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PRELIMINARY RESULTS ON THE CHEMICAL PREPARATION OF DINOSAUR EGGSHELLS

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ABSTRACT

Traditionally, techniques used in the preparation of fossil eggshells are based on the similarity of the matrix and the eggshells. Often, these techniques involve aggressive preparation and frequently lead to significant dissolution or abrasion of the eggshells. The damage often does not allow proper taxonomical studies, because eggshell features were potentially destroyed. Based on our experience, we propose a new method for the chemical preparation of fossil eggshells, specifically those composed of calcium carbonate.

Keywords: Dinosaur eggshells, chemical preparation, chemical agents, microscopy

RESUMO [in Portuguese]

Tradicionalmente, as técnicas usadas na preparação de cascas de ovo fósseis baseiam-se na semelhança entre a matriz e as cascas de ovo. Muitas vezes estas técnicas envolvem uma preparação agressiva e levam frequentemente à dissolução e abrasão significativas das cascas de ovo. Os danos muitas vezes impedem estudos taxonómicos adequados porque as características das cascas são potencialmente destruídas. Baseados na nossa experiência, propomos um método novo para a preparação química de cascas de ovo fósseis, especificamente as compostas de carbonato de cálcio.

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INTRODUCTION

This work summarizes preliminary results on cleaning fossil eggshells. These treatments can be used for the study of the surface under electronic microscope of both small fragments and complete fossil eggs.

Cleaning of the surface of fossil eggshells for electron microscopy is usually very difficult. In order to be able to study its microstructure under the microscope, the elimination of the matrix that covers the eggshells without damaging them is crucial. Eggshells cannot be protected with any resin layer to prevent them from being damaged by cleaning products, since it would prevent thorough cleaning of the eggshell surface, and it would not allow us to clearly observe the structure of the eggshell. The difficulty of the cleaning techniques used in these first tests lies in finding a good cleaning product that could not damage the eggshell. In our case, fossil eggshells are mainly composed of calcium carbonate, and thus very similar to the types of matrix that cover them, which are usually very rich in calcium carbonate (Quinn, 1994).

Traditionally, cleaning fossil eggshells for electron microscopy has been done using organic acids such as acetic acid (Jeppsson et al., 1985; Quinn, 1994; Rutzky et al., 1994; Shelton, 1994). However, this technique usually damaged the eggshells, and did not allow accurate observations of their surface.

The research team "Àrea de recerca del Mesozoic" of the Institut Català de Paleontologia Miquel Crusafont (ICP) is continuously studying dinosaur fossil eggshells and, for this reason, members of our team have been working for years trying to find the best cleaning techniques for these fossil eggshells that need to be studied after preparation. Our first studies on cleaning techniques were performed on Titanosaur fossil eggshells from the Fumanya area (Berguedà, Catalonia, Spain), which were embedded in marls with a composition of 50% calcium carbonate and 50% silicate (Val, 2007). During these first studies, we tried to dissolve the noncarbonated part of the matrix in order to prevent damaging the carbonate in the eggshells. To do so, we used chemicals that dissolve the silicate part of the matrix. The results obtained using these alkaline agents were better than other acid cleaning techniques. Highly alkaline agents dissolve silicates converting them into very soluble crystals (San Andres Moya and de la Viña Ferrer, 2004). Afterwards, we performed more cleaning trials with other types of matrix, the results of which were presented in Val et al. (2010). Those essays were performed with carbonated matrices, matrices with a high content of iron oxides, as well as matrices with a high silicic composition. The main feature common to all those matrices was their hardness and their resistance to many cleaning agents and techniques.

The herein presented preliminary study consists of an analysis made using an electron microscope to study the effects of various chemical treatments on eggshells.

METHODOLOGY

Preparation techniques were conducted on dinosaur eggshell fragments collected from several Late Cretaceous deposits (Tremp Fm.) in the South-Pyrenean basins (Catalonia, NE Iberian Peninsula; see Figure 1). Sampled localities occur in continental facies that include mudstones, marls, oncolite limestone and fine to medium, well-cemented sandstones. In most cases, eggshells are imbedded in a highly carbonated matrix that strongly hinders the removal of secondary deposits.

The different treatments were conducted with different volume concentrations, starting with 2% until reaching the percentage of optimal cleaning. All the samples were subjected to the same dilution percentage, at the same temperature and the same time of exposition. Following the first standard tests, we tried to find, in each case, the best percentage of dissolution and the best time of exposition of each one. In order to observe the results obtained for each treatment in detail, we used an environmental SEM (FEI Quanta 200) at the Serveis Cientifico Tècnics of the Universitat de Barcelona. We compared the damage suffered by the eggshells with each treatment. An image of a non-treated eggshell was included as control (Figure 2). The optimal cleaning was defined as the one that allowed us to identify the oxygenation channels properly, and where the morphology of the surface of the eggshell was not or minimally altered in comparison with the eggshell used as control.

