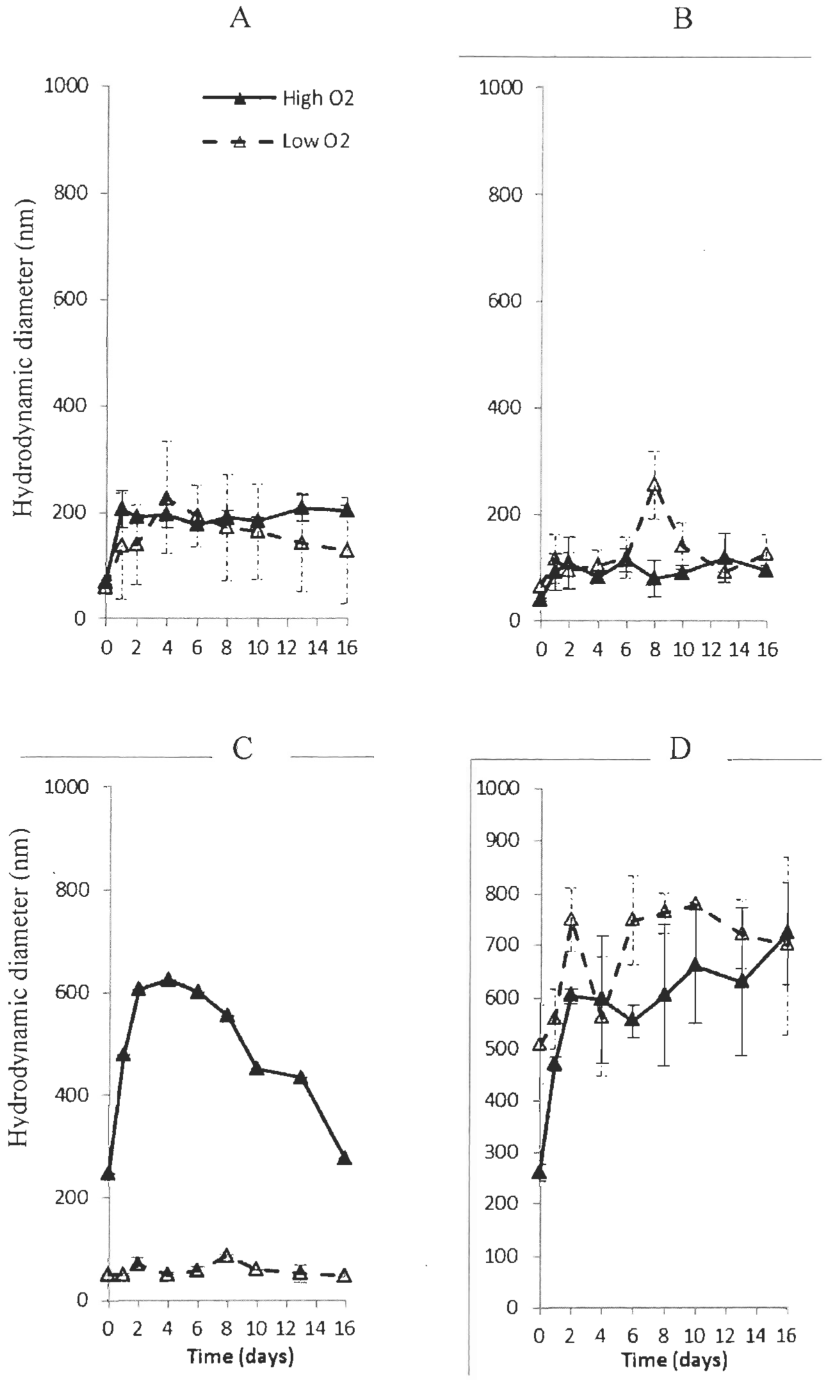


Figure 8 : Changes in hydrodynamic diameter (nm) Citrate-coated AgNP over 16 days (A) in deionized water (B) in river water (C) in diluted seawater and (D) in seawater.

3.3.3. PAAm-AgNP

The change in hydrodynamic diameter of PAAm-AgNP over 16 days is represented in Figure 9. In DW, nanoparticles were first observed around 240-340 nm for both low and high O_2 conditions (Figure 9A). For high O_2, the size did not vary greatly with an apparent slow increase up to 320 nm from day 10 to day 16. The general pattern is almost the same for the low O_2 conditions with slight variations of the observed size over the experimental time indicating an apparent stability of PAAm-AgNP in DW.

However, the mean size of PAAm-AgNP showed a high variability in RW with mean size ranging between 400 and 1,100 nm for high O_2, and from 500 to 1,300 nm for low O_2 conditions (Figure 9B). Taking in account a high variability between bottles with same O_2 content, both distributions as a function of time are not significantly different $p > 0.05$ (Table 7).

In DWS (Figure 9C), the size of PAAm-AgNP did increase slowly and constantly during the experimental period. For both conditions, a mean size of 70 nm was determined at the beginning (5 minutes after injection) and evolved closely except at the end of the experiment where PAAm-AgNP reached a maximum size of 290 nm for high O_2 and a minimum of 168 nm for low O_2.

In SW (Figure 9D), the mean size of PAAm-AgNP was 358 ± 47 nm for high O_2 conditions and 507 ± 90 nm for low O_2 conditions. In the first day, the mean size increased for both conditions. For low O_2 conditions from day 1 to day 13, the mean size decreased from 675 nm to 250 nm and then increased to 330 nm at day 16. For high O_2 conditions, the mean size fluctuated more than for the other condition, between 300 and 600 nm over the first 8 days, and then started to increase to reach 825 nm at day 16.

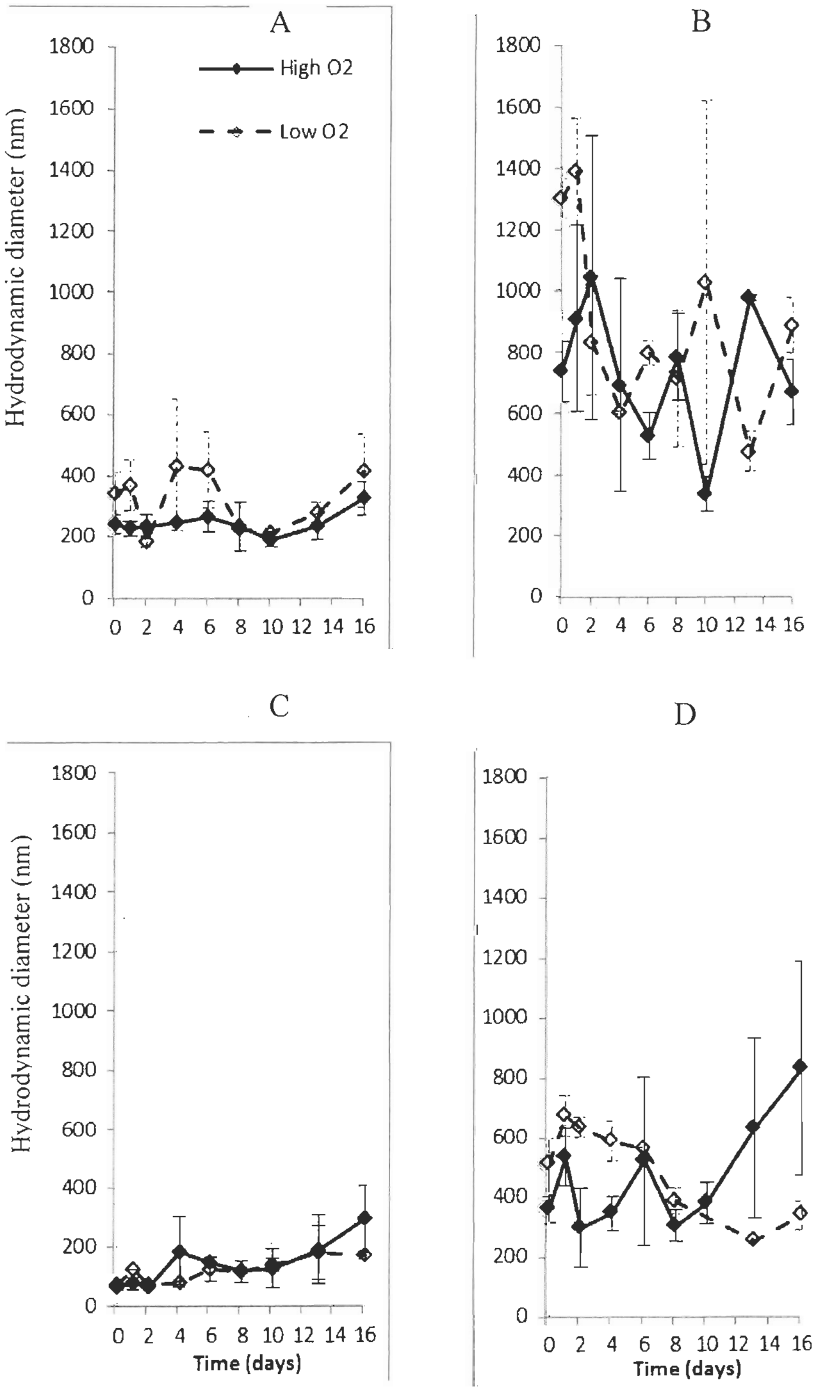


Figure 9 : Changes in hydrodynamic diameter (nm) PAAm-coated AgNP over 16 days (A) in deionized water (B) in river water (C) in diluted seawater and (D) in seawater.

