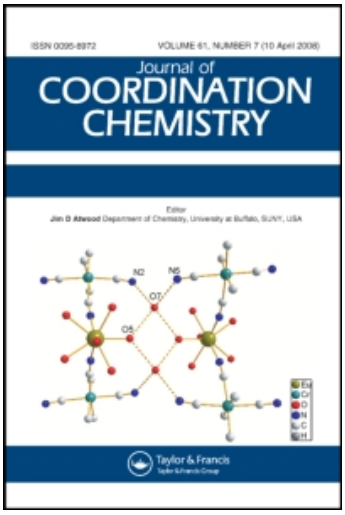

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LOW MOLECULAR MASS ALUMINUM COMPLEX SPECIATION IN BIOFLUIDS

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Formation constants for the aluminum phosphate system and particle size analyses for solutions containing this material are reported. From a database of (i) the total ligand and metal concentrations in saliva, stomach juice, small intestinal fluid, milk, blood plasma and intravenous fluid, and (ii) physicochemical constants for all feasible reactions involving low molecular mass complexes, a series of computer models was constructed and used to calculate the distribution of chemical species at equilibrium. The pie-diagrams of speciation indicate that some aluminum complexes exist as net-neutral charged species (which are potentially bioavailable) whereas others are charged and so are highly solvated and/or removed by the renal system. The chemical speciation knowledge produced in this research can be useful in researching aluminum intoxication, prevention and decontamination therapies.

Keywords: Aluminum, phosphate, biofluids, speciation, modelling

INTRODUCTION

We have previously reviewed the apparent relationship between aluminum in our environment, chemical speciation and our health.[1] Computer models of the low molecular mass (1mm) fractions of a range of milks were studied since the concentrations of aluminum species therein are below those analysable by known techniques. The work highlighted the desirability of re-establishing the formation constants for the aluminum-phosphate system (these are pivotal data for the modelling) and of applying such speciation modelling to other biofluids.

This paper reports the determination of the formation constants for the aluminum phosphate system and leads to a new solubility product for $AlPO_4$. The formation constant work and the subsequent modelling paid particular attention to net-neutral species since they either stay in aqueous solution and pose a tissue bioavailability threat, or else they precipitate and although spoiling the titration they usually reduce the health risk. Traditionally, precipitation during titrations has been observed as a cloudiness or from emf drift. However, as the presence of such neutral $AlPO_4$ species is pivotal to all of this work, the titration solutions were analysed using a particle analyser. The precipitate-free formation constant data was then used to model the aluminum species in saliva, stomach juice, small intestinal fluid, milk, blood plasma and an intravenous nutrition fluid.

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EXPERIMENTAL

Determination of formation constants

The potentiometric titrations of the aluminum phosphate system were performed using our established approach[2] (Table I) and data were treated using the ESTA suite of programs.[3] The resulting formation constants are given in Table II.

TABLE I

The aluminum-phosphate-proton system. Summary of titration data used in the formation constant calculations; initial total concentration of aluminum (C_{Al}), phosphate (C_{PO_4}), hydrochloric acid (C_{HCl}), pH range and the number of experimental points (n) for each titration. Concentrations are expressed in mmol dm^{-3}.

System	C_{Al}	C_{PO_4}	C_{HCl}	pH	n
Phosphate protonation		13.34	26.67	1.76–11.20	151
		10.00	20.00	1.83–11.25	125
		6.68	13.33	2.01–11.26	87
Aluminum phosphate	4.00	8.99	13.00	1.99–2.91	27
	3.33	9.99	13.33	2.02–2.95	24
	3.33	8.33	20.00	1.80–3.05	45
	4.00	8.00	20.00	1.79–2.94	45
	2.76	8.62	20.00	1.80–3.09	43
	6.76	6.67	20.00	1.76–2.82	40
	3.33	9.99	13.33	1.99–3.04	26

TABLE II

Formation constants determined in this study; $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$, I = 150 mmol dm^{-3} $[Cl^-]$

System	p	q	r	lg β	S.D.	O	R	n
Phosphate protonation	0	1	1	11.54	0.007			
	0	1	2	18.22	0.008	119.3	0.0025	363
	0	1	3	20.22	0.013			
Aluminum phosphate	1	1	0	15.32	0.052			
	1	1	1	17.79	0.011			
	1	1	2	20.93	0.044	218.6	0.026	250
	2	1	0	18.72	0.047			
	2	1	−2	12.58	0.053			

S.D. = standard deviation; O = objective function; R = Hamilton *R*-factor; n = number of data points.

