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BON(N)-STONES - PRODUCTION AND CHARACTERIZATION OF SYNTHETIC STANDARD STONES FROM NATURAL MATERIAL

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Abstract

Synthetic urinary stones, as we have known them so far, have consisted of artificial materials (e.g., chalk or dental cement) and have not corresponded with natural concrements either in their chemical composition or physical properties. For the purpose of scientific research on chemolysis, in combination with lithotripsy, and for tests on the efficacy of lithotriptors, we can no longer do without the use of standardized artificial stones made from natural materials. The successful production of these standardized stones (BON(N)-STONES) was achieved by a special coating procedure. Struvite, brushite and whewellite stones were produced as standardized BON(N)-STONES. Tests for the purity of synthetic urinary stones were performed by infrared spectroscopy. Scanning electron microscopy determined the crystal morphology and the function of gelatin used as organic matrix. Physical properties such as density and crushing strength may be compared to those of natural stones. These artificial stones allow systematic scientific research on chemolysis and lithotripsy.

Key Words: Standard urinary stones, natural material, physical properties, scanning electron microscopy.

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Introduction

Scientific research investigating the quality of stone analysis methods, the efficacy of lithotripsy procedures and the possibilities to dissolve urinary stones, requires standards of identical composition. Test results can only be compared when any number of test repetitions with standardized material is possible. Natural stones, however, differ in shape, size and composition and cannot be regarded as an appropriate standard material. In addition, complete natural stones have become a rarity in recent years owing to the advent of lithotripsy.

When the efficacy of lithotriptors was tested in the past, "stones" were used which were made of artificial materials such as chalk, spatula, plaster, dental cement, and ceramic material [1, 5]. This procedure was quite legitimate for the development and improvement of lithotriptors but the simulation of natural conditions was rather insufficient. The composition, the morphological structure, and the physical properties (such as density, crushing strength, elasticity) of the currently available artificial stones do not correspond to those of natural stones. This kind of test material has proved to be entirely unsuitable for investigations of stone analysis and lysis experiments. We have, therefore, developed a method which allows the production of standard urinary stones from natural materials: BON(N)-STONES. The letters BON(N) stand for the French word "bon" (in the sense of: good) and stones from the city BONN, Germany.

Materials and Methods

Authentic crystals of struvite, brushite and whewellite were used for the production of artificial urinary stones, BON(N)-STONES. Infrared spectroscopy confirmed their correspondence to natural urinary stone components [3]. Materials used: (1) Struvite $MgNH_4PO_4 \bullet 6\ H_2O$ (Riedel de Haen, Seelz, Germany, No. 04255); (2) Brushite $CaHPO_4 \bullet 2\ H_2O$ (Riedel de Haen, Seelz, Germany No. 04231); and (3) Whewellite $Ca(HCOO)_2 \bullet 1\ H_2O$ (Roth, Karlsruhe, Germany, No. 6145.1)

Production of BON(N)-STONES

Granulation of the basic substance 150 g of the crystals are gradually mixed with approximately 60 g of a 3% gelatin solution (at approximately 40-50°C) to form a moldable mass. This substance is then passed through a sieve with a mesh size of 0.8 mm, and is pre-dried for 3 hours at 30-40°C. The granules that have been formed by this procedure are passed through the sieve again and dried over a period of 24 hours at 30-40°C. The granules, now completely dry, are again passed through a sieve with a mesh size of 1 mm to remove large pieces and subsequently passed through a sieve with a mesh size of 0.2 mm to remove fine dust (Fig. 1).

Production of the stone core A press (KIS Kilian, Köln, Germany) is used to form stone cores of 3 mm x 3 mm in size. A mixture of granules with 1% micro-crystalline cellulose and 0.5% magnesium stearate was prepared.

Application of stone layers In order to increase the size, stone layers are added to the cores in a coating pan, as used for pharmaceutical purpose. Coating suspension: 25 g of solid substance (stone material and 3% gelatin) per 120 ml H_2O.

Thirty grams of stone cores (about 1,000 pcs) are filled into the coating pan. The speed of the coating pan is set at 30 rpm. Gradually, the coating suspension is added until the cores start sticking together. The cores are stirred several times with a wooden spoon and dried in a warm air stream at 50-60°C. Cores sticking together are separated by hand. Dried cores are then left to cool down. These steps are repeated until the stones reach the desired size.

About 1,000 layerings are necessary to produce BON(N)-STONES of 1 cm in diameter. Gelatin was used, both as a binding agent and as organic matrix, to which, when in its gaseous stage, formaldehyde was added for cross linkage.

Determination of physical properties

The density of the stones was determined according to the Archimedean principle using a Mohr'sche Waage (scales) (Kern, Albstadt, Germany).

Crushing strength was tested on a tablet tester (Heberlein & Co., Zürich, Switzerland).

Scanning electron microscopy investigations

Scanning electron microscopic (SEM) investigations were carried out of the basic material, the granulate, the stone surface, and the surface of fractured stones of all types. The studies were carried out after sputtering the samples with gold.

Infrared analysis

The samples were homogenized in an agate mortar and ground to a fine powder. 0.5-1.0 mg of the sample were mixed with 200-250 mg of potassium bromide in a vibrating ballmill and subsequently pressed to tablets under vacuum. Infrared spectra were recorded by FTIR spectrometer 1700 (Bodenseewerk, Perkin Elmer GmbH, Ueberlingen, Germany).

