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A new method of rock disintegration and foraminiferal extraction with the use of liquid nitrogen [LN_2]. Do conventional methods lead to biased paleoecological and paleoenviromental interpretations?

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ABSTRACT

An extremely fast, easy, and clean method of rock disintegration and foraminifera extraction from variously lithified porous rocks with the use of liquid nitrogen [LN_2] is proposed. This method markedly limits the time of rock disintegration from days to only minutes, is safe for foraminifera, and does not require special chemical labs. In the experiment, the LN_2 method was used to decompose rock samples and simultaneously to extract the foraminifera hidden within. The proposed method disintegrates the rocks to a finer fraction than conventional methods such as the Glauber's Salt method, allowing to collect more smaller planktonic and benthic foraminifera, resulting in marked changes in foraminiferal assemblages e.g., the planktonic/benthic ratio [P/B defined as P/P+B], leading to new conclusions. The comparison between the LN_2 and Glauber's Salt [GS] methods and obtained results are provided.

1. Introduction

There are many different methods of rock disintegration leading to microfossil extraction. The use of a particular method depends on the rock, especially its chemical composition and physico-chemical properties. It also depends on the microfossils that have to be extracted.

The extraction of microfossils from unconsolidated sediments is generally not a problem and will not be discussed herein. If the microfossils are hidden in more consolidated rocks, like chalk or opokas [= siliceous limestone], the sample must be disintegrated before wet sieving to obtain the fraction containing interesting microfossils. Additionally, the method must be safe for the microfossils.

The simplest way of rock disintegration is to repeat the natural erosion process—freezing and thawing, in lab conditions. The freeze–thaw methods can be generally summarized as follows: 1) saturating the rock with water; 2) placing the sample in a freezer or outside during winter; 3) formation of ice crystals in the pore system leading to disintegration to a finer fraction. In its general assumptions, this method has been known for over 120 years. It was used by Józef Grzybowski in the 1890's and was further described by Hanna and Church (1928) and Pojeta and Balanc (1989) among other.

A specific type of freeze–thaw method is the well-known and widely used Glauber's Salt method, especially for extracting foraminifera (e.g. Herrig, 1966; Surlyk, 1972; Schmid, 1974; Wissing and Herrig, 1999; Green, 2001). In this method, ordinary water is replaced by a saturated solution of sodium sulfate [$Na_2SO_4 \times 10H_2O$]. The formation of sodium sulfate crystals in the pore system disintegrates the rock in similar way as in the classic freeze–thaw method.

In all freeze–thaw methods repetition is required to obtain a sufficiently disintegrated rock sample. The above-mentioned methods are however very time consuming. Depending on the nature of the rock sample, the disintegration can take days or even weeks.

From a literature review (compare Hodgkinson, 1991) there are no reports on how the freeze–thaw methods damage specimens. Here we demonstrate that the use of a particular method is critical.

2. Liquid nitrogen method [LN_2]—procedure

Liquid nitrogen is ordinary nitrogen in a liquid state at very low temperature [−196 °C]. The LN_2 method falls into the category of freeze–thaw methods [a natural erosion process], it is, however, extremely accelerated, leading to microfossil extraction in a simple way. This method is cheap, easy, and does not require special chemical labs — only a well-ventilated room is necessary. Liquid nitrogen is easily available — it is used in almost all physical and chemical labs and is supplied in convenient containers called dewars [e.g. 20–50 l].

In the LN_2 method, the rock sample is treated with LN_2 and hot water until the rock is sufficiently disintegrated to a fraction

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containing microfossils of interest. The step-by-step procedure is as follows (Fig. 1):

1) Break the rock into fragments [0.5–1 cm].
2) Leave the fragments in the water for a couple of hours or overnight and then decant the water.
3) Add the LN_2 to cover the rock fragments. We put the rock fragments into an ordinary aluminum bowl, about 20 cm in diameter and 7–10 cm deep. For a 100 g sample, 100–150 ml of LN_2 should be enough for one cycle. Because the use of large dewars is difficult [because of the weight] we pour LN_2 into a smaller thermos bottle [~2 l].
4) Wait a few moments until the LN_2 vaporizes. This step takes about 1or 2 min.
5) Add boiling water to cover the deeply frozen rock sample; in this step, the rock fragments can be gently crumbled between the fingers. After a few cycles, the rock becomes more and more fragile and prone to crushing between the fingers.
6) Decant the suspension. Stir the water in the bowl to produce more suspension and then quickly decant by a sieve e.g., 63 μm. The suspension contains the finest fraction which is usually rich in small and delicate planktonic and small benthic foraminifera. This is a very important step because the foraminifera released from the rock after every cycle are not subjected to repeated freezing and heating in the following cycles.
7) Repeat steps 3–6 until needed. Our experience shows that 15–20 cycles are enough to obtain a satisfactory amount of fraction for microscope examination.