The solubility product for $AlPO_4$ was determined by taking the product of the $[Al^{3+}]$ and $[PO_4^{3-}]$ concentrations at the points where the titrations showed first signs of precipitation as emf drift (Table III).

TABLE III

Calculation of the solubility product, K_{sp}, for $AlPO_4$ at 37°C and I = 0.15 mol dm^{-3} [Cl^-]; $K_{sp} = [Al][PO_4]$.

Titration	pH	$[Al^{3+}]$ mmol dm^{-3}	$[PO_4^{3-}]$/ mmol $dm^{-3}/10^{-12}$	K_{sp}/ mmol $dm^{-3}/10^{-16}$
1	2.88	0.246	1.91	4.69
2	2.89	0.194	2.21	4.29
3	3.00	0.184	2.49	4.58
4	2.92	0.227	2.19	4.96
5	3.06	0.149	2.77	4.14
6	2.80	0.482	1.09	5.25
7	2.95	0.194	2.22	4.31

Average $K_{sp} = 4.6 \times 10^{-19}$ mol^2 dm^{-6}, *i.e.*, $-\lg K_{sp} = 18.34$.

The Climet particle analyser

Solutions precipitating and near precipitation were analysed using a Climet particle analyser (I-1000) and particle sensor (CI-1010). This enabled the solutions to be compared directly to that of doubly deionised and distilled water as reference for particle size and distribution data.

The solutions were sampled at ambient room temperature (20–22°C) under atmospheric pressure with no stirring and the sample volume being 1 cm^3 which was injected into the sensor at a rate of 20 cm^3 min^{-1}. A tare volume of the solution (0.2 cm^3) was used between each measurement in order to purge the system. Each solution was sampled in triplicate; some typical average values are tabulated in Table IV.

TABLE IV

Climet data for particle size distribution in a 1 cm^3 sample of the precipitated solutions and, for comparison, that of double deionized, distilled and degassed water (DDDD) at ambient temperature (20–22°C) and pressure.

	No. of particles of diameter (μm)						
Titration	13	16	19	20	22	25	Total
(DDDD)	6	2	1	0	1	1	11
1	43	16	4	6	5	17	91
2	95	58	15	28	35	102	332
3	53	37	8	13	13	35	159
4	36	19	2	4	4	9	74
5	39	21	3	8	9	19	99

Speciation modelling

In common with Berthon *et al.*[4] we studied the 1mm fraction of biofluids by computer simulation of the chemical speciation prevailing. This fraction has long been recognised as the most active fraction in terms of bioavailability. Our speciation

modelling experiments used our established MINEIR[5] and ECCLES[6] programs to model the equilibria occurring in a range of biofluids. The concepts are that low molecular mass (1mm) complexes are in equilibrium with metals reversibly bonded to circulating protein in a biofluid and that this protein effectively buffers the free metal ion concentrations. Provided that the table of output chemical speciation is expressed as a percentage of the 1mm species present, the results are independent of the total concentration of metal bound to the protein. Table V contains the formation constants for all the relevant species used in the calculations. The input data and major species computed to be present are shown in Tables VI–XII.

TABLE V

Lmm speciation in human and bovine milk (pH = 6.8). Total aluminum levels in human and bovine milk and 1.67×10^{-11} mol dm^{-3} and 2.33×10^{-6} mol dm^{-3}, respectively (these values are obtained from the ECCLES[6-8] computer program using free aluminum levels of 1×10^{-20} mol dm^{-3} for human milk and 7.9×10^{-16} mol dm^{-3} for bovine milk[9]; CTA = citrate; PO_4 = phosphate.

		% total LMM Al as species	
Species	Charge	Human milk	Bovine milk
$AlCTA(OH)_2$	−2	85.6	77.6
AlCTA OH	−1	10.5	9.5
$AlPO_4$	0	3.1	11.7
$Al(CTA)_2OH$	−4	0.7	1.0
$Al(CTA)_2$	−3	—	0.1
Minor		0.1	0.1

TABLE VI

Aluminum speciation in saliva (pH 6.8). Aluminum concentration ranges from $4.31 \times 10^{-3} - 4.31 \times 10^{-1}$ mol dm^{-3} (equivalent to 3.4–340 mg in 29 cm^3).[4]

Species	Charge	% Aqueous aluminum species
$AlPO_4$	0	57.03
$Al_2PO_4(OH)_2$	+1	36.91
$AlCTA(OH)_2$	−2	4.65
AlCTAOH	−1	1.22
Minor		0.19

The choices of input data were based upon published criteria.[6-8] The milk models are those prepared by Findlow[9] and the blood plasma data are those of May *et al.*[6-8] The intravenous nutritional fluid is a typical admixture of commercially available solutions. The saliva model was taken directly from the work performed by Hurford.[10] Other body fluids such as gastric, bile and pancreatic juices were developed from various literature sources.[11,12] It was assumed that stomach juices consist basically of saliva and gastric fluids and that intestinal fluid is made up of a mixture of stomach, bile and pancreatic juices.