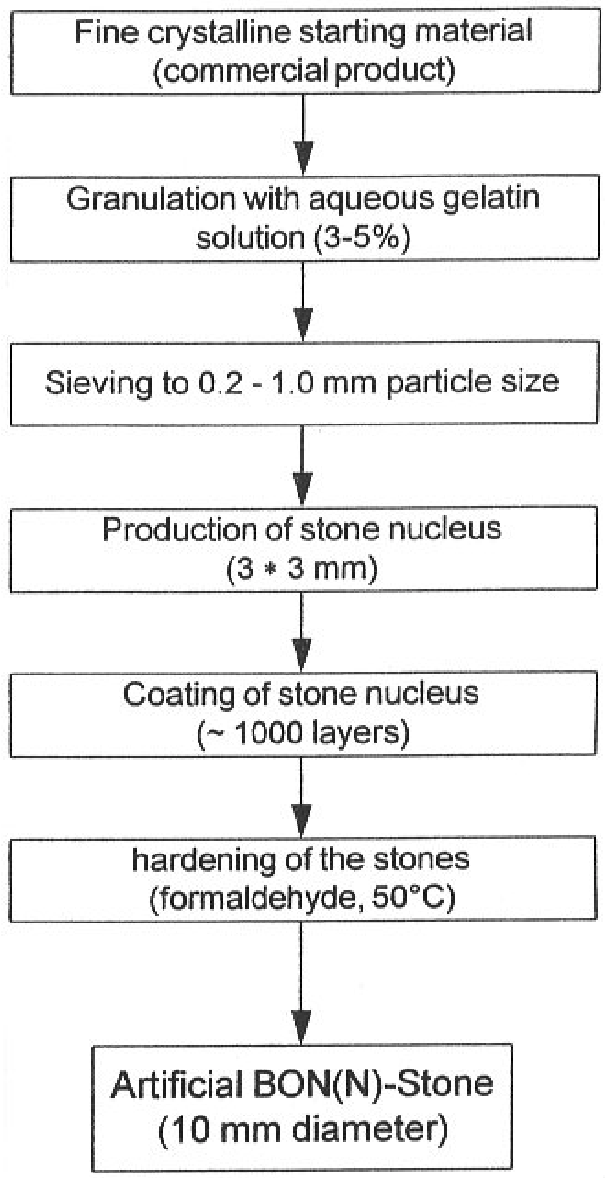

Figure 1. Outline of the production process of BON(N)-STONES.

Results

The stones produced are ball-shaped and quite uniform in diameter, volume and weight (Table 1; Fig. 2). A variation coefficient of the stone diameters amounting to

Figure 2. BON(N)-STONES made from struvite.

1.4% demonstrates that a good degree of standardization is achieved. The density and the crushing strength (measured in struvite stones, only) are within the range of scatter obtained from natural stones (Table 2). Values for crushing strength in natural stones vary between 2 and 17.5 kp, while standardized stones show values of 14.2 to 20 kp, thus ranging in the upper area of values found in natural stones (Table 3; Fig. 2).

Infrared spectroscopic analysis of synthetic stones shows a close conformity with natural stones of all 3 types of standard stones measured (struvite, brushite, and whewellite). Figures 3 and 4 show infrared spectra of struvite BON(N)-STONES and of natural struvite stones. Minor quantities of substances not exceeding 5% cannot be traced by infrared spectroscopy in most cases, so that the organic matrix in these stones cannot be identified.

The crystals of the struvite basic material (Fig. 5) were rather uniform in size (8-12 μm). They were coated by layers of gelatin in the granulation process (Fig. 6). The application of these layers and their linkage give struvite BON(N)-STONES a compact surface structure (Figs. 7 and 8). The layered structure of these stones is clearly visible from their fracture surfaces (Fig. 9). The crystals in their interior are loosely arranged (Fig. 10).

Crystals of the brushite basic material (Fig. 11) range in size between 8 and 40 μm. The granulation process produces large agglomerations (Fig. 12) which may be seen in a similar structure on the surface of the stones (Fig. 13). The coherence of crystals in the stone caused by the gelatin matrix is well demonstrated in Figure 14. Crystals and their agglomerations in the interior of the stones are similar to those of natural stones. Crystals resembling basalt columns, which are typical of brushite, are found (Figs. 15 and 16).

Crystals of the whewellite (Fig. 17) range in size between 2 and 10 μm. The granulation process links crystals with one another without forming discrete agglomerations (Fig. 18). Fibrous structures (seen left) must be attributed to the gelatin matrix. On the stone surface, crystals may be seen in an obviously loose arrangement (Figs. 19 and 20). The fracture surfaces demonstrate the solidity of the concrements. Figure 21 is a slight enlargement of the center of the stone after the removal of the stone's core. Figure 22, showing the stone's fracture surface, reveals the firm coherence of crystals, which retain their original structure.

