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Design of a phantom equivalent to measure bone-fluorine in a human’s hand via delayed neutron activation analysis

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Abstract
Fluorine is an element that can be either beneficial or harmful, depending on the total amount accumulated in the teeth or bones. In our laboratory, we have developed a non-invasive technique for the in vivo measurement of fluoride in bone using neutron activation analysis and performed the first pilot human study. Fluoride in humans is quantified by comparing the γ-ray signal from a person to the γ-ray signal obtained from appropriate anthropomorphic calibration phantoms. An identified problem with existing fluoride phantoms is contamination with aluminum. Aluminum creates an interfering γ-ray signal which, although it can be subtracted out, increases the uncertainty in the measurement and worsens the detection limit. This paper outlines a series of studies undertaken to develop a better calibration phantom for fluorine measurement, which does not have aluminum contamination.

Keywords: phantom, bone-fluorine, delayed neutron activation analysis

(Some figures may appear in colour only in the online journal)

Introduction
A common naturally-occurring contaminant and sometimes also additive of water and many foods is fluorine, which is an element associated with both benefits and problems in teeth and bone. Water, tea and some foods are the major sources of intake for fluoride (F⁻) (Yadav et al 2007, Johnson et al 2007). In North America and some other countries, there is still controversy as to whether fluorine should be added to drinking water (Ludlow et al 2007, Chamberlain et al 2012a). It is established that addition of about 1 ppm to water can be useful for the purpose of improved dental care (such as reduced caries), and at certain concentrations, fluoride has long been shown to reduce bone fracture risks (Chamberlain et al 2012b, Kurland et al 2007).
However, in some cases of high exposure, fluoride has been found to be toxic and harmful for consumers. Examples include people who have been found to have unusual habits such as swallowing fluoride containing toothpaste (Kurland et al. 2007). Bone deformities, and changes in children’s thyroid hormone levels are some of the health consequences that have been observed through ingestion of high doses of fluoride (Susheela et al. 2005). Another occasionally observed condition (due to the long half-life of fluoride in bone) for individuals exposed to elevated fluoride intake, is fluorosis (Tamer et al. 2007, Wang et al. 2007). Different approaches by nations to the issues surrounding fluoride in drinking water have been described (Khandare et al. 2005, Li et al. 2001, Vestergaard et al. 2008).

Fluorine accumulated in the skeleton interacts with bone by displacing the hydroxide content of the bone mineral. Bone is therefore potentially a useful site for measuring the fluoride concentration as a result of long term accumulation (Clarke et al. 1994, Chamberlain et al. 2012a). The concentration of fluorine in the skeleton has previously been measured using a reactor-based ex vivo technique, but obtaining a bone sample via biopsy is rarely possible (Krishnan et al. 1985). Our laboratory recently conducted the first series of in vivo measurements of bone fluoride using neutron activation analysis (NAA). The volunteers’ fluoride concentration was assessed non-invasively (Chamberlain et al. 2012b) and the measurement relied on the comparison of in vivo fluoride signals to those from anthropomorphic standard addition calibration phantoms.

Limitations with the phantoms used in our earlier studies were identified, including contamination by aluminum. This paper describes work that was undertaken to develop an improved anthropomorphic phantom that could be used for studies of fluoride in bone and potentially other elements as well. In total, eleven different possible phantom types were investigated, with one phantom type identified as being best for future studies.

Irradiation materials and methods

Sample (and in vivo) irradiation

An irradiation cavity was created for the in vivo analysis of toxic elements in a human hand using the accelerator-based 7Li(p,n)7Be neutron source at the McMaster Accelerator Laboratory (Aslam et al. 2008a). The irradiation facility has been previously described in the literature (Byun et al. 2007). Those elements whose neutron capture cross sections are sufficiently large can be analyzed by measuring the emission of γ-rays following neutron irradiation (Byun et al. 2007, Pejović-Milić et al. 2006). Measurement of the in vivo γ-ray signal, when compared with the γ-ray signal from an appropriate calibration standard, permits the level of the element in a person to be quantified.

The irradiation geometry means that the sample is totally enclosed within a cavity which has a port which allows hand access. This irradiation cavity setup has been used (Aslam et al. 2008a, 2008b, Davis et al. 2008) for the successful non-invasive measurement of several trace and minor elements in a series of studies of human volunteers. Elements studied have included magnesium, aluminum, manganese and fluorine (Davis et al. 2008, Aslam et al. 2008a, 2008b, Chamberlain et al. 2012a). This irradiation cavity and method was used to irradiate the phantoms described in this paper. In this set up the equivalent dose to the phantoms was determined to be 30 mSv (Chamberlain et al. 2012a).