The use of aluminum-containing preparations in medicine is widespread and this is particularly so for reducing the phosphate uptake in patients undergoing renal and parenteral therapy. The aluminum compound is usually given orally and modelling

the speciation along the route through the gastrointestinal tract could lead to potentially "less harmful" metals being used as possible phosphate binders in future preparations.

Doses of such medicine are usually matched to each patient's requirements. In order to cover the range of doses which could possibly be encountered, a dose of between 3.4 and 340 mg of aluminum was assumed, in 25 cm^3 of water. This is then mixed with 4 cm^3 of saliva.[13]

TABLE VII

Aluminum speciation in stomach solutions at pH 2, 4, and 6. Concentration of aluminum varies between $2.16 \times 10^{-3} - 2.88 \times 10^{-1}$ mol dm^{-3}, with the ratio of aluminum : phosphate ranging between 0.07 : 1 to 14 : 1.

			% Total aluminum species	
pH	Species	Charge	Al : PO_4: 0.07 : 1 → 14:1	
2	$AlPO_4$	0	1.63	0.01
	$AlPO_4H$	+1	4.8	0.03
	$AlPO_4H_2$	+2	76.2	0.45
	Al_2PO_4	+3	2.65	13.14
	Al	+3	14.7	86.25
4	$AlPO_4$	0	44.3	0.03
	Al_2PO_4	+3	0.1	0.2
	Al	+3	0.02	0.3
	$Al_2PO_4(OH)_2$	+1	4.8	13.9
	$AlPO_4$ solid	0	49.04	0
	$Al(OH)_3$ solid	0	0.00	85.6
6	$AlPO_4$	0	44.3	0.33
	$Al_2PO_4(OH)_2$	+1	6.3	1.21
	$AlPO_4$ solid	0	49.19	5.85
	$Al(OH)_3$ solid	0	0.00	92.59

TABLE VIII

Speciation of aluminum in small intestinal solutions at pH 4, 6, 8, with the aluminum concentration varying from 1.07×10^{-2} to 7.18×10^{-3} mol dm^{-3}, with the ratio of aluminum: phosphate ranging between 0.41 : 1 to 0.28 : 1.

			% Total aluminum species	
pH	Species	Charge	Al : PO_4: 0.41 : 1 → 0.28:1	
4	$Al_2PO_4(OH)_2$	+1	1.7	2.2
	$AlPO_4H$	+1	0.3	0.4
	$AlPO_4$	0	8.9	13.3
	$AlPO_4$ solid	0	89.6	84.4
6	$Al_2PO_4(OH)_2$	+1	2.0	3.2
	$AlPO_4$	0	8.9	13.3
	$AlPO_4$ solid	0	89.0	83.5
8	$AlPO_4$	0	0.08	0.12
	$Al(OH)_3$ solid	0	99.6	99.8

TABLE IX

Lmm aluminum speciation in blood plasma (pH = 7.40). The percentage distribution of species is identical for total plasma aluminum levels measured in neonates, both in the absence of (total Al = 1.85×10^{-7} mol dm^{-3}), and after (total Al = 1.37×10^{-6} mol dm^{-3}), administration of TPN. Total LMM aluminum is 20% of total plasma aluminum.[24]

Species	Charge	% of total LMM Al as species
$AlCTA(OH)_2$	−2	93.8
AlCTA(OH)	−1	2.9
$AlPO_4$	0	1.5
$Al(Ox)_2OH$	−2	1.4

TABLE X

Aluminum speciation in a typical pre-term infant TPN regimen (pH = 5.45, total Al = 2.79×10^{-6} mol dm^{-3}).

species	Charge	% of total Al as Species
$AlPO_4$	0	99.72
$Al_2PO_3(OH)_2$	+1	0.13
$AlHPO_4$	+1	0.10
AlF_2	+1	0.03

In considering the stomach model, it was assumed that the organ was empty prior to the introduction of gastric juice and saliva. Again, as wide a concentration range as possible was modelled using various mixes of saliva and gastric juice. Finally, in modelling the intestinal juices, the stomach fluid was modelled as being mixed with bile and with pancreatic fluids.

