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Identification of biogenetic calcite and aragonite using SEM

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ABSTRACT

Identification of calcite and aragonite is very important for studying different fossil or recent biomineralized skeletons. A problem occurs when scanning electron microscopy is used for studying calcite and aragonite present in the same part of skeleton. The same chemical composition of these two minerals produces the same contrast on SEM images. There are three possible ways to distinguish calcite and aragonite in such a mixture. (1) It is possible to recognize the crystal habits of these two minerals, if crystal faces are developed. (2) The geochemical difference can also be an important tool for distinguishing aragonite (containing large cations like Sr, Ba, or Pb) from calcite (containing small cations like Mg, Mn, Fe, Zn, or Ni). However, it is also possible that large cations remain in the calcite crystal structure after phase transformation from aragonite to calcite. (3) The third possibility for distinguishing calcite from aragonite is to use staining methods. Different staining methods used for SEM analyses have had varying degrees of success. SUZUKI et al. (1993) successfully used Meigen's staining method, but results provided by Feigl's staining method were unsatisfactory. The failure when using Feigl's staining method was caused by the erroneous application of the solutions.

Keywords: Calcite, aragonite, morphology, geochemical difference, Feigl's staining method, SEM

1. INTRODUCTION

Different methods have previously been used for distinguishing a variety of carbonate minerals at different scales – from hand specimens to thin sections and micro to nanoscale mixtures. Calcite and aragonite are the two most common polymorphs of calcium carbonate. They very often occur in mixtures within rocks and specially in different organism's carbonate skeletons – either recent or fossil ones.

Calcite $CaCO_3$ is a hexagonal mineral, point group $\bar{3}2/m$, space group $R\bar{3}c$, with a=4.9896(2) Å and c=17.0610(11) Å (ANTHONY et al., 2003).

The structure of calcite was determined by BRAGG (1914), redetermined by SASS et al. (1957) and refined by EFFENBERGER (1981). The customary way of describing the calcite structure is using sodium chloride structure as a starting point (BRAGG, 1937). If sodium chloride is set up so that a threefold axis is vertical and then compressed along the threefold axis until the edges which meet it make an angle of 101°55' with each other, and then sodium atoms are replaced by a calcium atoms, and chlorine atoms with CO_3 groups (BRAGG, 1937), the calcite crystal structure is obtained.

Aragonite, $CaCO_3$ is an orthorhombic mineral, point group $2/m\ 2/m\ 2/m$, space group *Pmcn*, with a=4.9611(4) Å, b=7.9672(6) Å and c=5.7407(4) Å (ANTHONY et al., 2003).

The crystal structure of aragonite is described as calcium atoms that lie approximately in the positions of a hexagonal close-packing lattice, deformed by compression along the hexagonal axis. In such an arrangement, each CO_3 group lies

between six calcium atoms (BRAGG, 1937). Calcium atoms in calcite and those in aragonite are arranged in approximate cubic and hexagonal close packing, respectively. In each case CO_3 groups occupy a position between six calcium atoms, but there is a difference. In calcite, the CO_3 group is placed in a way that each oxygen touches two calcium atoms. In aragonite, each oxygen atom touches three calcium atoms (BRAGG, 1937).

This difference in the crystal structure causes not only the different X-ray diffraction patterns, but also the various solubilities of these two polymorphs, which enables the use of several staining methods.

The crystal habit of calcite is extremely variable and some of the most common habits are: prismatic, thin to thick tabular, rhombohedral, or scalenohedral (PALACHE et al., 1951; GOLDSCHMIDT, 1913b). Figures 1A and 1B show the most widespread crystal habits of calcite. Untwinned crystals of aragonite are very rare. If they occur, they are usually short to long prismatic, very often needle like (PALACHE et al., 1951, GOLDSCHMIDT, 1913a). Figure 1C shows the prismatic crystal habit of aragonite. Crystal structure and chemistry are not the only factors affecting morphology. The chemical and physical conditions prevailing during crystal growth could also modify crystal habit (SPEER, 1983). During biomineralization, orthorhombic carbonates (among them aragonite) are used by different creatures to form a variety of structures to support, house and protect themselves (SPEER, 1983). Unfortunately, organisms produce skeletons of carbonate minerals mostly with close packing crystals, so, crystal habit in most cases cannot be observed in biomineralized skeletons. The crystal structure of calcite allows a wide range of compositional variation and different solid solutions, which include many divalent cations (like Mn, Fe, Mg, Zn, Co, or Ni).

