Different microscopic characterization techniques on hydroxyapatite powder

A. A. POINESCU[*], R. M. ION[a,b], R.-I. VAN STADEN[c], J. F. VAN STADEN[c], M. GHIUREA[b]
Valahia University of Targoviste, 2 Regele Carol 1 Street
[a]Valahia University of Targoviste, 2 Regele Carol 1 Street
[b] ICECHIM, Bucharest, Analytical Department, 202 Splaiul Independentei, Bucharest-060021, Romania
[c]National Institute of R&D for Electrochemistry and Condensed Matter, Electrochemistry Laboratory, Timisoara, 202, Splaiul Independentei, Bucharest-060021, Romania

---

The hydroxyapatite (HA) is one of the most used materials in medical applications, due to its similarity with human bones. A chemical synthesis method, and the characterization by atomic force microscopy (AFM) and scanning electron microscopy (SEM), has been chosen in this paper. The surface study on nm scale and the surface topography were evaluated by using AFM with tapping mode. The size distribution of the grown spherulite was carried out by AFM, operating in non-contact mode. The SEM pictures of the grown spherulite crystals shows many spherical agglomerations and few crystallites of 0.1 μm in size with pores.

(Received January 31, 2011; accepted April 11, 2011)

Keywords: Hydroxyapatite, Wet precipitation, AFM, SEM, FTIR

---

1. Introduction

Numerous biocompatible materials with a similar composition to bone tissue do contribute to creating conditions for the repair of bone structure functions. Various bioceramic materials like: hydroxyapatite (HA), calcium phosphate, ceramics of these phosphates, bioglasses, biopolymers and composites which are used in orthopedics, neurosurgery, and dentistry.

One of the most attractive materials for bone implant is hydroxyapatite [HAp, $Ca_{10}(PO_4)_6(OH)_2$] due to its compositional and biological similarity to host materials. It has unique biocompatibility feature among phosphate groups. However, due to lower strength of pure HAp, it has been difficult to use it as bone implant for a long time.

Mechanical strength of this material mainly depends on grain size, grain size distribution, porosity, and other microstructure defects. Bioactivity of the synthetic hydroxyapatite has been found to be strongly affected by structural crystallinity material.

Due to the chemical similarity between HA and mineralized bone of human tissue, synthetic HA exhibits strong affinity to host hard tissues.

Formation of chemical bond with the host tissue offers HA a greater advantage in clinical applications than most other bone substitutes, such as allografts or metallic implants (Itokazu et al 1998; Minguez et al 1990).

In the literature are many approaches to synthesize HA using several methods including wet-chemical method in aqueous solutions, sol–gel method, microwave irradiation, freeze drying, mechanochemical method, emulsion processing, spray pyrolysis, hydrolysis of a precursor.

Materials with various morphology, stoichiometry, and level of crystallinity have been obtained depending on the technique.

Modifications of these “classical” methods (precipitation, hydrolysis or precipitation in the presence of urea) or alternative techniques have been employed to prepare hydroxyapatite with morphology, stoichiometry, ion substitution or degree of crystallinity as required for a specific application.

Other techniques to prepare hydroxyapatite, the most important being: the aqueous precipitation and conversion from other calcium compounds.

Aqueous precipitation is most often performed by:

- the reaction between a calcium salt and a alkaline phosphate;

or

- the reaction between calcium hydroxide or calcium carbonate and phosphoric acid. [13].

2. Experimental

2.1. Materials

In the Fig. 1 are shown all the steps of the wet-chemical process to obtain HA powder.

Chemical reagents used in this work: $Ca(NO_3)_2$.4H2O and $(NH_4)_2HPO_4$ was dissolved in deionized water separately. The drop wise addition of $Ca(NO_3)_2$ aqueous solution to vigorously stirred $(NH_4)_2HPO_4$ solution at room temperature for about 1h produced a milky and somewhat gelatinous precipitate which was then stirred for 1h.