DISCUSSION

The overall formation constants for aluminum-phosphate are shown in Table II. The phosphate protonation constants are in fair agreement with previous literature data.[4,14]

Titrations of the aluminum phosphate systems gave evidence of precipitation in the pH range 3 to 4 for all the varying metal to ligand ratios (Table I).

Five species were characterized—ML, MLH, MLH_2, M_2L and $M_2L(OH)_2$—based upon the statistical information and graphical comparisons. This seems to be a considerably better fit than that achieved by Jackson and Voyi[15] when considering the same system in the pH range 2–4 and for which they reported a three constant set—MLH, MLH_2 and ML_2H.

The formation constants produced from this work also differ from those reported by Berthon *et al.* in two respects. First, we can describe our solution system in terms

of five constants, whereas reference 4 reports some six constants. Secondly, most of their group of six constants indicate somewhat stronger binding than found in our work. The overall effect of this is to suggest that relatively more of the total aluminum in the Berthon experiments is present in solution.

Our titrations were terminated as soon as precipitation was noted. If, however, precipitates were present—albeit as very fine particles—these could either be described in terms of a solubility product for the precipitate, or by having formation constants which are more numerous and of stronger binding capacity in order to compensate for the increase in the amount of titrant required.

We believe that there is a fundamental flaw in the approach used by Berthon *et al.* in that the possibility of precipitation occurring was not completely eliminated because autotitration equipment was employed, and because other workers such as Jackson[15] and ourselves ceased titrations at the first sign of precipitation at pH 3.8–4, whereas Berthon *et al.* titrated right through the pH scale up to pH = 10. As a consequence, it required extra and stronger formation constants in order to treat mathematically the aluminum which was producing fine particles of solid as if they were in solution.

In order to illustrate this dichotomy, we refer to Figure 1 of ref. 4. Effectively, our work has copied these titrations from a $\lg[PO_4^{3-}]$ figure of -17 to -14 but we have not been able to find precipitate-free titrations which cover the vast majority of Figure 1 that had more concentrated phosphate than 10^{-14} mol dm^{-3}.

Furthermore, we attempted to repeat the exact titrations reported by Berthon *et al.*, found precipitates which were analysed using a particle analyzer, photographed using magnification ($\times$ 100–1000), and samples of these precipitated solutions were delivered to Berthon *et al.* by the authors.

Figure 1 of ref. 4 also shows another surprising feature in that it contains a long, flat plateau between $\lg[PO_4^{3-}]$ -13 and -6, a feature not previously seen for straightforward metal ligand complexing in solution. This suggests that one rather special species is predominant over this extremely wide range of titrations; the actual species arising from the speciation plot appears to be $Al_2PO_4(OH)_2$, a species insufficiently special or powerful in terms of stability to explain this phenomenon. We have, however, been able to explain the plateau phenomena in terms of the solubility product of aluminum phosphate.

In summary, we believe our work is superior because

1. as distinct to Berthon, we did not separate our data into the precipitating titrations in order to isolate a K_{sp} and those *apparently* remaining in solution in order to obtain solution constants. Rather, we used all data together in order to give a set of solid and solution constants that were mutually self-consistent;
2. we used the Climet particle analyzer in order to detect our precipitates;
3. simulated data using our solution constants and solubility product were able to reproduce our titration data, whereas constants from Berthon were not able to simulate our system. We also used our constants and solubility product to simulate Berthon's system and found that we could reproduce his titrations by predicting solid throughout most of the pH range above pH = 4;
4. in the conclusions section of this paper, it will be noticed that our new constants give aluminum citrate bioavailability species which are biologically more reassuring, whereas the Berthon constants produce models in which approximately 90% of the aluminum was tissue bioavailable.

TABLE XI

Component total concentrations used as input data for computer simulation models. Input concentrations for the stomach were produced using a 1 : 1 and 2 : 1 ratio of saliva to gastric juice. The small intestine considered 1 : 1 : 1 : 1 and 1 : 1 : 2 : 2 ratios of saliva, gastric juice, pancreatic juice and bile.