Table 1. Diameter, volume and mass of BON(N)-STONES; n = 10; x ± standard deviation (SD); % - variation coefficient.

	struvite	brushite	whewellite
diameter	0.97 ± 0.04	0.97 ± 0.04	1.00 ± 0.05
(cm)	1.4%	1.4%	1.4%
volume	0.502 ± 0.104	0.385 ± 0.067	0.468 ± 0.064
(cm^3)	6.5%	5.5%	4.3%
mass	0.78 ± 0.11	0.73 ± 0.08	0.79 ± 0.10
(g)	4.5%	3.4%	4.1%

Table 2. Density and crushing strength of BON(N)-STONES; n = 10, x ± SD; % - variation coefficient.

	struvite	brushite	whewellite
density	1.56 ± 0.10	1.88 ± 0.11	1.69 ± 0.04
(g/cm^3)	2.0%	1.9%	0.81%
crushing	16.8 ± 2.9	> 20*	16 - 20*
strength	5.5%		
(kp)			

*The test apparatus was not capable of indicating the precise results of measurements when values exceeded 20 kp.

Table 3. Comparison of density and crushing strength of struvite BON(N)-STONES and natural struvite stones {x ± SD, (min-max)}.

	density	crushing strength
BON(N)-STONES	1.56±0.10	16.8±2.9
(n=10)	(1.43-1.77)	(11.0-20.0)
natural stones	1.7±0.1	8.6±4.7
(n=20)	5.5%	(2.1-17.5)

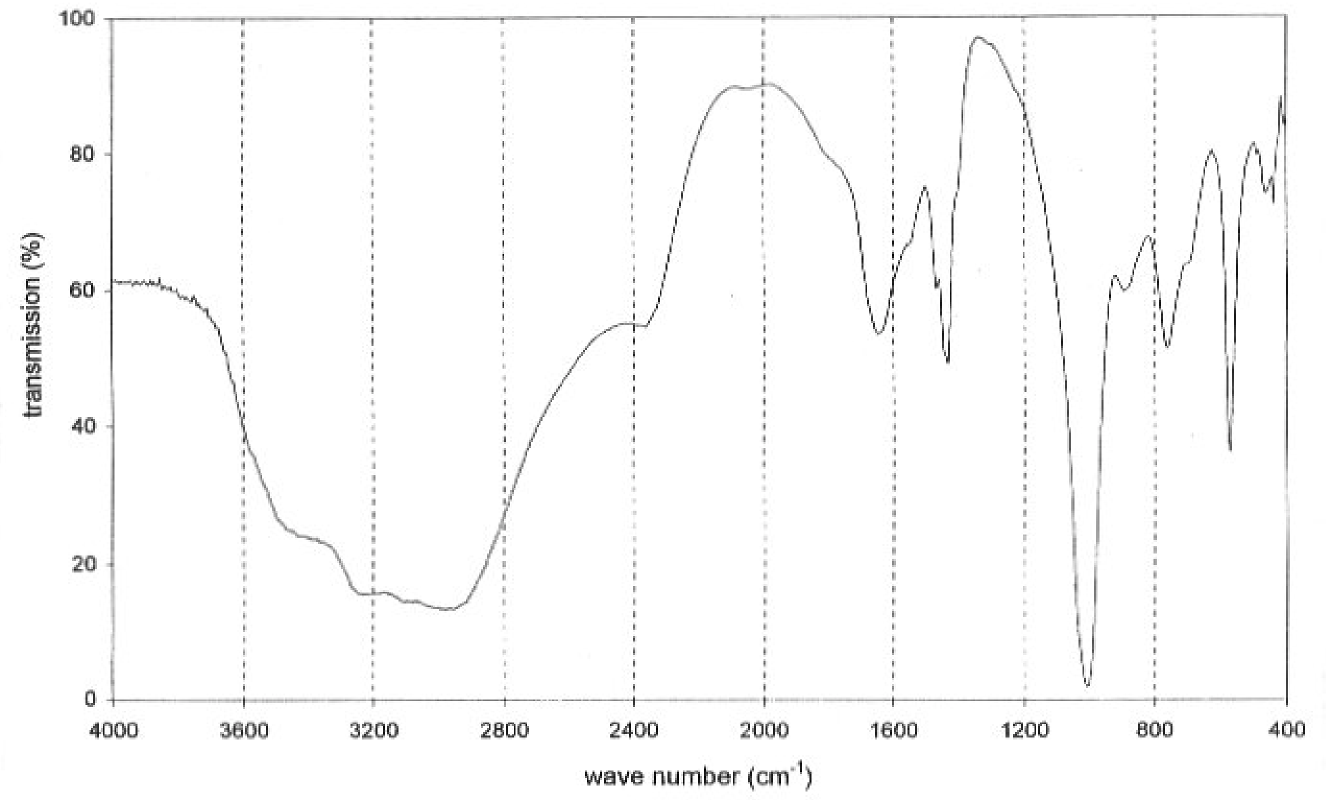

Figure 3. Infrared spectrum of a struvite BON(N)-STONE.

