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SORIN-CRISTIAN DOCA

PhD THESIS

NEW BIOACTIVE COMPOUNDS USED IN THE OSTEOGENESIS PROCESS

ABSTRACT

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KEY WORDS

Osteogensis, dentalimplants, biomimetic hydroxyapatite, bisphosphonates, biomimetic and bioactive composite

AIMS AND OBJECTIVES

THE AIM of the thesis is to study the conditions for obtaining a composite material adequate for prothetics and bone regeneration,with immediate applications in orthopaedics and dentistry. The composite material must satisfy the following conditions:

- to rely on hydroxyapatite and bisphosphonate;
- the synthetised hydroxyapatite (HA) must have a looser structure,similar to the one in human bones
- to have on the HA surface,either bound or absorbed,a quantity of BP which can stimulate at a local level the activity of the osteoblasts and inhibit the activity of the osteoclasts.

In order to obtain the desired mezostructure,we chose the HA synthesis in biomimetic fluid environments (with colagenic tissue). Through the template effect,the reaction medium matrix will determine the making of HA crystallites with an anisotrope orientation,resembling the natural orientation on the collagen matrix.

For the bisphosphonic component,we chose the sodium alendronate (ALE). By depositing on HA,we estimate a local biological action on the osteogenesis/osteoresorbtion balance without any significant spreading of the ALE throughout the entire body,which can create unpleasant side effects (see chapter 2)

ALE was also studied under the aspect of stability and compatibility with the usual excipients of solid forms. For comparison,we have studied the same aspects for sodium risendronate (Rise),to observe the heterocycle effect.

THE OBJECTIVES of the thesis are:

- obtaining and characterisation of some hydroxiapatites with high biomimetism in respect of the colagenic matrix;
- searching for a rapid method to characterise and identify biogenetic HA
- the study of ALE stability and its interactions with the excipients used in solid forms.
- obtaining and characterisation of highly biomimetic HA composites with HA bound on the surface
- comparison of the thermal behaviour of ALE and Rise

The stages of reaching these objectives are:

- Synthesis of HA in biomimetic fluids
- Characterising the HA
- Study of the thermal stability of ALE
- Study of the ALE interactions with the excipients used in solid forms
- Obtaining the composites HA/ALE and characterising them
- Study of the thermal behaviour of Rise in comparison to ALE.

These objectives can be reached in different stages,presented in their logical order in Table 3.1 together with the activities associated with each stage.

Table 3.1. Stages and activities of the objectives

Objective (Name of objective)	**Associated activities**
1.Synthesis of HA in biomimetic fluids	1.1preparing of biomimetic fluids
	1.2precipitation of HA precursors in ultrasonic field
	1.3.study of the thermal treatment of the precursors
2.Describing of HA	2.1.crystalographic analysis through RX
	2.2.IR analysis
	2.3.texture analysis through SEM, EDAX 2.4.describing the mezostructure through adsorbtion 2.5.publishing the results
3. The study of thermal stability of ALE and Rise	3.1.thermical analysis in non-isothermal regime 3.2.kynetic analysis of thermooxidation 3.3.publishing the results
4. The study of ALE and rise interactions with excipients used in solid formulas	4.1.gathering the thermoanalytical data for the excipients 4.2.thermal analysis of the binary mixtures BP/excipients. 4.3.thermical anlysis of FOSAMAX 4.4.publishing the conclusions
5. Obtaining the HA/ALE composites and describing them	5.1.depositing of ALE from aquaeos solutions in a microwave field 5.2.checking the deposit

Arborele de decizie corespunzător este prezentat în Fig. 3.1.