Components	Human milk/ mol dm^{-3}	Bovine milk/ mol dm^{-3}	Typical TPN solution/ mol dm^{-3}	Saliva/ mol dm^{-3}	Gastric juice/ mol dm^{-3}	Pancreatic juice/ mol dm^{-3}	Bile/ mol dm^{-3}	Blood plasma/ mol dm^{-3}
METALS								
Aluminum	1.7×10^{-11}	2.3×10^{-6}	2.8×10^{-6}					9.0×10^{-8}
Calcium	3.5×10^{-3}	4.1×10^{-3}	4.1×10^{-4}	1.5×10^{-3}	1.8×10^{-3}	1.7×10^{-3}	7.7×10^{-3}	1.5×10^{-3}
Cobalt				6.3×10^{-7}				
Copper(I)								1.9×10^{-8}
Copper(II)	1.1×10^{-9}	5.8×10^{-10}		1.0×10^{-15}	3.9×10^{-3}		8.8×10^{-5}	9.4×10^{-11}
Iron(II)								4.7×10^{-11}
Iron(III)	1.1×10^{-11}	1.1×10^{-11}	2.7×10^{-5}				1.0×10^{-4}	1.2×10^{-11}
Lead								6.6×10^{-11}
Magnesium	9.3×10^{-4}	1.6×10^{-3}	1.6×10^{-3}	2.7×10^{-4}	3.1×10^{-2}	5.0×10^{-4}		6.7×10^{-4}
Manganese			2.2×10^{-5}					1.8×10^{-12}
Nickel								1.5×10^{-14}
Potassium			2.1×10^{-2}	2.1×10^{-2}	1.2×10^{-2}	4.6×10^{-3}	1.4×10^{-2}	
Sodium			2.1×10^{-2}	1.0×10^{-2}	4.9×10^{-2}	1.4×10^{-1}	2.2×10^{-1}	
Zinc	4.9×10^{-6}	1.6×10^{-4}	1.1×10^{-6}	5.0×10^{-6}		1.9×10^{-5}		1.6×10^{-7}
LIGANDS								
Alanate	1.5×10^{-4}	2.1×10^{-5}	7.4×10^{-3}	1.4×10^{-4}	2.4×10^{-4}			3.7×10^{-4}
Aminobutyrate	1.5×10^{-5}	1.8×10^{-6}						2.4×10^{-5}
Ammonia	1.2×10^{-4}	4.8×10^{-4}		2.6×10^{-3}	6.1×10^{-3}			2.4×10^{-5}
Arginate	1.0×10^{-5}	1.2×10^{-5}	4.1×10^{-3}	3.8×10^{-4}	2.0×10^{-4}			9.5×10^{-5}
Ascorbate	4.3×10^{-5}	1.1×10^{-4}		8.0×10^{-6}	4.7×10^{-5}			4.3×10^{-5}
Asparaginate								5.5×10^{-5}
Aspartate	3.8×10^{-5}	6.3×10^{-5}	6.7×10^{-3}	1.1×10^{-5}	1.5×10^{-4}			5.0×10^{-6}
Carbonate	4.0×10^{-3}	4.4×10^{-4}		6.7×10^{-3}			1.9×10^{-2}	2.5×10^{-2}
Chloride			3.7×10^{-2}	2.9×10^{-2}	1.2×10^{-1}	7.7×10^{-2}	3.1×10^{-2}	
Citrate	1.0×10^{-3}	1.7×10^{-3}		5.4×10^{-5}				1.1×10^{-4}
Citrullinate	1.1×10^{-5}	1.7×10^{-6}						2.7×10^{-5}
Cysteinate			5.0×10^{-4}	1.0×10^{-5}	1.1×10^{-4}			2.3×10^{-5}

