

Is Precipitation of Aluminum Fast Enough to Explain Aluminum Deposition on Fish Gills?

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A peristaltic pump was used to mix environmentally realistic concentrations of Al in soft water (14–15°C) with 30 μM base (NH_4OH) to simulate the alkalization which occurs when acidic soft water passes over fish gills. Solutions were filtered under vacuum through 0.2 μm prerinsed filters using a polycarbonate filter apparatus. If final solution pH was ≤ 5.3 , only about 10% of total Al (159 $\mu\text{g}\cdot\text{L}^{-1}$) was filtered from solution. If solution pH was raised from pH 5.3 to pH 5.7 with base, about 35% of Al was removed from solution, 1–4 s after the pH was raised. Similar results were obtained for 335 $\mu\text{g}\cdot\text{L}^{-1}$ Al solutions. Production of additional Al retained by filters was fast enough to show that Al accumulation on fish gills may be partly due to polymerisation and precipitation of Al, as Al solubility is reduced in the more alkaline gill micro-environment.

On a utilisé une pompe péristaltique pour incorporer du Al dilué dans de l'eau douce (14–15°C) avec 30 μM de base (NH_4OH) afin de reproduire l'alcalinisation qui se produit quand des eaux douces acides traversent les ouies des poissons. On a filtré les solutions sous vide avec des filtres pré-rinsés de 0,2 μm à l'aide d'un appareil de filtrage au polycarbonate. Si le pH de la solution finale était inférieur à 5,3, moins de 10 % de la concentration totale de Al (159 $\mu\text{g}\cdot\text{L}^{-1}$) était filtrée de la solution. Si, par ajout de base, le pH passait de 5,3 à 5,7, environ 35 % du Al était enlevé de la solution de une à quatre secondes après l'augmentation du pH. On a obtenu des résultats semblables avec des solutions de 335 $\mu\text{g}\cdot\text{L}^{-1}$. La production de Al supplémentaire retenu par le filtre était assez rapide pour révéler que l'accumulation de Al dans les ouies des poissons peut être en partie le résultat de la polymérisation et de la précipitation du Al étant donné que sa solubilité est diminuée dans le milieu plus alcalin des ouies.

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Solubility of Al is dependent on water pH, falling exponentially as pH rises from pH 4, until minimum solubility is reached at about pH 5.8 (eg. Roberson and Hem 1969). Recently, we showed that the pH of acidic soft water increases as it passes over rainbow trout gills, because the fish's metabolism releases approximately 30 μM of base (ammonia plus unidentified titratable alkalinity; Playle and Wood 1989a). The extent of the pH rise depends on inspired water pH and the acidifying influence of CO_2 released at the gills. If inspired soft water contains 100–200 $\mu\text{g}\cdot\text{L}^{-1}$ Al, the pH increase at the gills could theoretically cause a loss of Al solubility at inspired pHs 4.8–5.8 (cf. figure 5 of Playle and Wood 1989b). Reduced Al solubility in the gill micro-environment would result in Al deposition on the gills. Accumulation of Al on fish gills appears to be the cause of disturbances in ionoregulation and respiratory gas transfer which may kill the fish (eg. Neville 1985; Neville and Campbell 1988; and others listed in Playle et al. 1989).

However, it was not known if Al could precipitate from solution fast enough to account for Al deposition on fish gills. In the past, researchers working with Al chemistry have usually been interested in geological time scales rather than in biological time scales, and have often measured Al precipitation from concentrated Al solutions over hours to days (eg. May et al. 1979; Parthasarathy and Buffle 1985). The fastest reported time

for Al precipitation is 1 min, for 540 or 1080 $\mu\text{g}\cdot\text{L}^{-1}$ Al solutions at 2–20°C (Tipping et al. 1988). As a result, we measured Al precipitation over a time scale approximating water residence time at fish gills, in acidic soft water of low Al concentration, with an alkalization of the water similar to that previously measured at fish gills.

Methods

Soft water was produced by passing dechlorinated Hamilton city tapwater through a deionising resin canister (J.W. Anderson Co. Ltd., Dundas, Ont.) or through a reverse osmosis unit (Culligan MP1000), and adding appropriate amounts of analytical grade NaCl and CaCl_2 (BDH, Toronto, Ont.). Water composition was: Ca^{2+} ~ 40 $\mu\text{eq}\cdot\text{L}^{-1}$, Na^+ ~ 50 $\mu\text{eq}\cdot\text{L}^{-1}$, Cl^- ~ 90 $\mu\text{eq}\cdot\text{L}^{-1}$, titratable alkalinity to pH 4.0 ~ 130 $\mu\text{eq}\cdot\text{L}^{-1}$, pH ~ 6.5 . Aluminum solutions were made in this water by adding a concentrated stock solution of $\text{AlCl}_3\cdot 6\text{H}_2\text{O}$ (0.39 $\text{g}\cdot\text{L}^{-1}$, pH ~ 4 , Sigma).