To determine if PAAm-AgNP aggregated, an examination of samples with TEM was performed for DW, RW and DSW. Seawater samples cannot be observed by TEM as the salt content was too high, and aggregates and salt crystals could not be differentiated accurately.

In DW, size distribution data from the Zetasizer showed that nanoparticles aggregates slightly grew from 337 nm to 400 nm at the end of the experiment. TEM image confirmed the presence of aggregates (Figure 10 (A) and (B)), where AgNP are close together and apparently trapped in PAAm. In RW, aggregation happened really fast with high variability in data. The aggregate images obtained with TEM are different from those observed in DW (Figure 10 (C) and (D)), as AgNP seem to be fused with each other and are not clearly trapped in the polymer. Aggregation was also observed in DSW and TEM (Figure 10 (E)) with nanoparticles that are fused together as in river water, but the shape of the aggregates is more spherical than in river water. The behaviour and mean size of PAAm-AgNP in these two types of water were different. The conductivity of the two waters being the same, only the composition of water can explain this difference. PAAm-AgNPs are more stable in DSW than in RW. PAAm-AgNP in seawater aggregated, but not as fast as in river water and also do not form larger aggregates.

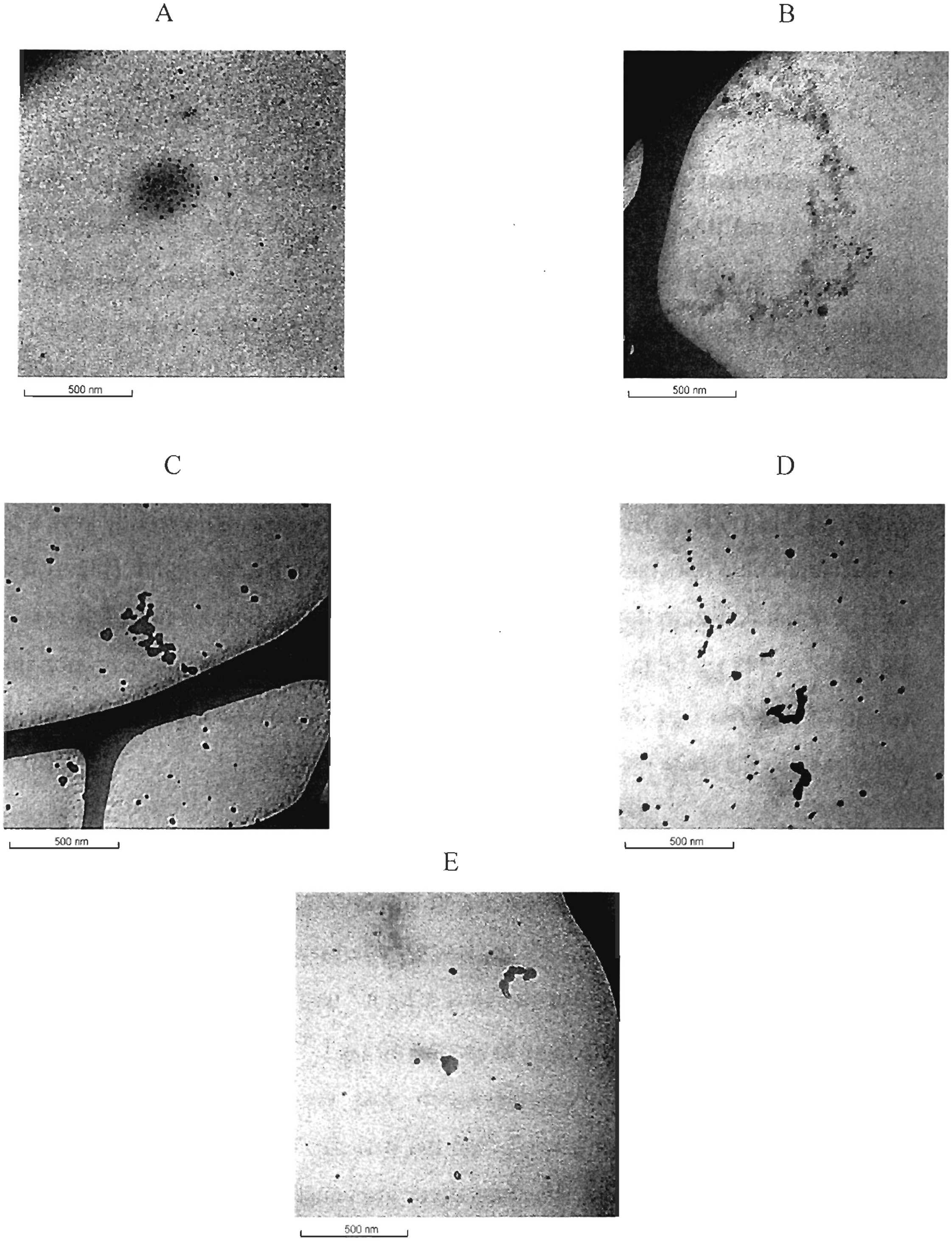


Figure 10 : TEM images of PAAm-AgNP in (A) DW with high O_2, (B) DW with low O_2, (C) RW with high O_2, (D) river water with low O_2 and (E) diluted seawater with low O_2

3.4. The free silver ion concentration

3.4.1 In deionized water (DW)

Concentrations of free silver ions in DW are illustrated for bare- and citrate-AgNP (Figure 11A) and PAAm-AgNP (Figure 12A). After a high initial peak, the concentration of free silver ions for bare-AgNP suspension decreased from 77 $\mu g \cdot L^{-1}$ to 25 $\mu g \cdot L^{-1}$ on the first day of the experiment and reached 3 $\mu g \cdot L^{-1}$ at day 6; the free silver ions then increased again to 25 $\mu g \cdot L^{-1}$ for water with high O_2. Both low and high dissolved oxygen concentrations behaved similarly; the difference is not significant ($p > 0.05$; Table 7). For citrate-AgNP in DW, the free silver ion concentration increased slowly from 2-3 $\mu g \cdot L^{-1}$ to 12-15 $\mu g \cdot L^{-1}$ in 16 days. The concentration seemed to increase more rapidly with a high O_2, but differences are not significant ($p > 0.05$; Table 7). For PAAm-AgNP (Figure 12A), the free silver ion concentration observed at the first sampling time (5 min) fell from around 700 $\mu g \cdot L^{-1}$ to 345 $\mu g \cdot L^{-1}$ in 4 days. In a second step, free silver ion concentration increased back to around 700 $\mu g \cdot L^{-1}$ and did not change for the rest of the experiment. The situation appeared to be the same for the high and low O_2 conditions.

3.4.2. In river water (RW)

The concentrations of dissolved Ag in suspensions of bare-AgNP dispersed in RW (Figure 11B) started at 27 $\mu g \cdot L^{-1}$ for the low O_2 condition and at 46 $\mu g \cdot L^{-1}$ for the high O_2 condition. Again a rapid decrease to about 5 $\mu g \cdot L^{-1}$ was observed in the first days with a slight increase in the following days to around 10 $\mu g \cdot L^{-1}$ for bare-AgNP under the high O_2 condition. The behaviour of citrate-AgNP was the same for both conditions, as concentrations started around 2-3$\mu g \cdot L^{-1}$ on the first day and slightly increased to 5 $\mu g \cdot L^{-1}$ near the end of the experiment. The free silver ion concentration of PAAm-AgNP (Figure 12B) started at 275 $\mu g \cdot L^{-1}$ for both conditions in RW. With a low O_2 concentration, the free silver ion concentration decreased to 190 $\mu g \cdot L^{-1}$ on the first day then increased back to 250 $\mu g \cdot L^{-1}$ and continued to decrease to around 135 $\mu g \cdot L^{-1}$ at the end of the experiment. For river water with a high O_2 level, the free silver ion concentration decreased slowly to 228 $\mu g \cdot L^{-1}$ in 2 days and then fluctuated from 278 $\mu g \cdot L^{-1}$ in day 4 to 221 $\mu g \cdot L^{-1}$ at day 8 and to its maximum of 287 $\mu g \cdot L^{-1}$ at day 10 before decreasing to 220 $\mu g \cdot L^{-1}$ at day 16.

3.4.3. In diluted seawater (DSW)

For the DWS and a low O_2 concentration (Figure 11C), the free silver ions of bare nanoparticles started at 50 $\mu g \cdot L^{-1}$ for high O_2 and at 38 $\mu g \cdot L^{-1}$ with low O_2. Both conditions behaved the same way with a rapid decrease to about 15 $\mu g \cdot L^{-1}$ after 24 h and a slower decrease to reach a plateau near 6-8 $\mu g \cdot L^{-1}$ at day 6. For citrate-AgNP, both conditions started at 2 $\mu g \cdot L^{-1}$ and behaved the same way. A fast increase of the free silver ion concentration to near 10$\mu g \cdot L^{-1}$ was observed over 4 days, followed by small changes between 7 to 11.5 $\mu g \cdot L^{-1}$ for both conditions until the end of the experiment. Dissolved Ag in bottles with PAAm-AgNP in DSW (Figure 12C) and a low O_2 started at 65 $\mu g \cdot L^{-1}$ and increased rapidly to 100 $\mu g \cdot L^{-1}$ by day 2. From day 2 to day 6, free silver ion concentration decreased rapidly to 38 $\mu g \cdot L^{-1}$ and slowly increased to reach 55 $\mu g \cdot L^{-1}$ at day 16. With a high O_2, the free silver ion concentration behaved nearly the same way. It started at 86 $\mu g \cdot L^{-1}$ and increased to 105 $\mu g \cdot L^{-1}$ over 2 days, then fluctuated between 44 $\mu g \cdot L^{-1}$ at day 13 and 85 $\mu g \cdot L^{-1}$ at day 16.