Aragonite forms solid solutions with strontianite, cerusite and witherite, so, the presence of large cations like Sr, Pb and Ba could be an indicator for distinguishing aragonite from calcite. This method is not completely accurate, because of the possible phase transformation from aragonite to calcite (ŠEBEČIĆ & SLOVENEC, 1990), after which large cations including Sr, Pb, or Ba could remain in the calcite structure. If a mineral grain contains small cations (Mg, Fe, Mn, Zn, Co, or Ni), it is surely crystallized as calcite.

Staining recipes and procedures as a useful tool for calcite-aragonite differentiation were used and tested by numerous authors (FEIGL & LEITMEIER, 1933; FRIEDMAN, 1959; AYAN, 1965; DICKSON, 1966). Solutions most widely considered and used as contrasting agents for the selective staining of calcite and aragonite in electron microscopy are Feigl's (SCHNEIDERMANN & SANDBERG, 1971; SUZUKI et al., 1993; KATO et al., 2003) and Meigen's stains (SUZUKI et al., 1993; KATO et al., 2003).

FEIGL & LEITMEIER (1933) described Feigl's staining test and its mechanism is based on the different crystal structures, i.e. dissolution rates of calcite and aragonite in water.

SCHNEIDERMANN & SANDBERG (1971) first described the use of Feigl's solution in discrimination between calcite and aragonite by scanning electron microscopy. SUZUKI et al. (1993) stained molluscan shells with Feigl's and Meigen's stains and compared results by SEM, BSE and EDS. They reported much better results with Meigen's solution, because using Feigl's staining method they "were unable to find a good, size-effect-free condition". The main disadvantage of Feigl's staining was the coarse granular appearance of the staining precipitate.

KATO et al. (2003) used both staining methods for the separation of calcite and aragonite for isotopic analyses.

