Calcium phosphate based biomaterials: solid state characterization

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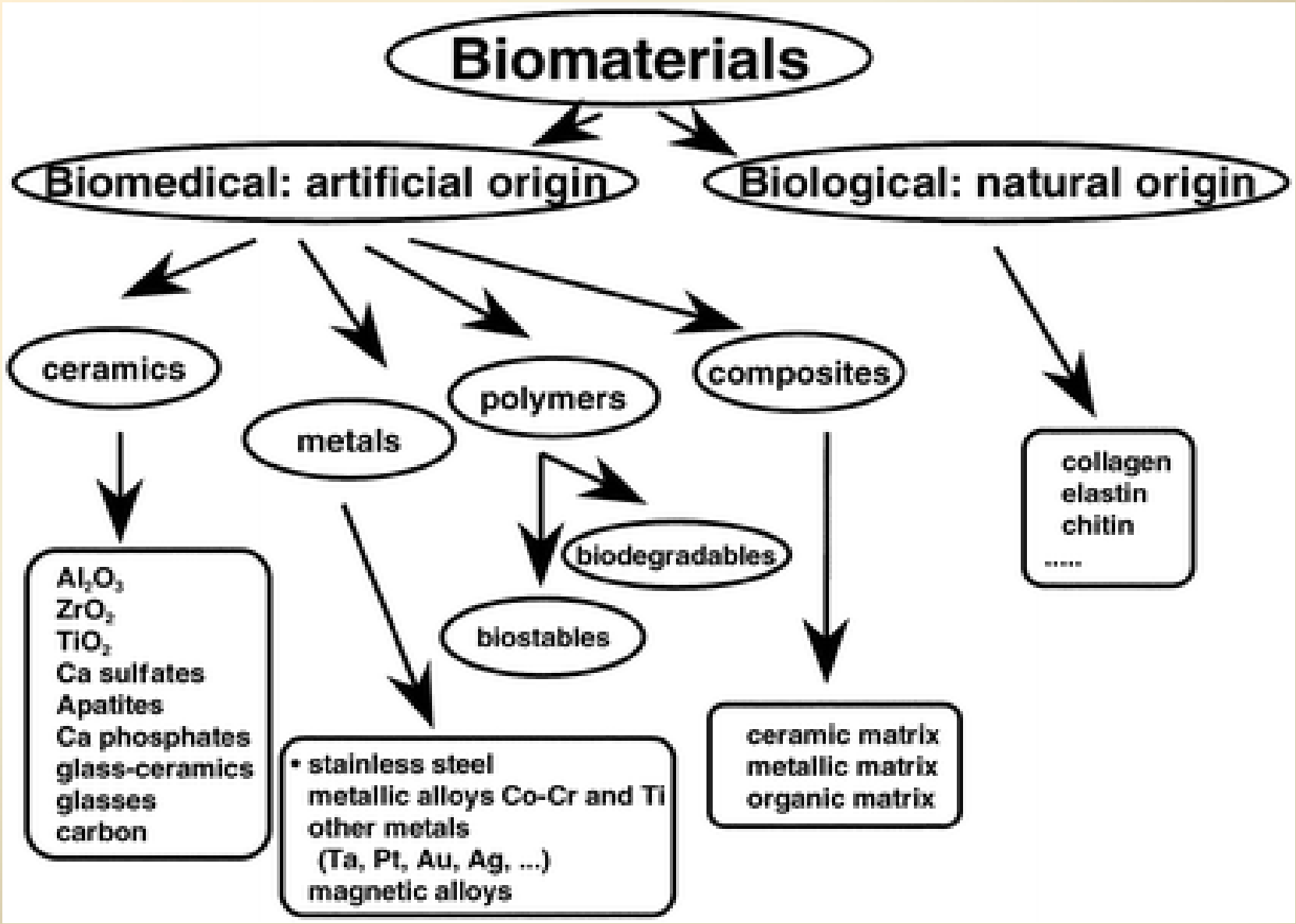
NIS Colloquium
Advances in biomaterials: combining simulations and experiments

Torino 28-29 November 2013


Biomaterials
Biomedical: artificial origin
Biological: natural origin
ceramics
metals
polymers
composites
collagen
elastin
chitin
.....
biodegradables
biostables
Al_2O_3
ZrO_2
TiO_2
Ca sulfates
Apatites
Ca phosphates
glass-ceramics
glasses
carbon
• stainless steel
metallic alloys Co-Cr and Ti
other metals
(Ta, Pt, Au, Ag, ...)
magnetic alloys
ceramic matrix
metallic matrix
organic matrix

Mineral phase is composed mainly of microscopic crystals of calcium phosphates, in which the hydroxyapatite (HA), whose chemical formula is $Ca_{10}(PO_4)_6(OH)_2$, is the most important.

Other mineral phases are dicalcium phosphate ($Ca_2P_2O_7$), dibasic calcium phosphate (DCP, $CaHPO_4$), tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) and some amorphous phases of calcium phosphate.

Biological apatites

$$(Ca,Na,Z)_{10}(PO_4,CO_3,Y)_6(OH,X)_2$$

Z = Sr^{2+},Ba^{2+},Pb^{2+}, K^+
Y= HPO_4^{2-}
X= F^-, Cl^-

⇨ Of all the calcium phosphate ceramics used as bone-replacement materials, HA most closely resembles the main inorganic phase of bone and teeth in humans.