3. Glauber's Salt vs liquid nitrogen methods

Both the LN_2 and GS methods fall into the category of freeze–thaw methods. The formation of ice crystals or sodium sulfate crystals in the pore system respectively, breaks up the rock leading to disintegration. The rocks generally disaggregate at their weakest points along cracks within the matrix and/or on the contacts between fossils and matrix. Thus, both methods should provide similar results regarding the composition of foraminifera and percent frequency of individual morphogroups, as well as possible damage to microfossils. However, the results are different. To explain the differences, we must look at what is happening during disintegration.

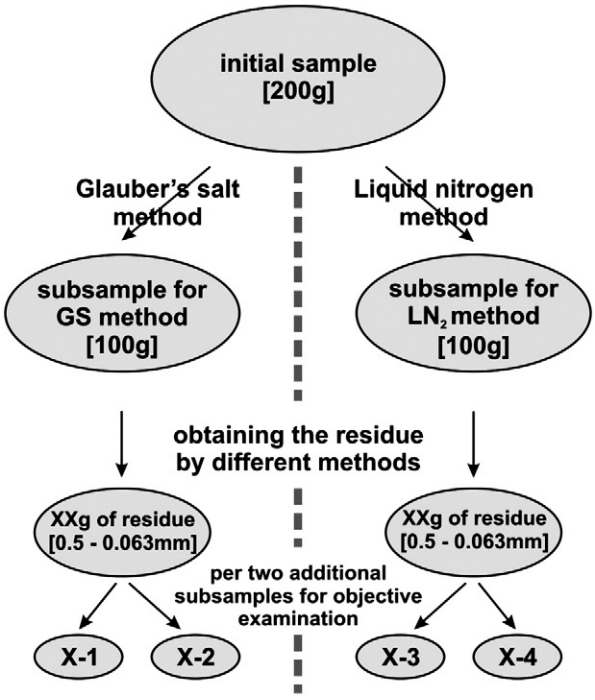

Fig. 1. Step by step experiment for comparing the GS and LN_2 methods.

3.1. Glauber's Salt method

It seems that in the Glauber's Salt method the disintegration process stops at a certain point and the repetition of the procedure does not provide further disintegration. The question is why? The most important in our opinion is the size of the rock particles and pores and the crystal growth process. Generally, crystals grow where they have enough space. In the GS method, the crystals grow relatively slowly (similarly to ice crystals in the classical freeze–thaw method). Because growth is slow, the forming crystals will push out the solution from the pore system. Additionally, if the rock particles and pores are small, crystals will grow outside the rock particles preventing further damage. Therefore, the residue will be disintegrated to a fraction small enough to provide some foraminifera, but too large to provide all of them, especially smaller ones.

3.2. Liquid nitrogen method

There is a synergic effect of two processes: 1) the ice crystal formation and 2) the thermal expansion of the rock matrix and microfossils. During the formation of ice crystals, the volume of water present in the pore system expands by approximately 10%. However, the most important factor, is the fact that freezing by LN_2 [−196 °C] is instantaneous in the whole volume of the rock fragments. The water, and consequently the forming crystals, does not have time and space to escape from the pore system, even from the smallest pores inside the rock particles, as would probably occur when the formation of crystals is slow.

The second very important process is the thermal expansion by the temperature amplitude of approximately 300 °C [−196 °C for LN_2 and ~100 °C for boiling water]. The thermal expansion is different for the rock matrix and different for the microfossils. Therefore, during every cycle of cooling and heating, the rock will be disaggregated along its weakest points [e.g. contact of matrix and microfossils]. Together with ice crystal formation, it will lead to further and further disintegration of the rock with simultaneous releasing of foraminifera from the matrix.

Additionally, in the LN_2 method, there is a step that does not occur in the GS method. After every single cycle of the LN_2 method, when the deeply frozen rock fragments are flooded by hot water, the suspension is decanted by a sieve [in our case 0.063 mm]. The suspension contains the finest fraction and is enriched in very small and delicate planktonic and small benthic foraminifera. This step is critical since the most delicate foraminifera are not subjected to repeated salt crystallization, preventing them from damage, as probably happens using the Glauber's Salt method.

