The chemical speciation of aluminium and silicon in human serum

A. Sanz-Medel

Department of Physical and Analytical Chemistry, University of Oviedo, 33006 Oviedo, Spain

Speciation of aluminium and silicon in biological fluids by complementary techniques is discussed. Non-chromatographic separation (ultrafiltration) with off-line graphite furnace AAS detection is compared with anion-exchange chromatography with low and high resolution ICP-MS.

Aluminium and silicon are two of the three most abundant elements in the earth crust. However, during human evolution it appears that both elements have been excluded from biochemical processes. Efforts directed to establish clear essential functions of Al or Si in humans have not been successful so far. In fact, their essenciality in healthy human people has not been proved yet, while most effective ways have been demonstrated in healthy individuals to excrete these elements after their entering the body [1]. Conversely, the toxicity of Al to renal failure patients is now worldwide recognised; aluminium accumulation in uremic patients results in dialysis-related diseases, including dialysis encephalopathy syndrome, non-Fe-related microcytic anemia and Al-induced bone diseases [1]. Even if many mechanism(s) of Al toxicity remain unknown, it seems that silicon can prevent aluminium toxicity "*via*" reduction of bioavailability of Al by formation of hydroxialuminosilicates under physiological conditions [2]. It should also be mentioned that aluminium and silicon have been controversially implicated as possible causative agents of Alzheimer's disease because both metals have been found in the form of

Article available at http://analusis.edpsciences.org or http://dx.doi.org/10.1051/analusis:199826060076

aluminosilicates in senile plaques and neurofibrilary tangles found in the brain of such patients [1,2].

In order to unveil the complex and varied mechanisms of aluminium toxicity, its transport in the body or its accumulation in "target" organs (bone, brain, muscle) where its detrimental health effects develop, speciation information is clearly needed. Concurrently, the proposed detoxifying effect of Si, by forming aluminosilicates [2], needs speciation data.

Thus, knowledge of the low molecular and high molecular mass biocompounds binding Al and Si in biological transport fluids (e.g. human serum transporting those metals to their "target" organs) is now paramount. Therefore, highly performant analytical techniques should be developed to achieve the chemical speciation of aluminium, and silicon, in human serum.

Unfortunately, Al speciation analysis in biological samples is beset with difficulties. One major difficulty is that "normal" Al tissue levels in healthy people (a concept that we will call, from now on, "basal" level of the element) are very low. Moreover, Al is an ubiquitous element in nature and so external contamination problems are almost unavoidable. In fact, it is believed today that "basal" Al concentrations in blood serum are around 2 µg L^{-1} or below, while 100 − 500 µg L^{-1} is the "basal" Si serum level. Typical concentrations in uremic sera are in the region 40 − 80 µg L^{-1} for Al and 800 − 1000 µg L^{-1}, indicating clearly that renal failure people fail to excrete adequately those two elements and so their body overloading occurs.

Particularly for speciation, where fractions of the sought element are analysed in each species in which it occurs, those low levels and the high risk of exogenous contamination will demand special analytical ability and care.

Having said that, in the following I will try to summarise the problems, techniques and results we have encountered on trying the speciation of aluminium (and Si) in human serum, an undertaken which we initiated more than ten years ago, when I started a fruitful cooperation with the medical research group of Dr. J.B. Cannata of the Dialysis Unit of the Hospital General of Asturias in Oviedo.

Speciation in biological systems calls for complemetary analytical techniques

The extreme complexity of biological systems and biocompounds renders very often extremely difficult a correct interpretation of results obtained by speciation analysis. This is particularly so when just a single analytical technique is employed. As stated before [3] such complexity calls for a knowledge and judicious choice of different principle-based separations assisted by complementary selective detectors.