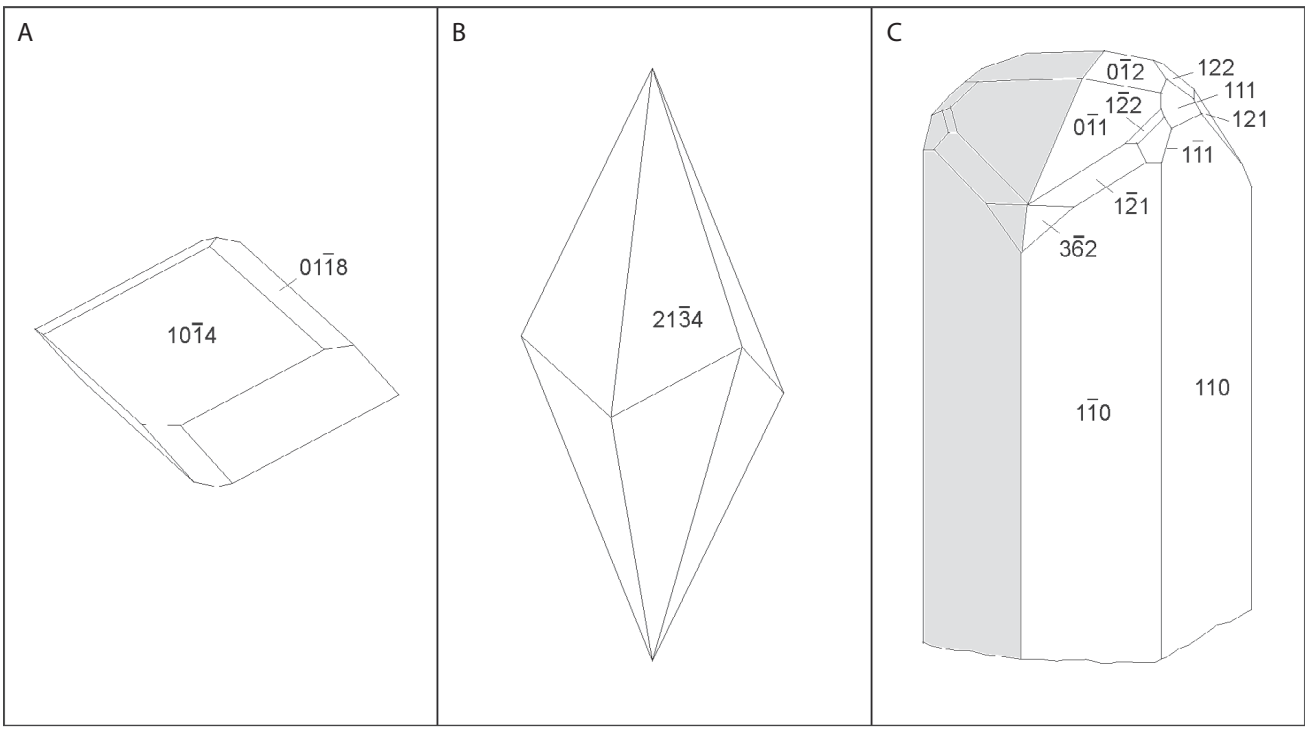

Figure 1: (A) Rhombohedral crystal habit of calcite. (B) Scalenohedral crystal habit of calcite. (C) Prismatic crystal habit of aragonite twins.

They concluded Meigen's solution was not effective for recrystallized or coarse-grained aragonites. Feigl's method was useful for such samples and investigations.

In this paper, in the description of the possible use of morphological observations, geochemical properties and Feigl's staining method are presented as useful tools for studying calcite and aragonite, separately or in mixtures, with scanning electron microscopy. Failure of the experiment described by SUZUKI et al. (1993) is the result of inconsistency in the solutions temperature, chemical composition and staining duration. Careful control of these parameters improves Feigl's staining method as a useful tool for identification of biogenetic calcite and aragonite using SEM.

2. MATERIALS AND METHODS

Two different groups of samples containing calcite and aragonite were studied. The first group consists of bryozoans *Pentapora* sp. skeletons from the Adriatic Sea, collected on Jabuka shoal from 40 to 50 m depth. A second group consists of white, sometimes macroscopically green stained sediment found around the hot well in Varaždinske toplice spa. This sediment precipitated from hot water, at least partially under the influence of some microorganisms.

Mineral phase determinations of the bulk samples were done using a Philips X'pert powder diffractometer, with CuKα radiation filtered with a graphite monochromator running at 40 kV and 40 mA. An X-ray diffraction data set was collected from 4 to 63°2θ.

Preparation of samples for SEM observations was not completely identical. Samples of bryozoans were first cleaned from their organic parts, and then fractured, finely ground, polished and mounted on the SEM stubs. They were not additionally etched prior to staining. Samples of hot well sediments were simply mounted on the SEM stubs. Both groups of sediments were then sputtered with carbon.

Morphological observations on the samples were carried out using a Tescan TS 5136 scanning electron microscope (SEM). For examination of the samples, the SEM was operating in back scattered (BSE) mode at an accelerating voltage of 20 kV and current of 10 mA. The same SEM microscope equipped with the Oxford energy dispersive spectrometer (EDS), coupled with INCA 250 system, was used for elemental distribution analysis in the samples. EDS qualitative analysis and elemental mapping was performed on the carbon coated samples at an accelerating voltage of 20 kV.

Morphological observations with SEM and elemental analysis with EDS on hot well sediment samples from Varaždinske toplice provided enough data to conclude the presence and relationship between calcite and aragonite. So, only the bryozoan samples were stained using Feigl's staining method.

The Feigl's staining method is based on the reduction of silver and manganese cations by hydroxyl anions released during aragonite dissolution, because aragonite is less stable than calcite; the solubility product of aragonite ($6.0 \cdot 10^{-9}$), represented by product of the concentration of ions (mol/dm^3) is larger than solubility product of calcite ($4.5 \cdot 10^{-9}$) (KRAUSKOPF, 1994).

The staining process could be described by the following equation (FEIGL, 1937):

Carbonate dissolution:

$$CaCO_3 + 2H_2O \leftrightarrow Ca^{2+} + H_2CO_3 + 2OH^-$$

Reaction of the cations with hydroxyl anions:

$$Mn^{2+} + 2Ag^+ + 4OH^- \leftrightarrow MnO_2 + 2Ag^\circ + 2H_2O$$

The result is precipitation of manganese dioxide and metallic silver on the carbonate surface, where the maximal concentration of hydroxyl groups lies. The precipitate is black in colour observed by naked eye or using a binocular microscope, (because both colloidal silver and manganese oxide are black), contrasting generally with white calcite and aragonite.