All trials were made with fragments of similar size (1 cm^2 approximately) and with a volume of 40 ml of dissolution for the different percentages of each chemical agent. Cleaning essays were performed using ultrasonic baths (with a duration of 15 min), which accelerated the cleaning process and increased the penetration capacity of the cleaning agents. It is important to emphasize that these cleaning techniques are useful for individualized eggshells that need to be studied under the microscope, but when cleaning complete fossil

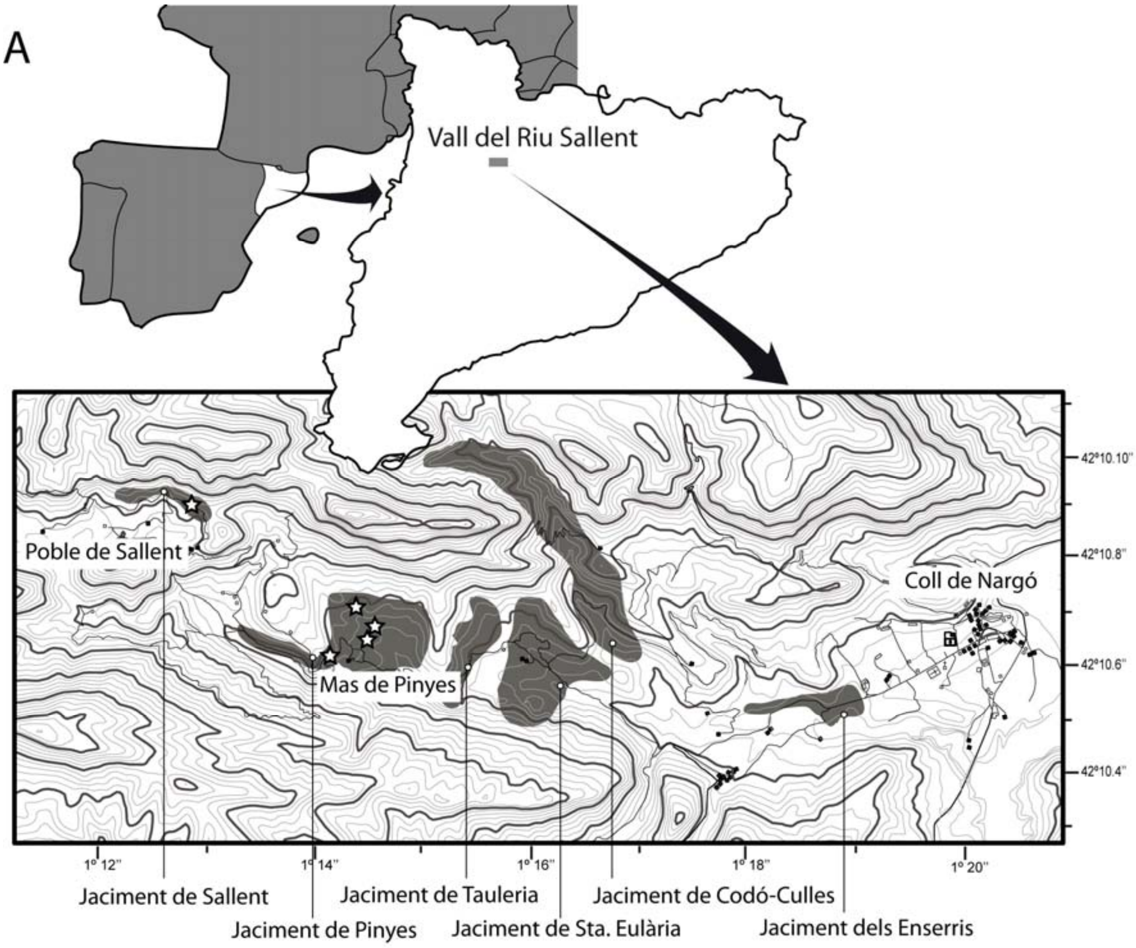

Figure 1: Map showing the area where the sites are located. Image by Albert García; Grup Mesozoic ICP.