⇨ HA: very insoluble compound and, under physiological conditions of temperature and pH, is the most insoluble calcium phosphate

Hydroxyapatite (HA) , $Ca_{10}(PO_4)_6(OH)_2$

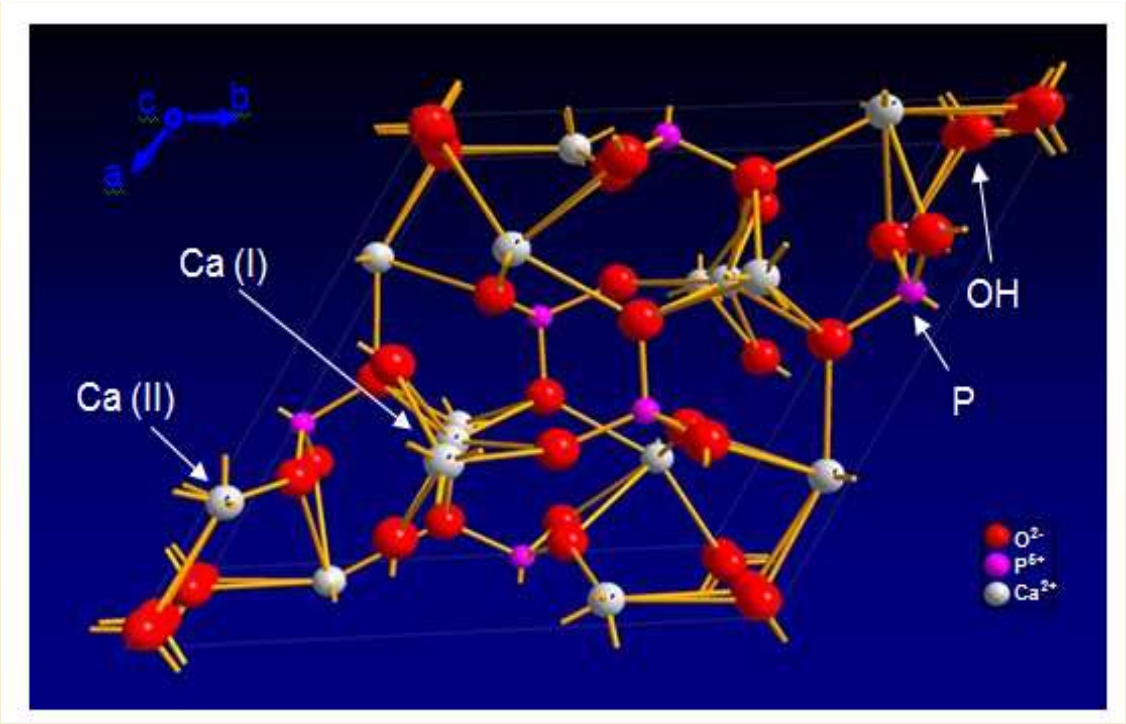
hexagonal system, although with some exception in a monoclinic system

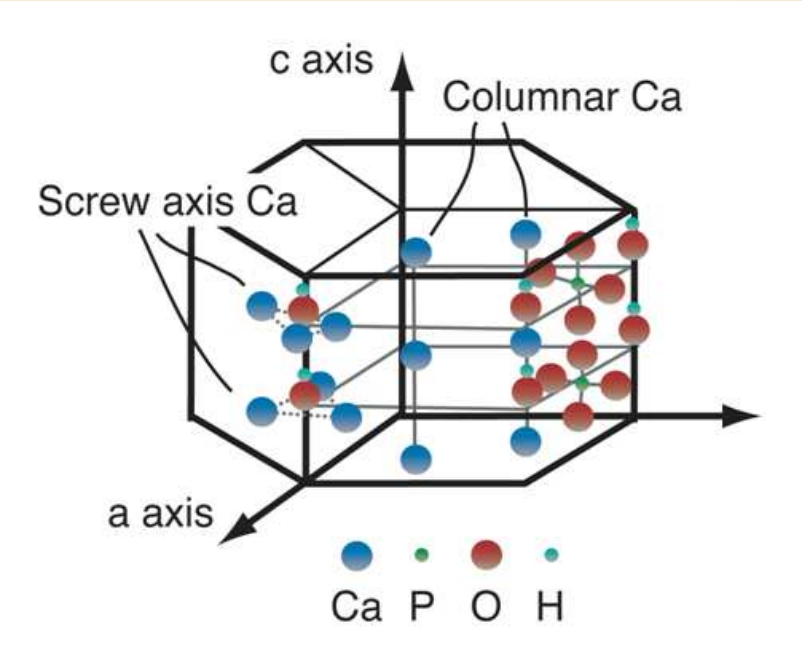
$P6_3/m$

a=b=9.418 Å ,
c=6.884 Å

•The structure can easly accomodate a great variety of substitutions, both cationic and anionic

•The incorporation of foreign ions affect the crystallinity, morphology, lattice parameters and as a consequence the stability of the structure


c
b
a
Ca (I)
Ca (II)
OH
P
O^{2-}
P^{5+}
Ca^{2+}


c axis
Columnar Ca
Screw axis Ca
a axis
Ca P O H

- Confocal micro-Raman spectroscopy
- Fourier transform infrared (FT-IR) adsorption spectroscopy; attenuated total reflectance (ATR) spectroscopy
- Scanning electron microscopy–energy dispersive spectroscopy (SEM–EDS)
- Specific surface area (SSA) measurements
- Thermal analysis (DSC/DTA)
- Transmission electron microscopy (TEM)
- X-ray photoelectron spectroscopy (XPS)
- X-ray powder diffraction (XRPD)

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Sr-containing hydroxyapatite: morphologies of HA crystals and bioactivity on osteoblast cells

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Strontium : improved solubility, antiresorptive activity, osteoclast apoptosis and osteoblast stimulation.

