Accumulation of dissolved silver and silver nanoparticles in liposomes used as a model membrane

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Abstract

The increasing presence of nanomaterials in consumer products has led the scientific community to study the environmental fate of these contaminants of emerging concern. Understanding their transformations and interactions with living organisms is a crucial step in the study of their impacts on aquatic ecosystems. Silver nanoparticles, used mainly for their antibacterial properties, are among the most common nanomaterials. How they interact with aquatic organisms, especially how they cross the biological membrane, remains uncertain.

In this project, we are studying the uptake of dissolved silver and silver nanoparticles by liposomes. These unilamellar vesicles composed of phospholipids have long been used to model natural biological membranes. This allows studying the potential uptake of silver by passive diffusion through the phospholipid bilayer.

The liposomes were synthesized in a pH 6 buffer using extrusion techniques and potential membrane leakage was monitored throughout. Size exclusion chromatography was used to remove the outer buffer and the liposomes were then exposed over time to silver under different conditions where Ag^+, $AgS_2O_3^-$ or $AgCl^0$ were the dominating species. At the end of the exposure, Ambersep GT74 cation exchange resin (0.3 g per sample, > 95% efficient) was used in order to bind the non-assimilated dissolved metal. Similar experiments were conducted with the complexes $HgCl_2$ and $Cd(DDC)_2$, both hydrophobic and known to diffuse passively through biological membranes.

The uptake kinetics of Ag^+, $HgCl_2$, $Cd(DDC)_2$ and AgNPs show no increase over time, unlike $AgS_2O_3^-$, $AgCl^0$ and tritiated water, which appear to go through the phospholipid bilayer. This seems in contradiction with our initial hypothesis that lipophilic Hg and Cd complexes would be able to cross the membrane whereas charged silver complexes would not.

Introduction

- The free metal ion (Ag^+) is normally the most chemically reactive form.
- However, small and hydrophobic complexes are present in natural waters.
- The involvement of neutral complexes in transmembrane transport is somewhat controversial (ex.: $AgCl^0$).
- The mechanism of interaction between silver nanoparticles and biological membranes is still unknown.

Q.: Is passive diffusion of silver complexes and silver nanoparticles through the phospholipid bilayer possible?

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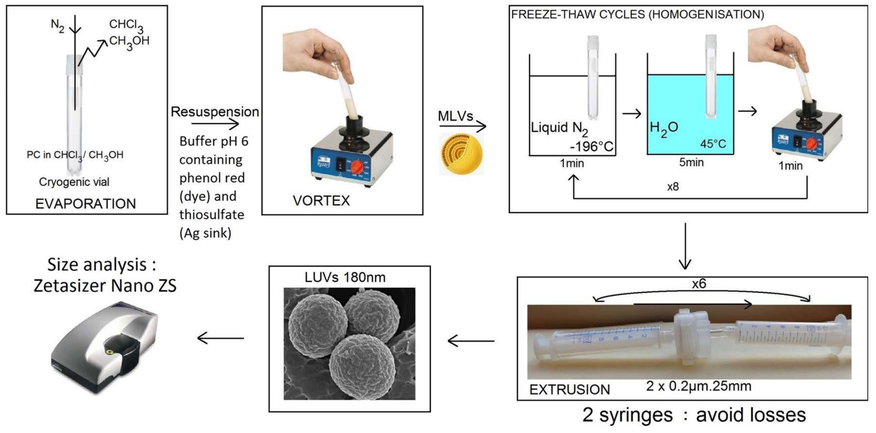
Methodology

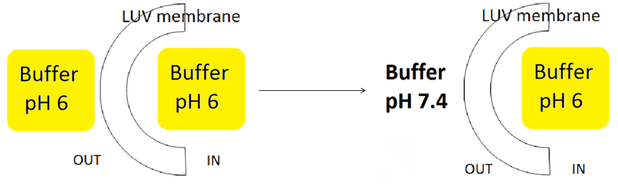
- Preparation of liposomes

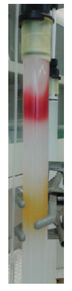
  Buffer pH 6 (I = 25 mM): Phenol red 6 mM, MES (2-(N-Morpholino)ethanesulfonic acid) 5 mM, $Na_2S_2O_3$ 1.33 mM, NaOH 2.4 mM, KNO_3 15.8 mM