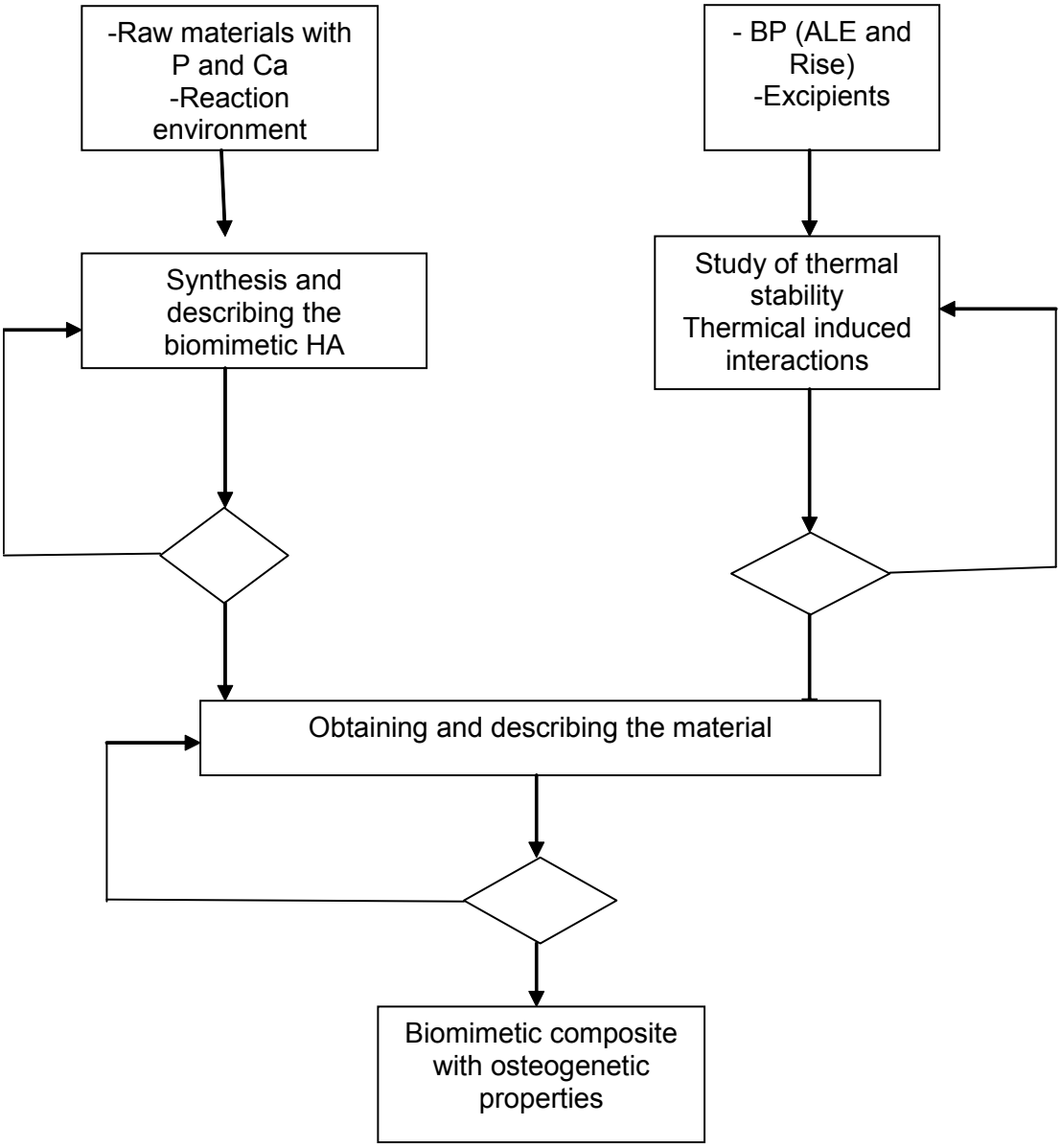


Figure 3.1. Decision plan.

The innovation in the thesis is exactly the idea of binding BP on HA synthetised in a biomimetic matrix.

The complexity of the objectives comes from the following:

- the use of unconventional technologies for obtaining HA (ultrasonic field) and depositing the BP (microwave field),technologies which require dedicated devices and specific expertise.
- describing of HA,ALE,Rise and the materials obtained through complex instrumental techniques (RX,EDAX,FTIR)

- the extended use of thermal analytics in nonisotherm regime,detecting emitted gases and kynetic analysis,methods which are applied in the field of the substances with biological action and highly promoted by the Timisoara school (UVT and UMF). The technique and theoretical base are complex and require special expertise.

The aim of the thesis is redefined by the three objectives,presented in their logical order: first the synthesis and describing the HA samples,after that and not at the same time the study of ALE and Rise stability as pure substances as well as mixtures with different excipients,finally obtaining and describing the composite samples.

Setting as a special objective the study of ALE and Rise stability and that of the excipients was necessary in order to obtain the data needed to describe the composite,data describing the farmacology of the product,but also to fill in a gap in literature.