Hyphenated techniques, combining a powerful separation with an atomic detector, are preferred to carry out the Al and Si speciation in biological fluids. Of course, the coupling of HPLC with ICP-MS for speciation purposes is here also highly performant (speciation of biological materials by HPLC-ICP-MS is rocketing in the last three years or so). However, unlike environmental speciation of organometallics (where the compounds expected are known), metal speciation in biological materials is much more complex because we can think of known and unknown dozens of species of a given element which has been integrated and metabolized by a living organism. This absence of detailed knowledge and of available pure biocompounds to confirm the presence of a suspected given species, makes the use of complementary separation/detection tecniques, providing a good matching of results, extremely useful at this stage of development of the trace element speciation field in biological material.

Another important aspect is that, notwithstanding the speciation power of expensive hybrid approaches as HPLC-ICP-MS, simpler and more widespread approaches can also be used to solve important speciation problems (if designed adequately to provide complementary speciation information). Moreover, speciation information obtained with cheaper approaches can afterwards be invaluable to guide our further research when more performant (expensive) speciation tools become available.

Let me illustrate these ideas by resorting to the progress made in my research group during the last ten years working on the problem of Al and Si speciation in human serum. It is interesting to revise the long way walked from our first off-line experiments with simple techniques (ultrafiltration and AAS measurements) to our latest findings on aluminium speciation with on-line HPLC-ICP-High Resolution-MS approaches.

Non-chromatographic separations: Ultrafiltrable silicon and aluminium in serum

On approaching trace element speciation in clinical samples, a most advisable first step is the fractionation of the metal-biocompounds into two broad groups: high molecular mass (HMM) and low molecular mass (LMM) type of species. Particularly in the case of Al toxicity, this "primary" or rough speciation could tell us if Al could be eliminated during an haemodialysis session of the uremic patient. That is, if Al-LMM species predominate in serum we could expect they could cross the dialysis membrane and so toxic aluminium eliminated in the dialysis fluid. In this line, ultrafiltration should "mimic" the dialysis process and so it was selected first for serum Al fractionation using Electrothermal-AAS detection of Al (and Si) in the ultrafiltrable fractions obtained.

First experiments showed that conventional ultrafiltration was plagued with Al contamination problems. Therefore, an Amicon MSP-1 ultramicrofiltration system [4] was finally chosen for fractionation, while Al and Si specific detection was accomplished by ET-AAS and ICP-AES. Our ultramicrofiltration results of human serum clearly showed that only 11 ± 2% of serum aluminium is ultrafiltrable, while for silicon this figure amounted to 45 ± 5% for fresh serum samples. Ultrafiltrable Al in serum did not showed dependence with factors such as age of serum, small pH increases, renal failure status or kidney transplantations. Ultrafiltrable serum silicon, however, depended upon all those factors (figures between 46 − 16% ultrafiltrable were observed) being the aging of serum the more critical one.

It is important to note that addition of desferrioxiamine (DFO), a low molecular mass drug used for Al and Fe detoxication, rendered 75 ± 2% of total serum aluminium into an

Al-LMM ultrafiltrable species. On the contrary, serum silicon at pH 7.4 did not change its fractionation pattern when DFO was added to serum (see Fig. 1 where observed fractionation of Al and Si in the same human sera has been plotted).

We had understood why DFO could be an Al-detoxifying drug in dialysis and demonstrated, as some other authors, that DFO administration is able to release Al from its binding proteins and also from target organs in the body (e.g. bones). It could have been thought that proper kidney function restauration (i.e. after successful transplantation) should also produce such benefitial functions. In a close collaboration with surgeon Dr. A. Martín de Francisco of the Valdecilla's Central Hospital in Santander, we measured the speciation of Al and Si in his transplanted kidney patients sera, at different times after kidney transplantation. Figure 2 shows the results which, for the first time, demonstrated experimentally how the transplanted kidney was able to eliminate slowly the two metals overloading in the patient's sera. Such clearance process is rather slow and can take over one year, however, fractionation of aluminium in Al-LMM and Al-HMM species was always similar in all the sera analysed.