The solution is prepared according to the original recipe (FEIGL & LEITMEIER, 1933). 1 g Ag_2SO_4 is added to a solution of 11.8 g $MnSO_4 \cdot 7H_2O$ and 100 ml distilled water. The mixture is boiled, cooled and filtered. It is very important to neutralize the mixture with dilute sodium hydroxide until a black precipitate starts to form. After neutralization, the solution must be re-filtered and kept in a dark bottle.

Samples of bryozans *Pentapora* sp. were not stained over a set period of time, but until formation of the first light colouration. Aragonite surface colour changed from white to grayish over one to three minutes; more prolonged staining resulted in a completely black aragonite surface.

It is very important to control the complete staining process under the binocular light microscope. During staining, the solution was at room temperature.

3. RESULTS

X-ray powder diffraction patterns proved that both the carbonate shell of the bryozoan *Pentapora* sp. (Fig. 2A) and hot well sediment from Varaždinske toplice (Fig. 2B) are composed of a mixture of aragonite and calcite.

Using the SEM equipped with back scattered electron detector it was possible to recognize both calcite and aragonite crystals (Fig. 3A) on the samples from Varaždinske toplice. Recognition was based on their crystal habits.

Using of EDS allow confirmation of calcite crystal(s) or its agglomerations (Fig. 3B) and aragonite (Fig. 3C), because calcite contains a small but important and recognizable amount of Mg, and aragonite contains a small but important and recognizable amount of Sr. Small amount of S precipitates together with calcite and aragonite in the natural environment of the hot wells.

Morphological observations on the bryozoan samples were not so successful, because the crystal habit of skeleton-bearing carbonate minerals could not be observed, due to close packing of the crystals. EDS qualitative analysis and elemental mapping show that Mg is present in the inner part of a shell. Bryozoan *Pentapora* sp. samples were then stained using Feigl's staining method.

Cross sections of bryozoan *Pentapora* sp. samples showed differential staining indicating a bimineral skeletal composition (Fig. 4).