Sample detection

Two methods were used to measure the samples described in this paper. Firstly, a system of eight 10.2 × 10.2 × 40.6 cm³ plus one 10.2 × 10.2 × 10.2 cm³ NaI(Tl) detectors
Phantoms

Eleven different types of phantom in total were studied. For the original fluoride in vivo study, hand bone phantoms with different concentrations of F had been fabricated in 125 ml cylindrical Nalgene (polyethylene) containers. However, subsequent work determined that the presence of aluminum contamination within the plastic of the bottles made the determination of fluoride more challenging. Figure 1 shows a phantom measurement spectrum and it can be seen that there are a number of $\gamma$-ray signals that either overlap or are in close proximity to one another. In particular, the $^{28}\text{Al}$ peak overlaps with the $^{20}\text{F}$ signal. Subtracting the $^{28}\text{Al}$ signal from the $^{20}\text{F}$ increases the uncertainty in the estimation of the fluorine signal. It is therefore preferable to try and eradicate the Al contamination from the phantoms.

Phantom type 1. Initially, we tried water-based phantoms in Nalgene bottles. However, the masses of powder, and the volumes of water required, meant that the materials did not mix well. It was not possible readily to obtain a homogenous solution of fluorine and calcium, and post-irradiation settling was found to occur.

Phantom type 2, 3 and 4. We next eliminated the water and used dry powder phantoms. These consisted of $\text{NaNO}_3$ (1.25 g of Na), $\text{NH}_4\text{Cl}$ (1.19 g of Cl), $\text{CaCO}_3$ (14.9 g of Ca) and varying masses of $\text{NH}_4\text{F}$ with a fluorine content that varied between 0.03 to 0.5 g (as per ICRP 1975).

Table 1 shows the elements which were added to the phantom in the specified concentrations so as to match a ‘reference’ man (ICRP 1975). Detection was of micrograms...
Figure 2. Comparison of HDPE Nalgene bottles with different proton energies.

**Table 1.** Elemental composition of hand bone (ICRP 1975).

<table>
<thead>
<tr>
<th>Elements</th>
<th>Ca($^{48}$Ca)</th>
<th>Na($^{23}$Na)</th>
<th>Cl($^{37}$Cl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected amount (ICRP)</td>
<td>14.9 g</td>
<td>1.25 g</td>
<td>1.19 g</td>
</tr>
<tr>
<td>Compound added</td>
<td>Ca(NO$_3$)$_2$</td>
<td>NaNO$_3$</td>
<td>NH$_4$Cl</td>
</tr>
<tr>
<td>Amount of compound (g)</td>
<td>88.06 ± 0.05</td>
<td>4.6 ± 0.01</td>
<td>1.80 ± 0.01</td>
</tr>
</tbody>
</table>

(μg) of F per gram (g) of Ca. Chlorine was required because of a spectral interference with the fluorine signal. Sodium is required because $\gamma$-rays from the $^{23}$Na(n,$\gamma$)$^{24}$Na reaction contribute significantly to the $\gamma$-ray background signal (Chamberlain et al. 2012a).

1. Phantom type 2’ were powder within high density polyethylene (HDPE) Nalgene bottles.
2. Phantom type 3’ were powder within low density polyethylene (LDPE) Nalgene bottles.
3. Phantom type 4’ were powder contained within Adanac Polyethylene bottles.

**Analysis and results for phantoms type 2, 3 and 4**

**Bottles**

In order to determine the level of aluminum in phantom type 2, empty 250 ml Nalgene bottles were irradiated with different neutron beam energies produced from proton energies of (2, 2.1, 2.2, 2.3 MeV respectively) with a proton current of 400 μA for 60 s (Byun et al. 2006) as performed in earlier studies (Chamberlain et al. 2012a, 2012b). The bottles and materials were measured for 300 seconds in anti-coincidence mode in the $4\pi$ detection system. Figure 2 shows the resulting spectra.

The lowest Al signal resulted from irradiation with the lowest proton energy. This is to be expected as the neutron yield is lower for lower energies. However, an important finding was that having a bottle filled with water did not show any significant difference in signal in comparison with the empty bottles. The extra moderation within the sample would appear to result in a negligible effect on the signal, probably because the scattering and pre-moderation within the cavity dominate the effect on the neutron flux at low energies. The bottle that showed
the most difference, in comparison to the one filled with water, was due to the activation of argon (Ar) in air. Less water meant there was more air in the bottle.

