

Considerations on Thermal Behaviour and Porosity of Renal Calculi in Bisphosphonate -Treated Postmenopausal Women

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Scientific data assess the connection between decreased bone mineral density, osteoporosis, and renal urolithiasis in postmenopausal women and point out the risk of recurrence in these stone-former patients. A longitudinal, randomized, five year follow-up clinical and experimental study was conducted during May 1, 2011 - June 30, 2016. A number of 125 eligible postmenopausal women (BMD T-score spine (-2.8 ± 0.5); BMD at the femoral hip (-2.6 ± 0.8), treated only with bisphosphonates, were enrolled in the study. The patient's age ranged between 60 and 65 years. All the women were stone formers, with more than five episodes of crystalluria in the last five years. Exclusion criteria: kidney chronic diseases, use of drugs known to affect bone metabolism and renal function, patients with multi-morbidities. The aim of our study was to assess the connection between postmenopausal osteoporosis and the risk of worsening calcium balance in urolithiasis, based on the complex thermogravimetric findings and porosity studies of renal stones eliminated by these patients. The study of renal-urinary concretions offers the possibility to amplify the specific effects of the thermal decomposition processes of substances, reflecting the sample's global composition. The research of calculi porosity gives information about the dissolution possibilities of the renal-urinary concretions. In conclusion, in postmenopausal women, proper administration of bisphosphonate therapy must accompany the supplementary medication with calcium and vitamin D, always correlated to the metabolic status of the osteoporotic patient.

Keywords: *postmenopausal osteoporosis, bone mineral density, bisphosphonates, urolithiasis, porosity, Thermogravimetry (TG), Differential Thermal Analysis (DTA)*

Physico-chemical techniques based on thermal analysis have important clinical contribution in understanding the structure of the osteoporotic bone and avoiding the risk of future fractures in postmenopausal osteoporotic women [1-5].

Thermogravimetric Analysis coupled to Mass Spectrometry, High-Temperature X-ray Diffraction, and Fourier Transform Infrared Spectroscopy are modern research tools, which highlight kidney stone composition and improve the knowledge of the bone mineral content, as noted in the studies they've published in recent years [3-11].

Cortical bone porosity, is valuable to reflect bone structure, and plays a major role in increasing distal fracture risk in elderly women [12]. However, these data are still controversial and difficult to study especially in patients with two chronic and unpredictable diseases, such as postmenopausal osteoporosis and recurrent urolithiasis [13-14]. The method of thermal analysis allows the monitorization of skeletal changes that interfere with the osteoporotic disease owing to therapeutic intervention, and its value to the practitioner lies in the fact that spectral data may be correlated with the microscopic and clinical data. The relationship between crystal size, bone turnover and remodellation is the most important aspect for monitoring postmenopausal osteoporosis, as revealed in our previous studies.

The aim of our study was to assess the connection between postmenopausal osteoporosis and the risk of worsening of calcium balance in urolithiasis, based on the complex thermogravimetric findings and porosity studies

of renal stones eliminated by these patients. Further research will be focused on porosity studies and on their correlation with bone remodeling markers in postmenopausal osteoporotic stone-formers.

Experimental part

Material and method

A longitudinal, randomized, five year follow-up clinical and experimental study was conducted during May 1, 2011 - June 30, 2016. A number of 125 eligible postmenopausal women - BMD T-score spine (-2.8 ± 0.5); BMD at the femoral hip (-2.6 ± 0.8), treated only with bisphosphonates, were enrolled in the study. The patient's age ranged between 60 and 65 years. All the women were stone formers, with more than five episodes of crystalluria in the last five years. Exclusion criteria: kidney chronic diseases, use of drugs known to affect bone metabolism and renal function, patients with multi-morbidities.

The study material was represented by twelve urinary calculi removed from women with postmenopausal osteoporosis under bisphosphonate therapy who were previously monitored for calcium microlithiasis. In order to be enrolled in the study, osteoporotic patients gave their written consent, in accordance with current medical ethics [15].