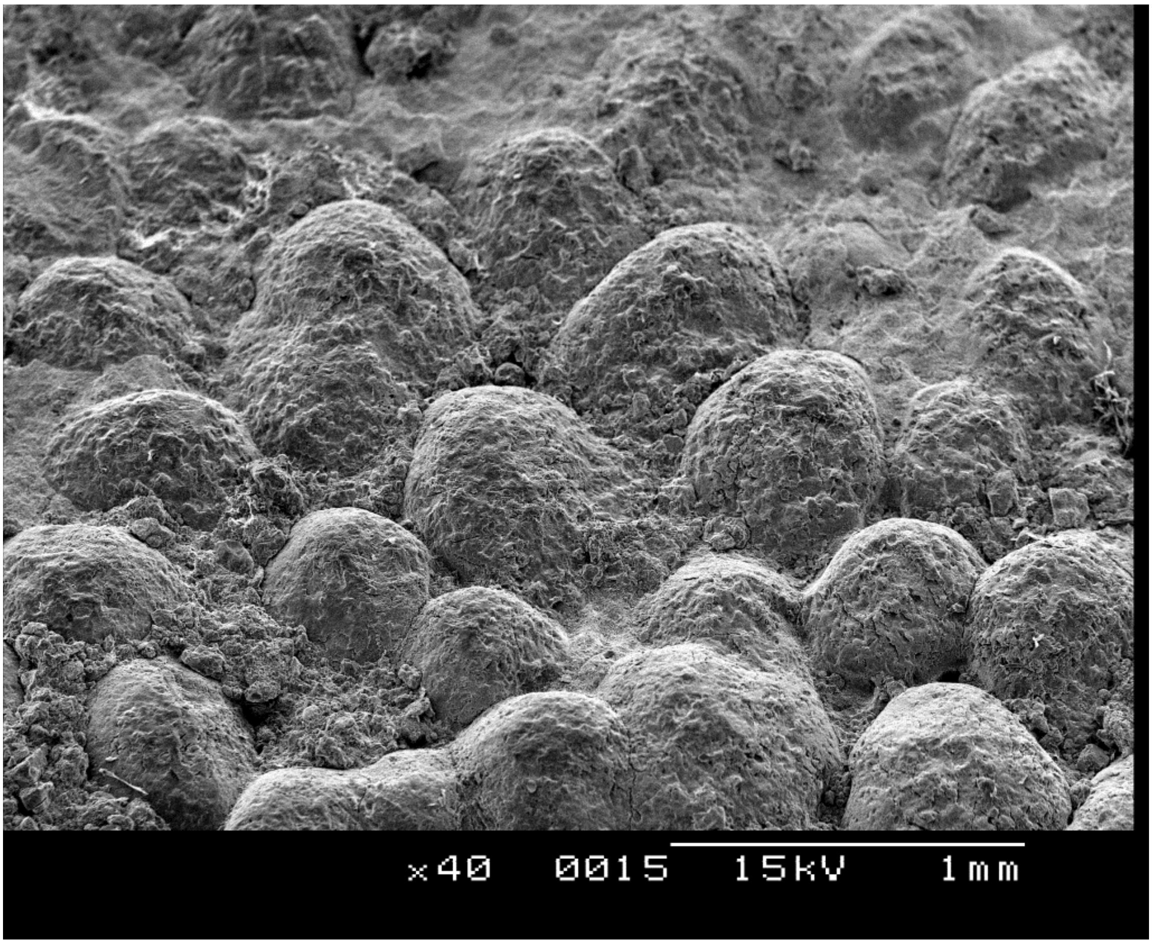

Figure 2: Non-treated eggshell. Impossible to see details of the relief or to determine the position of the oxygenation channels.

eggs, full immersion is not recommended because it could cause the eggshell to break to pieces. In the case of complete eggs, cleaning techniques are performed using bandages and easy-to-neutralize cleaning agents (Val et al., 2013). It is important to stress that any chemical treatment used must be neutralized in order to prevent future damage to the specimens.

During any chemical preparation, health hazards must be known and corresponding actions taken in order to prevent any risk. It is paramount to know the toxicity of the used chemical agents, and the products that can be created in various chemical reactions. Each chemical requires specific security equipment, but as a rule, we perform the tests under a ducted fume hood, and use personal protective equipment to chemical agents such as goggles, gas masks, gloves and acid resistant lab coats. Also, the health and safety regulations must be available for each product.

RESULTS

For this paper we have selected the more significant results obtained from all the essays performed. They have been grouped by the treatment used and the problems that arose during the different essays depending on the types of matrix involved.

Acids: for dissolving carbonated matrices in sandstones

The carbonates that make up the matrix can be dissolved by acids. Usually, the most commonly