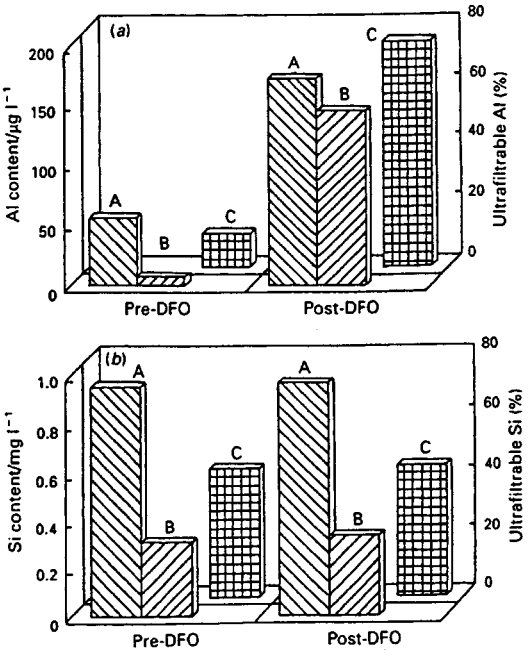

Figure 1. Influence of administration of DFO on A, total and B, ultrafiltrable levels of a) aluminium and b) silicon in serum; C, shows the ultrafiltrable percent of the element. Uremic serum samples, 7.4 < pH < 7.6.

Chromatographic separations: Which protein transports aluminium in human serum?

The above question stood up once LMM and HMM Al biocompounds had been separated and about 88% of the metal was shown to be bound to a high molecular mass biocompound. One of the best speciation tools to answer such question was probably the coupling of Liquid Chromatography with Atomic Detectors.

In our first approximation HPLC of sera with "off-line" fractions collection and final Al and Si detection by ET-AAS was used [5]. Silica-based ion-exchange and size-exclusion chromatography separations pointed to transferrin as the protein binding serum Al^{+3}. Parallel experiments measuring serum silicon showed that this latter element is only weakly bound to serum proteins (Fig. 3); in fact, silicates appeared to be adsorbed on albumin, transferrin and globulins of serum [6] and the extension of such Si-biocompound associations was dependent upon many external conditions (age of solutions before analysis, pH, etc.). Conversely, Al^{+3} seemed bound to transferrin only (Fig. 3) and this association was characterized by a strong binding constant. For satisfactory recoveries of the total serum Al^{+3} injected in the columns, however, we had to resort to the use of anionic exchangers based on polymeric columns (Protein-Pak DEAE - 5 PW, from Waters). This latter column and off-line ET-AAS detection proved to provide an excellent Al speciation approach to identify, confirm and determine Al-Desferrioxiamine, Al-Transferrin and, more controversially, Al-Citrate with just a single injection of the serum, diluted 1 + 4 with ultrapure water. The speciation observed by HPLC-"off-line" AAS (93 ± 4% for Al-Transferrin and 11 ± 6% for Al-Citrate) matched very well our previous ultramicrofiltration results. Moreover, further polyacrylamide gel electrophoresis (PAGE) experiments, carried out in collaboration with the biochemist Dr. Kazimierz Wróbel in Oviedo, showed conclusively that transferrin is the sole human serum protein binding realistic physiological levels of this metal in uremic patients [6].