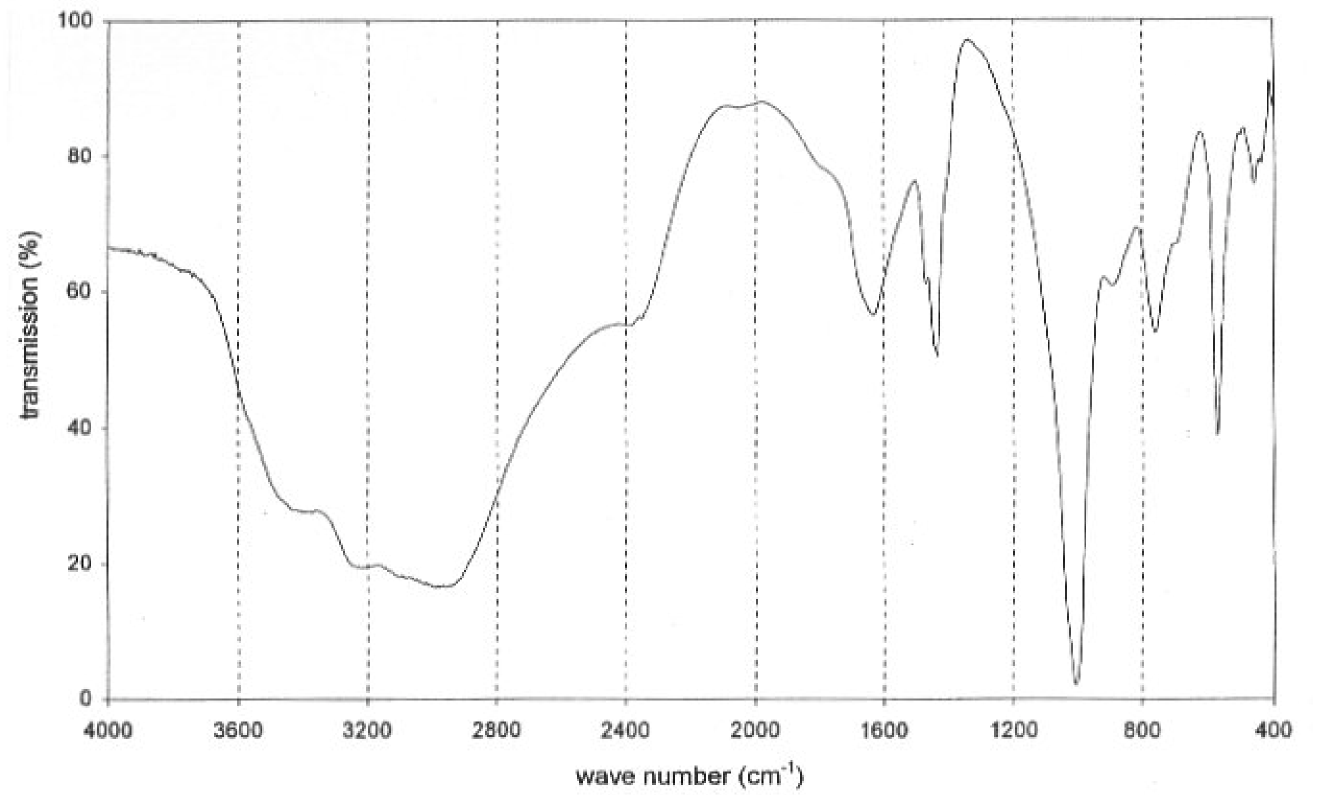

Figure 4. Infrared spectrum of a natural struvite stone.

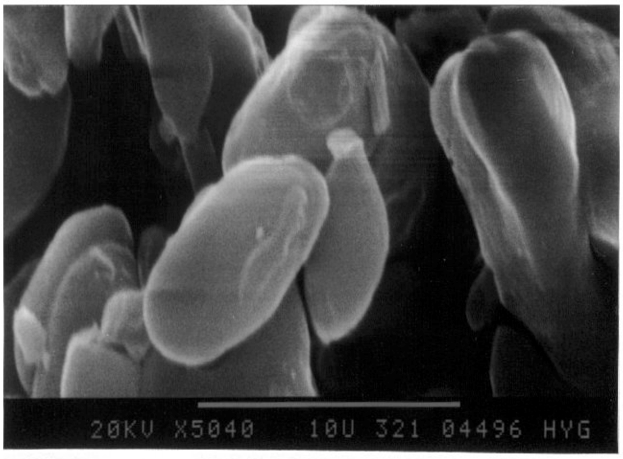

Figure 5. Basic material for the production of struvite BON(N)-STONES. Bar = 10 μm.

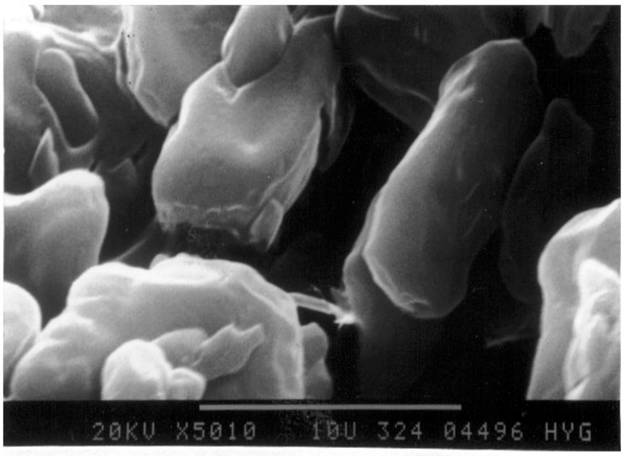

Figure 6. Struvite granulate: gelatin superimpositions. Bar = 10 μm.