RESULTS AND DISCUSSIONS

5.1.

In the articles presented in the thesis,we have tried the synthesis of HA in a polymer matrix,using the template effect. The idea was that,promoting conformations which can favour the recognition and fixing of proteins from the biological fluid from the crystallization phase,it can increase the biomimetic degree of the inorganic compound.

The synthesis reaction is given by the equation 4.1

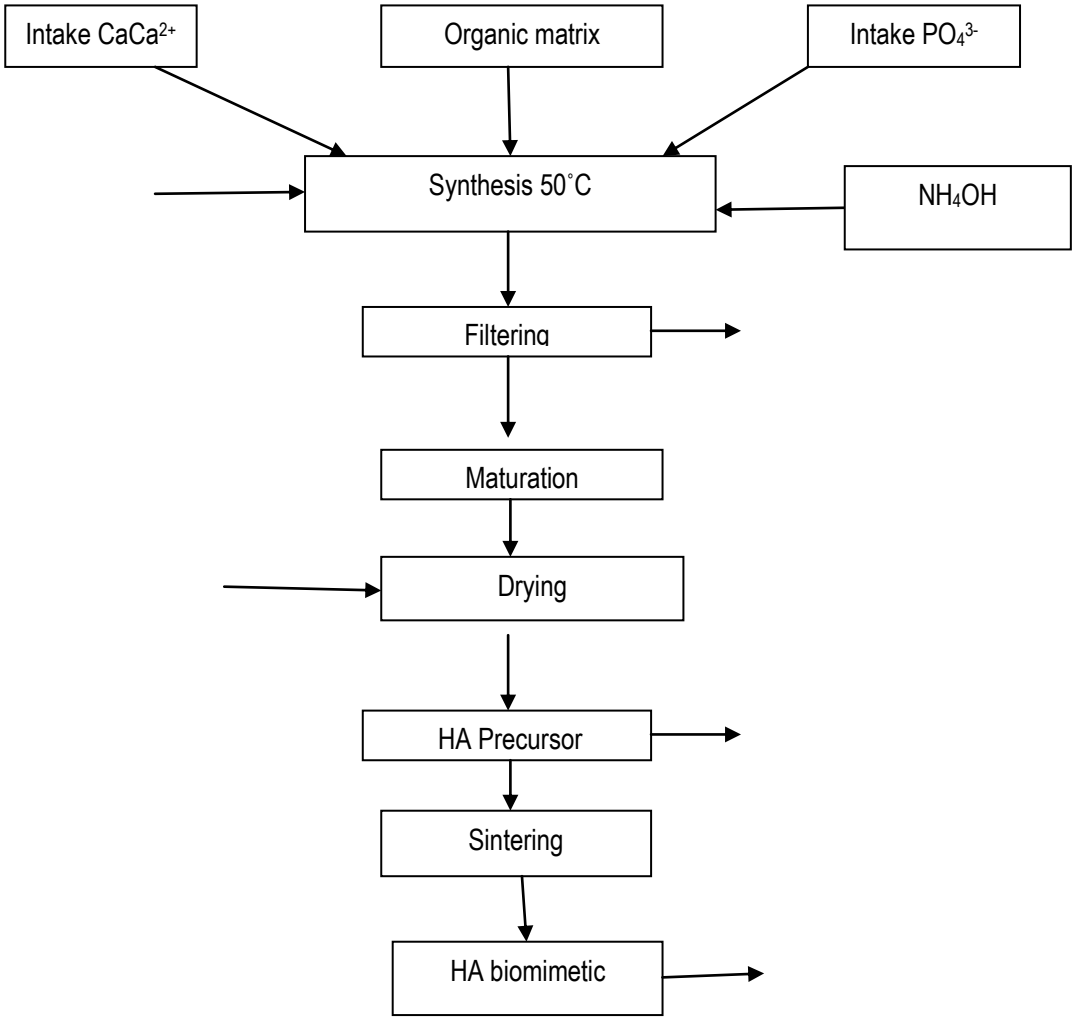
$$10Ca[NO_3]_2+6[NH_4]_2HPO_4+2NH_4OH \xrightarrow[-20NH_4NO_3]{10<pH<12} Ca_{10}[PO_4]_6[OH]_2$$

he synthesis reports are presented in Tab 5.1 and the synthesis process is given in Fig 4.1