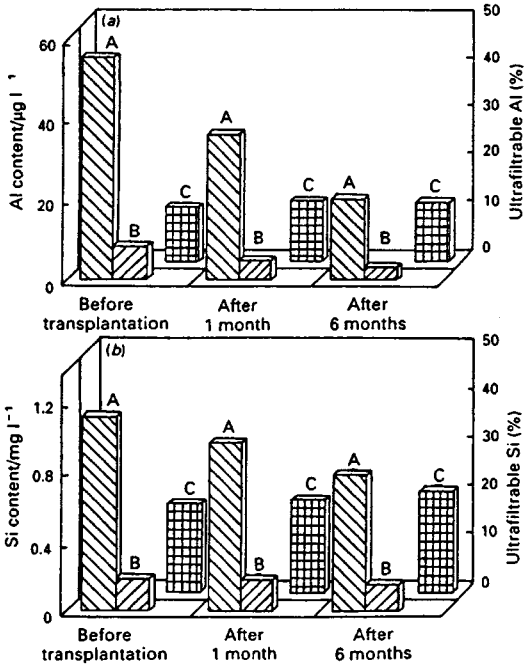

Figure 2. Influence of kidney transplantation on A, total and B, ultrafiltrable levels of a) aluminium and b) silicon; C, shows the ultrafiltrable percent of the element. Serum pH > 8.

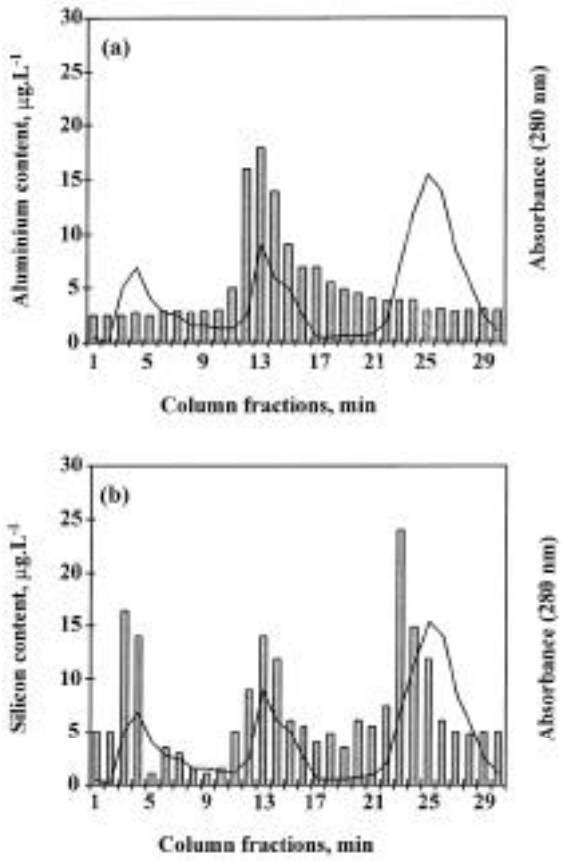

Figure 3. Elution profiles of proteins (detection at 280 nm) and a) aluminium and b) silicon (ETAAS) in diluted (1 + 4) serum sample (1.0 µg mL^{-1} Al, 0.1 mmol L^{-1} citric acid, 5.0 µg mL^{-1} Si).

However, the chromatographic peak for Al-Citrate in this separation was not conclusive as it overlaps with the huge Albumin band (this fact could explain some old claims that aluminium can also be bound to albumin). Therefore we also tried a different complementary separation: fast protein liquid chromatography (FPLC) in a column Mono Q HR 5/5 (Pharmacia, LKB, Barcelona, Spain) using UV-Vis spectrophotometry for detection of proteins and citrate and off-line ET-AAS for determination of aluminium [7]. Using such FPLC column transferrin elutes at 6.5 min, albumin at about 12 min. while the Al-citrate complex appeared at 10 − 11 min retention time. Aluminium determinations in each fraction collected (1 mL fractions) indicated a major Al peak at 6.5 min, both in spiked and unspiked uremic sera, overlapping the transferrin peak. In other words, the previously reported, by us and others, association of Al^{+3} with the HMM biomolecule Transferrin (80 000 Daltons) was again confirmed. Most importantly, this work (7) used an inert chromatographic system equipped with an "on-line" Al^{+3} - scavenger column (Kelex 100 - impregnated silica C_{18}, a packing for Al^{+3} preconcentration /separation in dialysis fluids, previously developed in our group). Such "inert" system avoids exogenous Al contamination and so allowed us to carry out for the first time speciation of this metal in unspiked sera of dialysis patients at low concentration levels [7]. Moreover, the observed recovery of total Al in such columns was of 95 ± 10% indicating the reliability of this separations and the absence of typical Al^{+3} column absorptions (often detected when using other types of stationary phases).