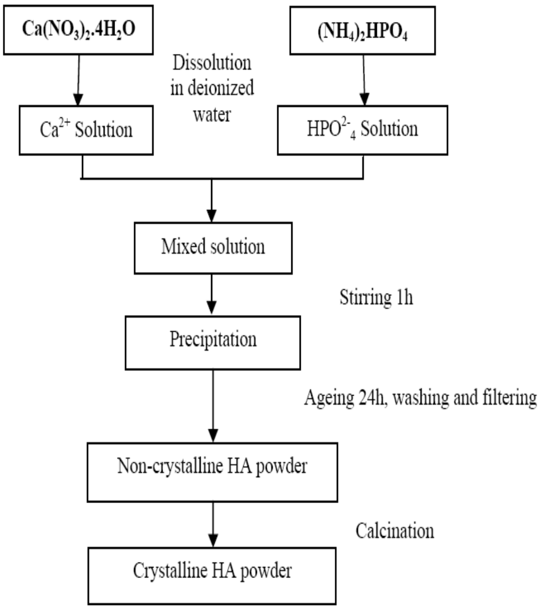

Fig. 1. Modified chemical precipitation route for HA powder preparation. (Adapted from Sung) [9]

The mixture was sintering at 100^{0}C for 24h. It was then washed and filtered in a filter glass.

After filtration the compact, sticky filter cake was dried at 80^{0}C in laboratory oven. Dried powders was crushed by using mortar and pestle and calcined in alumina crucible at 1000^{0}C for 1h [4].

Table 1.

Method	Sample	Temperature (^{O}C)	Concentration (M)
Reflux after mixing	HA1	800	0.1:0.06
Reflux after mixing	HA2	1000	0.1:0.06
Reflux after mixing	HA3	1200	0.1:0.06

2.2 Apparatus

Atomic force microscopy (AFM) investigations were carried out with an Agilent 5500 SPM system, described by PicoSPM controlled by a MAC Mode module and interfaced with a PicoScan controller from Agilent Technologies, Tempe, AZ, USA (formally Molecular Imaging). A multipurpose large scanner and Point Probe Plus Silicon SPM Sensor cantilevers (PPP-FM cantilevers), n$^+$- silicon material and none coating, of about 227 µm length, 1.8 N m^{-1} spring constants, with the tips oscillated near their resonant frequencies (NANOSENSORS) in air, of about 64 kHz were used for all measurements.

All AFM measurements (256 samples/line × 256 lines) were done by scanning the surface at a rate of 0.8-1.2 lines per second and were done at room temperature in tapping mode. The original images for the samples, the 3D topographical images and section analysis over the magnetite particles were performed using the PicoView SPM Software, version 1.6.2, Molecular Imaging.

Height image data obtained by the AFM is three-dimensional. The usual method for displaying the data is by using a color mapping for height, for example black for low features and white for high features. The images were processed by first order flattening in order to remove the background slope and the contrast and brightness were adjusted

Acoustic AC Mode (AAC mode, tapping mode) is an oscillating technique that is less sensitive than MAC Mode, but gentler and less destructive than contact mode. AAC mode excites the cantilever by vibrating the piezo where the cantilever holder is attached. The AAC mode option includes an AAC mode controller and an AAC mode scanner module.

The piezo causes the probe tip to oscillate, typically at or near one of its resonance frequencies, such that it taps gently on the surface. The tip is then raster-scanned over the region of interest while the amplitude of oscillation is monitored to produce images. Through this method, lateral forces on the tip are virtually eliminated, enabling higher resolution imaging than is possible with Contact Mode.

For AFM, the solutions of HA were freshly prepared before each experiment by suspending of the appropriate quantity of each sample in absolute ethanol purchased from Sigma-Aldrich. The AFM results in tapping mode showed the best resolution.

The scanning motion is conducted by a piezoelectric scanner which scans the tip in a raster pattern with respect to the sample (or scans the sample with respect to the tip). The tip-sample interaction is monitored by reflecting a laser beam off the back of the cantilever into a split photodiode detector. Tapping Mode AFM consists of oscillating the cantilever at its resonance frequency (typically ~300 kHz) and lightly "tapping" the tip on the surface during scanning. The advantage of Tapping Mode over contact mode is that it eliminates the lateral, shear forces present in contact mode

SEM analysis has been achieved with Quanta 200 Scanning Electron Microscope (SEM), which produces enlarged images of a variety of specimens, achieving magnifications of over 100000x providing high resolution imaging in a digital format. This important and widely used analytical tool provides exceptional depth of field, minimal specimen preparation, and the ability to combine the technique with X-ray microanalysis.