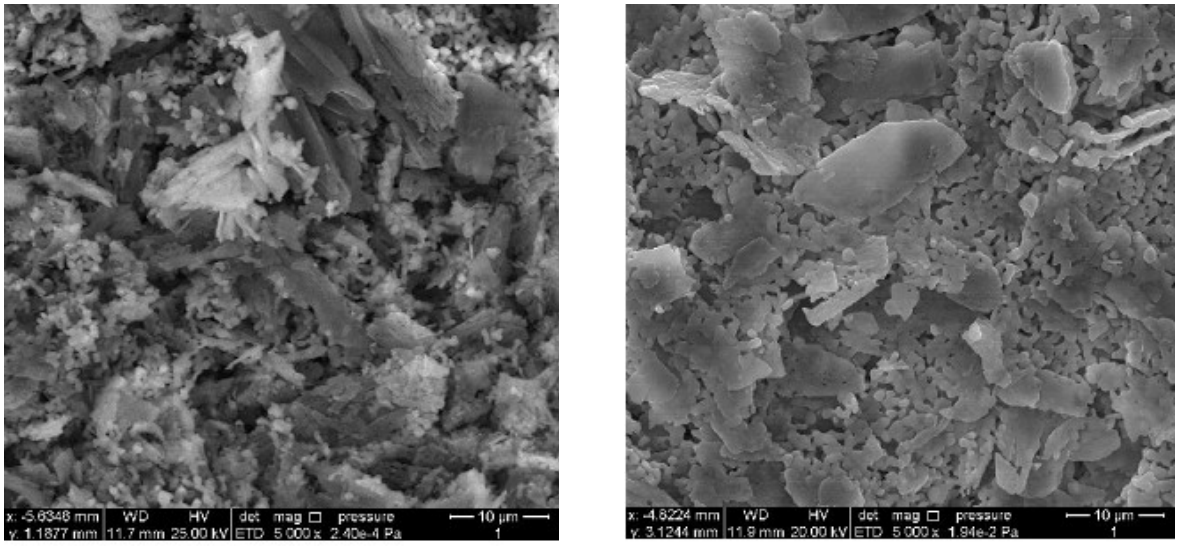
Table 5.1 Synthesis parameters

Symbol	HA Type	Synthesis reports		
		Atomic Ca/P	gelatine/phosphate g/mol	pectin/gelatine g/g
HAst	standard	1, 67	0	0
HAb1	biomimetic	1, 67	16, 66	0
HAb2	biomimetic	1, 67	16, 66	1:1
HAb3	biomimetic	1, 67	16, 66	1:2

Figure 4.1 Block diagram of HA synthesis



We found out that adding the gelatine (polypeptide) to the HA synthesis gives it a high biomimetism,by determining the formation of acicular crystals, whose orientation is influenced by the conformation of the biopolymer (Fig 5.7)



a) b)

Figura 5.7. SEM images after sintering for: a) HAb2; b) HAb3.

Adding the pectine (polysaccharide) enhances the formation of nanodimensional crystals. But a high intake of pectin determines a higher thermooxidiing rate for the organic matrix (in the sintering phase) which results in the collapse of the inorganic component conformation. As a consequence,we only keep the HAb3 as a substratum for obtaining the composite.

5.2.

We have created a rapid method for identifying and comparing the HA-type materials. The method is based on interpreting the Heat Flow curves of a mixture of HA and reactive (Precise Path/Giese Diagnostic Roma). As observed in Fig 5.19