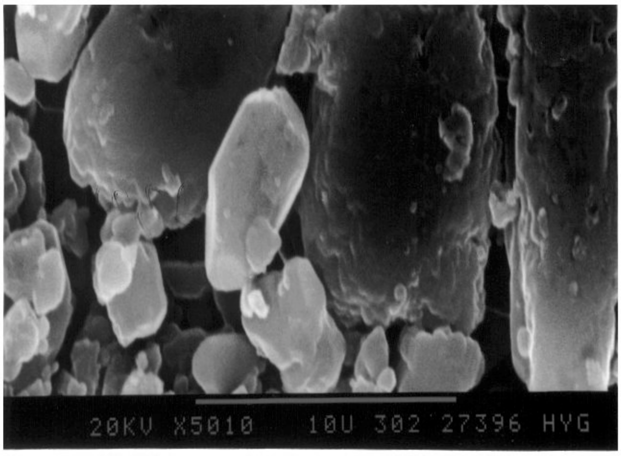

Figure 7. Surface of a struvite BON(N)-STONE: dense structure. Bar = 10 μm.

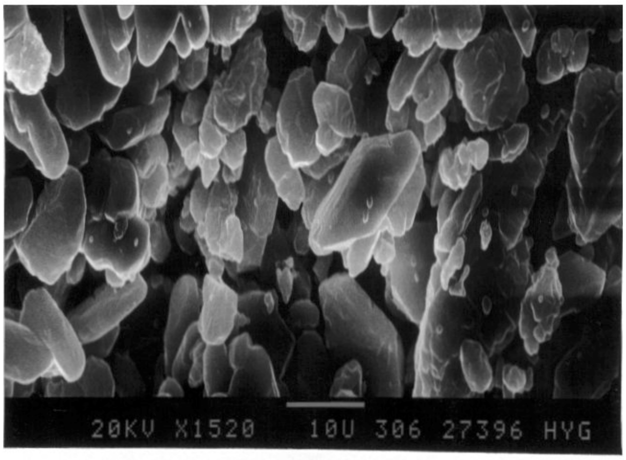

Figure 8. Surface of a struvite BON(N)-STONE. Bar = 10 μm.

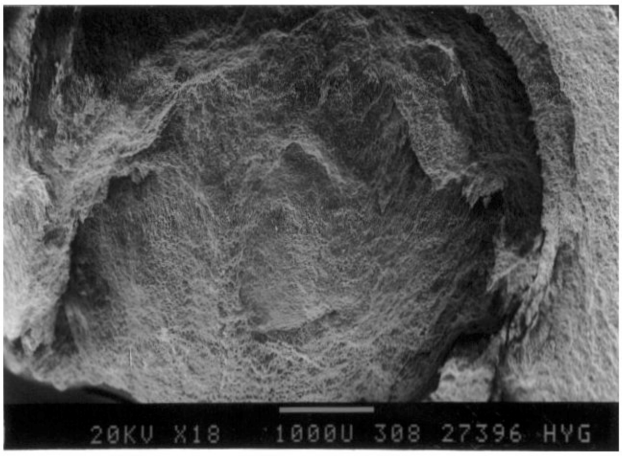

Figure 9. Fracture surface of a struvite BON(N)-STONE: shell-like structure. Bar = 1 mm.

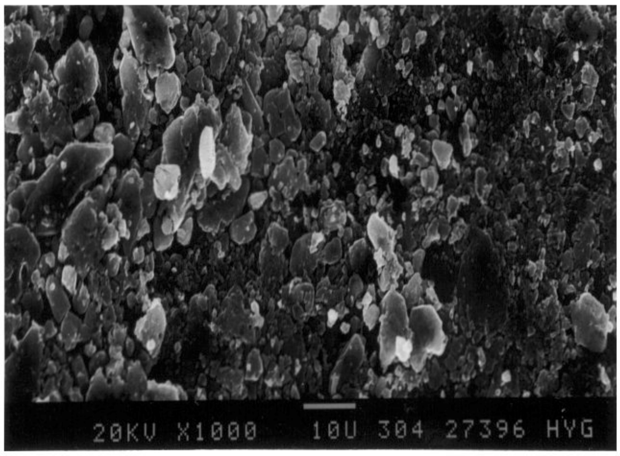

Figure 10. Fracture surface of a struvite BON(N)-STONE: rather isolated monocrystals. Bar = 10 μm.

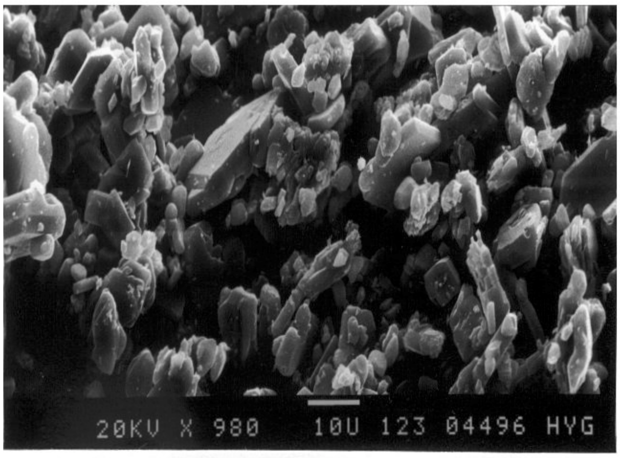

Figure 11. Basic material for the production of brushite BON(N)-STONES. Bar = 10 μm.