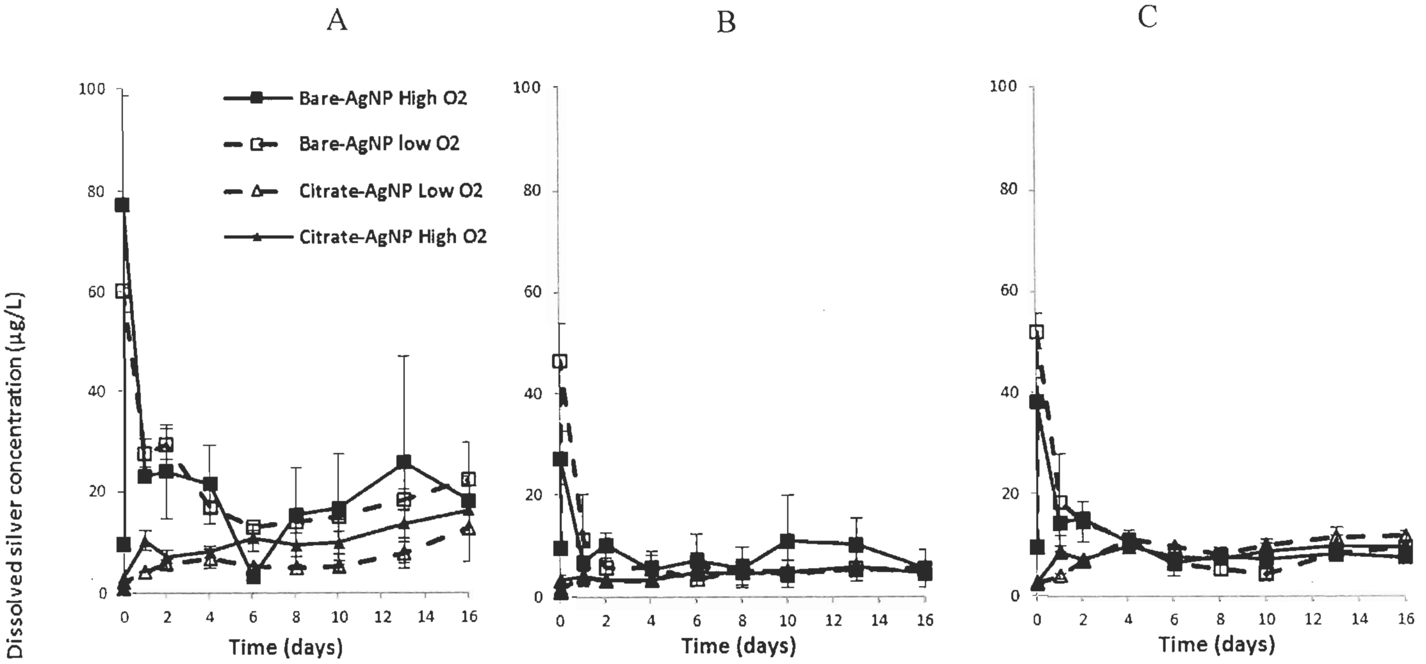


Figure 11 : Bare and citrate coated-AgNP dissolution profiles over 16 days (A) in deionized water, (B) in river water and (C) in diluted seawater.

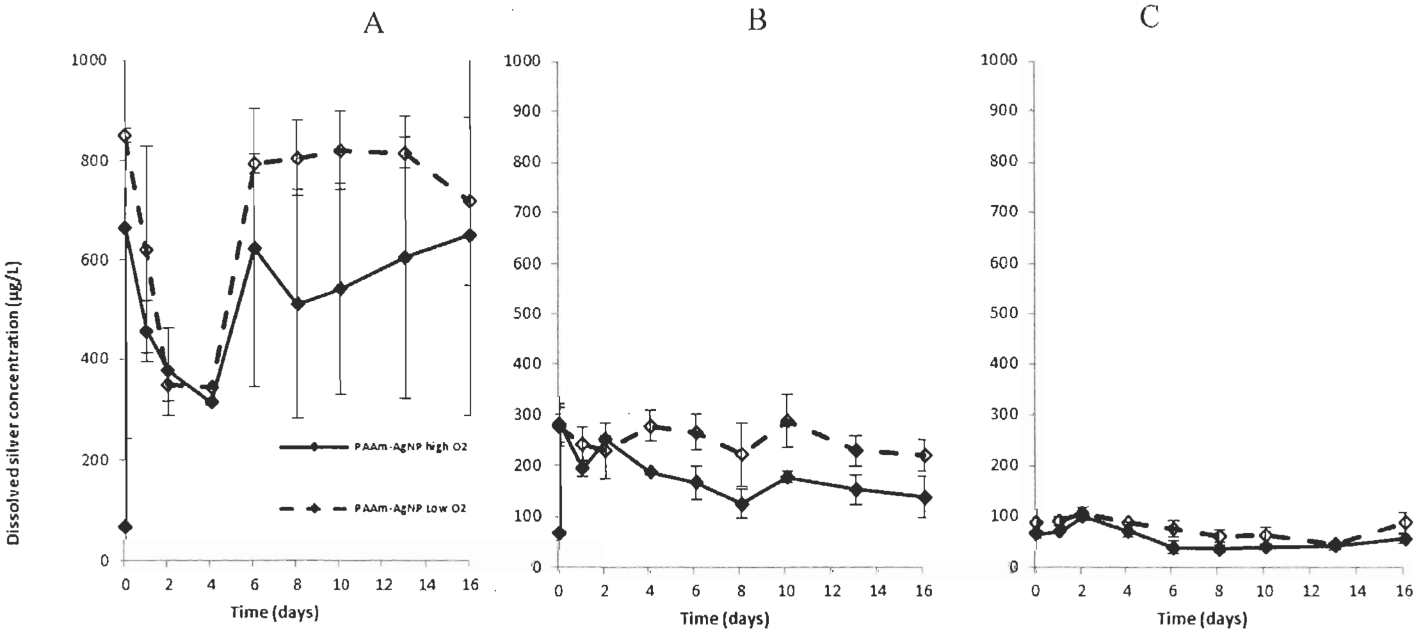


Figure 12 : PAAm coated-AgNP dissolution profiles over 16 days (A) in deionized water, (B) in river water and (C) in diluted seawater.

3.4.4. In seawater (SW)

The behaviour of all types of AgNPs in seawater was quite different from other water conditions described above and showed clearly a dissolution process (Figure 13). For bare-AgNP, the free silver concentration ranged between 41 $\mu g \cdot L^{-1}$ for high O_2 and 75 $\mu g \cdot L^{-1}$ for the low O_2 condition at t=0. Free silver then increased rapidly to reach 270 $\mu g \cdot L^{-1}$ on day 6 and varied between 275 $\mu g \cdot L^{-1}$ and 220 $\mu g \cdot L^{-1}$ at the end of the experiment for the high O_2 condition. In the case of the low O_2 condition, the free silver concentration increased slowly over the entire experiment and had attained 230 $\mu g \cdot L^{-1}$ on day 16. For citrate-AgNP in SW, free silver ion varied slightly between 9 $\mu g \cdot L^{-1}$ for high O_2 and 13 $\mu g \cdot L^{-1}$ for low O_2 at t=0. Both increased rapidly to reach 165 $\mu g \cdot L^{-1}$ on day 16 for low O_2 and 125 $\mu g \cdot L^{-1}$ for the high O_2 condition. PAAm-AgNP in seawater (Figure 13) showed a constant and fast increase of free silver for both conditions. The initial concentrations of free silver were 311 $\mu g \cdot L^{-1}$ and 322 $\mu g \cdot L^{-1}$ for the high O_2 and low O_2 conditions, respectively, and attained concentrations of 688 $\mu g \cdot L^{-1}$ and 553 $\mu g \cdot L^{-1}$ after 16 days.

3.4.5. Dissolved Ag vs total silver added

Because the starting concentrations of each type of AgNP were different from each other (Table 5), comparing the relative proportion of different AgNP that dissolved in the course of the experiment provides a better overview of the process (Figure 14). It becomes clear that citrate-AgNP dissolved more rapidly and with a higher proportion than PAAm- and bare-AgNP. Although less than 5% of the bare-AgNP and about 20% of the PAAm-AgNP had dissolved after 16 days, the dissolution of citrate-AgNP reached 60% for the low O_2 condition and close to 50% under the high O_2 condition. Dissolution of PAAm-AgNP was almost linear with time.