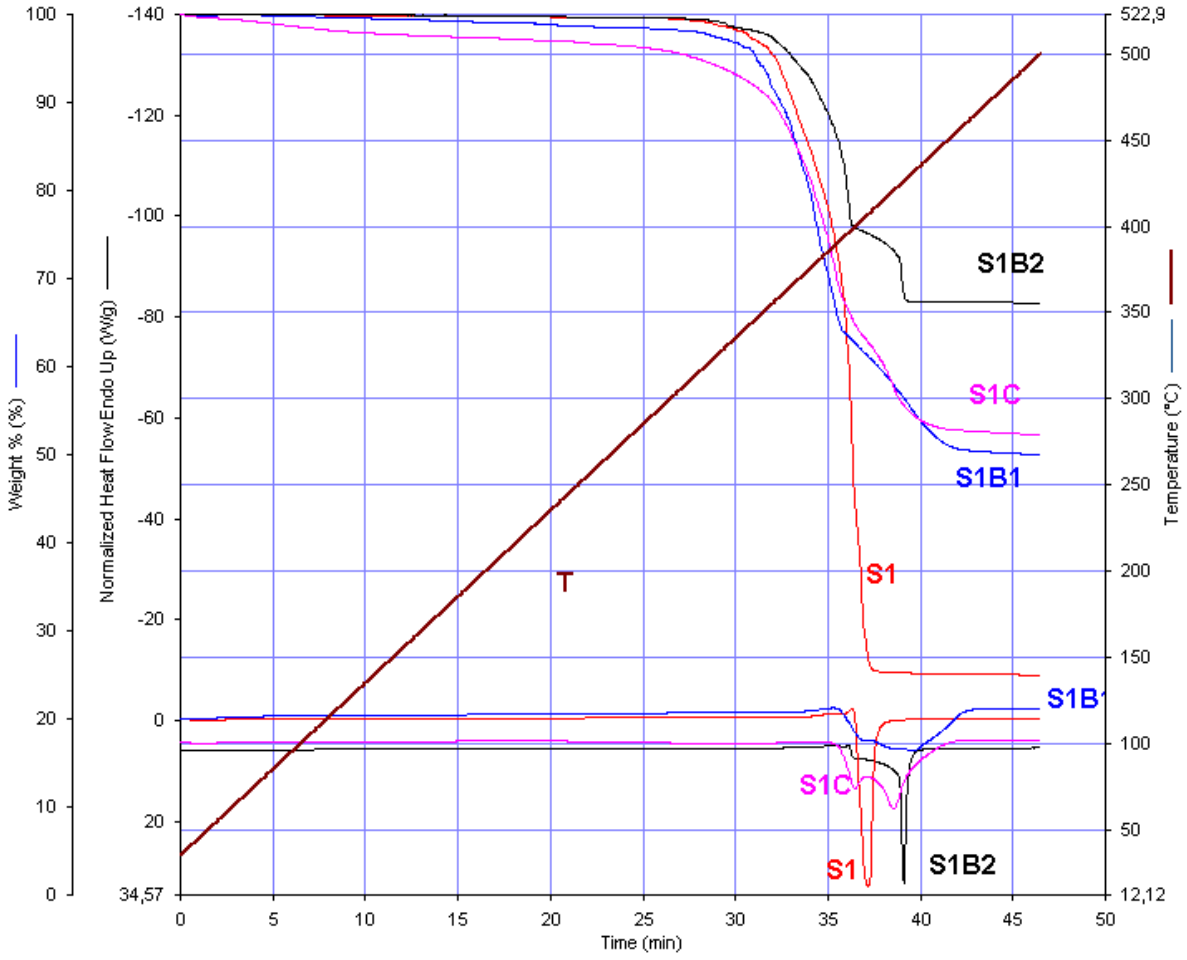
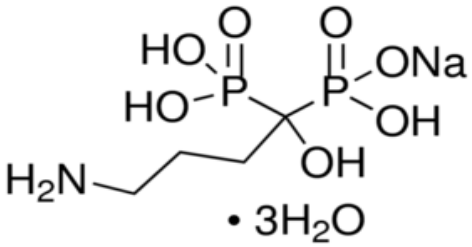


Figure 5.19. Heat Flow curves and TG for S1B1, S1B2 și S1C samples

the HF signals are the sample prints,being useful for identifying the hydroxiapaptites. The presence of HF signals in the same temperature range confirms the fact that the synthetised biomimetic HA,B2, has a similar mezostructure with tow biogene hydroxiapatites B1 and C.

5.3.

The Sodium Alendronate Trihydrate (Scheme 5.1) was studied



Scheme 5.1. Sodium Alendronate Trihydrate

under the aspect of stability,being the first candidate for crating the composite,due to its two essential properties:

- structural resemblance between phosphonic groups and the phosphate ions in HA
- the biological activity of the organic radical on inhibiting the osteocyte formation.

When the article was published (2016) it was the first to cover this subject. The suggested degradation mechanism (Fig 5.22) has contradicted the opinion accepted until then.