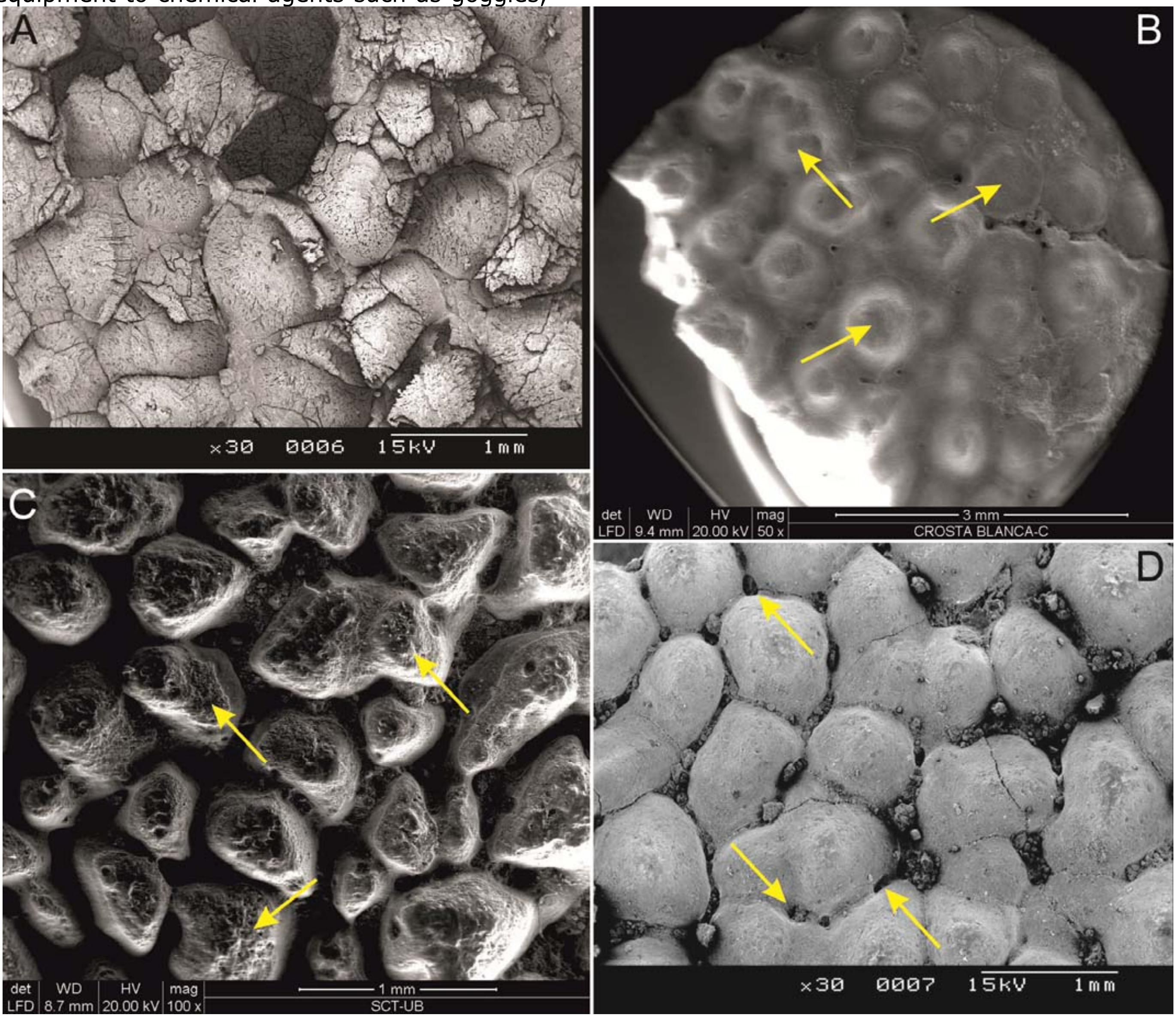

Figure 3: Eggshells treated with acids. A) Acetic acid at 10%. The relief of the eggshell has been damaged and eroded. B) Hydrochloric acid at 15%. The surface of the eggshell has been highly eroded (arrows). C) Oxalic acid at 10%. The relief of the eggshell could be observed, but the surface was slightly altered (arrows) and the oxygenation channels could not be detected. D) Sodium hexametaphospate $(NaPO_3)_6$ at 15%. The relief of the eggshell could be observed, its surface did not seem altered and the oxygenation channels could be detected (arrows).

used acids are organic, such as acetic acid CH_3-COOH ($C_2H_4O_2$) (Figure 3A) and formic acid H-COOH (CH_2O_2) (San Andres Moya and de la Viña Ferrer, 2004). In very hard matrices, inorganic acids have also been used. Some examples are hydrochloric acid (HCl), (Figure 3B) and sulfuric acid (H_2SO_4). We have also tested oxalic acid $H_2C_2O_4$ (Figure 3C), an organic acid 3000 times stronger than acetic acid, and commonly used for eliminating iron oxide concretions from archeological iron (Mourey, 1987). Nevertheless, there are other agents that can act on calcium carbonate in a less aggressive way, and that transform calcium carbonate into other carbonates that are more soluble in water, and thus easier to eliminate without using acids. One example is sodium hexametaphosphate $(NaPO_3)_6$ (Figure 3D), a salt that transforms calcium carbonate into sodium carbonate. This agent is commercially available with different pH. For the present study, we used the agent with a pH value of six, and thus slightly acidic. Therefore, we included it among the acids, even though it is not considered an organic acid. The treatment with $(NaPO_3)_6$ is widely used in other fields of heritage conservation and preparation, and it yielded excellent results when used for eliminating carbonate concretions. In our case, treatments performed using sodium hexametaphosphate $(NaPO_3)_6$ have been highly effective and poorly aggressive (Val, 2007; Val et al. 2010). Table 1 shows the concentrations working best for each treatment.