Cystinate	2.5×10^{-5}	9.2×10^{-6}	2.0×10^{-3}	1.3×10^{-5}				4.0×10^{-5}
Fluoride			2.7×10^{-5}	7.3×10^{-6}				
Gluconate			1.7×10^{-3}					
Glucose			3.6×10^{-1}					
Glutamate	9.3×10^{-4}	2.2×10^{-5}	1.4×10^{-2}		1.8×10^{-4}			4.8×10^{-5}
Glutaminate	1.7×10^{-4}	8.9×10^{-5}		8.2×10^{-5}				5.2×10^{-4}
Glycinate	8.1×10^{-5}	1.4×10^{-4}	6.1×10^{-3}	1.4×10^{-4}	1.9×10^{-4}			2.4×10^{-4}
Histamine	3.6×10^{-6}	2.7×10^{-7}		1.6×10^{-4}				1.0×10^{-8}
Histidinate	2.4×10^{-5}	4.7×10^{-6}	3.4×10^{-3}	8.5×10^{-5}	1.1×10^{-4}			8.5×10^{-5}
Hydroxyprolinate	5.3×10^{-6}	1.3×10^{-6}						7.0×10^{-6}
Isoleucinate	1.0×10^{-5}	4.0×10^{-6}	6.5×10^{-3}	4.2×10^{-5}	8.0×10^{-5}			6.5×10^{-5}
Lactate	6.3×10^{-4}	6.3×10^{-4}		1.4×10^{-6}	1.3×10^{-3}		4.1×10^{-3}	1.8×10^{-3}
Leucinate	2.5×10^{-5}	4.6×10^{-6}	8.8×10^{-3}	1.2×10^{-5}	1.3×10^{-4}			1.2×10^{-4}
Lysinate	2.5×10^{-5}	2.7×10^{-5}	5.8×10^{-3}	5.3×10^{-5}	1.1×10^{-4}			1.8×10^{-4}
Malate	3.5×10^{-5}	3.0×10^{-5}						3.5×10^{-5}
Methionate	4.4×10^{-6}	9.0×10^{-7}	2.8×10^{-3}	5.0×10^{-7}	1.1×10^{-5}			2.9×10^{-5}
Ornithinate	3.2×10^{-5}	1.0×10^{-5}						5.8×10^{-5}
Oxalate	3.2×10^{-6}	1.0×10^{-5}						1.2×10^{-5}
Phenylalanate	1.0×10^{-5}	8.7×10^{-6}	7.3×10^{-3}	9.4×10^{-5}	7.9×10^{-5}			6.4×10^{-5}
Phosphate	1.6×10^{-3}	1.1×10^{-2}	5.5×10^{-3}	3.7×10^{-3}	2.8×10^{-4}	8.0×10^{-4}	4.5×10^{-2}	3.8×10^{-4}
Picolinate	3.7×10^{-6}	5.0×10^{-6}						
Prolinate	5.0×10^{-5}	3.1×10^{-5}	1.5×10^{-2}	8.0×10^{-5}	2.2×10^{-4}			2.1×10^{-4}
Pyruvate	5.6×10^{-5}	1.2×10^{-4}						9.5×10^{-5}
Salicylate	5.0×10^{-6}	5.0×10^{-6}						5.0×10^{-6}
Serinate	8.1×10^{-5}	1.6×10^{-5}	1.6×10^{-2}	6.3×10^{-5}	1.9×10^{-4}			1.2×10^{-4}
Silicate	1.4×10^{-4}	1.3×10^{-4}						1.4×10^{-4}
Succinate	4.2×10^{-5}	4.0×10^{-5}						4.2×10^{-5}
Sulphate	4.4×10^{-4}	1.1×10^{-3}				4.2×10^{-3}		2.1×10^{-4}
Taurinate	3.5×10^{-4}	6.2×10^{-5}						
Thiocyanate	1.4×10^{-5}	1.7×10^{-4}		2.1×10^{-3}				1.4×10^{-5}
Threoninate	6.7×10^{-5}	1.1×10^{-5}	5.5×10^{-3}	2.5×10^{-4}	1.7×10^{-4}			1.5×10^{-4}
Tryptophanate	1.0×10^{-6}	1.0×10^{-6}		2.7×10^{-5}				1.0×10^{-5}
Tyrosinate	1.3×10^{-5}	1.1×10^{-6}	6.0×10^{-4}	3.3×10^{-5}	8.0×10^{-5}			5.8×10^{-5}
Valinate	5.2×10^{-5}	1.1×10^{-5}	8.0×10^{-3}	1.2×10^{-4}				2.3×10^{-4}
Urea				3.0×10^{-3}	3.3×10^{-4}	1.8×10^{-3}	5.4×10^{-3}	

Since precipitation occurred, it is also possible to calculate a solubility constant for aluminum phosphate (Table III).

The results of the particle size and distribution investigations found in Table IV indicate that the water used for the titrations is reasonably pure, some eleven particles per 1 cm^3 sample being noted. In addition, the solutions which did precipitate contain between 9 and 30 times the number of particles found in this water used as the point of first precipitation. This would seem to corroborate the initial inference that the solutions did indeed precipitate, irrespective of the ratio of aluminum to phosphate.

Chemical speciation in biofluids

Figures 1 and 2 show the major speciation in solution for human and for bovine milk. For both fluids the predominant species is that of hydroxycitrate which has a net negative charge and, therefore, is not readily bioavailable. The bovine milk speciation has considerably more neutral aluminum phosphate complex present compared with human milk and this suggests that the aluminum in that fraction is more bioavailable. More exact figures are given in Table V.

