Aluminium Speciation Studies in Biological Fluids. A New Investigation of Aluminium Hydroxide Equilibria under Physiological Conditions†

Marilyne Venturini and Guy Berthon *
Institut National de la Santé et de la Recherche Médicale U305, Laboratoire de Chimie Bioinorganique, Université Paul Sabatier, 38 rue des Trente-Six Ponts, 31400 Toulouse, France

Aluminium is prone to hydrolyse within a large range of physiological pH. Although this phenomenon has drawn much attention in the recent past, no reliable hydroxide formation constants have been produced so far that can be applied to physiological conditions of ionic strength and temperature. Prior to future aluminium–ligand equilibrium studies, aluminium hydroxide formation at 37 °C in aqueous NaCl (0.15 mol dm^{-3}) has been investigated. The following species have been characterized: $[Al(OH)]^{2+}$, $Al(OH)_3$, $[Al(OH)_4]^-$, $[Al_3(OH)_{11}]^{2-}$, $[Al_6(OH)_{15}]^{3+}$, and $[Al_8(OH)_{22}]^{2+}$

Aluminium was long considered a non-toxic element, but there is now a growing awareness that it is actually involved in many health disorders.[1,2] Major clinical concerns refer to its use as a phosphate binding agent in the management of long term renal dialysis[3–5] and as a constituent of antacids in the treatment of peptic ulcers.[6–9] In addition, its frequent occurrence as a pollutant in nutritive mixtures has recently emerged as a serious hazard for patients on long term total parenteral nutrition,[10] especially infants.[11,12]

Before specific answers to each of these problems can be brought to light, speciation studies are necessary to elicit the main factors that may affect aluminium bioavailability in the various biofluids involved. Among the several fractions of aluminium present in these biofluids, the different protein-associated pools can be assessed by analytical techniques.[13–16] Nevertheless, the main complexes of the low-molecular-weight (l.m.w.) fraction cannot be discriminated on an experimental basis, due to their extremely low concentrations and to the lability of their equilibria. Yet, l.m.w. species are of particular interest, these being the most active in terms of metal bioavailability.[17,18] In such a case, the only way of access to quantitative investigations exists in the simulation of the distribution of aluminium into its different l.m.w. complexes in relevant biofluids.

Such studies have already been initiated concerning the bioavailability of aluminium in natural waters,[19] and preliminary reports have recently been published on aluminium distribution in blood plasma.[20,21] However, relatively little information has been produced so far on which reliable simulation models can be built,[19,22–27] this being especially true for physiological conditions.[28,29] It may indeed be worth recalling that the reliability of a given simulation model crucially depends on that of the parameters fed into the corresponding database.[30]

As far as aluminium is concerned, its particular ability to give rise to hydroxides of various stoicheiometries within a large range of physiological pH makes it necessary to ascertain the exact nature and stability of such species under experimental conditions specific to complex equilibrium studies involving this metal.

The present work thus reports a new detailed investigation of aluminium hydroxide formation at 37 °C in 0.15 mol dm^{-3} aqueous sodium chloride.

Table 1. Summary of the titration data used: initial total concentrations of aluminium (c_{Al}) and hydrochloric acid (c_H) (mmol dm^{-3}), pH * range investigated, and number of experimental points (N) for each experiment

Expt.	c_{Al}	c_H	pH Range	N
1	10.20	19.71	2.62—11.26	59
2	10.19	9.63	2.01—11.08	61
3	10.27	9.70	2.00—10.35	49
4	5.19	4.80	2.32—11.16	63
5	3.82	3.61	2.45—10.70	67
6	2.55	2.41	2.62—10.70	52
7	2.56	2.41	2.62—10.26	46

* pH actually represents $-\log[H]$.

Experimental

Reagents.—To avoid any initial metal hydrolysis, the aluminium stock solution (0.1 mol dm^{-3}) was prepared by dissolving 99.997% pure aluminium chloride (Alfa Products) in aqueous hydrochloric acid (BDH, *pro analysi*), so that the resulting concentration of the latter was *ca.* 0.1 mol dm^{-3}. This operation was carried out in an Erlenmeyer flask which was constantly cooled to compensate for the exothermicity of the reaction, and to prevent any projection of matter. The metal content of this solution was checked by ethylenediaminetetra-acetate (edta) back titration against zinc using Eriochrome Black T as an indicator,[31] whereas its exact final acid titre was deduced from Gran titrations.[32]

Sodium hydroxide solutions were prepared by diluting the contents of BDH concentrated volumetric solution vials with freshly boiled doubly deionised water, which was saturated with purified nitrogen before use. These solutions, standardised against potassium hydrogenphthalate (Prolabo, Normapur) and proved to be carbonate-free from the features of the corresponding Gran titration plots,[33] were stored under a nitrogen atmosphere.