Table 1: acidic agents.

CHEMICAL AGENT		%	OBSERVATIONS
Formic acid	H-COOH (CH_2O_2)	10%	Surface highly damaged
Acetic acid	CH_3-COOH ($C_2H_4O_2$)	10%	Surface highly damaged (Figure 3A)
Hydrochloric acid	(HCl)	10%	Surface highly damaged (Figure 3B)
Oxalic acid	$H_2C_2O_4$	10%	Surface slightly eroded (Figure 3C)
Sodium Hexametaphosphate $_6$ (pH 6)	$(NaPO_3)_6$	15%	Optimal cleaning (Figure 3D)

Table 2: alkaline agents.

CHEMICAL AGENT		%	OBSERVATIONS
Potassium hydroxide	KOH	10%	Optimal cleaning % (Figure 4A)
Sodium hydroxide	NaOH	4%	Surface slightly eroded (Figure 4B)
Sodium hexametaphosphate	$(NaPO_3)_6$	15%	Surface slightly eroded

Alkalies: for dissolving silicates present in matrices composed of 50% carbonate and 50% silicate marls

Alkaline agents dissolve silicates present in the matrix without attacking the calcium carbonate of the eggshells. As can be observed in figures 4A and 4B, the most effective treatments are the ones using potassium hydroxide (KOH; Figure 4A), as shown in the absence of degradation in the surface and the good observation of all microstructures of the eggshell. However, they are difficult to apply and neutralize. In those cases, we highly recommend neutralization using an ultrasound bath twice with 80 ml of distilled water (double the volume) during 30 min (double the time of exposition). Moreover, we tested sodium hydroxide (NaOH; Figure 4B; Fernández et al., 2005), but in a lower percentage, due to its more aggressive character, and because we could note more abrasion on the surface and degradation of the microstructure of the eggshell. Finally, we used Sodium hexametaphosphate $(NaPO_3)_6$, with a pH value of eight, but contrary to the results obtained with the acidic version, the alkaline Sodium hexametaphosphate did not yield positive results. Table 2 shows the concentrations for obtaining the best results for each treatment.

Other chemical agents

Mixtures to dissolve carbonate in hard matrices with ferric iron

The dissolution of matrices containing high amounts of iron oxide is problematic, since there are no established protocols or guidelines for doing so. Thus, we test ed new and old treatments used in paleontological conservation/preparation (Rutzky et al., 1994), and also in other fields of conservation/restoration of cultural property (Mourey, 1987). This type of matrix is very hard and resistant to many treatments, including mechanical work. The most commonly used method working with this type of matrix is the Waller Method (see Figure 5A; Waller, 1980; Blum et al., 1989; Rutzky et al., 1994). The Waller method uses a solution of sodium