The Quanta 200 has 3 operating vacuum modes to deal with different types of sample:

- High Vacuum - the conventional operating mode associated with all scanning electron microscopes (pressure <1.3 Pa)
- Low Vacuum - when using uncoated, non-

conductive samples (pressure 10-130 Pa) - ESEM - when using moist samples dynamic experiments, hot/out gassing or dirty samples (pressure 130-2600 Pa) Accessories: the Peltier cold stage - allows wetting/freezing of the sample using the ESEM mode pressure range. Temperature range: -20 ÷ 80 °C

The operation of the SEM consists of applying a voltage between a conductive sample and filament, resulting in electron emission from the filament to the sample, in a vacuum environment. The electrons are guided to the sample by a series of electromagnetic lenses in the electron column.

Microstructure observations were performed with an environmental scanning electron microscope and equipped with analyzer and software for quantitative elemental analysis. For SEM analysis the powders were embedded in LR White resin. The surface of the resin blocks was ground to expose the powder; the blocks were then mounted on an aluminum stub and coated with carbon.

3. Results and discussion

There are a number of different ways to compare and contrast these two techniques. A comparison of SEM and AFM techniques will be conducted with respect to 3 factors: surface structure, composition and environment [10].

Although both SEM and AFM are similar in lateral resolution, there are situations in which one technique can provide a more complete representation of the sample surface, depending on the information desired.

One principle difference is in how the two techniques process vertical changes in topography.

Although the SEM measurement is destructive to the sample, the ability to image the undercuts of these lines is a useful application that AFMs are not typically designed to perform with the exception of the Dimension X3D Automated AFM.

One of the key advantages of the SEM with respect to other types of microscopy is its large depth of field. This ability makes it possible to image very rough surfaces with millimeters of vertical information within a single image.

Although the AFM can measure vertical surface variations below 0.5Å, its ability to measure a tall structure comes from how far the scanner can move vertically. Standard scanners typically have 5 to 6µm of vertical range, however, in some configurations the vertical range approaches 10µm or larger. For scanning areas that have heights of greater than 5 to 10µm's of variation, the SEM would be better suited for the analysis.

SEM is the only one of the two techniques which provides elemental analysis, however, both SEM and AFM are associated with techniques which can provide compositional information through analyzing materials and physical properties of the sample.

One of the primary differences between these two types of microscopy is the environment, in which the measurements are performed, SEM is only conducted in vacuum conditions. In addition to vacuum, AFM is conducted in an ambient, gas or liquid environment and in ethanol in our case.

There are several issues which make environment an important issue. First, there is a frequent need in fields such as biology and biomaterials to study techniques compensate for this need by different means: an environmental chamber for a SEM, and a fluid cell for the AFM. Second, the SEM is required to work in a vacuum environment due to the nature of the technique which brings up the issues of vacuum compatibility of the sample, the conductivity of the surface.

One of the primary attractions to the AFM is its ability to image insulating surfaces at high resolution in liquid.

Since AFM does not rely on conductivity, the image and scanning mechanism are not disturbed by the presence of the liquid. Common applications for AFM investigations in liquid are in the biological sciences, biomaterials, crystal growth, force interaction studies, and for investigating processes in-situ.

In the SEM images of HA, was depicted small crystals (<100 nm) in the agglomerated particles and the uniform grain size with a narrow size distribution corresponding to an improved crystallinity of HA powders, especially for that sample after calcination at 1000°C for 1h.

For a good correlation, the SEM pictures of the grown spherulite crystals shows many spherical agglomerations and few crystallites of 0.1 µm in size.

The size of subgrains was approximately 70 nm corresponding to the size of the synthetic HA powder [2].

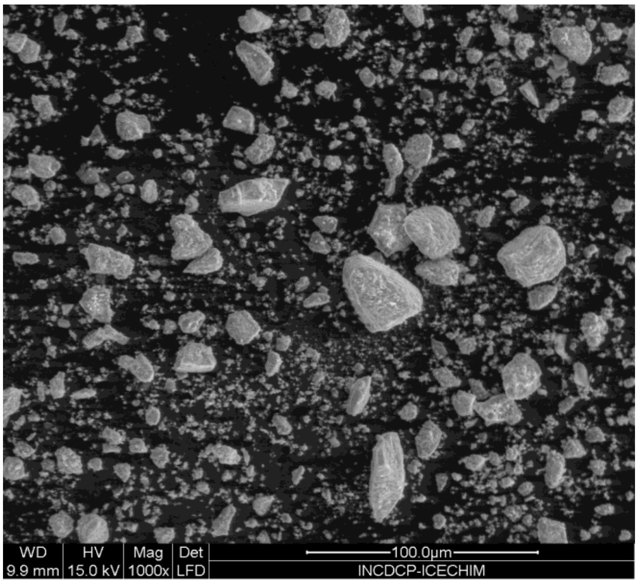

Fig. 2. SEM images of HA2 calcined at 1000^{0}C for 1h, Size magnification: 1000x