It shows that a great number of papers on the synthesis (with different methods) and physico-chemical characterization of Sr-HA have been reported, but with *often discordant data*

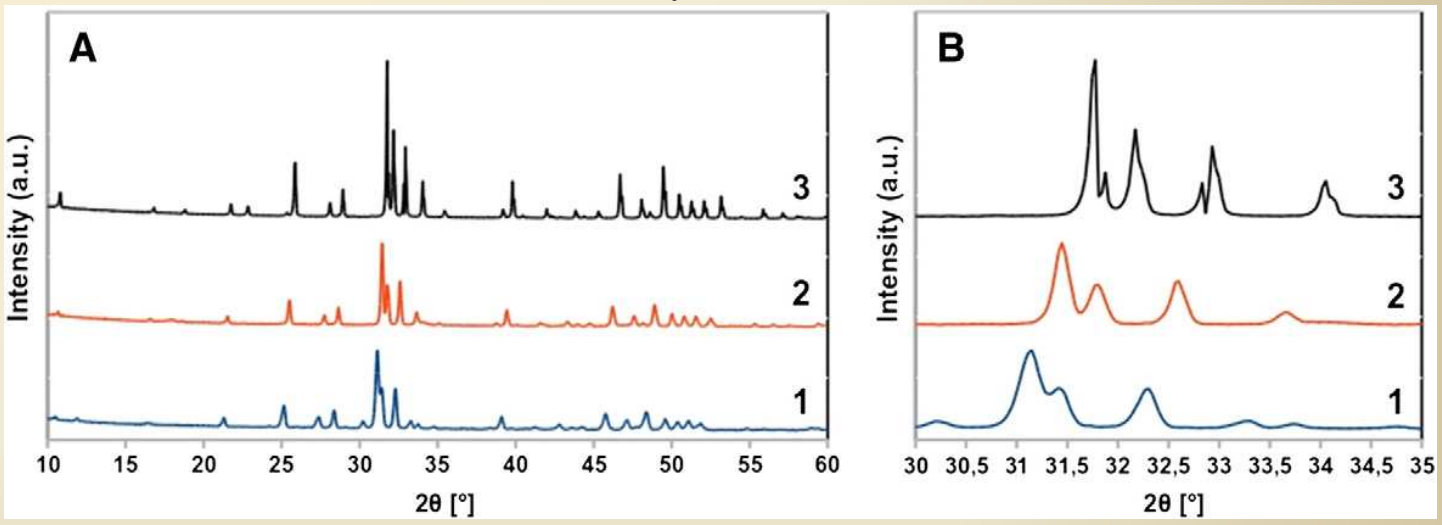
Sr-HA obtained by a solid state method

$Ca_{(10-x)}Sr_x(PO_4)_6(OH)_2$

Number of moles of reactants used and metal/phosphorus molar ratios in the solid-state synthesis of strontium substituted hydroxyapatite.

Desired composition	No. of moles of reactants used			M/P molar ratio (M = Ca + Sr)
	$CaHPO_4$	$CaCO_3$	$SrCO_3$	
$Ca_{10}(PO_4)_6(OH)_2$	0.06	0.04	–	1.67
$Ca_8Sr_2(PO_4)_6(OH)_2$	0.06	0.02	0.02	1.67
$Ca_6Sr_4(PO_4)_6(OH)_2$	0.06	–	0.04	1.67

XRD patterns



1) $Ca_6Sr_4(PO_4)_6(OH)_2$ 2) $Ca_8Sr_2(PO_4)_6(OH)_2$ 3) $Ca_{10}(PO_4)_6(OH)_2$

Sr-HA was formed for x=2

Sr-HA with a small amount of β-TCP for x=4

Data obtained by XRPD analysis.

Nominal composition	(*hkl*)	Intensity [counts]	*d* [Å]	Position [°2θ]	FWHM [°2θ]	*a* = *b* [Å]	*c* [Å]	Crystallite size *d* [Å]	Crystallinity degree (X_c) [% ± 4]
$Ca_{10}(PO_4)_6(OH)_2$	(2 1 1)	25611	2.81	31.84	0.10	9.413(3)	6.879(5)	817 ± 25	93
	(0 0 2)	8888	3.44	25.93					
	(3 0 0)	14533	2.72	32.97					
$Ca_8Sr_2(PO_4)_6(OH)_2$	(2 1 1)	13376	2.84	31.51	0.13	9.486(1)	6.959(2)	629 ± 15	87
	(0 0 2)	3885	3.48	25.57					
	(3 0 0)	7258	2.74	32.67					
$Ca_6Sr_4(PO_4)_6(OH)_2$	(2 1 1)	12594	2.87	31.19	0.23	9.546(4)	7.029(3)	354 ± 10	84
	(0 0 2)	3410	3.53	25.23					
	(3 0 0)	6454	2.77	32.37					

Rietveld Refinement

$$d = \frac{0.9\lambda}{w \cos\theta}$$

Crystallite size
Debye-Scherrer

w = full width at half maximum value (FWHM)
λ = 1.5405 Å
θ = diffraction angle at the (002) hkl reflection

$$X_c \approx 1 - \frac{V_{112/300}}{I_{300}}$$

Crystallinity degree, fraction of crystalline phase, Xc

I_{300} = intensity of (300) hkl reflection
$V_{112}/_{300}$ = intensity of the hollow between (112) hkl and (300) hkl reflections.