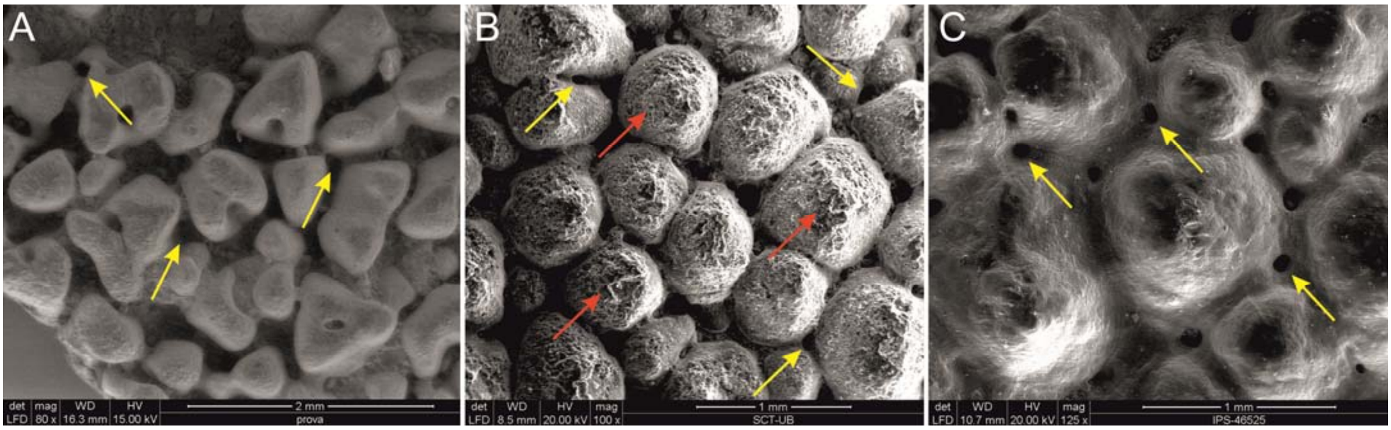

Figure 4: Eggshells treated with alkalies. A) Potassium hydroxide KOH at 10%. The relief of the eggshell could be observed, the surface has not been altered and the oxygenation channels were also detected (arrows). B) Sodium hydroxide NaOH at 4%. The relief of the eggshell could be observed, but the surface was slightly damaged (red arrows). The oxygenation channels were observable (yellow arrows). C) Potassium hydroxide KOH at 10%. The relief has not been damaged and the oxygenation channels (arrows) are perfectly observable.