At this point the "off-line" character of the ET-AAS detector should be confronted with the possibilities of "on-line" detection, as it is customary in HPLC work with traditional UV-Vis detectors. There is no question that on-line, real-time detection should had been much more desirable for Al speciation in the previous HPLC experiments. Of course, in that case the atomic detector selected should be highly performant, providing the required sensitivity/specificity for Al speciation at physiological levels in serum. Probably ICP-MS could offer such performance. In this line of thinking, we took advantage of the FPLC separation developed for ET-AAS [7] and coupled such column directly on-line with the ICP-MS nebuliser in an attempt to tackle the problem of "basal" Al speciation in human serum (around 2 µg L^{-1} of total element!). Considering the possibilities of ICP-High Resolution-MS present instrumentation (e.g. less spectral interferences at higher resolution powers and better sensitivity than quadrupoles when used at a low resolution setting) parallel experiments were carried out comparing the performance of quadrupole and double focusing ICP-MS for Al detection in serum.

Thus, separation of human serum proteins was accomplished with the Mono Q (HR 5/5) anion-exchange column. However, compared with ETAAS previous detection [7] the mobile phase was changed to an ammonium acetate gradient (0 − 0.25 mol L^{-1}) at physiological pH of 7.4 (0.05 mol L^{-1} buffer of Tris-HCl) more compatible with the ICP-MS liquid sample introduction. As usual, main serum proteins were detected spectrophotometrically, at 295 nm, on-line with the ICP-MS. Comparative parallel couplings were performed using Quadrupole-ICP-MS and Double Focusing-HR-ICP-MS for Al speciation at serum concentration levels below 5 µg L^{-1}. As figure 4a shows, the use of the low resolution instrument proved to be inadequate in this particular application due to CN^+ ions spectral interference at m/z = 27. Conversely, the coupling of the FPLC separation with the Double Focusing ICP-HR-MS detector solved the problem because that spectral interference is well separated at resolution power of 3000. In this way, such performant coupling offers the possibility of Al speciation studies in non-uraemic human serum samples, for the first time [8].

Work using Capillary Electrophoresis is in progress in our laboratory to demonstrate that the two chromatographic peaks observed containing aluminium (see Fig. 4b) are probably due to two different Al-Transferrin complexes: one via the C-terminal site and the other via the N-terminal site of the two lobules of Transferrin.

Regarding the speciation of Al-LMM forms in human serum, it seems demonstrated that Al-Citrate complex accounts for the vast majority of the ultrafiltrable 12 ± 5% Al fraction [6] and this has been recently confirmed by R. Milačič's group [9] using FPLC separations.

Of course, the separation and determination of DFO, Al-DFO and Fe-DFO in serum of haemodialysis patients is also important these days. Reversed phase conventional HPLC allows such speciation of possible Metal-LMM compounds in uremic serum samples, provided that a previous simple ultrafiltration step is carried out [10]. On-line spectrophotometric detection proved again to be a most useful complementary detector of atomic ones and so a simple and