Renal calculi samples

The small kidney stones were sectioned into three parts and the analysis of the mean sample was carried out. Bulky bladder stones were analyzed into three different parts, namely: the external *crust*, the *median* (intermediate part)

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Working conditions	Sample 1	Sample 2
Heating range	20 – 1000 °C	20 – 1000 °C
Sample mass	640 mg	560 mg
Balance sensitivity calibrated at 100 TG divisions	200 mg	500 mg
Reference material	Al ₂ O ₃	Al ₂ O ₃
DTA	1/5	1/10
DTG	1/10	1/10

Table 1
THERMAL DIFFERENTIAL
ANALYSIS WORKING
CONDITIONS FOR
SAMPLE 1 AND SAMPLE 2

and the *core* (inside part) and in these cases - one performed Rx diffraction study of successive phases.

Differential Thermal Analysis (DTA)

Complex differential thermal analysis was carried out using MOM F. Paulik, I. Paulik, L. Erdey equipment [16] and the atlas of thermoanalytical curves was used for compound identification [17]; the working temperature ranged between 20–1000°C, highlighting the thermochemical transformation of substances in samples which were partially investigated and detected by analyzing qualitative X-ray diffraction. Determinations were performed at the Faculty of Industrial Chemistry and Environmental Engineering belonging to the Politehnica University Timisoara.

In table 1 there are highlighted the working conditions for two representative samples:

Measurement of bone mineral density

Bone mineral density (BMD) in the lumbar spine region (L₂ through L₄) and at femoral sites was measured with dual-energy X-ray absorptiometry (DXA HOLOGIC). All scans were taken using general scan software at a scan speed of 10 mm/s and with pixel size 1.0x 1.0 mm². The scan width was 5 cm and the total effective dose remained <1μSv.

Results and discussions

Of all concretions were selected two cases as representative examples.

The urinary calculus originating from the patient N.A., 64 years old, depicts the following trades:

- Location: right renal pelvis;
- External appearance: brown-dark color, alternating with rare white disseminated spots, rough surface, with visible crystal structure;
- Sectional appearance: nonhomogenous, concentric layers, rare white spots;

- Dimensions: 0.6/0.4 cm;
- Weight: 0.168 g;
- Consistency: high hardness;
- Number: multiple calculi;
- Elimination way: spontaneous.

When performing the thermal analysis, this concretion was noted as sample 1.

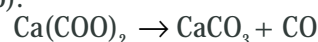
The urinary calculus originating from the patient N.C., 62 years old, presented the largest sizes and weight of all, revealing the following morphological characteristics:

- Location: concretion fixed in the bladder;
- External appearance: white-gray color, smooth round surface;
- Sectional appearance: homogenous;
- Dimensions: 2.5/0.8 cm;
- Weight: 1.907 g;
- Consistency: reduced hardness, comparable to that of grout;
- Number: single concretion;
- Elimination way: surgically removed.

When performing the thermal analysis, this urinary stone was noted as sample 2.

For sample 1, one revealed the following thermal decomposition stages (table 2).

- Free water loss within the temperature range 20–200 °C, with an endothermic effect (corresponding at a minimum speed of the process at 110°C), and of the crystallization water, with a minimum endothermic effect at 240 °C (fig. 1 a);
- Decomposition of calcium oxalate according to the reaction (fig. 1 b):



and was carried out at a temperature of 480°C.