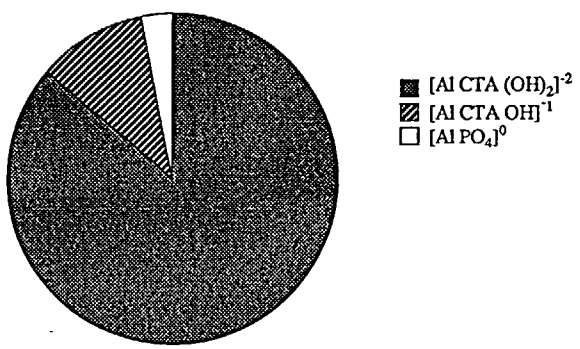

FIGURE 1 Aluminum speciation of human milk. Key to shading reads clockwise.

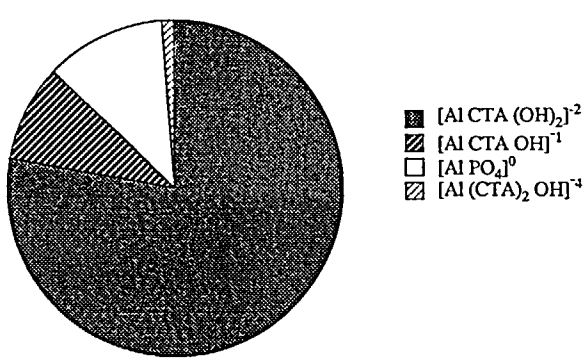

FIGURE 2 Aluminum speciation of bovine milk.

Saliva

Table VI and Figure 3 indicate the distribution of the major aluminum species present in the aqueous fraction of saliva. It must be stressed that the major portion of aluminum in saliva will precipitate as aluminum hydroxide. Of the aluminum remaining in solution, the major fraction is aluminum phosphate which is net-neutral and the remainder is predominantly distributed as charged complexes of phosphate and citrate.

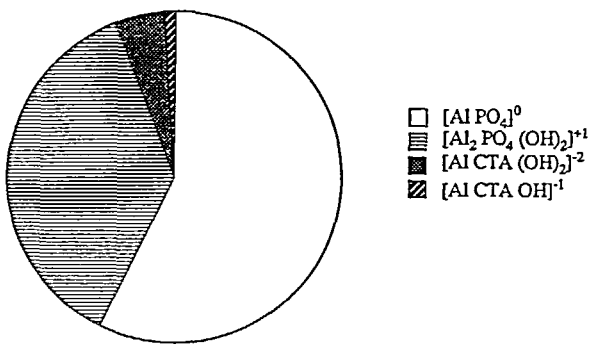

FIGURE 3 Aluminum speciation of saliva.

Stomach

Table VII and Figure 4 indicate a range of pH over which the stomach can be found depending on whether it is actively excreting acid or is in resting mode. Similarly, the ratio of aluminum : phosphate varies depending upon diet. The table shows that most of the aluminum is present as a protonated aluminum phosphate, $[AlPO_4H_2]^{2+}$, which is not expected to be bioavailable because of its charge. Indeed, the only bioavailable fraction present is aluminum phosphate and that represents but a small percentage of the aluminum present in solution. Thus, once again, any aluminum in the diet which reaches the stomach is not expected to be used as intake but rather to pass through to the intestine.

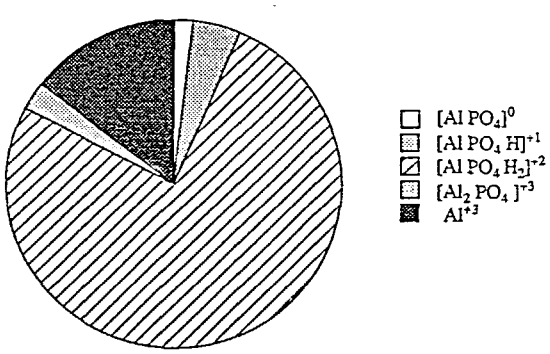

FIGURE 4 Aluminum speciation of the stomach.

Small intestine

In this organ, the pH is higher than the stomach and the speciation profile of aluminum shown in Table VIII and in Figure 5 indicates that it is either present as the hydroxide or as the phosphate, both being neutral.

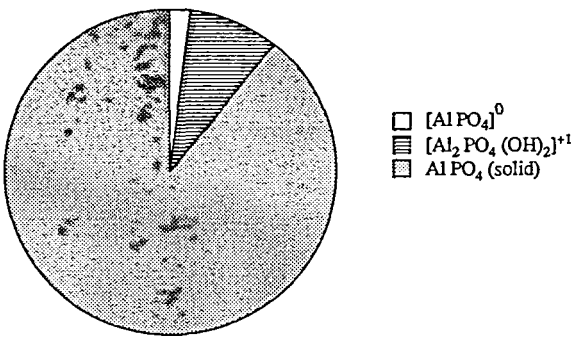

FIGURE 5 Aluminum speciation of the small intestine.