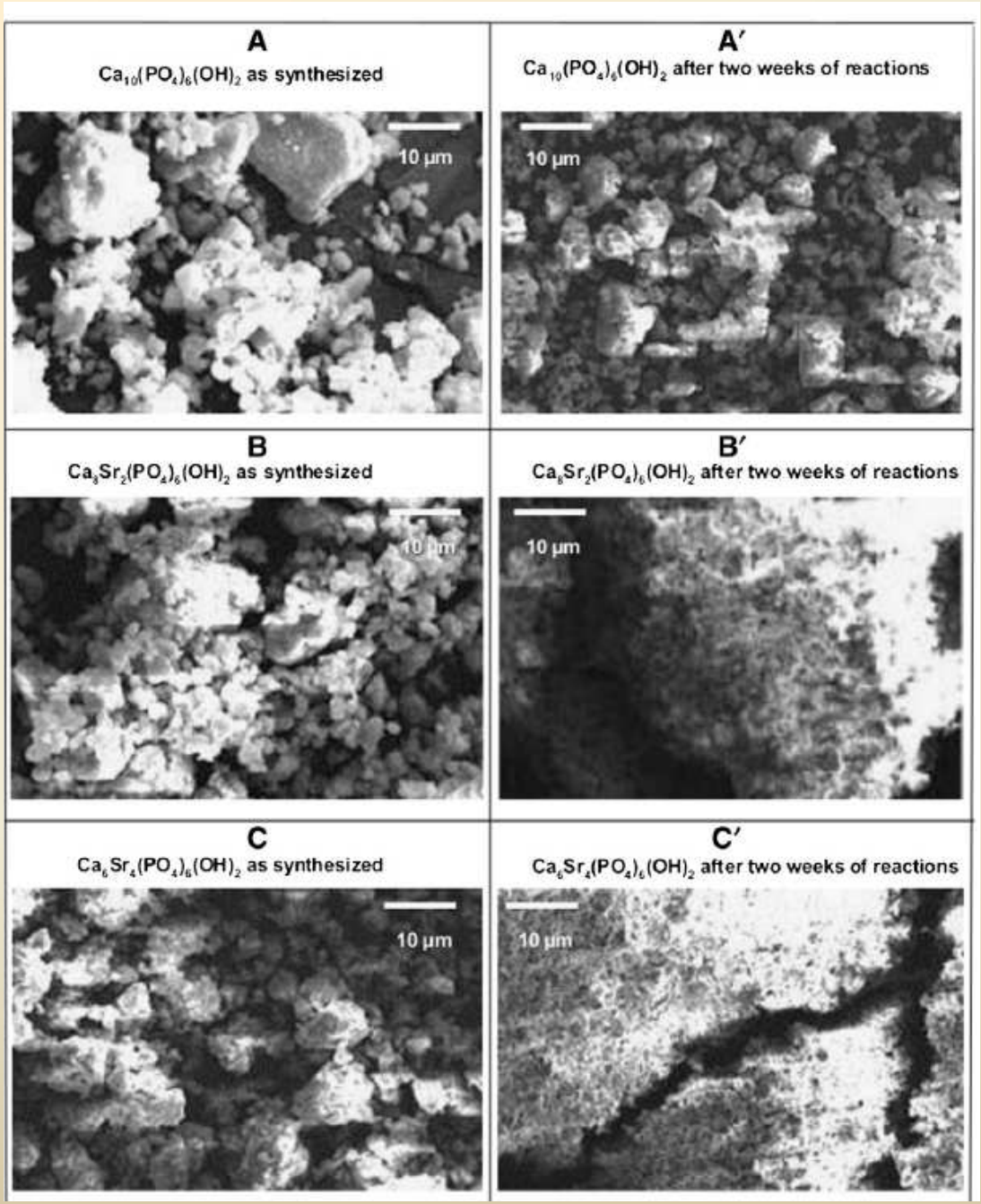
Effect of Strontium on HA structure

- Increase of lattice parameters

Sr^{2+} 118 pm
Ca^{2+} 100 pm

- Decrease in the crystallinity degree, consistent with the overall peak intensity decrease reported and also with Raman results

- Decrease in the specific surface area



Effect of Strontium on the morphology

After bioactivity test

large aggregates (hundreds of µm in size) of particles

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Magnesium- and strontium-co-substituted hydroxyapatite: the effects of doped-ions on the structure and chemico-physical properties

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Magnesium : fundamental element and prevents possible risk factors for osteoporosis in humans

Strontium : improved solubility, antiresorptive activity, osteoclast apoptosis and osteoblast stimulation

Several articles have already been published on the chemico-physical properties of apatites and substituted apatites, *only a few works were devoted* to careful investigation of Mg-HA and *no paper reports* the study of co-substitution of Sr and Mg in HA.

The substituted samples are synthesized by an aqueous precipitation method

Desired composition	Molar concentration of reactants				(Ca + X)/P molar ratio (X = Mg and/or Sr)
	$Ca(OH)_2$	H_3PO_4	$Sr(NO_3)_2$	$MgCl_2{\cdot}6H_2O$	
$Ca_{10}(PO_4)_6(OH)_2$	0.100	0.06	–	–	1.67
$Ca_{9.9}Mg_{0.1}(PO_4)_6(OH)_2$	0.099	0.06	–	0.001	1.67
$Ca_{9.5}Mg_{0.5}(PO_4)_6(OH)_2$	0.095	0.06	–	0.005	1.67
$Ca_9Mg_1(PO_4)_6(OH)_2$	0.090	0.06	–	0.010	1.67
$Ca_9Mg_{0.1}Sr_{0.9}(PO_4)_6(OH)_2$	0.090	0.06	0.009	0.001	1.67
$Ca_9Mg_{0.5}Sr_{0.5}(PO_4)_6(OH)_2$	0.090	0.06	0.005	0.005	1.67
$Ca_8Mg_{0.1}Sr_{1.9}(PO_4)_6(OH)_2$	0.080	0.06	0.019	0.001	1.67
$Ca_8Mg_{0.5}Sr_{1.5}(PO_4)_6(OH)_2$	0.080	0.06	0.015	0.005	1.67
$Ca_8Mg_1Sr_1(PO_4)_6(OH)_2$	0.080	0.06	0.010	0.010	1.67