Preliminary experiments on Al precipitation rates used a Hach 2100A turbidity meter, a Pye Unicam PU 8600 spectrophotometer, and a glass 300 mL filtering funnel (Millipore) with 0.2 or 0.45 μm membrane filters (Millipore). Aluminum concentrations from these experiments were measured using the pyrocatechol violet method (Dougan and Wilson 1974).

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Final experiments used a peristaltic pump (Gilson Minipuls 2) to mix the test Al solution ($7.2 \text{ mL} \cdot \text{min}^{-1}$) with deionised water or $300 \mu\text{M NH}_4\text{OH}$ ($0.7 \text{ mL} \cdot \text{min}^{-1}$ for both). The dilution resulted in about $30 \mu\text{M}$ base addition. Deionised water addition was the control. All solutions were kept at $14\text{--}15^\circ\text{C}$. Initial pH of the Al solution was adjusted with dilute H_2SO_4 . Final solution pHs (after mixing with base or water, but before being filtered) were measured in 8 mL polyethylene vials (stirred) using a Radiometer PHM82 meter and Radiometer GK2401C combination electrode. One minute was allowed for electrode stabilisation.

The Al solution and base (experimental) or Al and water (control) met at a polyethylene "T", then flowed along either 2 or 16 cm of polyethylene tubing, which took 0.8 or 3.2 s aftermixing, respectively. The solution then dripped onto a $0.2 \mu\text{m}$ Nuclepore filter under vacuum (700 mm Hg) and was collected in an 8 mL polyethylene vial suspended beneath the filtering apparatus. Total delay between mixing and arrival of a drop in the vial after filtration was estimated to be about 1.4 or 3.8 s. The filter funnel assembly was plastic (polycarbonate; Nuclepore), with a rubber O-ring and silicone gasket. The funnel was disassembled completely between each sample run, rinsed in 5% HCl, distilled water, then with deionised water. The re-assembled filtering apparatus and fresh $0.2 \mu\text{m}$ filter were rinsed with two 100 mL volumes of 5% HCl under vacuum, then with two 100 mL volumes of soft water acidified to $\text{pH} \sim 4.3$ with dilute H_2SO_4 , and finally rinsed for 2 min with the experimental or control solution dripping onto the filter, again under vacuum. This protocol eliminated Al contamination between samples. The third or fourth minute of filtered Al solution was the sample collected for analysis; filters did not clog in this time.

Samples (7 mL) from the final set of experiments were acidified with $20 \mu\text{L}$ concentrated HCl, refrigerated, then analysed without dilution for total Al using a Varian AA-1275 atomic absorption spectrophotometer with GTA-95 graphite tube atomizer. Ten μL of sample were analysed against standards at 309.3 nm . Operating parameters were as follows: 5 s at 80°C , 35 s at 95° , 10 s at 110° , 12 s at 1200° , and 2.7 s at 2500°C , N_2 gas. Unpaired Student's *t*-tests were used for statistical analysis of Al data.

Results

Initial experiments demonstrated that colloidal Al formed quickly in concentrated solutions brought to near neutral pH. For example, a $44 \text{ mg} \cdot \text{L}^{-1}$ Al solution brought from pH 4.0 to pH 6.4 with 1 N KOH (at room temperature) turned cloudy within 3 min. On the turbidity meter, turbidity increased by 1.2 FTU units 25 s after base addition. Absorbance (291 nm) on the spectrophotometer increased by 0.006 units about 15 s after base addition. However, it became clear that these methods were not sensitive enough or quick enough to measure fast Al precipitation in dilute solutions, so filtering was tried.

Filtering a concentrated Al solution ($44 \text{ mg} \cdot \text{L}^{-1}$) 10 min after partial neutralisation (pH 4.0 brought to pH 5.8 with 1 N KOH) removed 90–100% of total Al, whereas filtering the same solution at pH 4.0 removed only about 4% of the Al. A glass filtering apparatus and $0.45 \mu\text{m}$ membrane filter were used. With a more reasonable concentration of Al ($505 \mu\text{g} \cdot \text{L}^{-1}$ at pH 5.3, 15°C), filtration through a 0.45 or $0.2 \mu\text{m}$ membrane removed about 25 and 36% of total Al, respectively. About 93% of the Al was removed when the same solution was brought to pH 6.4