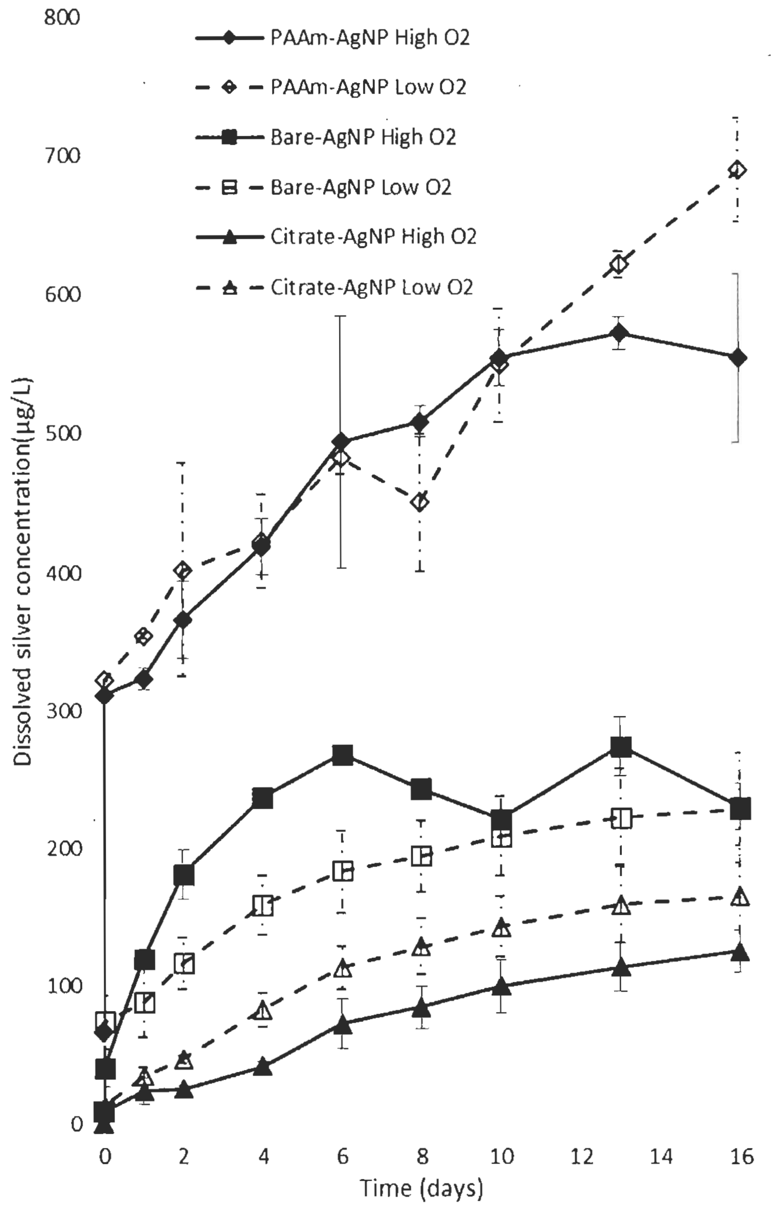


Figure 13 : Dissolution of bare, citrate and PAAm coated-AgNP in seawater over 16 days.

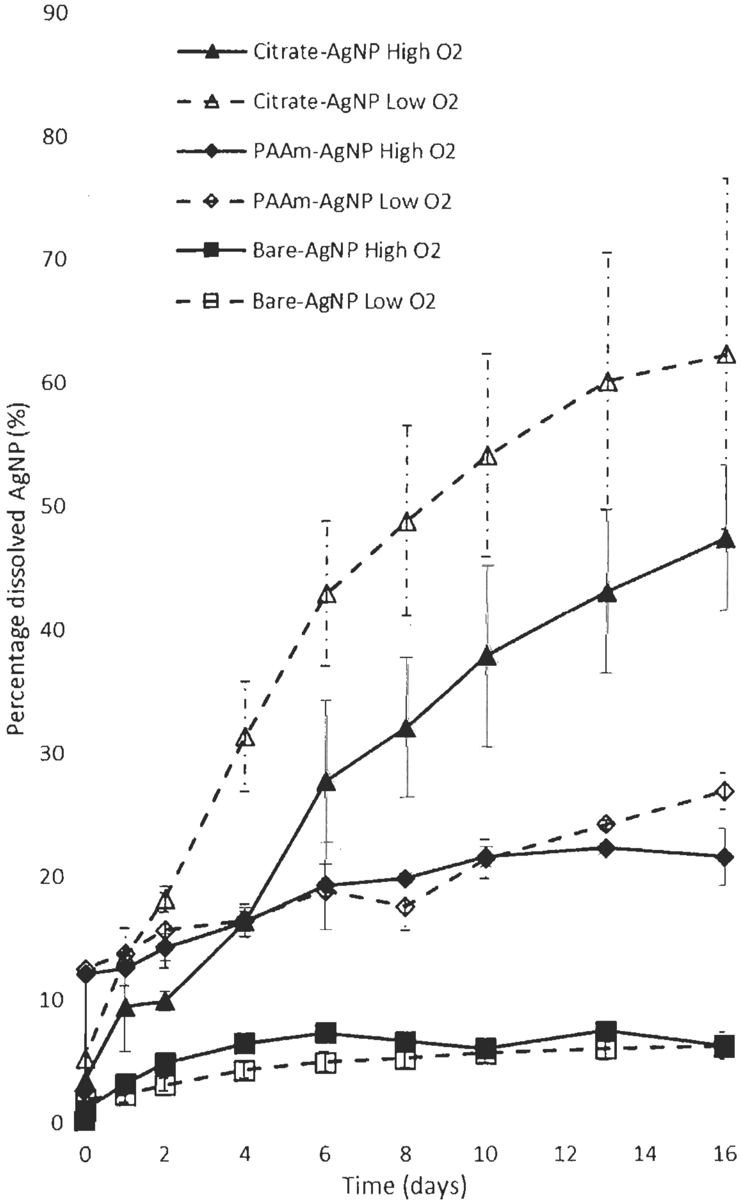


Figure 14 : Percentage of dissolved silver ions for bare, citrate and PAAm coated-AgNP in seawater over 16 days.

3.4.6. Statistic data

Statistic data reported in Table 6 show that the type/coating of nanoparticles and the water types have an effect on dissolution and aggregation behaviour ($p < 0.0001$). The concentration of dissolved oxygen has an effect on aggregation ($p = 0.0014$), but not on dissolution ($p = 0.2847$). The combination of water types and particles types has an effect on both dissolution and aggregation behaviour ($p < 0.0001$). Both combinations with dissolved oxygen (AgNP type with dissolved oxygen and water type with dissolved oxygen) play a role in aggregation behaviour ($p = 0.0008$) but do not have an impact on dissolution ($p = 0.2857$ and $p = 0.8293$, respectively). Combinations of the three factors have an effect on aggregation ($p = 0.0019$), but do not influence dissolution ($p = 0.8844$).

Factors	Dissolution p value	Aggregation p value
AgNP type	<0.0001	<0.0001
Water	<0.0001	<0.0001
Dissolved oxygen	0.2847	0.0014
AgNP X water	<0.0001	<0.0001
AgNP type X Dissolved oxygen	0.2857	0.0008
Water X Dissolved oxygen	0.8293	0.0008
AgNP type X Water X Dissolved oxygen	0.8844	0.0019

Table 6 : p values of repeated measures ANOVA for 3 factors: AgNP types, water types and dissolved oxygen concentrations.

Table 7 shows the result of statistical analysis of the impact of dissolved oxygen on aggregation and dissolution. From this Table, it can be observed that only dissolved oxygen influenced the dissolution in deionized water for citrate coated AgNP (p = 0.0520). For aggregation, dissolved oxygen has an impact for citrate coated AgNP in diluted seawater (p = 0.0133), for uncoated AgNP in deionized water, river water and seawater.

Dissolved oxygen effects		Dissolution p value	Aggregation p value
Citrate	Seawater	0.2023	0.2883
Citrate	Diluted seawater	0.7670	0.0133
Citrate	Deionized water	0.0520	0.7562
Citrate	River water	0.9563	0.4316
Bare	Seawater	0.1486	0.0177
Bare	Diluted seawater	0.1047	0.0204
Bare	Deionized water	0.4618	0.0293
Bare	River water	0.6615	0.7829
PAAm	Seawater	0.3663	0.9576
PAAm	Diluted seawater	0.1670	0.5274
PAAm	Deionized water	0.5530	0.2689
PAAm	River water	0.3446	0.2278

Table 7 : p values of repeated measures ANOVA for dissolved oxygen concentrations.

4. Discussion:

Each type of silver nanoparticles dispersed in different types of water (from deionized water to highly ionic seawater), with low or high dissolved oxygen, showed quite different aggregation and dissolution behaviours, most probably involving the nature of the capping material and complex interactions with dissolved gases, dissolved organic matter and ionic species. Aggregation is confirmed as an important factor for the behaviour of nanoparticles in the environment but much more was learned:

- Bare-AgNP did aggregate quite importantly in both DW and SW in less than 2 h. A strong aggregation was confirmed in long-term experiments in DW with high oxygen content but not observed in low O_2 bottles.