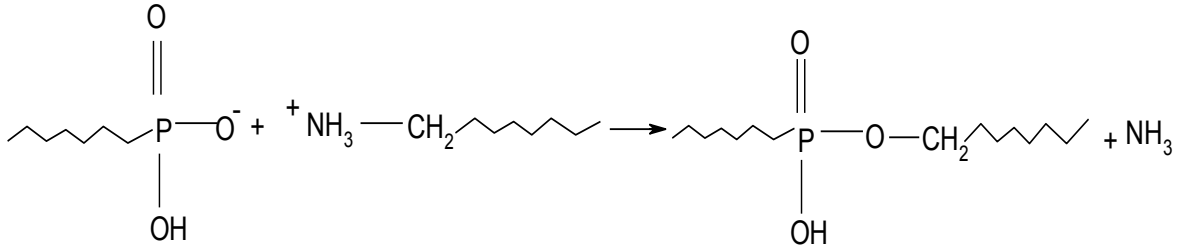


Figure 5.22.The deamination mechanism

It was also stressed that the dehydration stage can modify the biological action.

The quantitative data regarding the ALE stability were obtained through a detailed kynetic analysis.

5.4.

Through thermal analysis in in nonisothermal conditions,we managed to study the probable thermic inducted interactions between ALE and different excipients that can be used in creating the solid formulas. Microcrystalin cellulose,starch and gelatine don't interact with ALE. Malthose and magnesium stearate have low thermic stability,they decompose beneath the safety temperature of ALE,this is why it is recommended not to use the for solid formulas containing Alendronate.

The commercial product Fosamax was studied as well,observing that the degradation processes which interfere with those of the alendronate are caused by the lactose present in the excipient mixture (Fig 5.31).

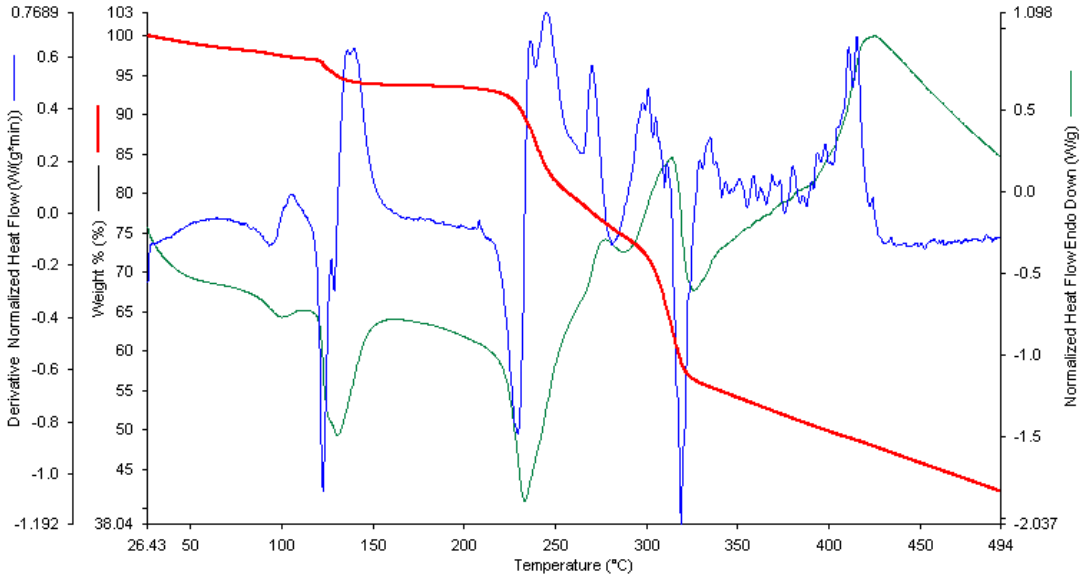


Figure 5.31. Thermograme for the FOSAMAX drug at a heating rate β=10^{0}C/min

5.5.