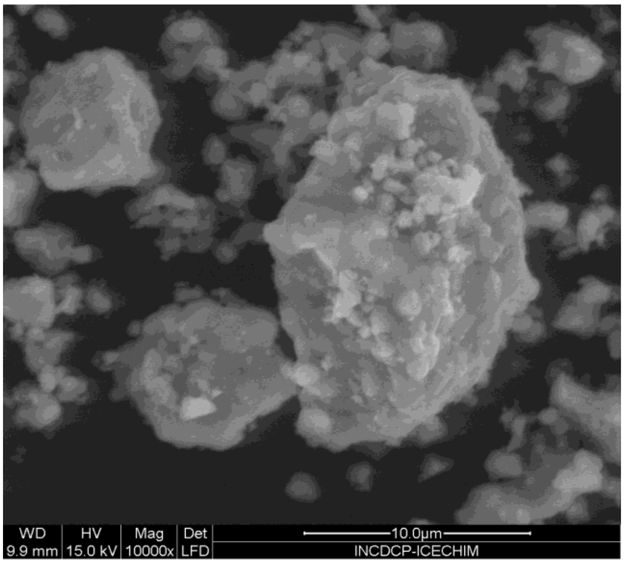

Fig. 5. SEM images of HA2 calcined at 1000^{0}C for 1h, Size magnification: 10000x

The determination of size distribution of the grown spherulite and sintered HA materials were carried out by atomic force microscopy.

Unlike SEM, the AFM can measure in all three dimensions (x, y, and z) with a single scan. A 3-D surface topography recorded on a 0.5-0.5 mm^2 surface area.

AFM revealed a rough surface architecture for HA, the predominant size of grains being in the range of 90-100nm.

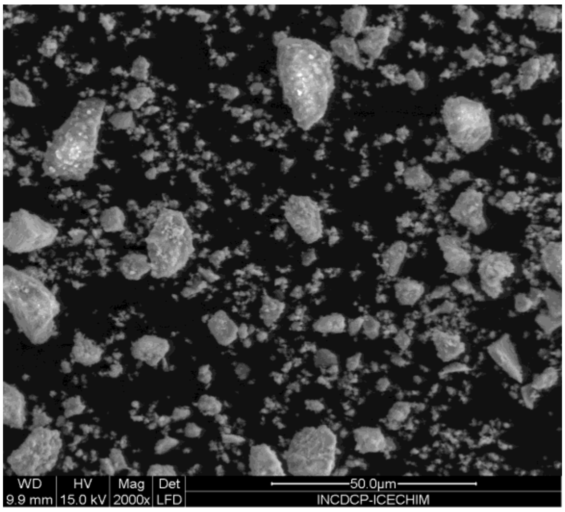

Fig. 3. SEM images of HA2 calcined at 1000^{0}C for 1h, Size magnification: 2000x.

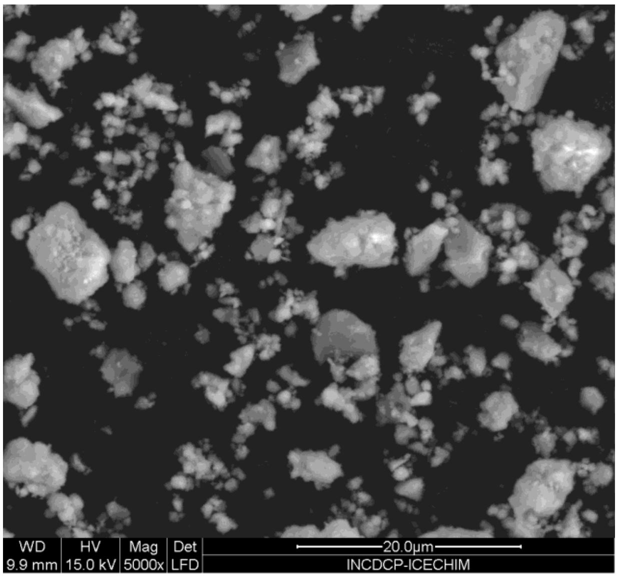

Fig. 4. SEM images of HA2 calcined at 1000^{0}C for 1h, Size magnification: 5000x

At higher temperature the deagglomeration of bulk phases and agglomeration of nano phases leads to the nano crystalline HA in this present study.