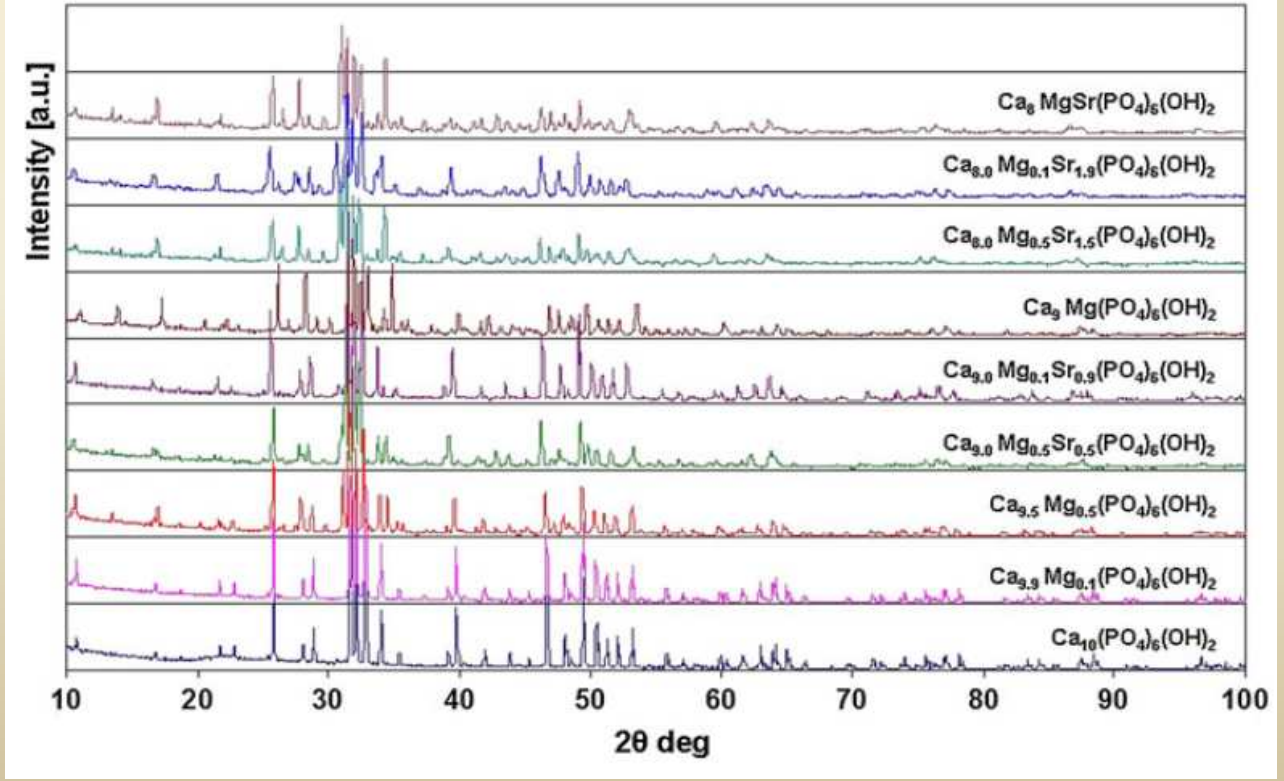

Table 2 Data obtained by XRPD analysis

Samples	Phases[a]	Intensity(counts)	d (Å) (I_{121})	Position (°2θ) (I_{121})	Crystallinity degree of HA *Xc* (% ± 4)	HA/ β-TCP ratio	$a = b$ (Å)[b]	c (Å)[b]
$Ca_{10}(PO_4)_6(OH)_2$	HA	7,806	2.82	31.77	96	–	9.422(3)	6.880(5)
$Ca_{9.9}Mg_{0.1}(PO_4)_6(OH)_2$	HA	6,555	2.82	31.77	94	–	9.421(2)	6.878(5)
$Ca_{9.5}Mg_{0.5}(PO_4)_6(OH)_2$	HA	5,181	2.83	31.63	93	0.74/0.26	9.437(3)	6.870(4)
	β-TCP	1,827	2.87	31.14			10.346(1)	37.162(3)
$Ca_9Mg_1(PO_4)_6(OH)_2$	HA	2,991	2.80	31.98	89	0.48/0.52	9.423(2)	6.877(3)
	β-TCP	3,279	2.84	31.52			10.360(2)	37.174(5)
$Ca_9Mg_{0.1}Sr_{0.9}(PO_4)_6(OH)_2$	HA	7,356	2.84	31.53	91	0.81/0.19	9.501(4)	6.840(5)
	β-TCP	1,682	2.86	30.85			10.377(3)	37.167(3)
$Ca_9Mg_{0.5}Sr_{0.5}(PO_4)_6(OH)_2$	HA	4,785	2.85	31.37	80	0.94/0.06	9.463(5)	6.911(5)
	β-TCP	281	2.88	31.01			10.440(1)	37.635(6)
$Ca_8Mg_{0.1}Sr_{1.9}(PO_4)_6(OH)_2$	HA	4,166	2.84	31.44	80	0.74/0.26	9.493(4)	6.924(4)
	β-TCP	1,427	2.92	30.63			10.495(2)	37.846(5)
$Ca_8Mg_{0.5}Sr_{1.5}(PO_4)_6(OH)_2$	HA	2,958	2.85	31.35	73	0.54/0.46	9.489(2)	6.905(2)
	β-TCP	2,495	2.89	30.94			10.463(1)	37.332(3)
$Ca_8Mg_1Sr_1(PO_4)_6(OH)_2$	HA	2,732	2.85	31.44	68	0.46/0.54	9.510(3)	6.889(4)
	β-TCP	3,176	2.88	31.03			10.409(2)	37.385(3)

[a] The structure model of these phases has been used for the Rietveld refinement

[b] Theoretical values for HA and β-TCP are, respectively: $a = b = 9.424(4)$ (Å), $c = 6.879(4)$ (Å) and $a = b = 10.429(2)$ (Å), $c = 37.380(3)$ (Å)

Rietveld Refinement

Effect of Magnesium on HA structure

- Reduction of peak intensity and crystallinity
- At increasing amounts (0.5, 1 mol), HA and β-TCP
- HA/β-TCP ratio: from ~3 (75/25 %) to ~1 (50/50 %)
- contraction of lattice parameters especially evident in the β-TCP Mg^{2+} (72 pm)

