

# Resorbable biomaterials based on calcium phosphates: Determination of in vitro solubility applying the ISO 10993-14 (first experiences)

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## Motivation

- Quantification of the degradation products by gravimetry as well as by ICP-OES analysis when applying the ISO standard
- Considerable differences between the results comparing both methods when applying the ISO standard to calcium phosphate materials (CPM)
- Modifying of the ISO standard procedure to overcome the differences.

## Materials and Methods

- Investigation of 5 CPM materials: resorbable - commercial  $\beta$ -TCP, RM1, RM2, long-term stable - commercial HA and LSM (composition: *Table 1*)
- Description of the preparation of RM1 and LSM, respectively, in previous papers [1, 2], preparation of RM2 by melting and subsequent crystallisation
- Storage of the granulated (315-400  $\mu$ m) material for the solubility test in TRIS-HCl buffer solution according to the ISO standard
- ISO standard procedure (M1): after 5 days separation of the granules from the solution by filtering, determination of the mass loss gravimetrically as well as by ICP
- Modified procedure (M2): Separation of the solution by decanting, washing of the granules 5 times with each 5 ml of water and combined with each other for the ICP-OES measurement, gravimetric determination of the mass loss, too.

Table 1  
Chemical composition and main crystalline phases of the tested materials

Sample code	Composition [mol%]								Crystalline phases
	CaO	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	MgO	Na <sub>2</sub> O	ZrO <sub>2</sub>	CaF <sub>2</sub>	SiO <sub>2</sub>	
HA	62.50	37.50	-	-	-	-	-	-	hydroxyapatite
$\beta$ -TCP	60.00	40.00	-	-	-	-	-	-	tricalcium phosphate
LSM1	57.58	26.17	-	-	-	10.47	5.78	-	fluorapatite, calcium zirconium phosphate
RM1	44.99	25.00	12.51	5.00	12.50	-	-	-	Ca <sub>2</sub> KNa(PO <sub>4</sub> ) <sub>2</sub>
RM2	61.62	25.27	0.97	2.39	7.40	-	-	2.35	Ca <sub>10</sub> [K/Na](PO <sub>4</sub> ) <sub>7</sub>

## Results

- Mass loss of the samples: from 0.2 mg\*g<sup>-1</sup> (long-term stable materials) up to 23 mg\*g<sup>-1</sup> (resorbable materials); relative error of the mass loss determination: from 2 up to 63%
- Agreement between the results obtained with M1 by ICP-OES or gravimetrically only sufficient for the higher resorbable CPMs, RM1 and RM2 (*Table 2, Figure 1*), disagreement regarding the long term stable materials
- Differences between ICP-OES and gravimetric values relatively decreased if applying M2 regarding the long-term stable materials
- In most of the cases, higher absolute values with method M2

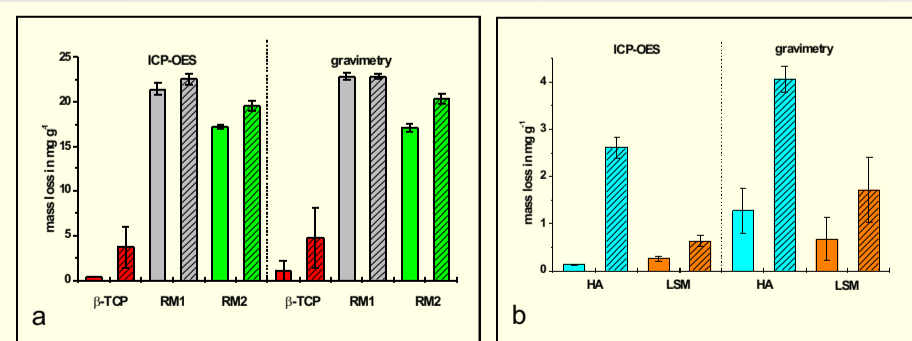


Figure 1  
Comparison of the mass loss of resorbable (a) and long-term stable (b) materials determined by ICP-OES or gravimetrically (M1-unicoloured bars, M2 - hatched bars)

Table 2  
Concentration of the leached ions after solubility test according to ISO 10993-14; average value from 7 ICP-OES measurements

Sample Code		Concentration in mg g <sup>-1</sup>						
		Ca	PO <sub>4</sub>	Mg	K	Na	Si	Zr
HA	M1	0.1	0.01	-	-	-	-	-
	M2	1.1	1.4	-	-	-	-	-
TCP	M1	0.1	0.2	-	-	-	-	-
	M2	2.2	3.2	-	-	-	-	-
RM1	M1	0.2	11	0.5	9.6	1.4	-	-
	M2	0.1	12	0.7	8.9	1.3	-	-
RM2	M1	0.3	10	0.02	1.4	5.1	0.1	-
	M2	0.4	11	0.02	1.3	4.9	0.1	-
LSM	M1	0.1	0.2	-	-	-	-	0.001
	M2	0.1	0.4	-	-	-	-	0.01

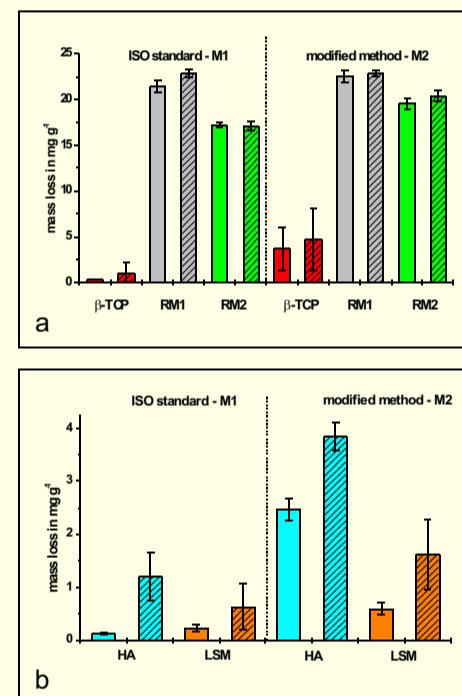


Figure 2  
Comparison of the mass loss of resorbable (a) and long-term stable (b) materials determined by M1 or M2 (ICP-OES - unicoloured bars, gravimetry - hatched bars)

## Discussion and Conclusion

- For nearly all materials, gravimetrically determined mass loss higher than that obtained by ICP-OES; no overcoming of that discrepancy if applying M2, but approach
- Only for highly resorbable CPMs no significantly different values with acceptable relative errors comparing M1 and M2 as well as ICP-OES and gravimetrically (*Figure 1 and 2*)
- No support of the assumption to get more irregular values from the resorbable materials due to the high mass-to-volume ratio (precipitation reactions) probably because of the relatively high content of alkaline ions (*Table 2*); best agreement for RM1
- Reproducibility of the mass loss determination of long-term stable materials decreased by M2 (*Figure 1 and 2*); further investigations necessary to clear up the disagreement of the results
- Very high error of all mass losses of  $\beta$ -TCP; material probably porous with some closed porosity, remarkable part of the granules swam on the surface of the solution; M2 not suitable for such porous materials

## References

- G. Berger, R. Gildenhaar, U. Ploska: Biomaterials 16 (1995), p. 1241-1246
- G. Berger, R. Gildenhaar, U. Ploska: KEM 240-242 (2003), p. 607-610