Figure 3 illustrates a comparison between phantom type 2 and phantom type 3 irradiated with a neutron beam produced by a 2.3 MeV proton energy on lithium (Chamberlain et al 2012a). The amount of aluminum inside the LDPE phantom is significantly lower than in the HDPE, but it is still a clear signal. LDPE is a better and more appropriate choice for creating a phantom, but is still not perfect. A further test of polyethylene phantoms (Phantom type 4) was performed to see if the Al signal could be completely eliminated. Adanac polyethylene was therefore examined through irradiation and activation, and the results from this test are shown in figure 4. Note that these bottles had a much lower volume than the previously used bottles.

The Adanac polyethylene shows a lower amount of aluminum than the previous two types of plastic container, and could be considered the best candidate for the phantoms. However, Adanac was not available in containers of sufficient volume for our studies of the human hand.
Non-contained phantoms

The possible use of phantoms without containers was studied. Phantom type 5. It was suggested that using pressure on the powdered elements used in the fluorine phantom could lead to a compressed stable phantom. However, a solid pellet could not be manufactured with the systems available, and the pressed powder was found to fall apart after approximately 5 min.

Phantom type 6. An attempt was made to heat the phantom materials to melting point, to then mix and cool to a solid. Unfortunately, calcium carbonate has a melting point of 825 °C. In our attempts, the compound disassociated, rather than melting.

Two attempts were made to create phantoms using wax as a binding agent. Similarly, two attempts were made using resin as a ‘binder’ to keep all fluorine phantom components together. Each binder needed to be tested for aluminum contamination. Phantom 7 tested DENTSPLY sticky paraffin wax. Phantom 8 tested regular paraffin wax. Phantom 9 was a test of dental resin. Phantom 10 was a test of polyethylene resin commonly used for car repair. Figure 5 shows the results for the two waxes. Figure 6 shows the results for the two resins. Irradiations were performed as before.
Table 2: Aluminum net counts for finding best composition of hand bone phantom. Materials were activated at MNR.

<table>
<thead>
<tr>
<th>Material name</th>
<th>Mass (g)</th>
<th>Irradiation time (s)</th>
<th>Aluminum net count</th>
<th>Al counts per g material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wax (regular paraffin)</td>
<td>1.83</td>
<td>10</td>
<td>205 ± 14.3</td>
<td>112 ± 5.8</td>
</tr>
<tr>
<td>Adanac polyethylene</td>
<td>1.62</td>
<td>10</td>
<td>1028 ± 32</td>
<td>634 ± 16</td>
</tr>
<tr>
<td>Nalgene polyethylene</td>
<td>1.77</td>
<td>10</td>
<td>2904 ± 53.9</td>
<td>1640 ± 24.7</td>
</tr>
<tr>
<td>Regular resin</td>
<td>1.65</td>
<td>10</td>
<td>12 599 ± 112.2</td>
<td>7635 ± 70.3</td>
</tr>
<tr>
<td>Quartz (silicon dioxide)</td>
<td>1.58</td>
<td>10</td>
<td>19 060 ± 138</td>
<td>12 063 ± 103.3</td>
</tr>
</tbody>
</table>

The amount of aluminum inside the DENTSPLY wax was lower than the paraffin wax, however, the aluminum concentration in both forms of wax binder was measurable and therefore still not fully acceptable. Figure 6 illustrates that the aluminum level in regular resin is comparable with wax, while the aluminum levels in dental resin are substantially lower. However, other elements in the dental resin meant that, as can be seen in the figure, the dental resin’s background signal is orders of magnitude higher at the energies of interest. This makes this material unsuitable for fluorine phantoms. The background signal is much higher so the detection limit in the calibration standards would be worse.

Signal comparison using the MNR

In order to quantify the differences in aluminum signal between phantom materials more accurately, the MNR pneumatic small-sample activation system was used to irradiate the following samples: Wax, Adanac Polyethylene, Nalgene Polyethylene, and regular resin. In addition, a quartz (silicon dioxide) sample was analyzed to test whether such a container would reduce the aluminum signal. A hyperpure germanium detector was used for $\gamma$-ray detection. Table 2 illustrated the number of aluminum 1.779 MeV $\gamma$-ray net peak counts for each of these samples.

As can be seen, paraffin wax provided the lowest level of contamination per gram of material by far. However, as previously discussed this signal, although low, is still observable.