- Bare-AgNP added to very low and low ionic strength waters (DW, RW and DSW) showed a high initial peak of «free silver» which disappeared over the first 4 days, followed by a very slow dissolution (or desorption) independent of the oxygen content. Bare-AgNP also dissolved slowly in SW without showing a peak of free silver at the beginning. Dissolution was not clearly related to aggregation.

- Citrate-AgNP did not aggregate importantly in either DW or SW over 6 h maybe due to a lower concentration. The O_2 content does not seem to have a significant effect on long-term aggregation. A much higher aggregation process (larger particles) was observed in SW.

- Citrate-AgNP added in low ionic strength waters showed a slow dissolution, mainly over the first 4 to 6 days. However, citrate-AgNP showed a high dissolution proportion in SW, with a significant higher proportion in low O_2 seawater. No relationship was found between aggregation and dissolution. Citrate-AgNP aggregated differently in (DW), (RW), (DSW) but dissolution was not affected.

- PAAm-AgNP showed highly scattered aggregation results over 6 h in both DW and SW and long-term behaviour was also highly variable, particularly with RW and SW. A part of the variability could be due to the limitations of the size determination technique (DLS).

- PAAm-AgNP did not dissolve in RW and DWS, but exhibited a very peculiar behaviour in DW (both high and low oxygen content) with a reduction of free silver followed by an important dissolution or liberation of captured silver. In seawater, the reduction of initial free silver was not observed and a slow dissolution took place.

- PAAm-AgNP dissolved in seawater with an almost linear relationship with time.

4.1. Bare-AgNP

Bare nanoparticles were prepared following the method of Li and Lenhart (2012) and the suspension in DW was carefully dialyzed to remove reactants in excess, such as ionic silver and maltose. Upon storage these particles did not aggregate and the analysis by ICP-MS of free silver after ultracentrifugation indicated that less than 1% of total silver was in an ionic form. According to Levard et al. (2012), these bare-AgNP are electrostatically stabilized against aggregation because they have negative charges on their surface layer (these charges coming from the presence of hydroxo-and oxo- groups on the particle surface) and aggregation should not be observed in DW as deionized water contains only small amounts of dissolved ions or molecules that could change the surface interactions between nanoparticles. In this study, bare-AgNP did slowly aggregate in DW over a 6-h period. An aggregation also occurred in both bottles containing DW bubbled with air at the beginning and also in the course of the 16-day experiment. Some authors have observed aggregation of bare-AgNP in DW containing different electrolytes (Li et al. 2010; Li and Lenhart, 2012; Delay et al. 2011). The absence of aggregation in DW bubbled with nitrogen is puzzling. The difference may come from the presence of CO_2 in air and a saturation of CO_2 in DW bubbled with air. The reaction of silver with CO_2 in solution has been documented (Hull et al. 2012; Gray et al. 2013) and the K_{so} of the resulting Ag_2CO_3 is given as 8.46×10^{-12} at 25°C indicating a very low solubility of this compound. We suggest that Ag^+ and/or Ag_2O entrapped at the surface of bare-AgNP could react with the surrounding dissolved CO_2 (present as H_2CO_3 in water at low pH) and form a thin coating of uncharged Ag_2CO_3 at the surface of bare-AgNP, promoting their aggregation. The absence of CO_2 in bottles bubbled with nitrogen did prevent any aggregation. A similar mechanism was proposed to explain the aggregation of AgNP in anoxic environments where sulphide is reacting with ionic silver to form Ag_2S and construct bridges between nanoparticles (Levard et al. 2011).

An unexpected dissolution (desorption) behaviour of bare-AgNP was observed in all low ionic strength waters at the beginning of the experiments. This behaviour could be attributed to entrapped silver (any water soluble form) present at the surface of the AgNP, which is freed by dilution in these waters. The free silver is then rapidly re-captured by particles. In bottles with air bubbling, free silver could react with CO_2 to precipitate as Ag_2CO_3 (as free silver carbonate nanoparticles or as coating on AgNP) and produce the observed reduction of free silver in solution within a few days. Air was bubbled in bottles after each opening (each day) to maintain the level of dissolved oxygen and incidentally the high level of CO_2. However, the re-adsorption of free silver on AgNP is also observed in bottles (DW, RW and DWS) bubbled with nitrogen and expected to be poor in dissolved CO_2. An alternative mechanism has to be suggested. Chloride ions may form low solubility AgCl which could also adsorb on the surface of AgNP. Li et al. (2012) also showed that free silver can be found on the surface of the oxic layer of bare-AgNP. Even after a long dialysis process to purify the suspension, some free silver could remain in close interaction with the surface. When diluted in the bottle, the free silver ions on the surface of the bare-AgNP would be released and this process could explain the initial peak of free silver.

It should be noted that the initial peak of free silver followed by re-adsorption is not seen in SW because the very large molar excess of chloride ions in seawater would capture ionic silver as soon as available and form soluble $AgCl_4^{3-}$, making re-adsorption impossible. The presence of dissolved organic matter in seawater would also stabilize free silver in solution.

4.2. Citrate-AgNP

Citrate-coated silver nanoparticles have been extensively studied in recent years and researchers have been using different synthesis methods and different laboratory designs, and have obtained more or less conflicting results (Table 6). The coating of AgNP with citrate increases the negative surface charge of the nanoparticles with hydroxo- and oxo-groups, and should provide a higher stability to their colloidal suspension (Levard et al.

2012). In an aquatic environment containing appropriate cation concentrations, these nanoparticles should be unstable and form aggregates.

The literature review shows that aggregation and dissolution behaviour for citrate-AgNP are favoured in an oxygenated environment. The synthesis process and starting size of citrate-AgNP do not change the fact that they aggregate and dissolve in all types of waters with dissolved oxygen (Ho et al. 2010; Kittler et al. 2010; Liu and Hurt, 2010; Li et al. 2011; Prathna et al. 2011; Zhang et al. 2011; Li and Lenhart, 2012; Angel et al. 2013; Baalousha et al. 2013; Dobias and Bernier-Latmani, 2013). Also, the aggregation tendency is stronger in seawater than in fresh water (Liu and Hurt, 2010; Prathna et al. 2011; Angel et al. 2013), but some results differ strongly depending on the studies. For example, Angel et al. (2013) and Kittler et al. (2010) showed conflicting results as one paper observed that the rate of dissolution was higher for citrate-AgNP compared to PVP-AgNP and the other one observed the reverse situation. The nanoparticles used in their experimental settings were different and this may explain the conflicting results. Kittler et al. (2010) were using a synthesis process with tri-sodium citrate as the reducing agent, and the nanoparticles provided by ABC Nanotech (Angel et al. 2013) were synthesized by an ICP method and then stabilized with a citrate solution. The study of Prathna et al. (2011) clearly showed differences in behaviour between two types of citrate-AgNP. The synthesis method strongly affects the behaviour of citrate-AgNP and often prevents direct comparison between papers seeking the same objectives (Table 8).

In the present work, citrate-AgNP aggregated in RW for both high and low O_2 conditions, but differently than in the case of bare-AgNP, as a plateau was reached at 200 nm, showing that the coating agent did help in stabilizing the nanoparticles in water. This result is however in contrast with results from Zhang et al. (2011) who observed an aggregation 3 to 8 times faster in the presence of dissolved oxygen than without oxygen for citrate-AgNP in a Hoagland medium, which is a hydroponic solution that is used to grow plants and contains a large number of elements. Their explanation was that a release of Ag^+ due to the exposure of dissolved oxygen could increase the ionic strength and promote aggregation via other unknown mechanisms.The difference could be in the ionic composition of the water. Ballousha et al. (2013) showed with citrate-coated AgNP that divalent cations have a stronger effect on aggregation than monovalent cations and also that aggregation in mixtures of monovalent and divalent cations is additive.

Liu and Hurt (2010) prepared very small citrate-AgNP (<10 nm) using $NaBH_4$ and observed that salt did not affect the oxidation kinetics for these nanoparticles and reported more dissolution in DW than in SW. The authors explained that the aggregation process increased the size of the nanoparticles and protected the surface area of the aggregated nanoparticles from reacting with dissolved oxygen. In the present study, using larger AgNP at a different concentration, the dissolution of citrate-AgNP was more pronounced in seawater than in fresh water and even faster with low dissolved oxygen concentrations, which is in contradiction with the results reported by Liu and Hurt (2010). The citrate-coating did not stabilize the AgNP, as a fast aggregation for both O_2 conditions was observed in SW and the size of the aggregates was the same as for bare-AgNP. Proportional dissolution was higher than for bare- and PAAm-AgNP and also faster with a low dissolved oxygen concentration. This observation also differs from the results of Liu and Hurt (2010) as they observed no dissolution from citrate-coated nanoparticles with deoxygenated water.

An explanation for all the differences in results with the literature might be related to the method used to synthesize the AgNP. Researchers synthesize their own suspensions or buy them from manufacturers. There are several ways of preparing citrate-AgNP as shown in Table 8. In our experiment, citrate-coated AgNP were synthesized from bare AgNP. The surface of the AgNP may only be partially covered with citrate or citrate may be partially attached and thus be easily removed from the surface of the nanoparticles. Prathna et al. (2011) showed that the surface stabilizing agent plays a role in the behaviour of AgNP, as they compared two types of citrate-AgNP synthesis, one biological and the other chemical, and results were different.