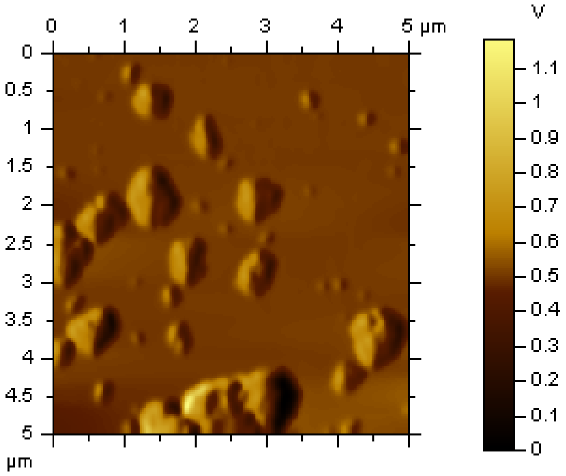

Fig. 6. Atomic Force Micrograph on sintered HA powder at 1000^{0}C

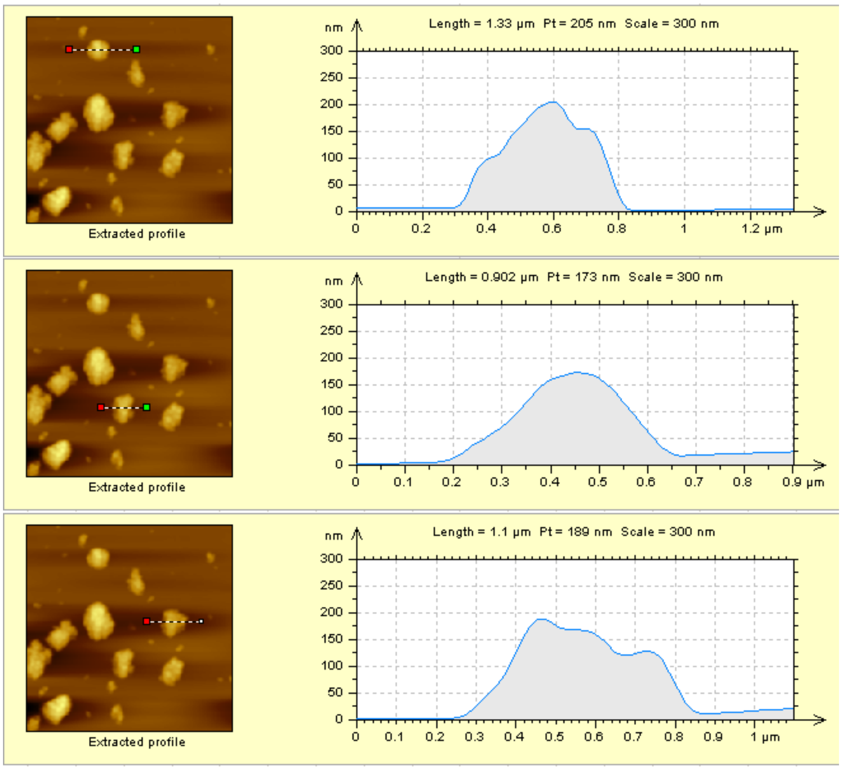

Fig. 7. AFM analyse on hydroxyapatite.

Table 2. Image information.

Grain	1	2	3
Length[µm]	1,33	0,903	1,1
Pt[nm]	205	173	189
Scale[nm]	300	300	300

Fig. 7 is given information of the AFM image and show three different lengths of the grains taken into analysis. The higher surface roughness values for the 205nm scans, compared with the 173nm scans, were attributed to the presence of the wide gap formed by the dissolution between grains.