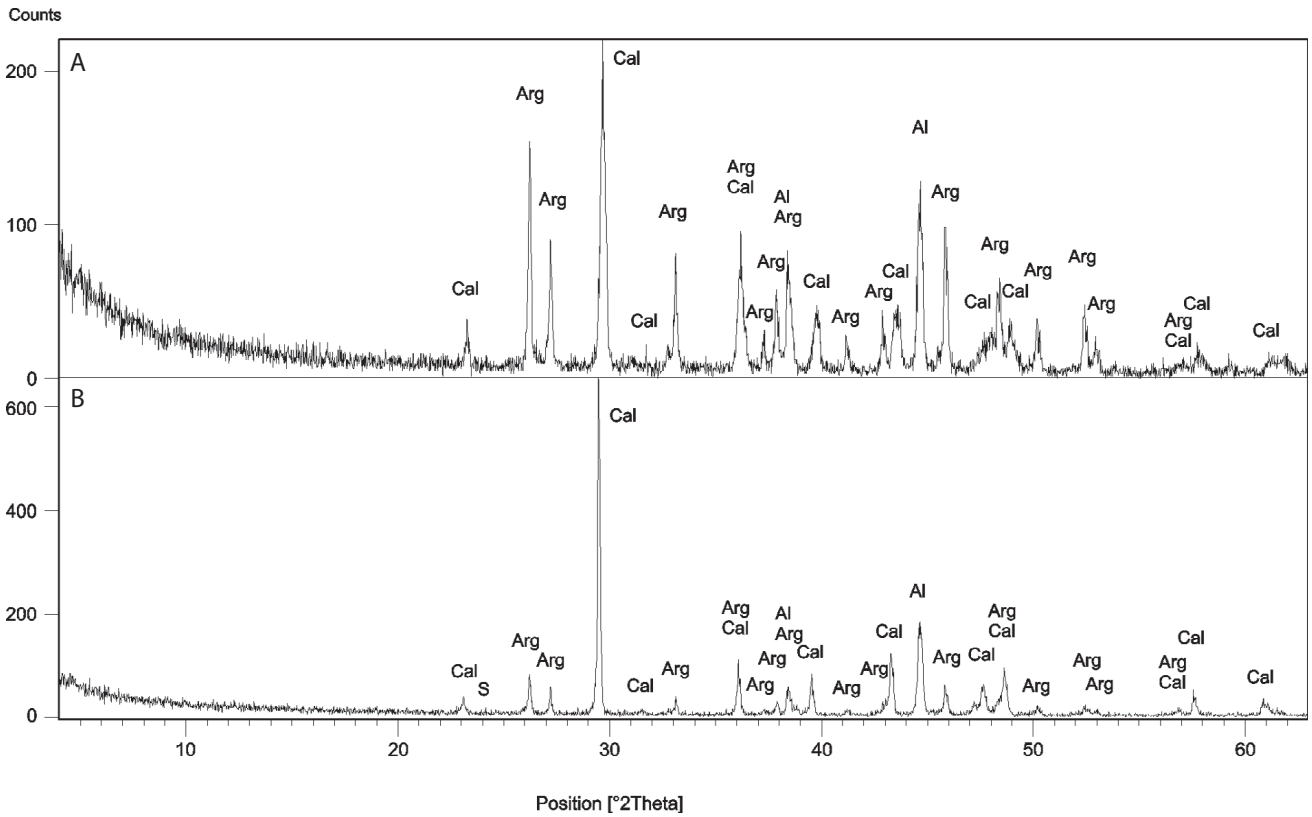

Figure 2: (A) XRD pattern of the sample *Pentapora* sp.; (B) XRD pattern of the sample from Varaždinske toplice; both showing a mixture of calcite and aragonite. Cal – calcite, Arg – aragonite, S – traces of coprecipitated sulfur, Al – aluminum from the sample holder.