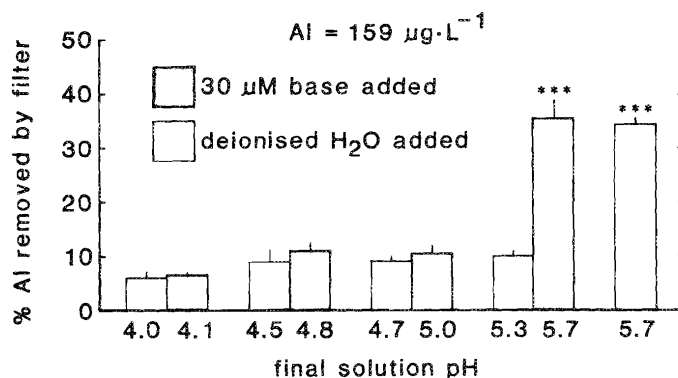


FIG. 1. Effects of solution pH on the amount of Al filtered from a $159 \mu\text{g} \cdot \text{L}^{-1}$ Al soft water solution, expressed as a percentage of total Al. $14\text{--}15^\circ\text{C}$. Clear bars: percent of total Al removed from solution when filtered through a $0.2 \mu\text{m}$ filter 1.4 or 3.8 s after mixing with deionised water (control). Combined data from 1.4 and 3.8 s tests. Adjacent dark bars: percent of total Al removed when solution pH was raised from control values by mixing with base ($30 \mu\text{M NH}_4\text{OH}$). Combined data for 1.4 or 3.8 s tests. About 35% of total Al was removed by filtration if final solution pH was 5.7; only about 10% was removed if pH was ≤ 5.3 . *** = $P < 0.001$ (*t*-test), vs. pH 5.3 (control). For solution pHs ≤ 5.0 , $n = 6$; for pH 5.3, $n = 16$; for pH 5.7 (dark bar) $n = 18$; and for pH 5.7 (clear bar) $n = 10$. Error bars: 1 SEM.

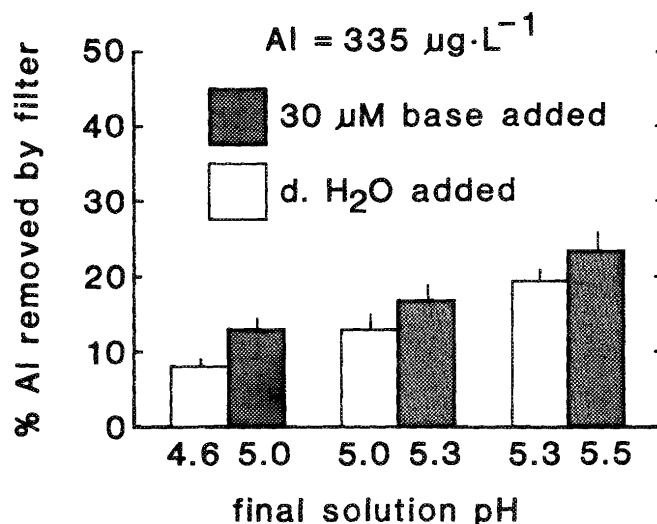


FIG. 2. Effect of solution pH on the amount of Al filtered from a $335 \mu\text{g} \cdot \text{L}^{-1}$ Al soft water solution, expressed as a percentage of total Al. Delay after base or water addition to the solution and filtering was 1.4 s. Other details as in legend of Fig. 1. The trend was more Al removed as solution pH increased. For all bars, $n = 3$.

and filtered within 3–5 min. However, for lower concentrations of Al, it became apparent that Al was being removed from solution by the glass filtering apparatus and glass porous filter support, as well as by the filters, so a plastic filtering unit was tried.

Final experiments used a plastic filtering apparatus, and a peristaltic pump to reduce the time between base addition and filtering. For a $159 \pm 2 \mu\text{g} \cdot \text{L}^{-1}$ Al solution in soft water, $\sim 10\%$ of total Al was removed by filtering if the final pH of the solution was ≤ 5.3 (Fig. 1). If final solution pH was 5.7, or was raised to that pH by $30 \mu\text{M NH}_4\text{OH}$ addition, the amount of Al removed by filtering increased to about 35% of total Al. This effect was seen just 1.4 s after the pH rise induced by NH_4OH addition. There was no indication that the amount of Al

removed from solution depended on the delay between base and Al solution mixing and filtration, therefore results from the 1.4 and 3.8 s delay tests have been pooled in Fig. 1.

To see if the same pattern of Al removal occurred from a more concentrated solution of Al at comparable pHs, experiments with a $335 \pm 5 \mu\text{g}\cdot\text{L}^{-1}$ Al solution were run. The pattern of increased Al removal from solution after base was added was still evident (Fig. 2), and, if the amounts removed are expressed as concentrations, more Al was removed from the more concentrated Al solution at a given pH. However, the trend of greater Al removal at higher pH was less pronounced, partly because of the small number of replicates run, but also because of smaller increases in pH as $30 \mu\text{M NH}_4\text{OH}$ was added.