Phantom type 11 (Mowiol 4-88)

Mowiol 4-88 is a high quality organic anti-fade medium that is used for immunofluorescence, as well as other molecular biological and geological applications. It is a material which hardens and which has the same refractive index as immersion oil. If used for immunofluorescence applications the addition of glycerin is recommended (Polysciences Inc. 2008). Mowiol 4-88 was prepared by following the manufacturer instructions in the following series of steps:

1. 4.8 g Mowiol 4-88 and 12 g glycerol were added to a 100 ml beaker.
2. The material was mixed well using a stir bar.
3. 12 ml of double distilled H$_2$O was added and the stirring continued for several hours at room temperature.
4. 24 ml 0.2 M Tris HCl (ph 8.5) was added and stirring continued. The mixture was heated occasionally to 50 °C in a water bath for approximately 10 min and stirring continued until the Mowiol 4-88 was dissolved.
5. Once dissolved, the solution was centrifuged at 500 × g for 15 min to clarify the solution.
6. The supernatant was carefully removed and aliquots stored at −20 °C (Polysciences Inc. 2008).
After preparation, the Mowiol 4-88 material was tested as previously described, using a proton energy of 2.3 MeV with 400 $\mu$A current. Irradiations were performed for 60 seconds, and the detection time in the $4\pi$ NaI(Tl) detector was 300 seconds in anticoincidence mode.

Mowiol was found to be the most successful material for use as a phantom binding agent in terms of limited aluminum contamination as can be seen in figure 7. The Mowiol 4-88 was found to be effectively aluminum free, which thus makes it appropriate for making fluoride bone phantoms.

Phantoms were then created using the Mowiol 4-88 as the binding agent. The Mowiol 4-88 was mixed with NaNO$_3$, NH$_4$Cl, CaCO$_3$ and NH$_4$F. In order to obtain solid fluorine phantoms, the Mowiol 4-88 and additives were left to sit and harden at room temperature for three weeks.

**Calibration data**

In order to determine whether robust homogeneous flouride-doped Mowiol phantoms could be created that produced calibration data that were comparable with the previously used phantom set, a new set of five phantoms with different masses of fluoride, 0.03–0.25 g F, was created. The phantoms were found to have remained very robust three weeks after adding the Mowiol to the components, and they showed sufficient hardening and stability to be irradiated.

The new phantoms were irradiated and measured as per the previously described method. On the same day, phantoms in the same mass range from the previously used ‘old’ phantom set (phantom type 2) were also irradiated and measured. Both sets of phantoms were made as standard addition phantoms from the same batch of ammonium fluoride, but were made several years apart. As ammonium fluoride is a compound that is hygroscopic, a drying experiment was performed to determine how much water had been absorbed by the compound. A small sample was weighed out, then placed in a relatively low temperature oven at 63 °C for several hours. The sample was found to have reduced in mass to 95.9% of the original mass. The masses, and hence concentrations, of fluoride in the new phantoms were therefore corrected by this factor.

Figure 8 shows the calibration lines of fluoride signal normalized to Ca signal versus mg of F per gram of Ca obtained from both the new fluorine phantoms and the old fluorine phantoms. The phantoms were compared over a concentration range from approximately 1–17 mg F per
gram Ca. Previous studies in our laboratory had measured levels in environmentally exposed volunteers from Southern Ontario in the range from 0 to 10 mg F per g Ca: this phantom calibration range therefore encompasses this range of in vivo data.

The new and old phantoms were found to have the same calibration slope and intercept to within uncertainties.

For the ‘old’ phantoms, the slope of the regression was 0.246 ± 0.012 (r² = 0.995, p = 0.002)

For the ‘new’ phantoms, the slope of the regression was 0.250 ± 0.006 (r² = 0.998, p < 0.001)

For the ‘old’ phantoms, the intercept was found to be −0.10 ± 0.12

For the ‘new’ phantoms, the intercept was found to be −0.04 ± 0.05

The slopes and intercepts of the two sets of phantoms were found to be the same to within uncertainties, as would be expected. The measured fluorine signals from the phantoms are normalized to the calcium signals. This should mean that the accuracy of the measurement is independent of factors such as target quality, variation in beam current or positioning of the phantom in the cavity. (The precision of the measurement will however depend on these factors.) The reason for the creation of these new phantoms was to eliminate the aluminum signal and thus reduce the uncertainty on the measurement. This seems to be borne out by these data, as the slope and intercept uncertainties are less for the new phantoms than the old.

Conclusion

We have developed a new type of hand-bone phantom for the determination of fluorine concentration by in vivo NAA. Previously used phantoms contained a small level of aluminum contamination, which contributed to the uncertainty on the fluorine measurement. A new phantom that uses Mowiol 4-88 as the binder has been developed and found to have non-detectable levels of aluminum. The phantom has more involved preparation requirements, but is robust, and produces a linear calibration signal that is comparable with the previously used phantoms. It eliminates the problem of aluminum contamination and thus appears to reduce measurement uncertainties. This phantom is suitable for NAA studies of fluoride in bone, and may also be a suitable phantom material for in vivo studies of other elements.


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