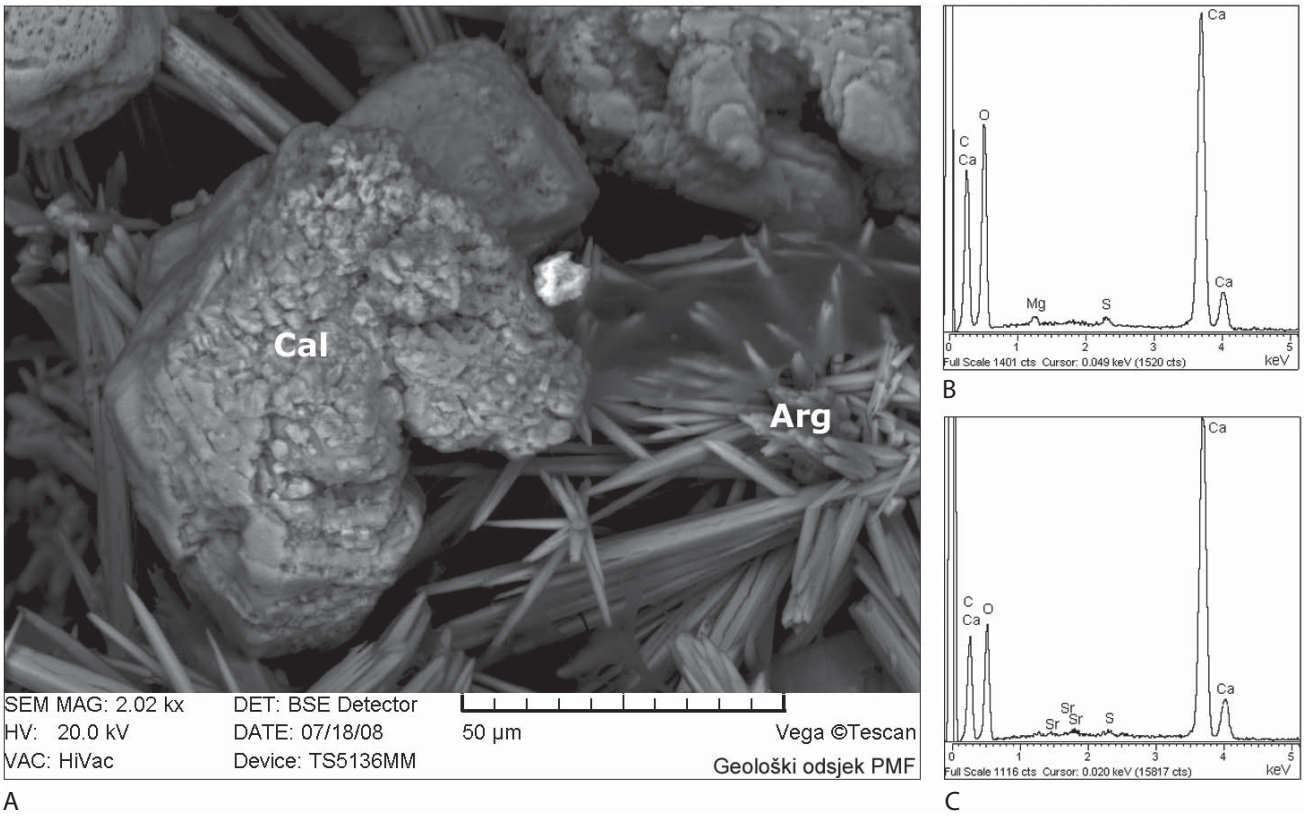

Figure 3: (A) Typical mixture of calcite and aragonite developed around the hot water well, in Varaždinske toplice under extreme conditions for living microbial organisms. Cal – rhombohedral crystals of calcite, Arg – sharp, needle like, crystals of aragonite. (B) EDS spectrum of calcite with minor content of Mg. (C) EDS spectrum of aragonite with increased content of Sr. Small, but measurable amount of sulfur also precipitate on the surface of these carbonates.

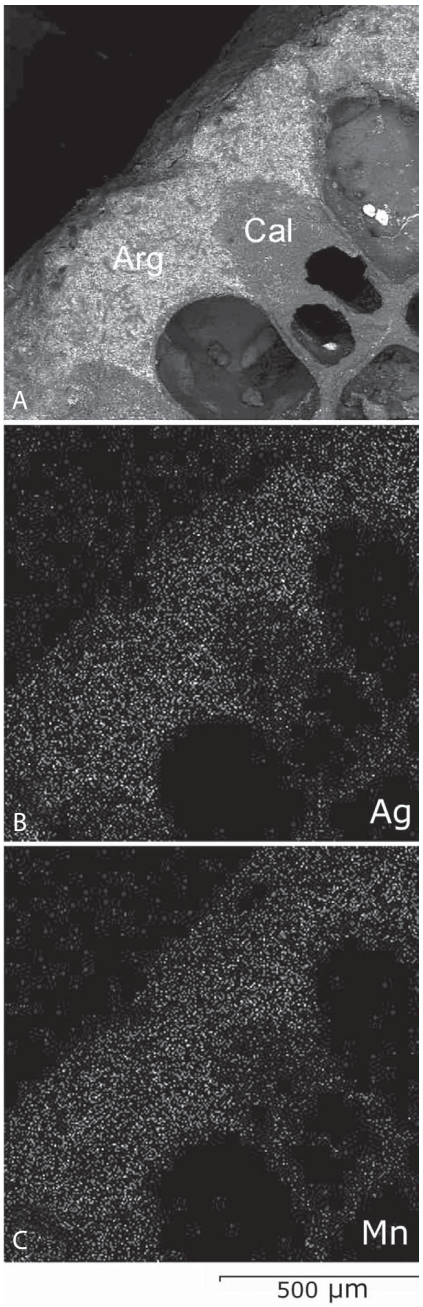

Figure 4: (A) SEM BSE image of the stained cross section of bryozoan *Pentapora* sp. with aragonite in the outer part and calcite inside the carbonate shell. Such images highlight the fine texture (smaller than a few tens of a micrometre), of the calcite and aragonite mixture of the outer shell. (B) Map of Ag. (C) Map of Mn. There is no significant difference between silver and manganese oxide deposition on the smooth aragonite surface.

Stained aragonite areas are covered with nanogranular manganese and silver precipitates, which do not completely cover the bryozoan microstructure, allowing very detailed examination. The calcite part of the shell was almost without any cover.

The black stain is easily recognizable macroscopically and under binocular light microscope, but is also easily recognizable using back scattered electron detector (BSE), because bright surfaces, rich in silver and manganese, on images indicate aragonite. Using light microscope allow to recognize presence of aragonite and/or calcite, but using of electron microscope it is possible to recognize the fine textures, for example the structure of outer bryozoan shell on the SEM BSE image. Mapping of such stained areas shows that there is colloidal silver and manganese oxide deposited homogeneously on aragonite (Fig. 4).

4. DISCUSSION

Many shells or skeletons have a bimineral composition. The ratio of calcite and aragonite could be determined by X-ray investigation (ANDERLE et al., 1998).

Such organisms usually have an aragonite inner and calcite outer layer of the shells. However, this could also be the opposite way around. It is not possible to recognize this arrangement using X-ray diffraction, except if it is combined by careful and sometime impossible separation. Nevertheless, it is possible to stain the sample by Feigl's solution. Using Feigl's staining method in combination with SEM BSE observation provides the opportunity to recognize not just aragonite distribution inside the shell wall, but also any fine textures.