The subgrain boundaries mostly rounded, appear to have evolved along the z axis resulting in the formation of crystallites aligned in a certain direction.

4. Conclusion

Hydrothermal techniques give hydroxyapatite powders with a high degree of crystallinity and better stoichiometry having a wide distribution of crystal sizes. Nanometer sized crystals can be obtained at temperatures lower than 100 °C with precipitation techniques.

The chosen methods was the wet precipitation because the wet-chemical precipitation route is the most talented route owing to its ease in experimental operations, low working temperature, high percentages of pure products and inexpensive equipment requirement.

In the SEM images of HA, was depicted small crystals (<100 nm) in the agglomerated particles and the uniform grain size with a narrow size distribution corresponding to an improved crystallinity of HA powders, especially for that sample after calcination at 1000°C for 1h. For a good correlation, the SEM pictures of the grown spherulite crystals shows many spherical agglomerations and few crystallites of 0.1 µm in size. The size of subgrains was approximately 70 nm corresponding to the size of the synthetic HA powder [2].

The determination of size distribution of the grown spherulite and sintered HA materials were carried out by atomic force microscopy. Unlike SEM, the AFM can measure in all three dimensions (x, y, and z) with a single scan. By AFM, 3-D surface topography has been recorded on a 0.5-0.5 mm^2 surface area.

AFM revealed a rough surface architecture for HA, the predominant size of grains being in the range of 90 - 100nm. At higher temperature the deagglomeration of bulk phases and agglomeration of nano phases leads to the nano crystalline HA in this present study.

The crystal size distribution attained depends on the size of the critical nucleus under the supersaturation condition, rather than on standard crystal growth, since the aggregation of very small particles was observed.

References

[1] A. Ruban Kumar, S. Kalainathan, Cryst. Res. Technol. **43**(6), 640 (2008).

[2] Dong Seok Seo, Jong Kook Lee, AFM analysis of anisotropic dissolution in dense hydroxyapatite, Ultramicroscopy **108**, 1157 (2008).

[3] N. Monmaturapoj, J of Metals, Materials and Minerals, **18**(1), 15 (2008)

[4] Aurora Anca Poinescu, Rodica Mariana Ion, Inna Trandafir, Elena Bacalum, Constantin Radovici, Obtaining and characterization of a calcium hydroxyapatite, The XV-th International Scientific Conference “Tehnomus”, May 8-9, (2009), Suceava Romania;

[5] Maria Helena Santos, Marise de Oliveira, Luciana Palhares de Freitas Souza, Herman Sander Mansur, Wander Luiz Gerais, Materials Research, **7**(4), 625, (2004);

[6] Dong Seok Seo, Jong Kook Lee, Ultramicroscopi **108** 1157 (2008).

[7] T. Anee Kuriakose, S. Narayana Kalkura, M. Palanichamy, D. Arivuoli,Karsten Dierks, G. Bocelli, C. Betzel, Journal of Crystal Growth **263,** 517 (2004).

[8] A. Lopez-Macipe, R. Rodriguez-Clemente, A. Hidalgo-Lopez, I. Arita, M. V. Garcia-Garduno, E. Rivera, and V. M. Castano, Journal of Materials Synthesis and Processing, Vol. 6, No. I. (1998).

[9] Naruporn Monmaturapoj, Journal of Metals, Materials and Minerals, **18**(1), 15 (2008).

[10] Phil Russell, Dale Batchelor College of Engineering North Carolina State University, John T. Thornton Veeco Instruments Inc., Chadds Ford, PA, SEM and AFM: Complementary Techniques for High Resolution Surface Investigations, www.veeco.com;

[11] Thin Calcium Phosphate Coatings for Medical Implants, Betty Le ´on l John A. Jansen, ISBN: 978-0-387-77718-4;

[12] N. A. Zakharov, I. A. Polunina, K. E. Polunin, N. M. Rakitina, E. I. Kochetkova, N. P. Sokolova, V. T. Kalinnikov, Calcium Hydroxyapatite for Medical Applications, Inorganic Materials, **40**(6), 641 (2004).

[13] B. D. Ratner, A. S. Hoffman, F. J. Schoen, J. E. Lemons, Biomaterial Science – An introduction to materials in medicine, Elsevier Academic Press ISBN-13:978-0-12-582463-7.

---

[*] Corresponding author: poinescua@yahoo.com