Synthesis method	Nanoparticle size	Experiment design	Results	Authors
Reduction of silver nitrate with $NaBH_4$.	(TEM) 5.4 nm (TEM) 106 nm (TEM) 20.5 nm	Kinetics and mechanism of oxidative dissolution of AgNP by H_2O_2 in deionized water.	First order dissolution of AgNP. Surface coating plays an important role in dissolution.	Ho et al.(2010)
Reduction of silver nitrate with citrate.	(DLS) 85 nm	Dissolution of citrate AgNP in dialysis tube in ultrapure water over 125 days.	Partial dissolution of AgNP. Dissolution rate depending on coating of AgNP. Dissolution rate lower than for PVP-AgNP	Kittler et al. (2010)
Reduction of $AgClO_4$ with citrate and $NaBH_4$.	(DLS): 1.9 nm (TEM): 4.8 nm	Time dependent release of dissolved silver using centrifugal ultrafiltration and atomic absorption spectroscopy.	No dissolution of AgNP in deoxygenated water. Dissolution in air-saturated water. Salt concentrations not affecting AgNP oxidation kinetics. Higher dissolution in DI water than in seawater.	Liu and Hurt (2010)
Reduction of $AgNO_3$ with D-maltose, then coated with citrate.	(DLS) 82 nm	Aggregation and dissolution of citrate AgNP with different electrolytes; experiment over 15 min.	Dissolution of AgNP. Citrate capping does not inhibit dissolution.	Li et al. (2011)
Biological synthesis with citrus lemon and chemical synthesis by reduction of silver nitrate with citrate.	Biological method: (DLS): 153 nm (TEM): 25-50 nm Chemical method: (DLS) 78 nm (TEM): 20 nm	1: Aggregation in deionized water with pH from 3 to 10 and ionic strength from 0 to 100.mM. 2: Aggregation in lake water and seawater and biological matrices. Sampled directly after injection of AgNP.	Biological AgNP: not changed in diameter with ionic strength. Addition of ions decreases aggregation. Chemical AgNP: Diameter increased with ionic strength. Aggregation greater in seawater for both particles.	Prathna et al. (2011)

Synthesis method	Nanoparticle size	Experiment design	Results	Authors
From Ted Pella Inc. (Redding CA). Reduction of $AgNO_3$ with citrate.	(TEM) 20nm (TEM) 40nm (TEM) 80 nm	Influence of dissolved oxygen on stability of AgNP in plant growing aqueous environment.	AgNP aggregated 3-8 times faster in presence of O_2. Small nanoparticles aggregate faster than bigger ones.	Zhang et al. (2011)
Reduction of $AgNO_3$ with D-maltose, then coated with citrate.	(DLS) 82 nm	Stability and dissolution of AgNP in river water in the presence or absence of sunlight. Experiment over 15 days.	Increase in particle size (82 nm to 500-800 nm) in 6 h. Dissolution of AgNP in river water. Aggregation of AgNP.	Li and Lenhart (2012)
From ABC Nanotech (Daejon City, South Korea). Synthesis method not reported.	(DLS) 14 nm	Stability, dissolution and toxicity of citrate and PVP-AgNP in synthetic freshwater and seawater with natural organic matter.	AgNP not stable in seawater rapidly forming large particles. Dissolution rate higher than for PVP-coated AgNP.	Angel et al. (2013)
Reduction of silver nitrate with citrate and $NaBH_4$	(DLS) 2-10 nm (5 ± 1.5% intensity) (DLS) 20-100 nm (95 ± 5.0% intensity)	Influence of different electrolytes on aggregation of citrate AgNP	AgNP aggregated. Divalent cations have stronger influence on aggregation than monovalent cations.	Baalousha et al. (2013)
From nanoComposix (San Diego CA). Synthesis method not reported.	(TEM & DLS) 10 nm (TEM & DLS) 50 nm	Release of Ag from nanoparticles of various sizes exposed to river and lake water. Nanoparticles embedded in 4% low melt agarose. Experiment over 4 months.	Dissolution of AgNP in river water.	Dobias and Bernier-Latmani (2013)
Reduction of AgNO3 with D-maltose then coated with citrate.	(DLS) 46 ± 9 nm (TEM) 40-45 nm	Aggregation and dissolution of citrate AgNP in DI water, river water, DI water +0.5% seawater and seawater with high and low dissolved O_2. Experiment over 16 days.	Dissolution and aggregation of citrate AgNP in all waters with high or low O_2. Slow dissolution in DI water, river water and DSW. Dissolution is faster in seawater.	Current study

Table 8 : Synthesis methods, nanoparticle sizes, experimental designs and results of dissolution experiments for citrate-coated AgNP reported in the literature between 2010 and 2014.

The biological synthesis partially coated the citrate-AgNP whereas the chemical synthesis completely coated the citrate-AgNP. Both types of citrate-AgNP aggregated in water, but chemically synthesized AgNP were more stable against aggregation in natural waters. Not only is the type of coating important in the behaviour of AgNP, but also the synthesis by which the AgNP are made for research, each study used a different type of AgNP, suspension can be bought from companies, and there are many different syntheses to obtain citrate coated-AgNP. Nanoparticles obtained from different synthesis may not have the same behavior which could explain the different results obtain by studies as in Table 8.

4.3. PAAm-AgNP

PAAm-AgNP have been used in our laboratory as a model of polymer coated silver nanoparticles to study bioaccumulation in biological tissues (Al-Sid-Cheikh et al. 2011). As the high molecular weight PAAm (~65,000 Da) is used as the reducing agent and each long polymer chain has many reducing sites, it is assumed that PAAm molecules are integrated inside silver nanoparticles and form «hairy nanospheres» with a complex and thick coating of each nanoparticle by PAAm molecules bearing amine functional groups (NH_2) along their carbon chains. Amines have one electron doublet available and can capture cations such as Ag^+ and exchange cations within the surrounding solution. In this particular case, the polymeric coating is very stable and cannot be displaced away from the surface of the nanoparticles by competing species. As a drawback, the excess of PAAm is difficult to remove after synthesis and the proportion of «free silver» remains relatively high (2.5%) even after a long cleanup process. PAAm-AgNP suspensions are stable for months (without aggregation or dissolution) when stored at high concentration in DW. Determining the size distribution for PAAm-AgNP suspensions was a technical problem as the presence of PAAm interfered quite strongly with DLS measurements. As an example, the mean size of PAAm-AgNP in the stock suspension was determined to be 337 nm by DLS, but only about 20 nm as determined by electron microscopy (TEM) (Appendix 1). The aggregation of PAAm-AgNP was clearly observed in all types of water but PAAm-AgNPs were more stable in DSW than in RW.

When PAAm-AgNP are diluted in low ionic strength waters (DW, RW, DSW), a relatively large amount of «free silver» is released in water and then re-captured in particulate form, a phenomenon particularly clear in DW due to the absence of competing anions (Figure 13A) (Dobias and Bernier-Latmani, 2013). PAAM-AgNP did not dissolve in DSW or RW as they are well protected by their polymeric coating. Accordingly, dissolved oxygen and nitrogen bubbling did not change the patterns of dissolution or recapture. However, the situation is slightly different in seawater where a slow and constant increase of «free silver» is observed over time. It cannot be stated if this increase of free sliver is due to a slow release of Ag^+ previously captured by PAAm, under the action of competing cations, or to a true dissolution of the silver nanoparticles under the action of dissolved oxygen. As dissolution seems not to be enhanced by a high O_2 content in bottles with seawater, a slow release of captured Ag^+ is suggested as the main mechanism.

5. Conclusion

This study illustrates the complexity of the behaviour of silver nanoparticles in water and questions the simplistic models previously described in the literature. The water composition and the coating of nanoparticles play together a major role in controlling the aggregation and dissolution processes. In water samples with a low ionic strength, AgNP did not show a clear dissolution process as free silver ions already present in solution at the beginning of the experiment were re-adsorbed on AgNP in a few hours and then, slowly released over the following days. The dissolved oxygen concentration did apparently play a determining role in dissolution of citrate-AgNP in seawater, but was not clearly identified as a governing factor in all the other cases, a result in conflict with the results of previous researchers who associated dissolved oxygen with a dissolution process. All types of AgNP aggregated in all water samples, especially in seawater (a result that is attributed to its high ionic strength). In addition to ionic strength, the chemical composition of the solution seems to play an important role in aggregation as a difference was observed between diluted seawater and river water which had the same ionic strength, but different ionic compositions; in addition, the dissolved organic matter was also probably of a different nature. This study is among the first to explore the behaviour of AgNP in filtered natural waters including diluted seawater.