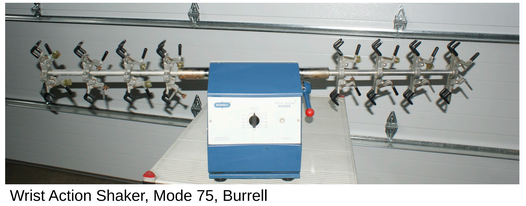
- Buffer exchange: size exclusion chromatography (Sephadex G50)

  Buffer pH 7.4: MOPS (4-Morpholinepropanesulfonic acid) 7.5 mM, NaOH 5.6 mM, KNO_3 19.65 mM





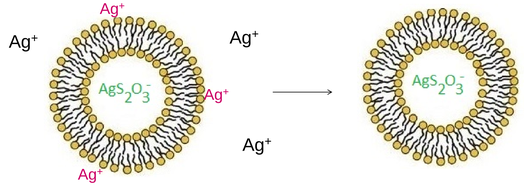
- Interaction with metals and silver nanoparticles



Wrist Action Shaker, Mode 75, Burrell

Short term exposure (kinetics study)

- Time: 100 min
- Δ [Ag]external solution < 10 %
- Δ $[S_2O_3^{2-}]$free in the internal solution < 10 %

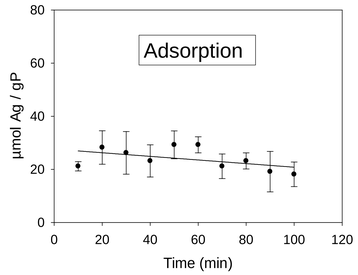


Treatment with a cation exchange resin (Ambersep GT 74)

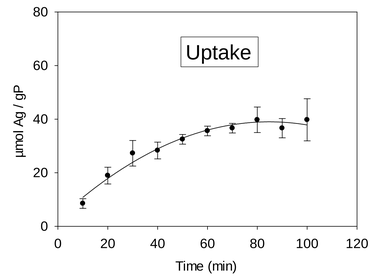
- Time: 5 min
- Resin weight : 0.3 – 0.5 g
- Aim: trapping non-internalized metal ions

Analysis of the supernatant on a gamma counter (dissolved metals) or ICP-MS (AgNPs).

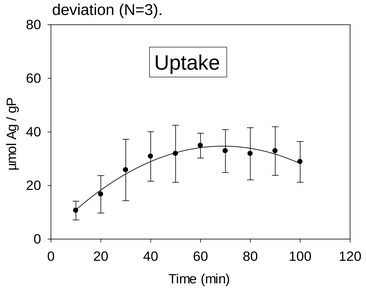
Results



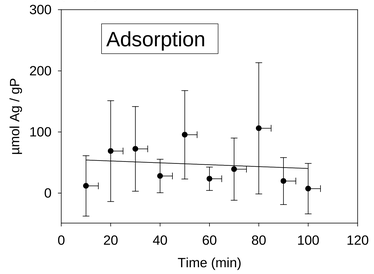
Accumulation of Ag^+ by liposomes over time. $[Ag_{tot}]_{init}$ = 23.9 ± 1.4 nM. Resin efficiency = 96.5 ± 0.7 %. Adsorption on walls = 5.3 ± 0.7 %. Mean ± standard deviation (N=3).



Accumulation of $AgS_2O_3^-$ by liposomes over time. $[Ag_{tot}]_{init}$ = 24.2 ± 1.2 nM. Resin efficiency = 98.3 ± 0.3 %. Adsorption on walls = 12.1 ± 0.7 %. Mean ± standard deviation (N=3).



Accumulation of $AgCl^0$ by liposomes through time. $[Ag_{tot}]_{init}$ = 24.5 ± 1.9 nM. Resin efficiency = 98.3 ± 0.5 %. Adsorption on walls = 3.7 ± 2.3 %. Mean ± standard deviation (N=3).



Accumulation of AgNPs-PVP 5 nm by liposomes through time. $[Ag_{tot}]_{init}$ = 70.8 ± 0.2 nM. Resin efficiency = 98.2 ± 1.1 %. Adsorption on walls = 77.6 ± 5.6 %. Mean ± standard deviation (N=3).

Conclusion

- Results suggest a passive diffusion of $AgCl^0$, but not of AgNPs.
- Some limits to the model: unexpected uptake of $AgS_2O_3^-$, lipid bilayers could be more permeable to anions than to cations. Hydrophobic $HgCl_2$ and $Cd(DDC)_2$ complexes surprisingly did not diffuse through our model membranes.
- Extrapolation to biological membranes should be made with caution.

Future work

- Influence of the vesicle curvature on membrane permeability: same experiments, larger liposomes.