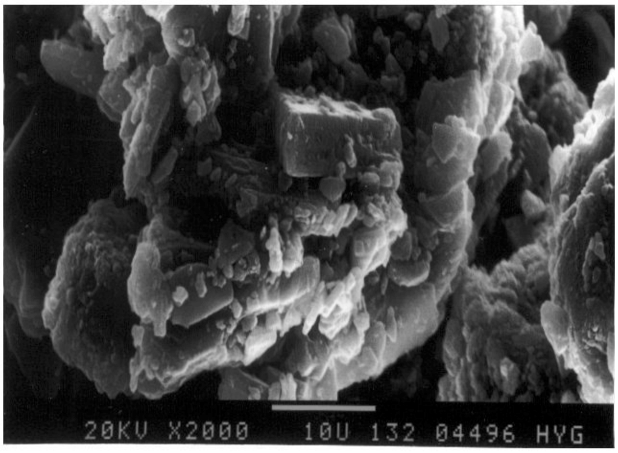

Figure 12. Brushite granulate: formation of large agglomerations. Bar = 10 μm.

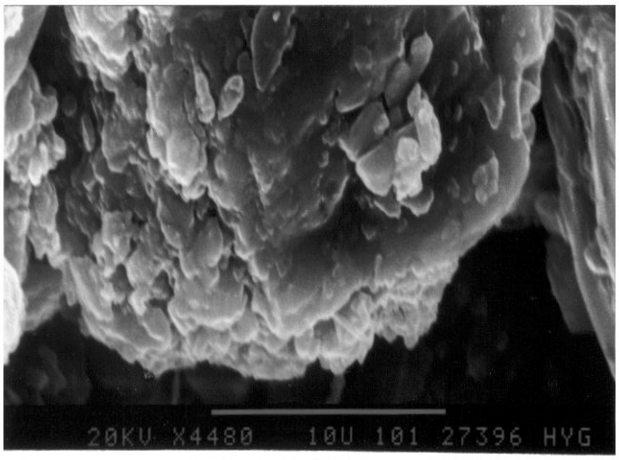

Figure 13. Surface of a brushite BON(N)-STONE: agglomerated structure. Bar = 10 μm.

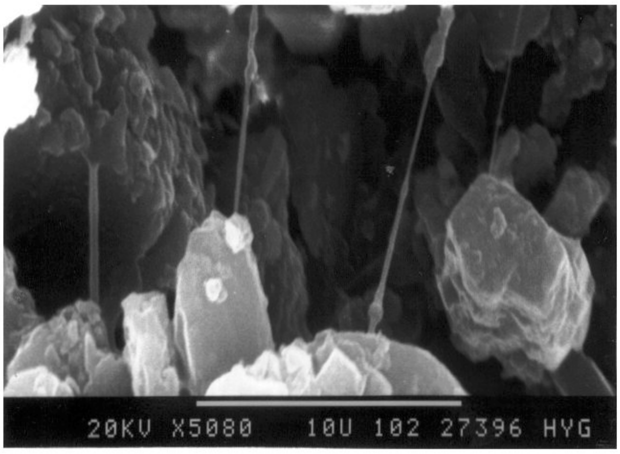

Figure 14. Surface of a brushite BON(N)-STONE: fibrous gelatin matrix. Bar = 10 μm.

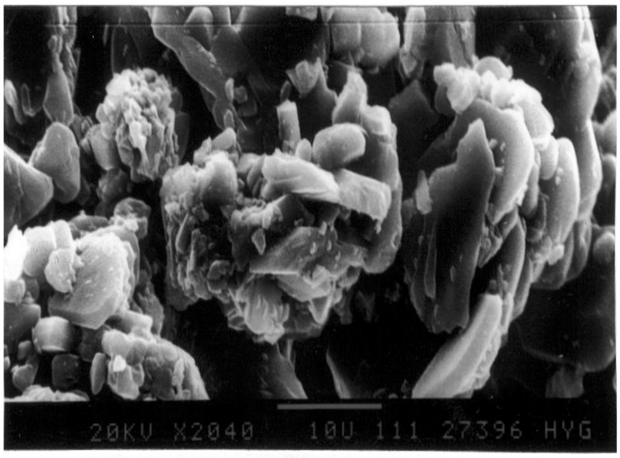

Figure 15. Fracture of a brushite BON(N)-STONE: monocrystals resembling basalt columns and large agglomerations. Bar = 10 μm.

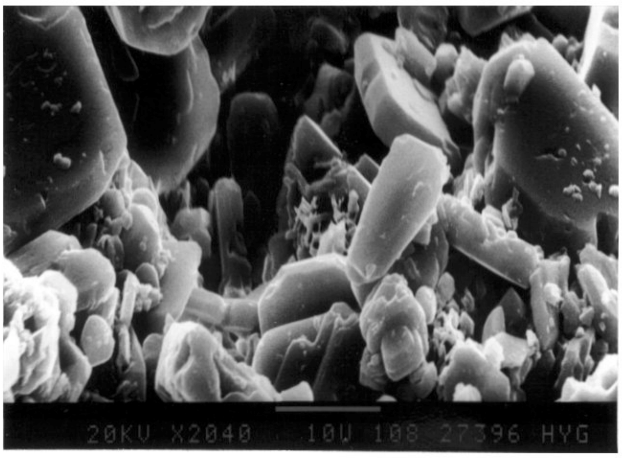

Figure 16. Fracture surface of a brushite BON(N)-STONE: typical monocrystals resembling basalt columns. Bar = 10 μm.