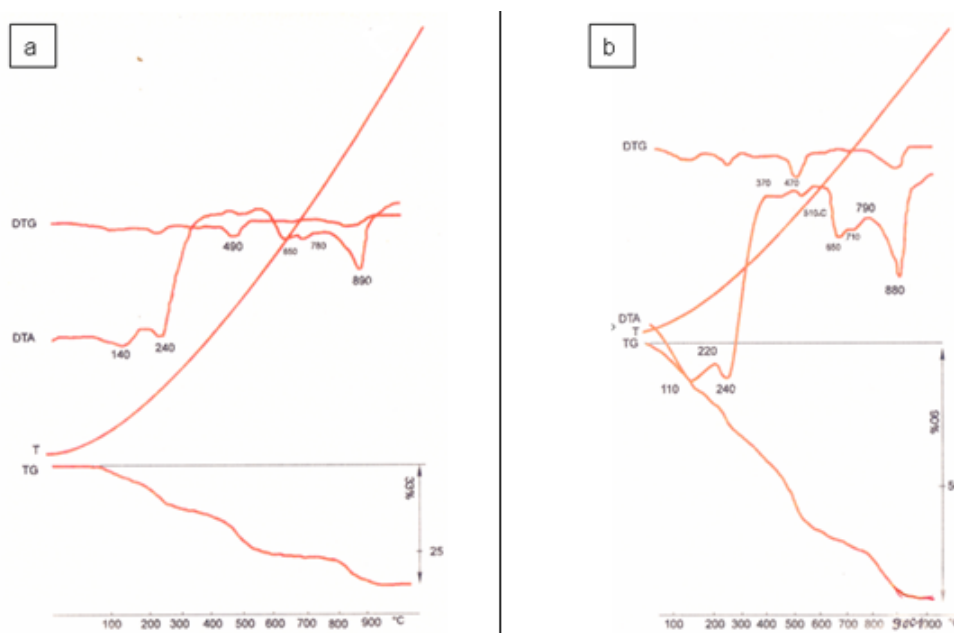


Fig. 1. Differential thermal analysis curves from kidney stone eliminated spontaneously - patient N.A., 64 years old, with postmenopausal osteoporosis (T score = - 3.0). Sample 1 (a and b)

- 1a - Endothermic effects of decomposition at 110°C and 240 °C
- 1b - Exothermic effect of decomposition at 480°C and endothermic effect at 880 °C

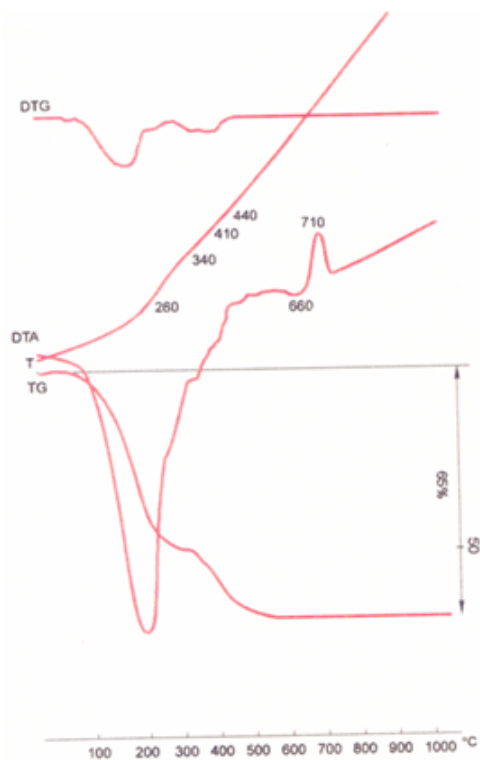


Fig. 2. Differential thermal analysis curves from bladder stone removed surgically - patient N.C., 62 years old, with postmenopausal osteoporosis (T score = - 2.8). Sample 2

Table 2
THE STEPS OF DIFFERENTIAL THERMAL ANALYSIS PROCESS OF SAMPLE 1

No. of steps	No. of divisions (TG sensitivity)	Temperature range (°C)	Mass (mg)	%	Process
I	22	20 - 200	44	6.87	Removing the adsorbed water
II	8	200 - 240	16	2.81	Eliminating the crystallization water from oxalate
III	19	240 - 455	38	5.94	Decomposing the matrix
IV	11	455 - 510	22	3.43	Releasing the CO from calcium oxalate
V	14	510 - 800	28	4.38	Decomposing the matrix
VI	15	800 - 1000	32	5.00	Decomposing CaCO ₃

A very important indication of the mentioned transformation is the fact that over a process that marks the weight loss on TG and DTG curves corresponding at a temperature of 480°C, on the DTA curve appears a slightly exothermic effect (an abnormality for the decomposition), explainable by the fact that carbon monoxide (CO) from the reaction shown above oxidizes easily at this temperature by an exothermic effect, according to the reaction:



Decarbonization of CaCO₃, corresponding at a minimal endothermic effect at 880°C (fig. 1b).