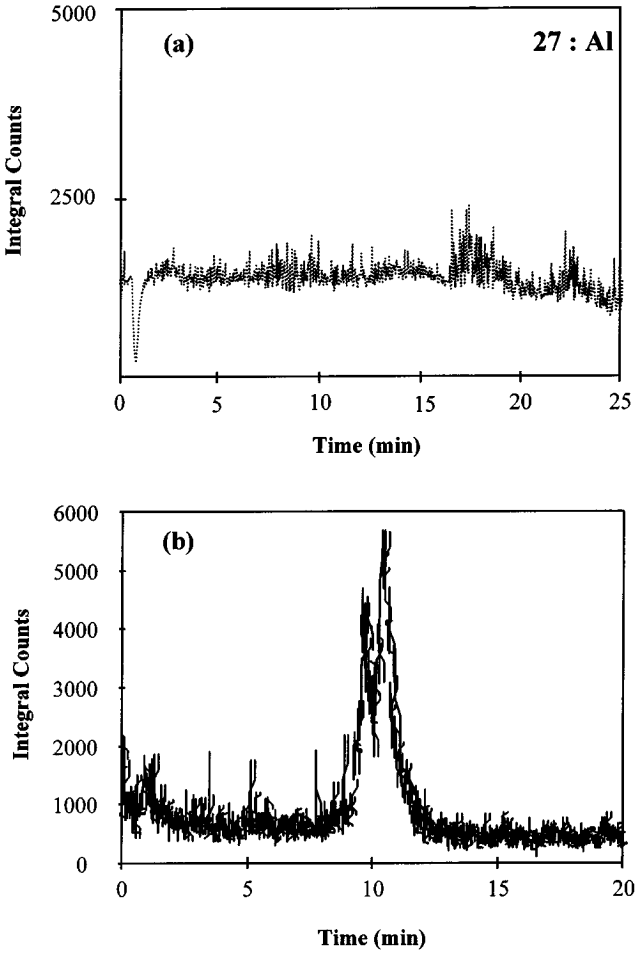

Figure 4. Chromatogram of normal unspiked human serum (Al content 2.5 µg L^{-1}), after FPLC separation of proteins using: a) Quadrupole-ICP-MS detection of Al; b) HR-ICP-MS (*R* = 3000) detection of Al.

convenient HPLC technique was proposed to study actual removal of aluminium from their deposits in renal failure patients treated with DFO [10].

Conclusion

As a final conclusion of our work, I would stress the importance today of actually using complementary analytical techniques and seek collaboration with complementary people. In order to advance efficiently in the field of speciation and scientific knowledge of metal biomolecules we should learn about and use complementary techniques for speciation problems if analytical "internal" validation of results is considered.

Moreover, "external" validation of speciation results has also to be aimed at, through cooperative work with potential users of such results including medicine, biology, biochemistry and clinical chemistry professionals, more and more interested in this basic knowledge of metal biomolecules in living organisms.

References

1. Massey, R. C.; Taylor, D. Aluminium in Food and the Environment, Royal Society of Chemistry, London, 1988.
2. Birchall, J. D. *Chem. Britain* **1990**, *26*, 141.
3. Sanz-Medel, A. *Analyst* **1995**, *120*, 799.
4. Pérez Parajon, J.; Blanco Gonzalez, E.; Cannata, J.; Sanz-Medel, A. *Trace Elem. Med.* **1989**, *6*, 41.
5. Sanz-Medel, A.; Fairman, B.; Wróbel, K. "Al and Si speciation in Biological Materials of Clinical Relevance", Chapter 7, in Element Speciation in Bioinorganic Chemistry, Caroli, S. Ed., John Wiley & Sons, Inc, 1996.
6. Wróbel, K.; Blanco González, E.; Wróbel, K.; Sanz-Medel, A. *Analyst* **1995**, *120*, 809.
7. Soldado Cabezuelo, A. B.; Blanco González, E.; Sanz-Medel, A. *Analyst* **1997**, *122*, 573.
8. Soldado Cabezuelo, A. B.; Montes Bayón, M.; García Alonso, J. I.; Blanco Gonzalez, E.; Sanz-Medel, A. *Analyst*, **1998**, *123*, 865.
9. Bantan, T.; Pihlar, B.; Milacic, R. *Talanta*, **1998**, *46*, 227.
10. Menéndez, P.; Blanco, E.; Sanz-Medel, A.; Cannata, J. B. *Talanta* **1997**, *45*, 25.