Experimental Conditions.—Sodium chloride (0.15 mol dm^{-3}) was chosen as a background electrolyte to maintain constant activity coefficients and to ensure isotonicity with blood plasma. Thermodynamically speaking, this salt may be considered as far from ideal due to the possible association of chloride with metal ions. On the other hand, its use offers the advantage of being more realistic from a physiological point of view, more especially concerning the influence of the biological medium implicitly accounted for by the formation constants to be used in computer simulation models referring to blood plasma.

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† *Supplementary data available* (No. SUP 56665, 36 pp.): full titration data and formation plots. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1987, Issue 1, pp. xvii—xx.

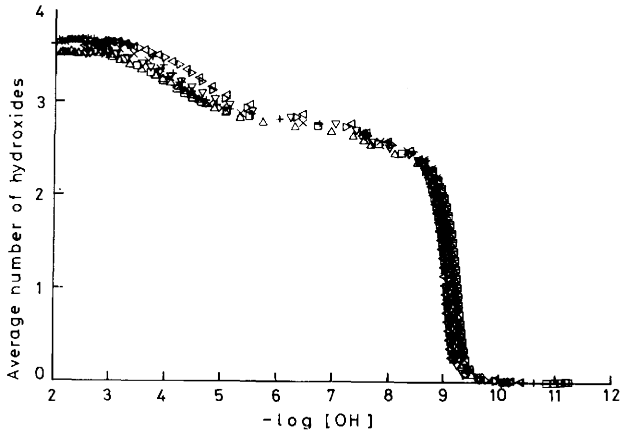

Figure 1. Experimental formation curves for aluminium hydroxides. Symbols +, ×, □, △, ▽, ◁, and ▷ correspond to experiments 1—7 of Table 1 respectively

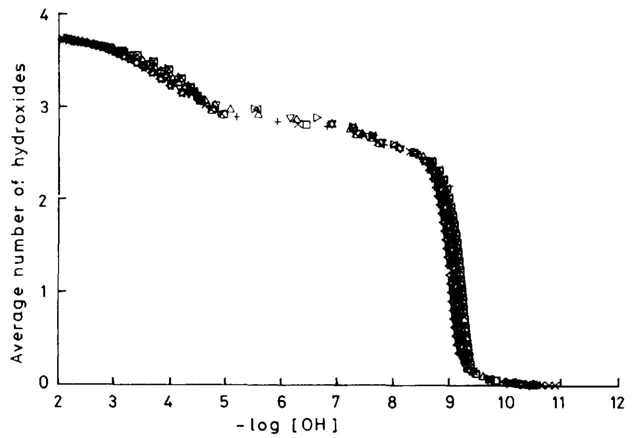

Figure 2. Aluminium hydroxide formation curves as obtained from PSEUDOPLOT simulation based on the data in Table 2 (*i*); key to symbols as in Figure 1

Potentiometric titrations were thus carried out using electrochemical cells of the type (1), opposing a Beckman glass

$$\text{g.e.}|\text{Al}^{3+}, \text{NaCl } (0.15 \text{ mol dm}^{-3})| \quad \text{NaCl (s.c.e.)}|\text{Hg}_2\text{Cl}_2\text{–Hg} \qquad (1)$$

electrode (g.e.) to a saturated NaCl Corning calomel electrode (s.c.e.). E.m.f.s were monitored with a Beckman model 4500 millivolt meter whereas successive aliquots of titrating sodium hydroxide were delivered by a Radiometer ABU12 autoburette.

The temperature was maintained at 37 °C in the thermostatted Ingold cell system, and a stream of purified nitrogen was bubbled through the test solutions.

The electrode system was calibrated in terms of concentrations, in accordance with the most recent specifications.[34] The corresponding logarithmic value of the ionic product of water was determined to be -13.25 under the present conditions using the ESTA program library,[35] the electrode slope being found equivalent to the expected theoretical one.