6. Acknowledgments

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1

CHAPITRE 2
SOMMAIRE ET CONCLUSION

Chaque type de nanoparticule (AgNP), dispersé dans les différents types d'eau (de l'eau déionisée à l'eau de mer ayant une force ionique élevée), avec une faible ou une forte concentration en oxygène dissous, a montré un comportement d'agrégation et de dissolution différent. Ces différences peuvent impliquer la nature du recouvrement de la nanoparticule et les interactions complexes avec les gaz dissous, la matière organique et les espèces ioniques du milieu. L'agrégation a été confirmée comme un facteur important dans le comportement des nanoparticules dans l'environnement, mais d'autres informations ont été acquises dans cette étude :

- Les nanoparticules sans recouvrement se sont beaucoup agglomérées dans l'eau déionisée et l'eau de mer en moins de 2 h. Une forte agrégation a été confirmée tout au long de l'expérience dans l'eau déionisée, avec une forte concentration en oxygène dissous, mais pas avec une faible concentration.
- Les nanoparticules sans recouvrement dans un milieu ayant une faible force ionique (eau déionisée, eau de rivière et eau de mer diluée) génèrent une forte concentration en ions argent au départ, qui diminue brusquement dans les quatre premiers jours. Puis, une faible dissolution (ou désorption) a lieu et est indépendante du contenu en oxygène dissous. Dans l'eau de mer, les nanoparticules sans recouvrement se sont

dissoutes lentement et n'ont pas présenté une forte concentration en ions argent au départ. Aucun lien direct entre la dissolution et l'agglomération n'a été observé.

- Après 6 h, les nanoparticules recouvertes de citrate ne se sont pas fortement agglomérées dans l'eau déionisée et dans l'eau de mer. La concentration en oxygène dissous ne semble pas avoir d'effet sur l'agglomération au long terme; une forte agglomération et de gros agrégats sont observés dans l'eau de mer.

- Dans les eaux de faible force ionique, les nanoparticules recouvertes de citrate se sont dissoutes très lentement durant les 4 à 6 premiers jours. Dans l'eau de mer, par contre, les AgNP-citrate se sont dissoutes rapidement, surtout avec une faible concentration en oxygène dissous, ce qui apparaît étonnant. Aucune relation n'a été observée entre l'agglomération et la dissolution contrairement à des travaux déjà publiés.

- Les nanoparticules recouvertes de PAAm ont montré des changements de diamètre hydrodynamique très dispersés sur 6 h dans l'eau déionisée et dans l'eau de mer. Les résultats sur le long terme ont été très variables, particulièrement dans l'eau de rivière et l'eau de mer. Une partie de cette variation peut être liée à la méthode de mesure des nanoparticules (DLS).

- Les nanoparticules recouvertes de PAAm ne se sont pas dissoutes dans l'eau de rivière et dans l'eau de mer diluée, mais elles ont montré un comportement particulier dans l'eau déionisée avec une réduction de la concentration en ions argent libres, suivie d'une forte dissolution ou désorption de l'argent libre capturé. Dans l'eau de mer, la diminution des ions argent au départ n'est pas observée et une dissolution lente a eu lieu et s'est poursuivie sur la durée de l'expérience.

Selon Levard et al. (2012), les nanoparticules sans recouvrement sont électrostatiquement stabilisées contre l'agglomération à cause des charges négatives sur leur couche de surface. Donc, aucune agglomération ne devrait être observée dans l'eau déionisée puisque celle-ci ne contient que très peu d'ions ou de molécules organiques qui pourraient changer l'interaction de surface entre les nanoparticules. Dans cette étude, les nanoparticules sans recouvrement se sont agglomérées dans l'eau déionisée sur 6 h et sur 16 jours. Plusieurs auteurs ont observé une agglomération de nanoparticules sans recouvrement dans de l'eau déionisée contenant différents électrolytes (Li et al. 2010; Liu et al. 2011; Delay et al. 2011). L'absence d'agrégation des nanoparticules sans recouvrement dans l'eau déionisée et bullé à l'azote soulève certaines questions. La différence pourrait venir du bullage à l'air, car la présence de CO_2 dans l'air bullé pourrait avoir saturé la bouteille en CO_2. L'ion Ag^+ où le composé Ag_2O à la surface des nanoparticules a pu réagir avec le CO_2 dissous et former une fine couche d'$AgCO_3$ à la surface des nanoparticules ce qui aurait favorisé une agglomération. La dissolution des nanoparticules nues a eu un comportement particulier dans les eaux à faible force ionique. Une hausse de la concentration en ions argent libres dès l'ajout des nanoparticules a été observée et est attribuée à la présence d'ions argent ou d'autres formes solubles d'argent capturés à la surface des nanoparticules et qui auraient été libérés lors de la dilution dans l'eau. Les ions argent libres se sont ensuite associés de nouveau à la phase particulaire. Dans les bouteilles bullées à l'air, les ions argent peuvent réagir avec le CO_2 pour former un précipité $AgCO_3$, ce qui pourrait expliquer la baisse des ions argent libres dans les bouteilles au début du processus. Cependant, le même phénomène a aussi été observé dans les bouteilles bullées à l'azote qui ne devraient pas contenir un excès de CO_2.

Le recouvrement des AgNP avec du citrate augmente la charge négative de surface. Donc, les nanoparticules recouvertes de citrate offrent normalement une meilleure stabilité contre l'agrégation. D'après la littérature, l'agrégation et la dissolution des AgNP citrate sont favorisées dans un milieu oxygéné. L'agrégation dans l'eau de mer a été observée par plusieurs auteurs (Liu et Hurt, 2010; Prathna et al. 2011; Angel et al. 2013). Prathna et al. (2011) ont montré que selon le type de synthèse de nanoparticules (citrate dans l'étude), le comportement est diffèrent. Donc, la méthode de synthèse des nanoparticules affecterait fortement le comportement et expliquerait aussi les différents résultats d'études ayant l'objectif commun de comprendre la dissolution des AgNP.

Le recouvrement citrate semble mieux stabiliser les AgNP dans l'eau déionisée et l'eau de rivière car les agrégats ne dépassaient pas 200 nm, comparativement aux agrégats des nanoparticules sans recouvrement beaucoup plus gros. Ces résultats diffèrent de ceux de Zhang et al. (2011), qui ont observé une agrégation 3 à 8 fois plus rapide des AgNP citrate en présence d'oxygène dissous dans une solution de nutriments hydroponique (Hoagland medium). Nous avons observé que la dissolution des AgNP-citrate est plus prononcée dans l'eau de mer et aussi plus rapide avec une faible concentration en oxygène dissous. Cette observation va à l'encontre des résultats de Liu et Hurt (2010). Le recouvrement citrate n'a pas stabilisé les AgNP puisqu'une forte agglomération a eu lieu dans l'eau de mer et la taille des agrégats était similaire aux nanoparticules sans recouvrement. Liu et Hurt (2010) ont aussi observé que les AgNP citrate ne se dissolvent pas dans l'eau désoxygénée. Or, dans notre étude, les faibles concentrations en oxygène semblent favoriser la dissolution des nanoparticules ce qui apparait paradoxal à première vue, mais indique plutôt un manque de compréhension de l'ensemble des facteurs qui sont mis en jeu.

Les nanoparticules recouvertes de polyallylamine (AgNP-PAAm) sont utilisées dans notre laboratoire comme des nanoparticules recouvertes d'un polymère modèle pour étudier la bioaccumulation dans des tissus biologiques (Al-Sid-Cheikh et al. 2011). Le poids moléculaire du PAAm est de 65 000 Da. Il est utilisé comme agent réducteur et chaque chaine du polymère a plusieurs sites de réduction. Les molécules de PAAm ont des groupements amines le long de la chaine de carbone. Les amines ont un doublet d'électrons libres et peuvent facilement capturer des cations comme l'Ag^+ et échanger des cations avec le milieu. La détermination de la taille moyenne des AgNP-PAAm par DLS a été problématique puisque l'excès de PAAm interférait avec les mesures. La taille moyenne de la suspension d'AgNP-PAAm a été déterminée proche de 337 nm par DLS, mais une observation au MET montre que les AgNP-PAAm avaient une taille moyenne de 20 nm.

Les AgNP-PAAm dans l'eau déionisée se sont agglomérées d'une manière particulière. En effet les images au MET montrent que les nanoparticules sont proches les unes des autres et sont emprisonnées dans le PAAm. Pour l'eau de rivière, l'agglomération est plus rapide et les agrégats sont des nanoparticules collées les unes aux autres. De même pour l'eau de mer diluée, les agrégats sont des nanoparticules fusionnées les unes aux autres, mais ils semblent plus gros que dans l'eau de rivière. Le comportement entre ces deux types d'eau est différent et les résultats montrent que la composition de l'eau influence probablement le comportement des nanoparticules puisque la conductivité des deux types d'eau était similaire.

Les AgNP-PAAm diluées dans les eaux de faible force ionique ont montré une forte concentration d'ions argent libres, qui ont ensuite été re-capturés, possiblement par le PAAm. Ce phénomène est d'ailleurs clairement visible dans l'eau déionisée. Ce phénomène de relargage d'ions argent a été observé par Dobias et Bernier-Latmani (2013) et est considéré comme normal. On pose l'hypothèse que des ions argent seraient d'abord emprisonnés dans le recouvrement polymérique, mais une fois que la suspension est diluée, ces ions argent sont libérés au moins en partie. Les AgNP-PAAm ne sont pas dissoutes dans l'eau de mer diluée ou l'eau de rivière, le recouvrement PAAm les ayant protégées. L'oxygène dissous et le CO_2 dissous n'ont pas affecté la dissolution ou le phénomène de recapture des ions argent. Le comportement dans l'eau de mer apparaît différent puisqu'une augmentation lente et constante d'ions argent a eu lieu. Ce phénomène serait dû à un relargage d'ions argent capturés dans le PAAm due à la compétition avec des cations dans l'eau de mer plutôt qu'à une dissolution, quoique les deux phénomènes peuvent être présents simultanément. Le fait que la concentration en oxygène dissous n'influe pas sur ce mécanisme appuie l'idée que ce n'est pas une dissolution qui a été observée.