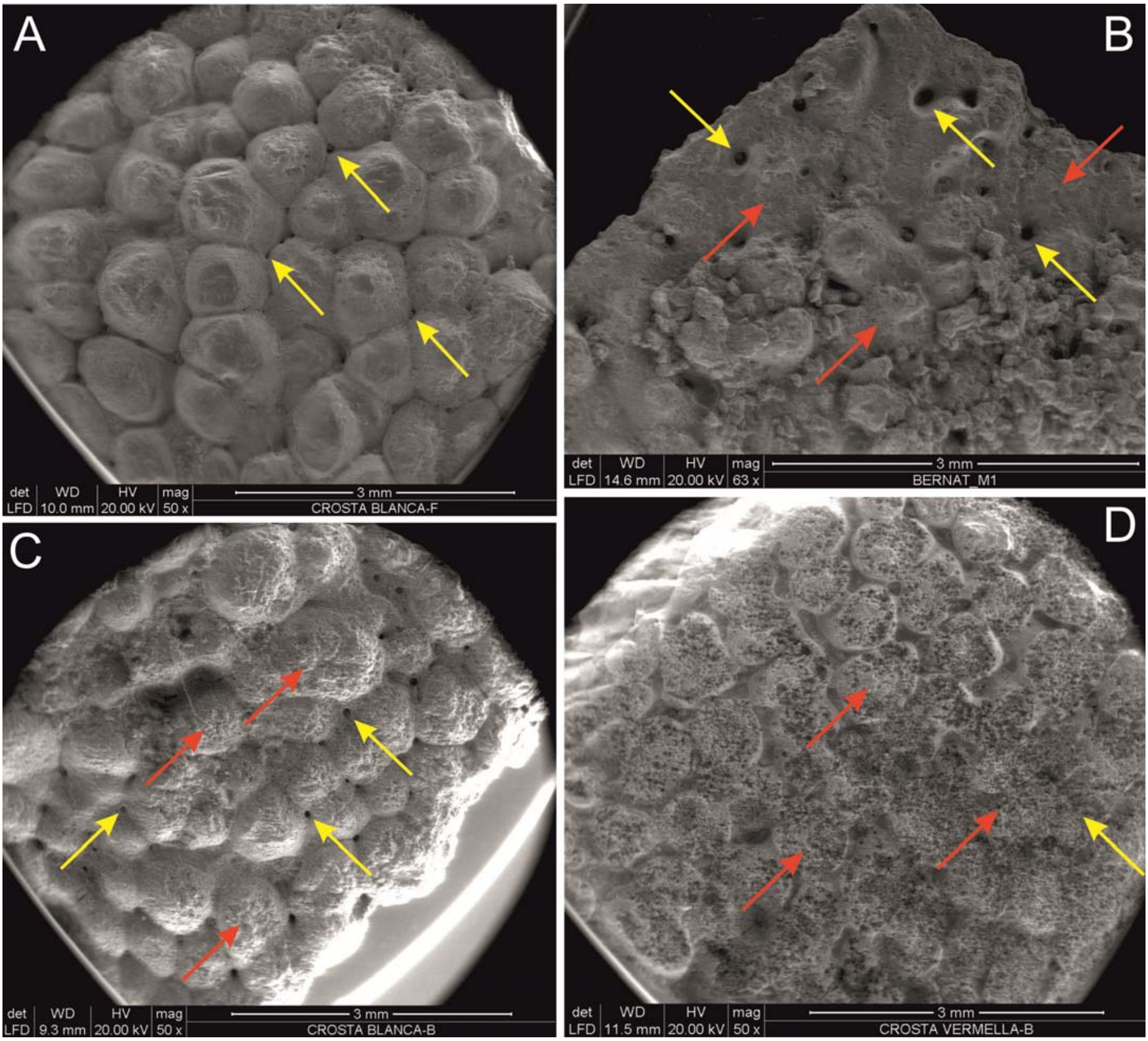

Figure 5: Eggshells treated with mixtures: A) Waller Method: Sodium citrate 71gr + Sodium bicarbonate 8,5gr + Sodium dithionite 20gr. It is possible to observe the oxygenation channels (arrows) and the relief perfectly. B) Ethylenediaminetetraacetic acid (EDTA) at 5%. The oxygenation channels could be detected (yellow arrows) but the relief was highly eroded (red arrows). C) Ethylenediaminetetraacetic acid (EDTA) at 5% with Sodium hydroxide at 4%. The relief of the eggshell could be observed, its surface was somewhat altered (red arrows) and the oxygenation channels could be detected (yellow arrows). D) Sodium hexametaphospate at 15% + Waller Method. The relief has been partially eroded (red arrows).

citrate, sodium bicarbonate and sodium dithionite. This method does not use acids, and therefore, dissolution of the calcium carbonate of the eggshell is avoided. Dithionite reduces ferric iron to ferrous iron, which is soluble; citrate sequesters ferrous iron; and bicarbonate buffers the pH to maintain the solution neutral. Additional treatments for dissolving concretions of iron oxides, and tested herein include Oxalic acid ($H_2C_2O_4$) and ethylenediaminetetraacetic acid (EDTA; see Figure 5B; Mourey, 1987). However, due to the hardness of these matrices, we had to test EDTA at 5% with sodium hydroxide at 4% (see Figure 5C), in order to dissolve them (Fernández et al., 2005). Sodium hexametaphosphate $(NaPO_3)_6$, when used in combination with the Waller Method, had to be diluted at 15% (see Figure 5D), in order to make the products more reactive. Table 3 shows the concentrations and results for each treatment.

Table 3: mixtures for carbonate in hard matrices with ferric iron.

CHEMICAL AGENT		%	OBSERVATIONS
Waller Method: Sodium citrate 71 gr. Sodium bicarbonate 8.5 gr. Sodium dithionite 20 gr.		-----	Optimal cleaning (Figure 5A)
Ethylenediaminetetraacetic acid (EDTA)	$C_{10}H_{16}N_2O_8$	5%	Not effective cleaning (Figure 5B).
Ethylenediaminetetraacetic acid (EDTA) Sodium hydroxide	$C_{10}H_{16}N_2O_8$ NaOH	5% 4%	Surface slightly eroded (Figure 5C).
Sodium hexametaphospate Waller Method	$(NaPO_3)_6$	15% ----	(Figure 5D).
Oxalic acid	$H_2C_2O_4$	10%	Surface slightly eroded

Table 4: organosulfurs.

CHEMICAL AGENT		%	OBSERVATIONS
Dimetilsulfoxide DMSO	CH_3SOCH_3	5%	effective cleaning (allows to do an study of the surface) (Figure 6)

Table 5: agents for hard silicate matrices.

CHEMICAL AGENT		%	OBSERVATIONS
Hydrofluoric acid	(HF)	5%	Surface highly damaged (Figure 7)

Organosulfur: for Oncolitelimestones (dissolving very hard matrices)

Oncolitelimestone matrix is highly carbonated and its dissolution is very difficult without using strong acids. In this case we have used a chemical agent not used in any previous analysis. It is an organic solvent (Dimetilsulfoxide (DMSO): CH_3SOCH_3) that has been used for the dissolution of very compacted and lithified matrices (Triplehorn, 2002; Triplehorn et al., 2002). Its disadvantage is that it can take weeks to break up the matrix. However, it was the only treatment that worked well with this type of matrix (Figure 6). Table 4 shows the concentration for obtaining the best result with this treatment

Agents for hard silicate matrices

It is known that hydrofluoric acid (HF) is a good silicate solvent. However, it did not provide very good results during our essays. Table 5 shows the concentration and the result with this treatment.

DISCUSSION AND CONCLUSIONS

Carbonated matrices in sandstones

For this kind of matrix, the best option for its dissolution with acids proved to be the treatment with Oxalic acid ($H_2C_2O_4$; Figure 3C). This kind of chemical agent is a good alternative to the traditional organic acids used for dissolving carbonated compounds, like acetic and formic. The effect of Acetic acid (CH_3-COOH ($C_2H_4O_2$)) is stronger and more harmful compared to oxalic acid (Figure 3C). However, oxalic acid is difficult to neutralize completely, and thus remains somewhat harmful on the surface of the eggshell (Figure 3C). The worst result was obtained with HCl (Figure 3B). On the other hand, the use of Sodium hexametaphosphate $(NaPO_3)_6$ with a pH value of six has proved to be a good cleaning method. It is better than Oxalic acid, because less damage is induced to the surface (Figure 3D).