We have obtained a composite(powder material) through a controlled deposit of the sodium alendronate on the HA surface. The controlled deposit was realised through spectroscopic techniques (e.g. the presence of nitrogen from the primary amine in the EDX spectrum) and the deposited quantity,22% mass was determined through thermogravimetry

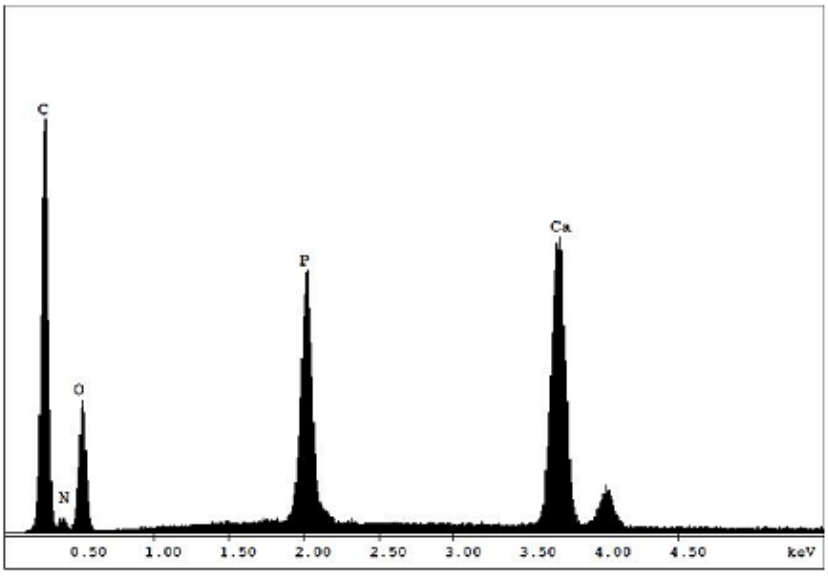


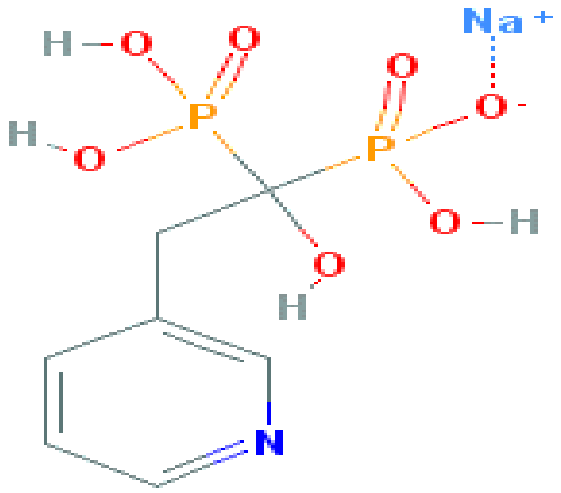
Figure 5.32. EDX spectrum for the composite.

The advantages of the composite

- HA was synthetised in a polymer matrix which offers it a high biomimetism
- ALE inhibits the resorbtion action of the osteoclasts
- The biological action manifests just at the surgical site,avoiding the spreading of ALE throughout the entire body,limiting and even eliminating its side effects.

5.6.

Rise(Scheme 5.2),with a biological activity ten times higher than ALE is a future candidate for creating bioactive composites as well as solid formulas



Scheme 5.2. Rise structure

Rise has a lower thermic stability than ALE. Thermoanalytical data and kinetic analysis have confirmed a range of complex degradation processes (Tab 5.7).

Table 5.7. Thermoanalytical data for Rise at 10°C/min

Stage	Experimental mass loss(%)	Calculated molecular mass,according to mass loss	Temperature range °C
I	5.6	17	---85
II	4.0	12.2	144-167
III	2.0	6	179-218
IV	3.38	10.3	220-255
V	6.3	19.2	255-264
VI	2	6	Over 380
Total	23.28	71	

Out of the studied excipients,we came to the conclusion that microcrystalin cellulose and starch do not influence the stability of Rise,whereas manitol and magnesium stearate make it significantly worse. Takong into account the fact that Rise has a mediocre stability,it is recommended to use biocompatible polymers for the solid formulas.

FINAL CONCLUSIONS

1. The aim of the thesis was to obtain a HA-type composite material with use in bone augmentation as a preparation stage for dental implants.

2. The new things brought by this thesis is creating this material in very similar conditions to the natural osteosynthesis product. This way,the synthesis process takes place in the presence of a protein matrix and using bisphosphonate-type fixing agents. Therefore,the bone will adapt to the new material faster and more efficient.

3. In achieving the thesis objectives,we've used a series of modern and complex technologies and methods,such as:infra-red spectrometry with Fourier transformed,attenuate total reflection,X-ray difraction,electronic imaging,thermical analysis.