For aluminium hydroxide formation studies, the main technical difficulty arises from the very slow reaction process, which never actually seems to reach true equilibrium.[27] Fortunately, the reaction rate in the presence of a ligand is largely improved, making investigations of aluminium complex equilibria much easier.[36]

Several quantitative studies dealing with the problem of aluminium hydrolysis refer to the species model postulated by Baes and Mesmer:[37] $[\text{Al(OH)}]^{2+}$, $[\text{Al(OH)}_2]^+$, Al(OH)_3, $[\text{Al(OH)}_4]^-$, $[\text{Al}_2(\text{OH})_2]^{4+}$, $[\text{Al}_3(\text{OH})_4]^{5+}$, and $[\text{Al}_{13}(\text{OH})_{32}]^{7+}$. Testing the existence of the main hydrolytic complexes among these various possibilities, Ohman and Forsling[24] concluded the predominance of $[\text{Al(OH)}]^{2+}$ $[\text{Al}_3(\text{OH})_4]^{5+}$, and $[\text{Al}_{13}(\text{OH})_{32}]^{7+}$ in the presence of carbonate under their particular conditions. On the other hand, Motekaitis and Martell[38] made allowance for the presence of $[\text{Al(OH)}]^{2+}$, $[\text{Al(OH)}_2]^+$, Al(OH)_3, $[\text{Al}_2(\text{OH})_2]^{4+}$, $[\text{Al}_3(\text{OH})_4]^{5+}$, and $[\text{Al(OH)}_4]^-$ in their recent computations. Recently, another group of authors[27] have proposed $[\text{Al(OH)}]^{2+}$, $[\text{Al}_2(\text{OH})_5]^+$, $[\text{Al}_4(\text{OH})_{10}]^{2+}$, and $[\text{Al}_6(\text{OH})_{15}]^{3+}$ for their own conditions, whereas Brown *et al.*[39] characterised $[\text{Al(OH)}]^{2+}$, $[\text{Al(OH)}_2]^+$, and $[\text{Al}_3(\text{OH})_4]^{5+}$ as predominant species, but failed to define the stoicheiometry of a coexisting high molecular weight polymer.

In view of these conflicting results, it may be concluded that, given the slow kinetics of the reaction, the formation of particular hydrolytic complexes largely depends on reactant concentrations as well as on the nature of the anions present. Thus it is imperative to define an operational protocol likely to yield hydrolysis formation constants that can then be used as a reliable reference for aluminium complex formation studies carried out under the same experimental conditions.

In view of this, several series of experiments were planned, in order to assess the influence of the kinetic factor on (*i*) the stoicheiometries of the potential hydroxides and (*ii*) the accuracy of their corresponding formation constants. Table 1 summarises the common experimental conditions used for these investigations, the only parameters allowed to vary being the extent and the frequency of sodium hydroxide additions.

Extreme kinetic conditions, of approximately equivalent duration, were applied for each experiment, and which involved: (*i*) frequent additions (every 2 min) of small aliquots of hydroxide and (*ii*) additions of larger aliquots of hydroxide, the electrode potential being allowed to evolve until its variation was less than 0.1 mV within 1 min. Details of these various measurements may be found in the supplementary data.

In the light of all the information collected from the above investigations, it eventually appeared that the stoicheiometries of the aluminium hydroxides constituting the 'best' set of species in terms of numerical and graphical coincidence (see below) were not affected by the experimental kinetic conditions, and that the corresponding formation constants varied only to a very limited extent.

It was thus decided to select among the different experiments those which corresponded to the smallest aliquots of hydroxide with potentials being read every 2 min. The main reason for this choice was the closest similarity of these kinetic conditions with those relative to aluminium–ligand complex equilibria.

Calculation Procedures.—Our usual approach involving both optimisation and simulation steps[40] was applied to the selection of the 'best' set of species. First, estimates of the constants deduced from experimental hydroxide formation curves expressing $\bar{q}$ (average number of hydroxides per metal ion)

$$\bar{q} = (c_{\text{OH}} - c_{\text{H}} - [\text{OH}] + [\text{H}])/C_{\text{M}} \qquad (2)$$

[equation (2)] as a function of $-\log[\text{OH}]$ were refined by means of the MINIQUAD program.[41] Then, the final discrimination between sets displaying similar numerical fits (*S* and *R* factors) was done on a graphical basis using pseudo-formation curves obtained with the PSEUDOPLOT program.[42] All calculations were run at the C.I.C.T. (Toulouse)