The rates of dissolution of calcite and aragonite in the Feigl's solution control the intensity of manganese dioxide and metal silver precipitation and the size of deposited particles on the crystal surfaces. Aragonite dissolves more rapidly in the staining solution and will be stained by silver and manganese oxide, while calcite reacts slower and remains unstained.

The speed of carbonate dissolution and staining is altered if the staining solution concentration or temperature is changed. However, the speed of dissolution also depends on carbonate crystal size. Dissolution speed decreases with increasing size of the mineral grains. The rate of dissolution also varies with the crystal orientation, because of anisotropy.

Improper use of Feigl's method (e.g. too fast or too slow dissolution) produces coarse grains of metal (Ag and MnO_2)

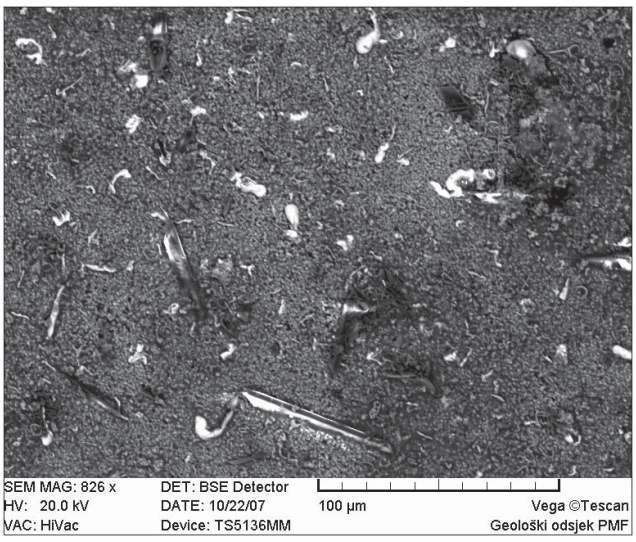

Figure 5: SEM BSE image of the manganese-oxide and metal silver precipitate on the aragonite surface of *Pentapora* sp. The precipitate is very coarse (up to 100 µm long) because of rapid aragonite dissolution in the very acidic Feigl's solution. Such preparation is inadequate for the microstructural observation of aragonite microstructures. White fragments are silver particles and gray particles are manganese oxide.

up to a few μm in size. The grains of silver and manganese oxide are too big for observing fine texture and cover both minerals – aragonite as well as calcite. This will happen when the solution is too acid and the procedure is too rapid. Such conditions caused failure of the method in the case of SUZUKI et al. (1993). If the pH is too low, dissolution of both calcite and aragonite produce an inconsistent and inadequate stain, as can be seen in Fig. 5.

Meigen's solution containing HCl acts more aggressively on aragonite dissolution than Feigl's stain. This means that Feigl's staining method allows preservation of more of the delicate mineral features of the bryozoan mineral textures (up to 10 μm). If the staining procedure is carried out carefully, with binocular light microscope control, it is possible to obtain a black manganese oxide and silver precipitate of nanometer size. If Feigl's solution is not neutralized properly, biogenetic carbonates will be corroded on the surface and this process will be easily visible using SEM BSE. So, SUZUKI et al., (1993) dismissed Feigl's staining method without any reason and favoured the less successful Meigen method.

5. CONCLUSION

Identification of aragonite and calcite is very simple by X-ray diffraction methods. But, it is not possible to describe the textural distribution of these carbonates using such methods.

The first and most logical aragonite-calcite differentiation by SEM BSE is on the basis of crystal morphology. Biogenetic carbonates, i.e. those mostly precipitated under microbial control, could sometimes be recognized (Fig. 3A). However, in most of the biomineralized skeletal structures, the typical crystal morphology is not developed.

Discrimination of two carbonate phases could be based on the large and small cation (usually strontium and magnesium) content of aragonite and calcite respectively, determined by EDS if these elements are present (Figs. 3B, 3C).

If none of the above-mentioned methods could be applied, staining methods alone will be useful. Staining with Feigl's solution can be successfully used for observations in mixtures in biogenetic carbonates using SEM BSE examination. Feigl's solution cannot be used as a standard procedure with fixed staining time or prescribed solution temperature. Because of this, binocular light microscope control is necessary. The difference in crystal structure of aragonite and calcite produces varying dissolution rates and without carefully monitored staining procedure, they will show inconsistent results or a coarse precipitate.

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