Samples	Phases[a]	Intensity(counts)	d (Å) (I_{121})	Position ($°2\theta$) (I_{121})	Crystallinity degree of HA *Xc* (% ± 4)	HA/ β-TCP ratio	$a = b$ (Å)[b]	c (Å)[b]
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	β-TCP	1,427	2.92	30.63			10.495(2)	37.846(5)
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	β-TCP	2,495	2.89	30.94			10.463(1)	37.332(3)
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	β-TCP	3,176	2.88	31.03			10.409(2)	37.385(3)

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Rietveld Refinement

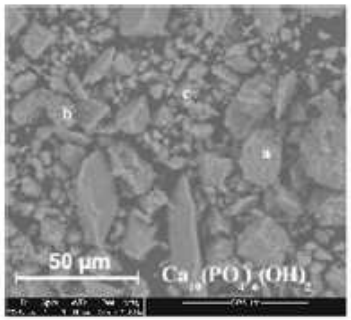
Effect of Magnesium and Strontium on HA structure

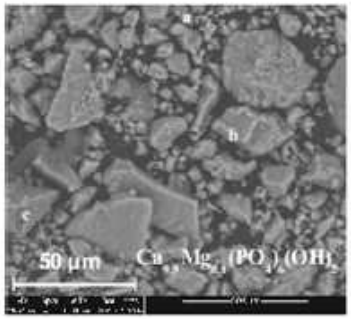
$Ca_9Mg_{0.1}Sr_{0.9}(PO_4)_6(OH)_2$ **$Ca_9Mg_{0.5}Sr_{0.5}(PO_4)_6(OH)_2$**

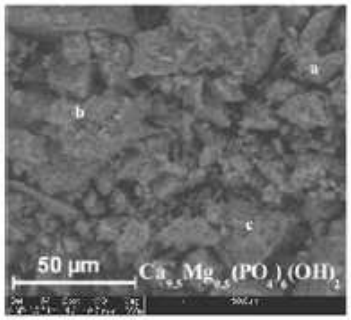
- HA , β-TCP
- decreases the crystallinity degree of the HA phase
- increases HA/β-TCP ratio

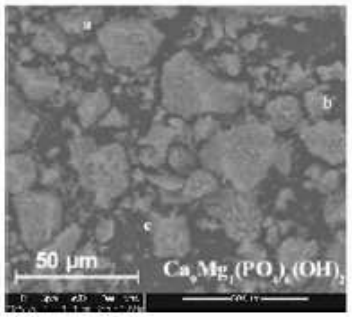
- $Ca_9Mg_{0.1}Sr_{0.9}(PO_4)_6(OH)_2$

-Lattice parameters: increase for the HA (effect of strontium), decrease for the β-TCP (effect of magnesium)



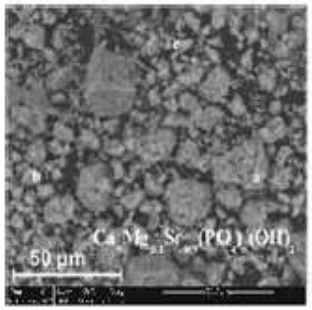


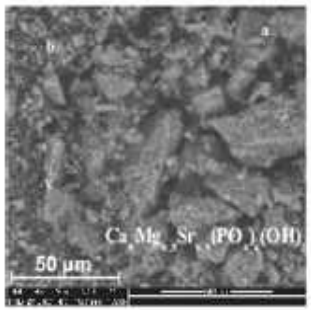


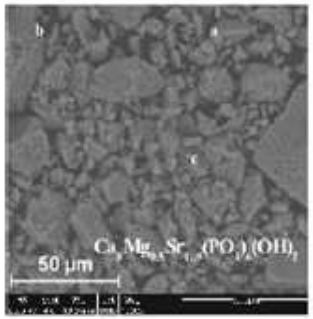


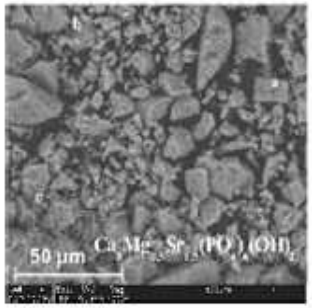
		Ca/P experimental/theoretical	Mg/P
$Ca_{10}(PO_4)_6(OH)_2$			
	a	1.68/1.67	/
	b	1.66/1.67	/
	c	1.70/1.67	/
$Ca_{9.9}Mg_{0.1}(PO_4)_6(OH)_2$			
	a	1.66/1.65	0.02/0.02
	b	1.41/1.65	0.03/0.02
	c	1.84/1.65	0.01/0.02
$Ca_{9.5}Mg_{0.5}(PO_4)_6(OH)_2$			
	a	1.62/1.58	0.09/0.08
	b	1.35/1.58	0.11/0.08
	c	1.77/1.58	0.04/0.08
$Ca_9Mg_1(PO_4)_6(OH)_2$			
	a	1.49/1.50	0.15/0.17
	b	1.26/1.50	0.18/0.17
	c	1.60/1.50	0.07/0.17

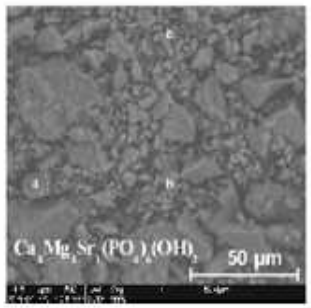
the morphology is quite irregular in term of dimensions (5–40 lm) and shape (generally, rectangular and very sharp)