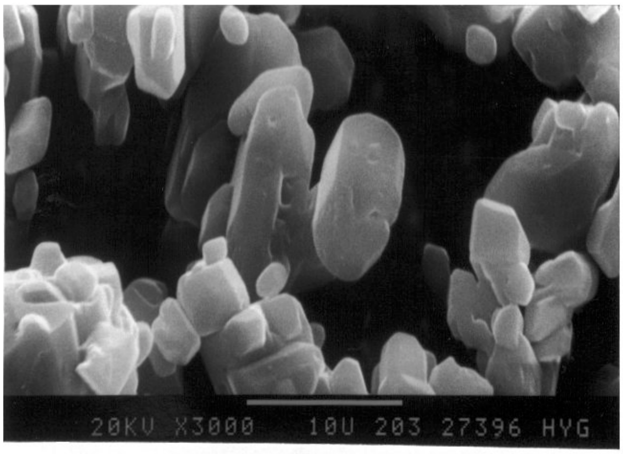

Figure 17. Basic material for the production of whewellite BON(N)-STONES. Bar = 10 μm.

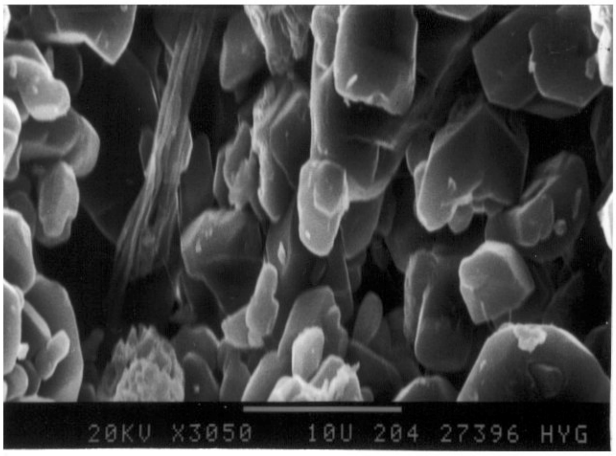

Figure 18. Whewellite granulate: fibrous gelatin matrix visible to the left. Bar = 10 μm.

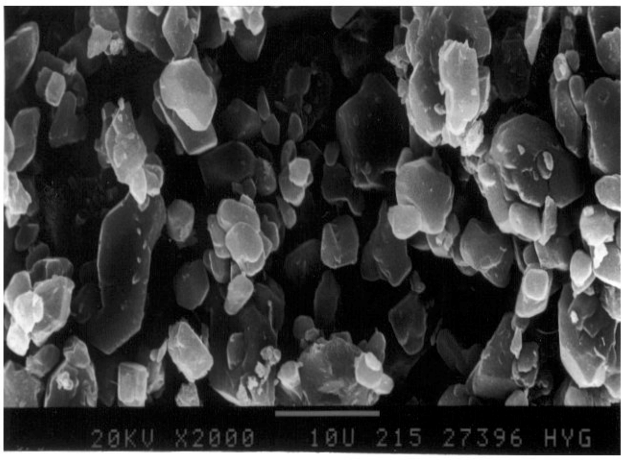

Figure 19. Surface of a whewellite BON(N)-STONE. Bar = 10 μm.

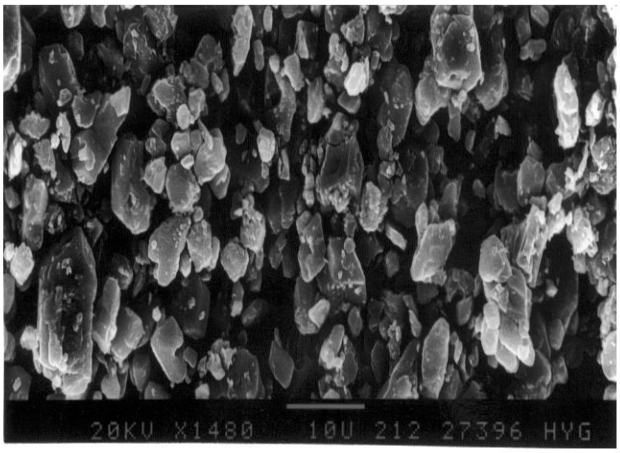

Figure 20. Surface of a whewellite BON(N)-STONE. Bar = 10 μm.

Figure 21. Center of a whewellite BON(N)-STONE. Bar = 10 μm.

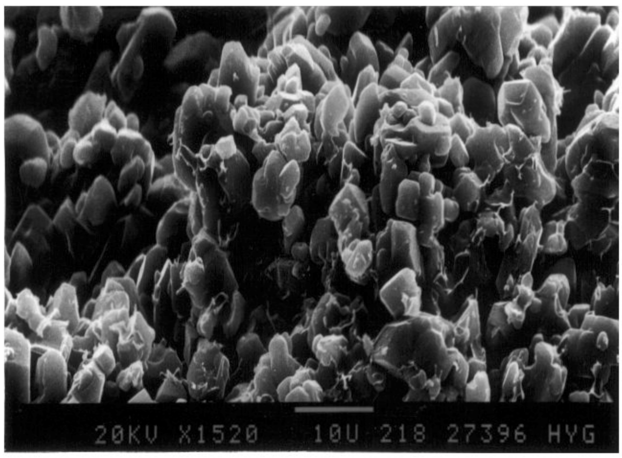

Figure 22. Fracture surface of a whewellite BON(N)-STONE: solidly packed monocrystals. Bar = 10 μm.