4. Liquid nitrogen and Glauber's Salt methods — an experiment

The main goal of this experiment was to compare the results of the two methods — the Glauber's Salt and liquid nitrogen. Before the final experiment, many variously lithified Upper Cretaceous rocks [mainly opokas] were processed by the LN_2 method to test its effectiveness, especially for extracting foraminifera. After achieving positive results, three samples of upper Campanian and lower Maastrichtian opokas [= siliceous limestones] were selected for the experiment. In the first step, the goal was to obtain the residue in the fraction 0.063–0.5 mm that contains both benthic and planktonic foraminifera and compare the time required for this process. In the second step of the experiment, the examination of the residue was carried out.

4.1. First step—disintegration

Each sample [200 g] came from one piece of rock [samples from Raj N and Kłudzie S are of Late Campanian age; Bliżów sample is of Early Maastrichtian age]. The rock was crushed into smaller fragments [0.5–1 cm] and then, samples were subdivided into two subsamples, 100 g each, and subjected to further processing by 1) the conventional Glauber's Salt method and 2) the LN_2 method (Fig. 1).

Each subsample from individual locations provided a similar amount of residue in the fraction of interest [0.063–0.5 mm] for microscope examination (Fig. 2). However, the LN_2 method provided additionally about 10 g of very fine fraction, obtained during decantation of the suspension after every cycle of freezing and thawing (Fig. 2). This step does not occur in the GS method. The number of cycles is higher in the LN_2 method (Fig. 2) but this difference means nothing when comparing the time required to obtain the residue. Here the difference is colossal — in all cases the LN_2 method took about 1.5 h whereas the GS method took from 10 to 15 h, which translates into at least 2–3 days of processing (Fig. 2).

4.2. Second step—residue examination

In this step of the experiment the degree of disintegration, state of foraminifera preservation, diversity of foraminifera, and the planktonic/benthic ratio [P/B] were estimated. To maintain essential objectivity during microscope residue examination, one of us [Z.D.] did a blind test. Z.D. did not know how the samples had been prepared. Additionally, each of the six residue samples (3 for GS and 3 for LN_2) was again subdivided per two blind subsamples [X-1, X-2 etc.; compare Fig. 1]. The subsamples were left unlabeled in order to avoid any potential influence on the final results. From 12 blind subsamples, six were chosen by Z.D. as better disintegrated [all treated by LN_2 method]. In all study cases, the P/B ratio calculated from the samples treated by LN_2 methods gave completely different results, as summarized in Fig. 2. The state of foraminiferal preservation is similar in both methods. The state of preservation of foraminifera is shown in Fig. 3.

The calculated P/P + B ratio for Raj N sample treated by LN_2 is markedly higher than the results obtained with the GS method: the P/B ratios are 47% and 19% respectively (Fig. 2). In the case of the Kłudzie S sample, the GS method gave a P/B ratio of 22% whereas the LN_2 method gave a completely opposite result with a P/B ratio 74% (Fig. 2). The P/B ratios calculated for the Bliżów sample, at first glance are similar in both methods [P/B = 2% and 11%], however the difference is significant, since there are about six times more planktonic foraminifera when the LN_2 method is used (Fig. 2).

5. Conclusion

The use of the LN_2 method is fast, clean, and saves a lot of time [compare Fig. 2]. The synergic effect of thermal expansion by the temperature amplitude of approximately 300 °C and instantaneous crystallization of ice crystals in the whole volume of rock fragments leads to very fast disintegration, along the surfaces predisposed to crack [e.g. contact between matrix and microfossils]. These processes systematically release the microfossils from the rock matrix.

The rock samples are better disintegrated to a finer fraction by the LN_2 method in comparison to the Glauber's Salt method. This allows the extraction of a significantly higher number of smaller benthic and planktonic foraminifera. It makes the assemblages more realistic and assemblage's parameters such as the P/B ratio more reliable.

The Glauber's Salt method does not provide a sufficient amount of finer fraction. Moreover, it cannot be excluded, that this method can be destructive for fragile planktonic foraminifera, since they are subjected to repeated salt crystallization.

Comparison of the two methods shows how the choice of the method [Glauber's Salt vs LN_2] may considerably influence the final results and conclusions. The use of the GS method may markedly underestimate the number of small benthic and planktonic foraminifera (Fig. 2) in comparison to the LN_2 method. It simultaneously leads to biased calculations of the proportion of individual morphogroups and assemblage parameters such as the P/B ratio. Our experiment shows, that the method used, highly influences the obtained foraminiferal assemblages, which can lead to opposite paleoecological and palaeoenviromental interpretations, as in case of Kłudzie S sample (Fig. 2).