Cette étude illustre toute la complexité du comportement des AgNP dans les eaux naturelles et questionne certains modèles simples présentés par la littérature. Le comportement varie surtout en fonction de la composition de l'eau et du type de recouvrement des nanoparticules. Dans les eaux à faible force ionique, les nanoparticules ne se dissolvent pas, mais un relargage rapide d'ions argent a eu lieu, suivi d'une re-adsorption lente (Tableau 9). La concentration en oxygène dissous semble influencer la dissolution dans certain cas précis comme la dissolution des AgNP citrate dans l'eau de mer. Tous les types d'AgNP s'agrègent dans l'eau. Les agrégations les plus rapides et les plus gros agrégats se retrouvant dans l'eau de mer (Tableau 9). La force ionique élevée de celle-ci contraint les trois types de nanoparticules à s'agréger. Il serait intéressant de répéter les expériences de dissolution dans des eaux de faible force ionique sur 24 h ou 48 h avec un délai d'échantillonnage plus court au début de l'expérience pour observer le phénomène de relargage des ions argent. Des expériences plus longues dans l'eau de mer pour savoir si la dissolution des AgNP est totale ou partielle seraient aussi souhaitables. L'interaction des nanoparticules avec la matière organique dans les eaux devrait aussi influencer le comportement des AgNP. Cette étude contribue à mieux comprendre le comportement des AgNP dans des eaux naturelles, mais les travaux ont été effectués dans des conditions de laboratoire sans lumière, ni changement de température ou de force ionique en cours d'expérience. De plus, il faut rappeler que la concentration des AgNP-citrate était environ 10 fois plus faible que celles des deux autres espèces ce qui a pu influencer certains de nos résultats. Des travaux faisant intervenir diverses concentrations en AgNP pourraient répondre à cette incertitude. Il reste encore beaucoup de choses à apprendre sur le comportement des nanoparticules dans le milieu naturel pour savoir si elles peuvent persister dans l'environnement et porter atteinte aux espèces et aux écosystèmes aquatiques. Les interactions multiples avec plusieurs types de nanomatériaux en même temps ainsi que la présence de métaux traces toxiques restent à explorer (Tong et al. Under Press).

Type de Nanoparticules	Conditions		Agrégation	Dissolution (-)/ Adsorption (+)
NUES	Eau Déionisée	O_2	++	++
		Peu d'O_2		++
	Eau Riviere	O_2	++	++
		Peu d'O_2	++	++
	Eau de mer diluée	O_2	+	++
		Peu d'O_2	n.d.	++
	Eau de mer	O_2	+++	--
		Peu d'O_2	+++	--
CITRATE	Eau Déionisée	O_2	+	-
		Peu d'O_2	+	-
	Eau Riviere	O_2	+	-
		Peu d'O_2	+	-
	Eau de mer diluée	O_2	+	-
		Peu d'O_2	n.d.	-
	Eau de mer	O_2	+++	----
		Peu d'O_2	+++	----
PAAM	Eau Déionisée	O_2	++	+
		Peu d'O_2	++	+
	Eau Riviere	O_2	+++	+
		Peu d'O_2	+++	+
	Eau de mer diluée	O_2	+	+
		Peu d'O_2	+	+
	Eau de mer	O_2	+++	---
		Peu d'O_2	+++	---

Tableau 9 : Tableau synthèse des résultats.

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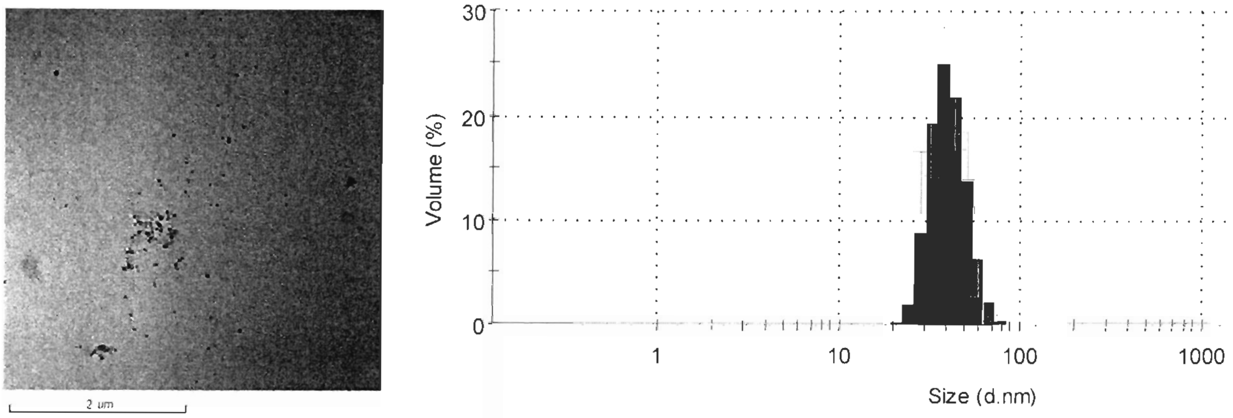
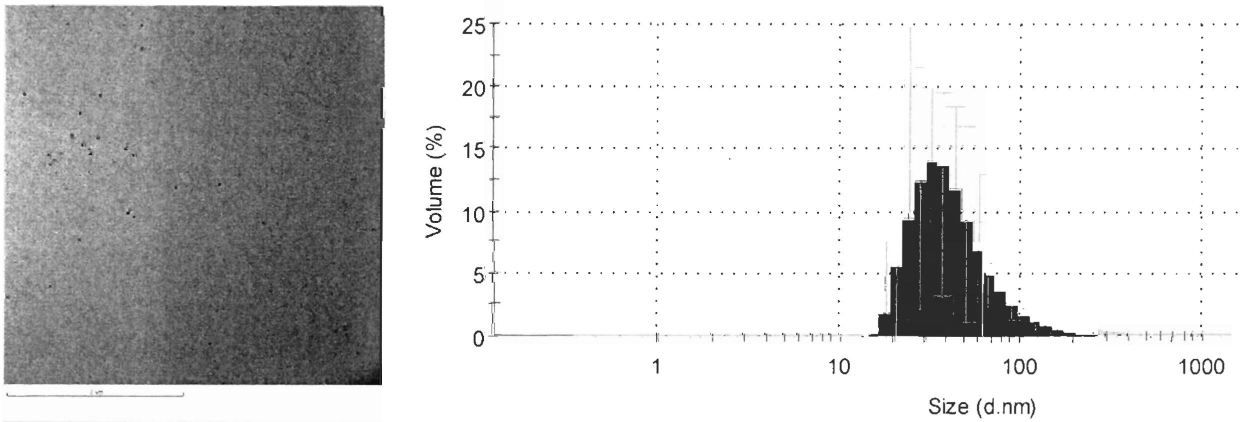
ANNEXE I

TEM images and size distribution (by DLS) of three suspensions of nanoparticles. (A) Bare AgNP, (B) Citrate AgNP and (C) PAAm AgNP

A


2 um
30
20
10
0
Volume (%)
1
10
100
1000
Size (d.nm)

B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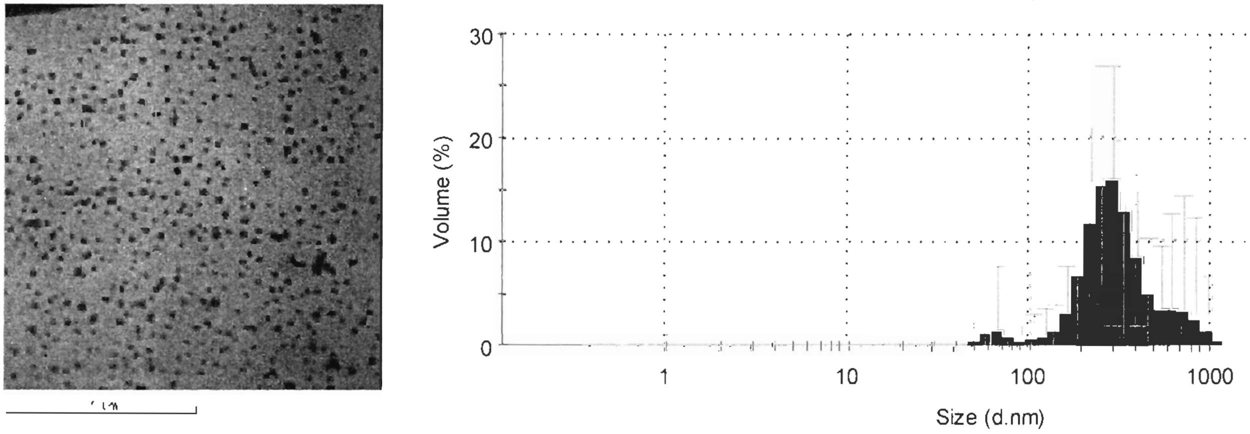
20
15
10
5
0
Volume (%)
1
10
100
1000
Size (d.nm)

C


30
20
10
0
Volume (%)
1
10
100
1000
Size (d.nm)