Determining Residual Calcium In Demineralised Cortical Bone By Three Methods

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INTRODUCTION:

According to American Association for Tissue Banks (AATB) Standards, a bone to be classed as demineralised bone must contain less than 8% residual calcium by weight using a standard method[1]. Methods for measuring calcium content in bone are titrimetric assay (TA)[2], atomic absorption spectrophotometer (AAS)[3,4], Calcium Assay Kit[5] and inductively coupled plasma optical emission spectrometry (ICP-OES)[6]. The study aimed to determine residual calcium in demineralized cortical bone by using the conventional method of TA, which was performed in-house and AAS method, which can easily be outsourced. Both measurements were compared to the most precise measured by Neutron Activation Analysis (NAA).

MATERIALS & METHODS:

Femurs were obtained from screened cadaveric donors (n=3) (UMMC Ethics No: 1037.8), cleaned from soft tissues, cut into small cubes (5x5x5 mm) and immersed in 70% ethanol for 3 hr. The cubes were washed with water and soaked in 0.5M HCl for 0, 2, 4, 6, and 8 hr. The demineralised cortical bones were subjected to a series of washing until the pH was neutral and then freeze dried to lower the water content to 5%. Residual calcium was measured by TA at the bank while by both AAS and NAA at Malaysian Nuclear Agency. The readings were expressed as weight % and in triplicate.

RESULTS:

Residual calcium of the demineralised bone was slightly but not linearly decreased with increasing immersion times as measured by all methods (Table 1). Readings by TA were average 71% and 79% lower than AAS and NAA, respectively. Calcium content in the untreated cortical bone measured by NAA at 21.9% was comparable to those reported earlier at 24.42%[3] and 23.12%[4]. The % recovery of calcium by NAA was higher than AAS. Readings measured by TA and AAS were consistently comparable to NAA (Table 2).

Table 1: Residual calcium of demineralised bone after varying immersion time in HCl measured by 3 methods.

Immersion time (hr)	Residual calcium (wt %)		
	TA	AAS*	NAA*
0	4.57 ± 0.02	13.4 ± 2.97	21.9 ± 0.28
2	3.36 ± 1.56	13.2 ± 0.57	20.2 ± 0.64
4	4.17 ± 0.30	14.7 ± 0.99	18.8 ± 1.48
6	4.10 ± 0.00	12.4 ± 1.98	18.7 ± 2.19
8	3.96 ± 0.26	13.1 ± 1.20	18.1 ± 1.06
%Recovery	N/A	70	90

**Standard used was CRM animal bone (H-5) with known % calcium concentration.*

Table 2: Comparable residual calcium of demineralised bone measured by TA and AAS to NAA.

Immersion time (hr)	%		
	TA	AAS	NAA
0	20.87	61.19	100
2	16.63	65.35	100
4	22.18	78.19	100
6	21.93	66.31	100
8	21.88	72.38	100
Average	20.70	68.68	100
Conversion Factor	**4.83**	**1.46**	

DISCUSSIONS:

All three methods can be used for residual calcium measurement. Unlike NAA, lower readings by TA and AAS might be due to inefficient sample digestion. Even though NAA method is most precise, its service is limited and only available in the Nuclear Agency.

CONCLUSION:

UMMC Bone Bank will consider AAS for routine measurement of residual calcium in demineralized bone. However the % recovery needs to be improved for the readings to be comparable to those measured by NAA.

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