Table 2. Formation constants for aluminium hydroxides: (*i*) for the whole titration data, and (*ii*) for data restricted to pH <8 (see text). General complex formula is $Al_p(OH)_q$; *n* = number of experimental observations, *S* = sum of squared residuals, and *R* = crystallographic factor (see ref. 41)

Data	*p*	*q*	log β	*S* (× 10^{-5})	*R*	*n*
(*i*)	1	1	8.577 ± 0.020	6.8	0.0095	373
	1	3	26.138 ± 0.035			
	1	4	29.044 ± 0.120			
	3	11	91.025 ± 0.099			
	6	15	149.278 ± 0.015			
	8	22	214.973 ± 0.184			
(*ii*)	1	1	8.642 ± 0.020	5.9	0.0145	249
	3	11	93.178 ± 0.155			
	6	15	149.312 ± 0.016			
	8	22	215.377 ± 0.184			

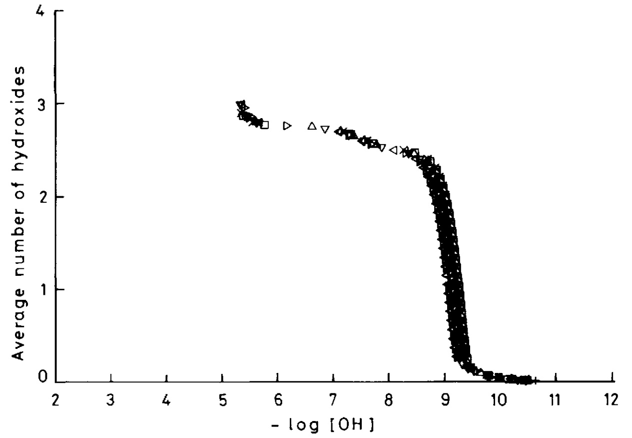

Figure 3. Aluminium hydroxide formation curve as obtained from PSEUDOPLOT simulation based on the data in Table 2 (*ii*); key to symbols as in Figure 1

and C.I.R.C.E. (C.N.R.S. Orsay) computing centres, through the E.A.R.N. network.

Results and Discussion

Figure 1 shows the experimental aluminium hydroxide formation curves as determined under conditions defined in Table 1 and above. It is noteworthy that for pH values above 8, a slight opacity appeared in the solutions, irrespective of the initial aluminium concentration. This phenomenon was later shown to correspond to initiation of the precipitation of $Al(OH)_3$. The investigation of the hydroxide species present in the solutions was thus carried out in two steps: (*i*) all the experimental points shown in Figure 1 were first taken into account and (*ii*) once the 'best' model was characterised, it was then tested on the range of experimental points limited to pH values less than 8.

39 Different species combinations were tried in the first step of this approach, whose results are given in the supplementary data. The 'best' result is to be found in Table 2, and the pseudo-formation curve simulated on the basis of the corresponding constants shown in Figure 2.

The testing of this 'best' constant set on the limited data range defined above led to the result reported also in Table 2, the related pseudo-formation curve being shown in Figure 3.

As may be seen in Table 2, constants related to the range of data restricted to pH <8 do not benefit from a better accuracy. This tends to indicate that, as none of the present data can be obtained at true equilibrium, taking the whole range investigated into account in the calculations is to be considered as more realistic from a practical point of view. Indeed, the examination of species distribution curves not presented here clearly shows that the highest percentage of $Al(OH)_3$ corresponds to pH values around 8, which explains the better accuracy obtained for the corresponding formation constant.

The stoicheiometries of the species characterised differ significantly from the sequence proposed for other conditions by Baes and Mesmer,[37] but all except $[Al_8(OH)_{22}]^{2+}$ have already been mentioned by other authors (see above). It is noteworthy that the existence of $[Al_8(OH)_{22}]^{2+}$ was always found necessary to simulate the experimental curves, regardless of the kinetic features of the titrations (see supplementary data).

In conclusion, we thus recommend the use of the larger set of constants in Table 2 for determining aluminium complex formation constants under the present experimental conditions. Nevertheless, as has frequently been done by previous authors,[24] if some of the corresponding hydroxides were to represent a significant fraction of aluminium within the pH range investigated, it would undoubtedly be advisable to refine their constants again together with those of other metal complexes definitely characterised in the relevant solutions.

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