Discussion

The exact mechanisms leading to the increases in Al retained by filters at $\text{pH} > 5.3$ (Fig. 1) are not known. As pH increases and Al solubility is reduced, monomers of Al may polymerise and form filterable complexes, or Al monomers may adsorb to the filter surface, polymerise, and form a precipitate (Dentel and Gossett 1988). These processes presumably occur at fish gills, as suggested by Baker and Schofield (1982). The increase in pH of ~ 0.4 units is about the difference between inspired and expired water pH seen in rainbow trout in similar soft water (Playle and Wood 1989a,b), and is large enough to theoretically result in Al precipitation if initial Al concentration is $> 100 \mu\text{g}\cdot\text{L}^{-1}$ (Playle and Wood 1989b).

Residence time of water at the gills is 0.4–2 s (Randall 1970), and may be longer because of unstirred boundary layers, so the time scale of the filtration experiments was appropriate. Background removal of Al by filtration was about 10% of total Al, similar to the loss of Al from a standard solution filtered through rinsed, $0.4 \mu\text{m}$ filters (Campbell et al. 1983). Background removal of Al probably represents adsorption of positively charged Al species to negative charges known to exist on membrane filters (eg. Bisio et al. 1980; Johnson et al. 1989). Similarly, negative charges are assumed to exist on branchial surfaces, because of carboxyl groups on mucus (Satchell 1984). The filtration method used here, although crude, was able to simulate chemical conditions currently thought to exist near fish gills, in magnitude of pH change, time scale, and even surface charge.

The solubility of Al is lower, and reaction rates faster, in water of low ionic strength because activity coefficients are greater at lower ionic strength (eg. Bache 1986). The low ionic strength of the soft water used ($\sim 10^{-4}$ M) would be expected to maximise Al precipitation rates. Aluminum filtered from solution was about 10% of total Al at $\text{pH} \leq 5.3$, increasing to 35% whether the solution was brought to pH 5.7 just seconds before filtration, or was prepared at pH 5.7 and filtered minutes later (Fig. 1). In contrast, Chappel and Birchall (1988) reported only about 20% retention of Al at pH 5.7 compared to about 10% at pH 5.3 (20 h after solutions were prepared, $0.2 \mu\text{m}$ filters, $\sim 3 \text{mg}\cdot\text{L}^{-1}$ Al solution). These workers, however, used water of ionic strength about 10^{-2} M, which would have an Al activity coefficient about two-thirds that in the soft water used here (extended Debye–Hückel equation, from Stumm and Morgan 1981). This could explain their apparently higher Al solubility even after 20 h.

The 14–15°C conditions used in our experiments would have slowed Al polymerisation and precipitation rates compared to rates at room temperature. However, Al precipitation as deter-

mined by filtration still occurred within seconds. Aluminum precipitation would be slowed further in very cold water (eg. 0–4°C), which would represent conditions encountered by fish during springmelt pulses of Al and acidity, but it is not known whether pH changes at the gills would be the same at these temperatures as at 15°. For example, fish not eating at cold temperatures might release more ammonia at the gills due to catabolism of endogenous protein, which would worsen Al precipitation. Alternatively, they might release less ammonia due to lowered metabolic rates. Clearly this question deserves more attention before chemical simulations of the gill micro-environment are done at lower temperatures.

As expected, more Al was filtered on an absolute basis from the $335 \mu\text{g}\cdot\text{L}^{-1}$ Al solution at a given pH compared to the $159 \mu\text{g}\cdot\text{L}^{-1}$ Al solution (Fig. 2). $335 \mu\text{g}\cdot\text{L}^{-1}$ is close to saturation at pH 5.0 (eg. Roberson and Hem 1969). Certainly at $\text{pH} \geq 5.3$ the Al solution is well above saturation, as indicated by the 17–24% removal of Al by filtration (Fig. 2). For the $335 \mu\text{g}\cdot\text{L}^{-1}$ Al solution at pH 5.3, the addition of $30 \mu\text{M}$ base raised its pH to 5.5, whereas for the $159 \mu\text{g}\cdot\text{L}^{-1}$ Al solution, pH increased to 5.7. This difference was likely due to the extra pH buffering of more Al in solution, because the formation of Al-hydroxides consumes base (eg. calculations in Playle and Wood 1989b).

Reduced Al solubility in the gill micro-environment can explain higher gill Al accumulation at inspired pH 5–6, but does not explain Al accumulation in more acidic water (eg. Neville 1985; Playle and Wood 1989b). Below pH 5, adsorption and complexation of positively charged Al species onto branchial surfaces and mucus better explain the smaller amount of Al accumulating on fish gills. The relative contribution of polymerisation, precipitation, adsorption, and complexation to gill Al deposition would be influenced by factors such as pH of the gill micro-environment, Al concentration, solution ionic strength and buffer capacity, negative charges on mucus and gill surfaces, the amount of mucus produced at the gills, and gill surface area.

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