	Ca/P	Mg/P	Sr/P
	experimental/theoretical		
$Ca_9Mg_{0.1}Sr_{0.9}(PO_4)_6(OH)_2$			
a	1.49/1.50	0.02/0.02	0.14/0.15
b	1.25/1.50	0.02/0.02	0.17/0.15
c	1.56/1.50	0.03/0.02	0.02/0.15
$Ca_9Mg_{0.5}Sr_{0.5}(PO_4)_6(OH)_2$			
a	1.48/1.50	0.04/0.08	0.06/0.08
b	1.32/1.50	0.21/0.08	0.13/0.08
c	1.78/1.50	0.06/0.08	0.16/0.08
$Ca_8Mg_{0.1}Sr_{1.9}(PO_4)_6(OH)_2$			
a	1.29/1.33	0.01/0.02	0.27/0.32
b	1.21/1.33	0.02/0.02	0.24/0.32
c	1.60/1.33	0.01/0.02	0.30/0.32
$Ca_8Mg_{0.5}Sr_{1.5}(PO_4)_6(OH)_2$			
a	1.31/1.33	0.09/0.08	0.22/0.25
b	1.50/1.33	0.07/0.08	0.21/0.25
c	1.05/1.33	0.05/0.08	0.19/0.25
$Ca_8Mg_1Sr_1(PO_4)_6(OH)_2$			
a	1.34/1.33	0.17/0.17	0.16/0.17
b	1.29/1.33	0.34/0.17	0.15/0.17
c	1.30/1.33	0.12/0.17	0.15/0.17

non homogeneous distribution of the elements that is consistent with the presence of two phases

The combined use of XRPD, FTIR and Raman spectroscopies provides complementary data and indicates clearly phase formation and transformation as well as related structural changes resulting from the ions substituted in the HA structure

Magnesium and strontium, interact with both HA and β-TCP in terms of variation of lattice parameters and the degree of crystallinity of HA

A careful evaluation of the different molar ratios of the ions allows to obtain biphasic materials (BCPs) with selected ratios of HA and β-TCP, with also a controlled crystallinity degrees of HA

BCPs are favoured for clinical applications because their resorption rate can be tuned to match the bone healing rate allowing to obtain a suitable balance between implant degradation and bone regeneration

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Synthesis and Characterisation of Strontium and Magnesium Co-Substituted Biphasic Calcium Phosphates

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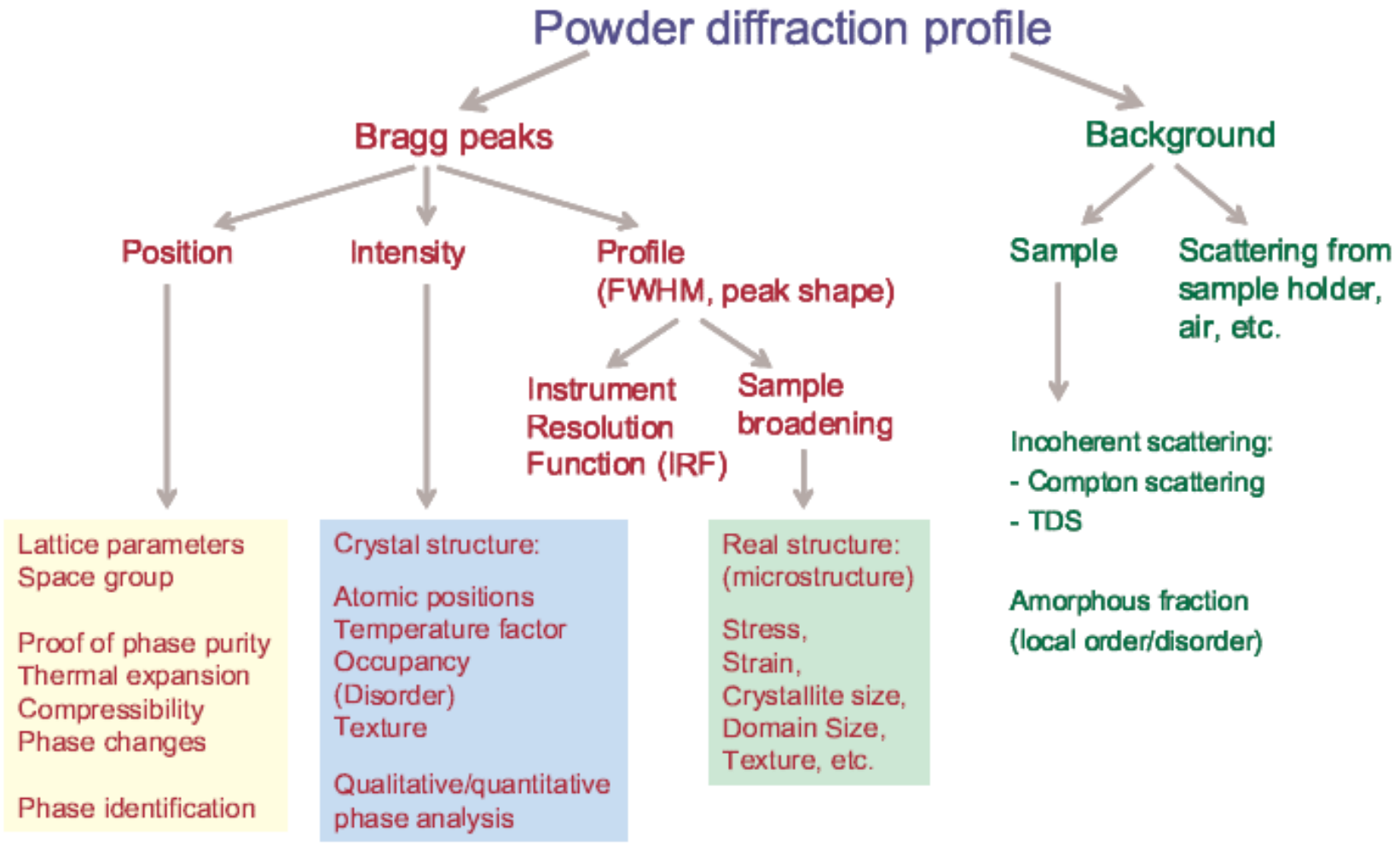
Information in a powder diffraction pattern



Rietveld Method

•Based on Whole Powder Profile Fitting (WPPF):
all categories of observables (d_{hkl} , I_{hkl} , background.....)
are considered together to build a model which is used to generate a full powder diffraction pattern