Like it is mentioned in the specialty literature [18], it results that during the analyzed period the hydroxylapatite does not suffer any changes.

From the DTA one calculated (with an error margin of 1%) the analyzed calculation's percentage composition:

Adsorbed water	6.87%
Organic matrix	10.33%
Calcium oxalate monohydrate	17.92%(2.21% crystallization water)
Hydroxylapatite	64.88%

By comparing the obtained results with the specialty literature [19-20], for the same type of concretions, the following conclusions regarding the method can be taken:

- the slight displacement of the thermal effects from the

analyses are due to the specific work conditions: the weight of the analyzed sample, the heating speed, the interaction between the sample's respective components, etc.

- while the quantitative analysis is less influenced by the above mentioned parameters, the balance's sensibility must be optimally chosen, the case where the methods' error margin is under 1%;

- the obtained duration curves is very close with the one mentioned in the specialty literature [21];

- the decomposition of the organic matrix is achieved over a large temperature range (stages III-V), over which the process of calcium oxalate decomposition with release of CO is overlapped;

- due to the complexity of the organic compounds which compose it and give it a range of overlapping effects the detection of the matrix's quantitative composition is very difficult to achieve.

Concerning the adsorbed quantity of water, following successive analyzes of the same calculation, it was observed that if the sample milled regarding keeping analyzes in laboratory conditions, the quantity of adsorbed water increased spectacularly. In the specialty literature, it is mentioned that a fresh calculi has a much greater quantity of water in comparison with a calculi kept in dry conditions for a prolonged period of time and at a normal temperature [22].

Even though the process of adsorbed water elimination is particularly evident in some ATD analyzes, it does not affect the chemical composition of the studied calculi,

essentially being a problem related to the storage conditions.

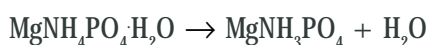
This observation allows for the highlighting of the chemical percentage composition of sample 1 (fig. 1 b):

Organic matrix	11.09%
Calcium oxalate monohydrate	19.24%
Hydroxylapatite	69.67%

For the second sample we concluded that the DTA curves are very similar with the one presented in the specialty literature [8], for $(\text{NH}_4)\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$, as a pure chemical compound. This aspect is also confirmed by the chemical percentage composition calculation on the sample made in the conditions specified in table 3.

There are five stages of the of the thermal decomposition process of the sample:

- Eliminating five crystallization water molecules from $(\text{NH}_4)\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$, which begins at 100°C and has an endothermic maximum at 160°C;
- Eliminating the residual water molecule until 300°C:



- Eliminating NH_3 from the phosphate's composition, over which the decomposition of the organic matrix until 440 °C overlaps;

- The transformation of the magnesium phosphate into pyrophosphate, according to the reaction:



at the temperature of 440°C;

- An exothermic effect that corresponds with the crystallization of the amorphous pyrophosphate, at the temperature of 710°C.

Based on the data from table 2 and from table 3 (column 2), one determined the percentage chemical composition of the second calculi:

$(\text{NH}_4)\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ (struvite)	98%
Organic matrix	2%

As previously mentioned, the DTA duration curves anticipated a higher concentration of struvite. This higher concentration of struvite from the sample (98%), makes the endothermic and exothermic peaks from the DTA analysis to be very close to those of the pure substance. The TG curve shape – suitable for the decomposition of the organic matrix, predicts that it is not incredibly complex or that the combined effect of the NH_3 decomposition is strongly masking it.