Discussion

The standard materials which resemble natural stones are necessary for testing the efficacy of lithotripsy methods, chemolysis procedures, and methods for the analysis of urinary stones. This applies to the composition of standard stones as well as to their physical properties such as density and crushing strength. All studies dealing with lithotriptors and laser devices have so far been carried out with varying artefacticious materials [1, 5, 6, 7, 8, 9]. These may serve as a standard for comparing the efficacy of various lithotripsy methods but is of no relevance to natural stones. In addition, these reference materials do not offer any possibility for carrying out studies on the chemolysis of various types of urinary stones. Nor are they appropriate for the verification of the quality of methods for urinary stone analysis.

The application of natural materials for the investigations mentioned has so far not been possible owing to the lack of artificial stones having the properties of natural stones. The method we have developed for the production of standardized artificial stones from natural crystals [4] allows the production of stones of any composition and size. This new method creates a stone core with numerous layers coated around it until it assumes the shape of a ball. Gelatin was used, both as a binding agent and as organic matrix, to which, when in it's gaseous stage, formaldehyde was added for cross linkage. This process supplies artificial stones with a solidity corresponding to that of natural stones.

These stones have an average mass of 0.7 g and an average diameter of 1 cm. So far, it has only been possible to make comparative tests with sufficiently complete natural struvite stones. Their density is in the same range as the density of struvite BON(N)-STONES. On the other hand, their crushing strength varies widely (Table 2; Fig. 2). The sizes, shapes, and crystal morphology of natural stones do not have the same degree of uniformity as the BON(N)-STONES. The values for the crushing strength of struvite BON(N)-STONES cover the upper borderline area as far as this type of stone is concerned.

Scanning electron microscopic (SEM) investigations have revealed crystalline structures in all of the standardized stones. The size of the crystals found here equals the size of crystals as may be found in urinary sediment. The granulation process forms agglomerations, which are of great importance to stone formation, including standardized stones.

Scanning electron micrographs also reveal differences among the various types of stones: the structure of struvite stones is characterized by the presence of a cross-linked organic matrix. Agglomerations can be identified in brushite stones, which retain their typical monocrystals resembling basalt columns. Both the outer surface and the fracture surface of the whewellite BON(N)-STONES reveal that their original monocrystals are packed very closely together. There is no evidence of agglomerations. The gelatin matrix can be identified as a fibrous substance that is found in between the crystals.

The standardized artificial stones made of natural crystals, which have been produced for the very first time - BON(N)-STONES - are rather uniform in their structure and physical properties. With regard to lithotripsy, chemolysis and for testing methods of stone analysis, we have been able to achieve a comparability of other prototypes (namely, apatite stones) with natural stones [2].

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Discussion with Reviewers

H.-G. Tiselius: My general impression from the description

of the procedure necessary to get these stones is that a lot of experience is required to get an acceptable product. Do you have any plans to produce and sell BON(N)-STONES?
Authors: The production of these BON(N)-STONES is indeed costly in both time and effort. Approximately 1000 layers are mounted onto the core, and production takes about 2 weeks. However, 300-500 stones can be produced in one batch. We now have perfected the technique for the following pure stones: apatite, struvite, brushite, whewellite, uric acid and cystine. The method of manufacture has been patented and is to be offered to a company to produce.

H.J. Arnott: Have the BON(N)-STONES been tested in lithotripsy experiments?
Authors: Yes. They have identical properties to those of natural stones, and we use them for checking the efficiency of lithotriptors.

A. Rodgers: The morphologies of the struvite, whewellite and brushite crystals in your artificial stones do not resemble those observed in natural stones (e.g., struvite crystals are normally trapezoidal, brushite crystals are columnar and whewellite crystals are coffin-shaped or dumbbell shaped). To what do you attribute these differences?
Authors: For the production of the BON(N)-STONES commercially available synthetic material was employed. These products are manufactured from aqueous solutions, and they are of fine crystalline structure. Consequently, certain crystal surface structures are missing which occur in natural urinary stones. Nevertheless, the BON(N)-STONES enjoy a high degree of crystallinity, and the typical basalt columnar crystals are detectable in the brushite stones (Fig. 16, center). The whewellite crystals in Figure 20 are typical and occur in the natural stone and in the urinary sediment, too.

A. Rodgers: Do you foresee the possibility of your procedure being used to test the efficacy of crystallization inhibitors by including them at some stage in the preparative steps?
Authors: That is a good ides, but the BON(N)-STONES are not produced in a crystallization process. The layers are created by the application of a suspension from crystals. The stone model is used to test chemolysis in liquids and to check inhibitors.

A. Rodgers: Could your method be used to produce uric acid stones? If so, why did you not do so? If not, why not?
Authors: We have now progressed to manufacturing uric acid and cystine stones as BON(N)-STONES, and they have been subject to appropriate testing.

K.M. Kim: Crushing strengths of brushite and whewellite were much higher than that of struvite (Table 2). What is the reason?
Authors: Natural stones of brushite or whewellite also have greater strengths than natural struvite stones. The intermeshing of the crystals is different.

K.M. Kim: Why is the coherence pattern of whewellite different from the other two BON(N)-STONES?
Authors: Synthetic, finely crystalline material was used for producing the BON(N)-STONES. The various products exhibit different properties during granulation. The whewellite crystals are less visibly linked through the matrix (gelatine). Their strength comes from the crystalline mesh.