•Calculated pattern is compared to the observed pattern and modified by least squares in order to minimize the differences between observed and calculated

•Function minimized by least square is

$$S_y = \sum_i w_i (y_{oi} - y_{ci})^2$$

$w_i = 1/y_i$
y_i =observed intensity
y_{ci}= calculated intensity

•**Least squares refinements are carried our until the best fit is obtained** between the entire observed powder diffraction pattern taken as a whole and the entire calculated pattern

$$y_{ci} = s \sum L_k |F_k|^2 \varphi (2\theta_i - \theta_k) P_k A + y_{bi}$$

y_{ci}	calculated intensity at the i^{th} step
s	scale factor
k	the Miller indices, *h*, *k*, *l*, for a Bragg reflection
L_k	multiplicity, Lorentz and polarization factor
F_k	structure factor for the k^{th} Bragg reflection
φ	reflection profile function
P_k	Preferred orientation
A	absorption factor
y_{bi}	background intensity at the i^{th} step

Rietveld refinement fits the whole pattern and refines

- atomic positions
- lattice parameters
- profile parameters
- background parameters
- instrumental parameters

Reasons to perform a Rietveld refinement

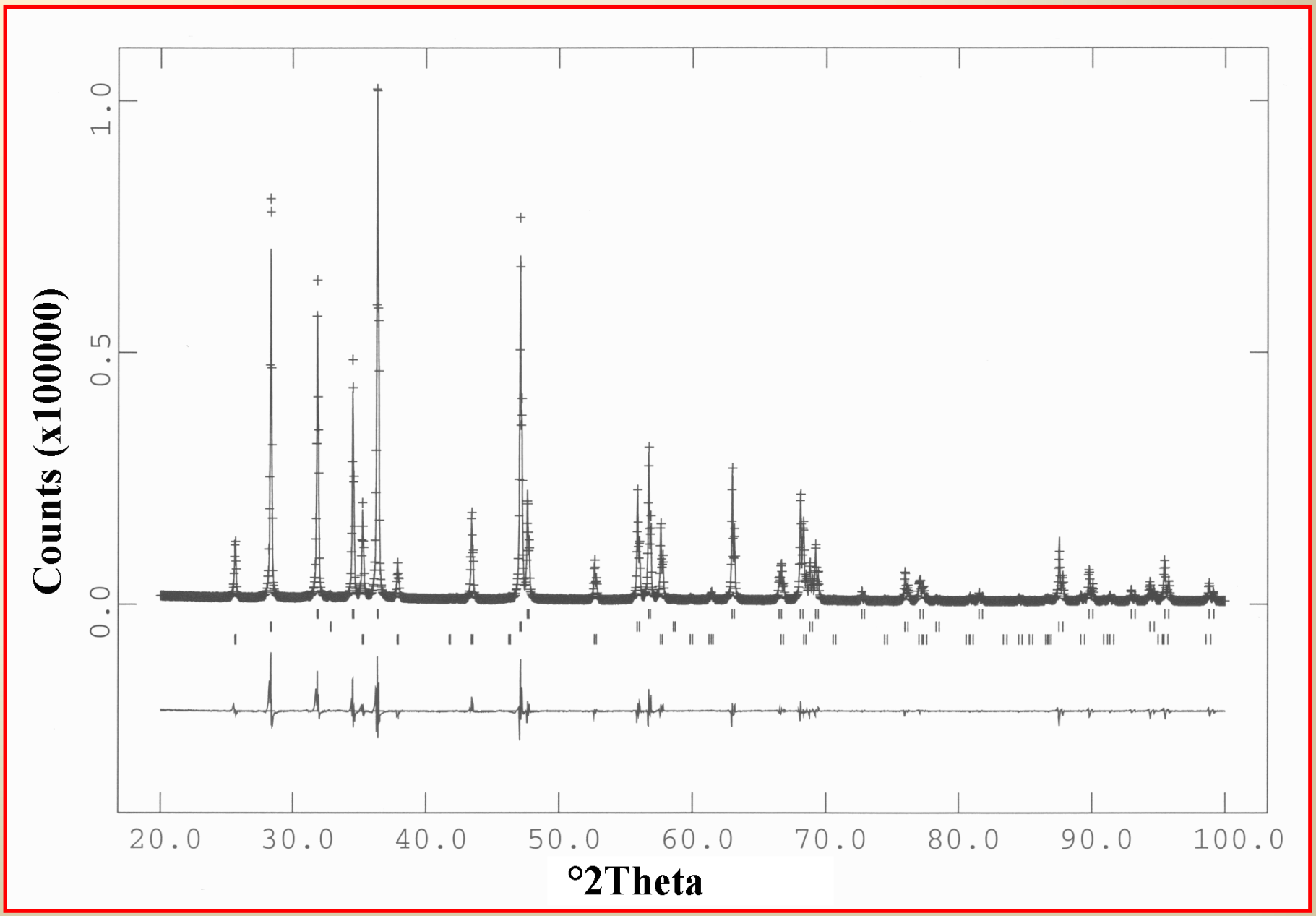
- solving an unknown crystal structure

- quantitative determination of the percentages of different phases and quantification of amorphous content (QPA)

Rietveld Method in Practice

Basic requirements

1. Accurate diffraction data
2. A reasonable starting structural model
   a. Space group symmetry
   b. Approximate atomic positions
   c. model may be from: isostructural materials, theoretical simulations, high-resolution atomic imaging
3. A Rietveld refinement program
   a. GSAS (Larson and von Dreele)
   b. Fullprof (Rodriguez – Carvajal)
   c. Others: BGMN (Bergmann), DBW (Wiles and Young), LHPM-Rietica (Hunter), MAUD (Lutterotti), Rietan (Izumi) Simref (Ritter)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30.0
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60.0
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90.0
100.0
°2Theta

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Thank You!!