The answer to the question raised in the title is positive. At least in some cases the paleoecological, paleoenvironmental, and paleobathymetrical conclusions obtained with conventional methods, like Glauber's Salt, will have to be rethought, in the light of the data generated by our comparison of the two processing methods.

sample	Raj N		Kłudzie S		Bliżów	
lithology	marly opoka; upper Campanian		very hard opoka; upper Campanian		hard opoka; lower Maastrichtian	
method	Glauber's Salt [100g]	LN2 [100g]	Glauber's Salt [100g]	LN2 [100g]	Glauber's Salt [100g]	LN2 [100g]
total residue [0.5 - 0.063mm]	47.8g	35.37g	33.24g	28.42g	47.80g	48.67g
residue from decantation [only for LN2]	0g	~10g	0g	~10g	0g	~10g
cycles	11	18	17	20	11	18
time	10h	1.5h	14h	1.5h	10h	1.5h
P/B ratio	19%	47%	22%	74%	2%	11%
P/B ratio plots	Raj N: P 19%, B 81% (Glauber's Salt); P 47%, B 53% (LN_2)		Kłudzie S: P 22%, B 78% (Glauber's Salt); B 26%, P 74% (LN_2)		Bliżów: P 2%, B 98% (Glauber's Salt); 11% P, B 89% (LN_2)	

Fig. 2. Results obtained from the Glauber's Salt method (white column) and liquid nitrogen method (gray column). Plots showing the percentage of planktonic [P] and benthic [B] foraminifera obtained by the GS and LN_2 methods; n > 200 specimens in each sample.

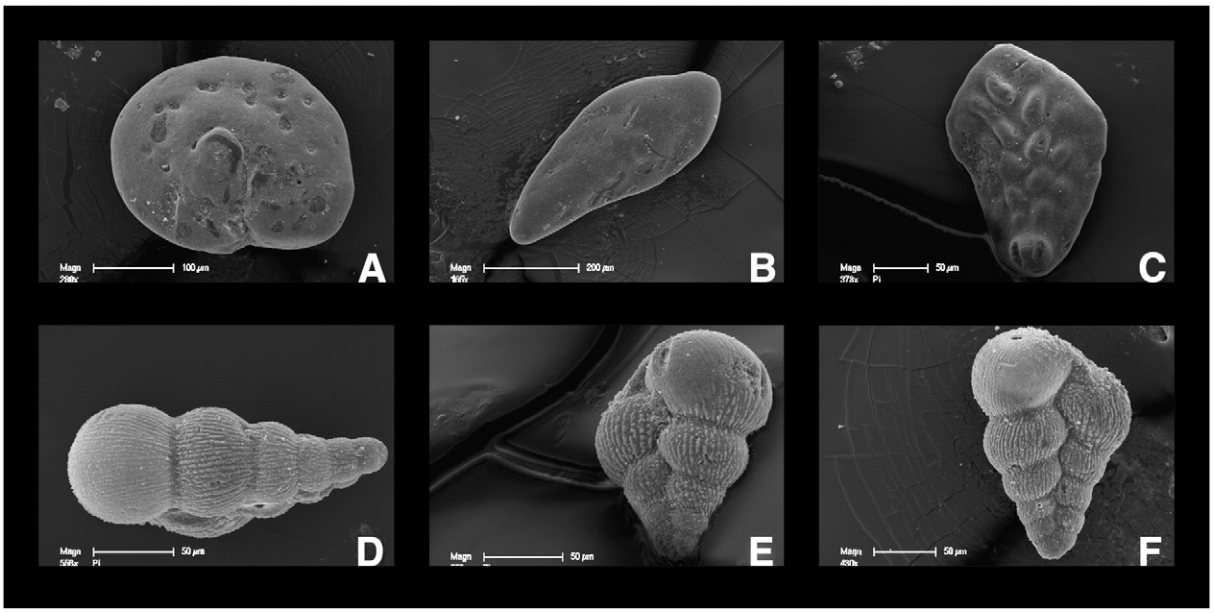

Fig. 3. Typical foraminiferal preservation extracted by the liquid nitrogen method [Kłudzie S sample]. Very well preserved planktonic and benthic foraminifera with all details of the morphology (e.g. ornamentation, aperture, sutures etc.) required for correct taxonomic classification. A—*Cibicidoides voltzianus* (d'Orbigny); B—*Coryphostoma incrassata* (Reuss); C—*Bolivinoides* sp.; D–F—*Heterohelix striata* (Ehrenberg).

6. Warning

Liquid nitrogen is safe when used safely. Because of its low temperature [−196°], the LN_2 can be dangerous in direct contact — it can burn skin. The processing with LN_2 must be always carried out in a well-ventilated room. An experienced person should be asked how to use it safely.

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