No. of steps	No. of divisions (TG sensitivity)	Temperature range (°C)	Mass (mg)	%	Process
I	40	20 - 200	200	35.71	The elimination of 5 crystallization water molecules
II	8	200 - 300	40	7.14	The elimination of the residual crystallizing water molecule
III	12.5	300 - 440	65	11.61	The elimination of NH_3 and the decomposition of the organic matrix
IV	4	440	20	3.57	The transformation of phosphorus into pyrophosphate

Table 3
THE STEPS OF DIFFERENTIAL THERMAL ANALYSIS PROCESS OF SAMPLE 2

Sample	1	2
Absorption (%)	11.99	20.43
Apparent volume (cm^3)	3.32	1.58
Apparent density (g/cm^3)	1.33	1.45
Apparent porosity (%)	20.18	37.50

Table 4
THE POROSITY OF SAMPLES 1 AND 2

The obtained result concerning the organic matrix (2%) fits with the specialty literature data and evidences the fact that in the struvite constituted calculi, 1.5 up to 10% of their weight is represented by organic components [23].

The method allows for the differentiation of certain important components and frequently discernable in the renal-urinary concretions from osteoporotic women, respectively:

- the calcium oxalate monohydrate (whewellite) and the calcium oxalate dihydrate (weddelite), an aspect often commented on in the specialty literature [24];
- the uric acid and 2.8 dihydroxiadenin [25].

Through microthermalanalysis procedures it is possible to research small size calculi, often found in osteoporotic stone formers; this aspect is very important as 55% of calculi weigh less than 0.1 g, and 15% - are even under 0.01 g [26].

Also, the method may as well be applied for researching renal microlithiasis [27]

Research Concerning the Porosity of Reno-urinary Concretions

Taking into account that the stability of reno-urinary calculi in their forming environment depends on a series of environmental factors: pH, the concentration of some ions in the contact liquid, the solubility product of the calculi, but also some characteristics of the solid body which the calculi constitutes, we proceeded to investigating the porosity of the samples through absorption without fragmenting them.

The pores represent spaces in the structure of a solid body, filled with gases or liquids [28].

The geometric shape of the pores is variable by simplifying they resemble spheres, cylinders, ellipsis, etc.

The total porosity is expressed by the ratio between the pore's volume and the total volume of the solid body. For characterizing a solid body the dimensional distribution of the pores is very important.

The more porous a solid body is, its mechanical resistance decreases (it can more easily fragment), and the characteristic surface that it offers liquids with which it is in equilibrium grows, greatly increasing the speed of some double exchange reactions.

Absorption is the property of porous materials to absorb and retain liquids and is expressed by the quantity of liquid absorbed or by the volume of the pores filled with liquid.

The determining porosity through absorption results on the two types of samples are presented in table 4.

As observed, the porosity of the second sample is almost

double in contrast with the first sample, which means that it offers an equilibrium surface with a liquid environment which surrounds it with a much increased penetrance, which favors its dissolution.

An accurate identification of minor components with their location in the stone is clinically relevant to assess environmental factors involved or to explain the lithogenic process (for example crystallization of calcium oxalate from Randall's plaque) [29]. Moreover, it could highlight a new lithogenic process in postmenopausal women with recurrent crystalluria.

Conclusions

The research of the 12 renal-urinary concretions analyzed through the method of thermal differential analysis revealed several significant observations:

The thermogravimetric analysis in its different forms presents numerous advantages for the study of renal-urinary concretions: - it allows for a very good deceleration of the types of substances that compose calculi, reflecting the sample's global composition; - it offers the possibility to amplify the specific effects of the thermal decomposition processes of substances taken in certain work domains; - the remarkable precision of the method is noted, the error margin of the composition being under 1%; - in contrast with the X ray diffraction analysis, the development of standards for compositional calculi is not necessary; - it exactly specifies percentage share of the organic matrix located in the composition of the renal-urinary concretions; this aspect constitutes a significant advantage of the thermogravimetric analysis in comparison with other complementary procedures of studying renal-urinary calculi.

The research of calculi porosity, through the data referring to the dissolution possibilities of renal-urinary concretions emphasizes the therapeutic implications of the presented method of study. In this regard, the struvite calculi, secondary recurrent urinary tract infections in postmenopausal osteoporotic women, present an increased porosity, which facilitates their dissolution.

These researches bring important contribution for the practitioner by guiding and improving the calcium balance and the osteoporotic treatments in postmenopausal osteoporotic women who are also stone-formers.

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