4. The unconventional HA synthesis,presented in the thesis,uses,as the only source of energy for heating as well as stiring an ultrasound field and it applies the template effect to orientate the crystalites in the synthesis/precipitation process. The necessary matrix was provided by gelatine and gelatine/pectin solutions. The role of gelatine is to simulate the fragments in collagen composition and that of pectin is to modify the hidrophobe/hidrophil equilibrium on the growth interfaces of crystals

5. The results of the thesis were to obtain a new material for bone augmentation "Biomometic Hydroxyapatite", a material similar with a biogenetic one, consecvently with high biocompatibility and efficiency for the osteointergration of the inorganic components of the bone.

6. The results were also published in 3 articles which add up to an impact factor Thompson of 5,860.

7. Verifying the efficiency of the material was made by comparison with the bone tissue sample,using the method of fixing the reference serum proteins. The experiments have shown that the new material fixes the proteins in quality and quantity in the same way as the bone tissue

8. The advantages of using instrumental techniques in the non-clinical phases of developing new drugs and biomaterials were proven. Especially the thermal analysis has to be mentioned for its versatility in the study of solid formulas which can be promoted in a higher stage of the non-clinical studies.

9. Together with the creation of the material “Biomometic Hydroxyapatite”,possible excipients needed for solid formulas with bisphosphonates were studied,obtaining a stable formula which contains the following excipients:silica,starch,crystaline microcellulose and gelatine;manitol and magnesium stearate are not recommended in this case

10. The results obtained in the thesis “New Bioactive Compounds Used In The Osteogenesis Process” opens up the possibility of personalising the materials used in biological processes by simulating the natural processes during their synthesis. This way,the classic HA,which makes the bone tissue adapt in a non-physiological way,was replaced with a structured HA from the begining on a protein matrix,making the integration of this HA in the collagen matrix very easy and natural

OWN CONTRIBUTIONS

1. A new product was obtained,based on a new method of wet synthesis of HA in the presence of a protein matrix,followed by the controlled deposit of a bisphosphonate

2. A study on HA used in orthopaedy and dentistry was made. Also,a list of literature entries on bisphosphonates as materials with direct intervention in the osteoblast/osteoclast equilibrium was made.

3. An idea was formulated to create a composite based on high biomimetic HA on which ALE is deposited so that this can be biologically active in the surgical site,without the side effects affecting the rest of the body

4. More HA synthesis in unconventional conditions were made:the intake of energy necessary to heat and stir was obtained from an ultrasound source and the mezostructure similar to the biogene HA was secured by the template effect of gelatine and pectin

5. Gathering the data from different instrumental techniques: thermogravimetric analysis,X-ray difraction,IR spectrometry,SEM imaging,EDX spectrometry. Interpreting these data allowed guiding the synthesis towards a material with the structure and texture close to the biogene one.

6. A deep study on the thermal behaviour of ALE and Rise was made. Matching TG and HF data assured suggesting the reaction mechanisms for the two thermical induced degradation stages: dehydratation and deamination. Gathering the TG and DTG data led to a detailed kynetic analysis of the thermodegradation

7. The study of the thermical behaviour of the binary mixtures bisphosphonates-excipients,allowed identifying the effects of more than seven excipients on the stability of the bioactive compound. These data are essential for guiding the solid formulas in the non-clinical stages of developing drugs.

8. The thermal behaviour of the FOSAMAX drug was studied and we could identify the active substance-excipient interactions,which affect the stability of LAE

9. Samples of biomimetic HA-ALE were created using a non-conventional technology based on ultrasounds and modifying the polarity of the solvent in the depositing phase. The samples were described form a physic-chemical point of view and can be forwarded towards clinical studies on animals

10. A rapid method for identifying the biocompatibility of HA-type materials,using the thermal analysis technique and a control serum common in clinical laboratories

PUBLISHED AND PRESENTED ARTICLES

1. Doca, S. C., Ceban, I, Vlase, T, Albu, P, Vlase, G, Biomimetic hydroxyapatite / biopolymer matrix composite:Synthesis,characterization and kinetic analysis, CCTA 12, 6-10 Sept.2015, Zakopane, Polonia, p.424.

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3. Albu, P., Doca, S.C., Anghel, A., Vlase, G., Vlase, T., Thermal behavior of sodium alendronate; a kinetic study under non- isothermal conditions, J. Therm. Anal. Calorim., 127(2017)571-6.

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