Silicates present in marls

For dissolving the siliceous part of the marl, the best option resulted to adopt alkaline treatments. These chemical agents do not attack the carbonated part of the matrix, and does thus not attack the calcium carbonate of the eggshells. We have obtained the bests results by using Potassium Hydroxide (KHO), which is a great alternative to traditional acid treatments on this kind of matrix. We can observe the microstructure of the eggshell with clarity (Figure 4A), observing even the holes of the oxygenation channels of the eggshell (Figure 4C).

Ferric iron in lithified matrices

It is difficult to find a good dissolution agent for matrices rich in ferric iron. We have done tests with both traditional methods and methods of other fields of preparation, as archeological preparation of iron objects. For this reason we tried Oxalic acid, which is useful to dissolve the matrix, but also attacks the surface of the eggshell. The best result was obtained with the Waller Method (Figure 5A), where we could observe a good dissolution of the matrix and little damage on the surface of the eggshell.

Oncolitelimestones

The eggshells included in this type of matrix were the most difficult to clean, due to the hardness of the sediment. It was necessary to use an inorganic acid because it is more reactive. However, this poses a serious risk to the conservation of the microstructure of the eggshell. For this reason, we tried to find an alternative method like dimethylsulfoxide (DMSO; Figure 6). This method allowed us to clean the surface of the eggshells, without inducing too much damage.

Silicates in lithified matrices

In the last case, due to the composition and hardness of the matrix, we had to use Hydrofluoric acid (HF) 5%, but this did not show satisfactory results (see Figure 7), because the surface of the eggshell was highly damaged after this treatment. Further research is therefore needed for this kind of matrices. Moreover, use of this acid is not recommended due to its high risk for the health.

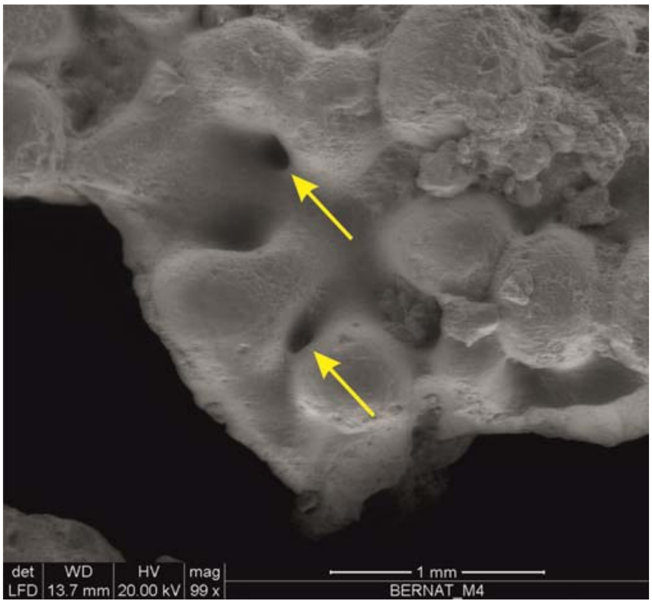

Figure 6: Eggshell treated with Dimetilsulfoxide DMSO at 5% (one week). It is possible to observe the relief and oxygenation channels (arrows).

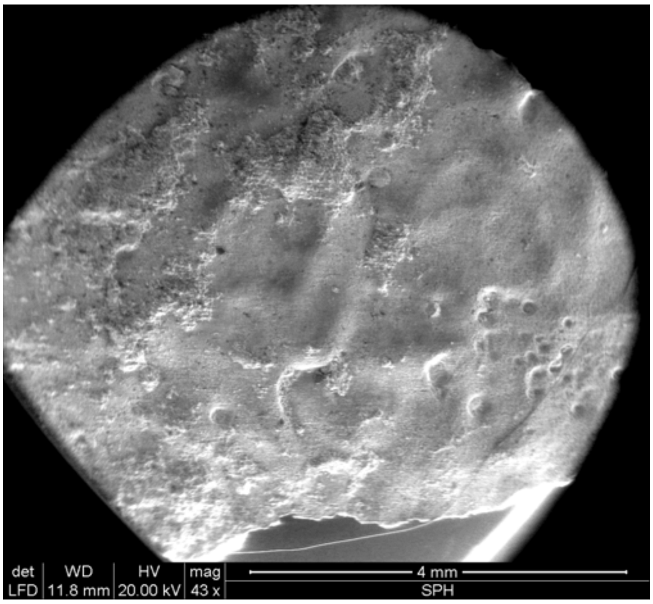

Figure 7: Eggshell treated with Hydrofluoric acid 5%. The eggshell relief has been completely lost.

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