Blood plasma

Any aluminum that does get taken in from the diet into blood plasma is seen to be present predominantly as a net negatively charged citrate species (Table IX and Figure 6) which is subject to excretion *via* the kidneys. Indeed, the amount of neutral aluminum phosphate complex present in normal blood plasma is seen to be less than 2% of the total load. Thus, very little aluminum will pass through to tissues and to the brain when the kidneys are working normally.

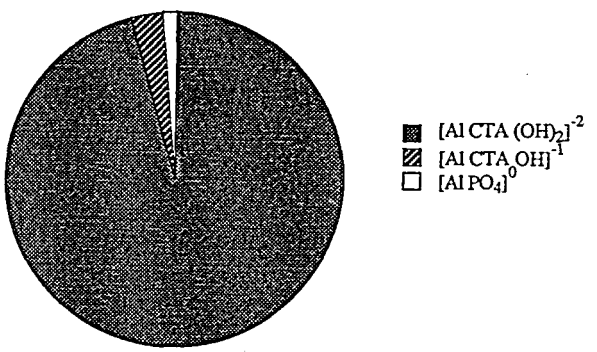

FIGURE 6 Aluminum speciation of blood plasma.

Intravenous nutrition fluid

The speciation calculations indicate that aluminum is present almost totally as the net-neutral soluble species $AlPO_4$ in this fluid (Table X and Figure 7). Slow infusion

of the nutrition mixture, however, does rapidly result in a change to the speciation profile of blood plasma. Thus, the negative species that are predominant in plasma allow renal excretion of the contaminant aluminum introduced by the intravenous fluid.

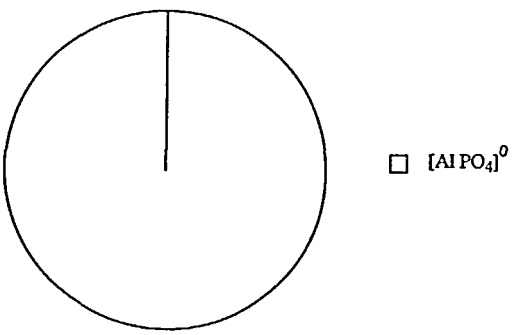

FIGURE 7 Aluminum speciation of TPN solution.

Of the pie diagrams shown in Figures 1–7, the distribution of the portions of the pie are not dependent upon the total aluminum present as a general statement but, of course, the size of the pie depends upon the total amount of aluminum present in the biofluid. Thus, a lack of knowledge concerning the proportion of aluminum that is bound to protein does not affect these distribution models. The exception to this generalisation is when phosphate is present as it may well precipitate some of the aluminum from solution.

The form in which aluminum is present in the gastrointestinal tract affects absorption. Kaehny found that the increase in plasma aluminum was greatest after aluminum hydroxide, lowest after aluminum carbonate, and not significant after aluminum phosphate ingestion.[16] Slanina *et al.* reported elevated aluminum levels in rats fed a diet containing aluminum citrate or even citrate alone.[17] Our modelling confirms that the ingestion of aluminum phosphate alone does not pose the threat of excessive aluminum absorption because of the low solubility of aluminum phosphate. However, the ingestion of aluminum in association with foodstuffs may create a more hazardous uptake and it has been reported that total serum aluminum concentrations were increased in both humans and in rats with simultaneous oral aluminum and citrate ingestion.[17–19]

This work indicates that less than 2% of the total 1mm aluminum is present as a net-neutral charged species in blood plasma. The remainder are charged species which are able to be excreted from the body and this substantiates the *in vivo* observations made on mice[20] that increasing plasma concentrations of citrate creates larger urinary excretions of aluminum. The model of Berthon,[4] however, predicts 92% of the low molecular aluminum complexes to be net-neutral in blood plasma and, therefore, rather than being predicted as being excreted, much would pass into tissue.

Clearly, these computer simulation models of aluminum speciation which rely upon a large database of all the competing reactions are now applicable to many research areas and may be used to explore a wide range of postulates concerning aluminum biochemistry. This work is continuing. The next stage of model develop-

ment would be to mix speciation models of food such as milk[1] with biofluids such as stomach or intestinal juices. Some databases concerning foods and the products of intestinal enzymes have already been reported.[21,22]

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