

New approaches in palynological processing: tackling calcium fluoride formation

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NEW APPROACHES IN PALYNOLOGICAL PROCESSING: TACKLING CALCIUM FLUORIDE FORMATION

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Abstract. Palynological processing comprises both traditional and non-traditional protocols, designed to recover representative assemblages of the depositional environments. In this context, the quality of palynological preparations is critical to ensure reliable and representative results. In this study, three different methodologies were implemented, each involving modifications to conventional palynological protocols, with the aim of removing or preventing the formation of insoluble calcium fluoride colloids generated following hydrofluoric acid treatment. The first treatment consisted of removing the already formed gel by the process of the organic residue with hydrochloric acid followed by washes with a sodium pyrophosphate solution. The second treatment included a deflocculation step using sodium pyrophosphate prior to the initial hydrofluoric treatment, modified from previously proposed protocols, in order to prevent colloid formation. The third treatment involved increasing the number of neutralization washes between acid treatments, also adapted from published palynological protocols, performing the final hydrochloric acid treatment in a heated water-bath. The results indicate that the most effective approach was to allow the formation of insoluble colloids and subsequently treat the residue with hydrochloric acid in heated water baths. In contrast, the intermediate deflocculation step, commonly recommended for such cases, proved unsuitable due to the scarce recovery of organic residue and the formation of colloids.

Key words. Palynology. Processing techniques. Calcium fluoride.

Resumen. NUEVAS PERSPECTIVAS EN EL PROCESAMIENTO PALINOLÓGICO: ABORDANDO LA FORMACIÓN DE FLUORURO DE CALCIO. El procesamiento palinológico comprende tanto protocolos tradicionales como no-tradicionales, destinados a recuperar asociaciones representativas de su ambiente de deposición. En este contexto, la calidad de los preparados palinológicos resulta crítica para garantizar la obtención de resultados confiables y representativos. En esta contribución se implementaron tres metodologías diferentes, cada una basada en modificaciones de protocolos palinológicos tradicionales, con el objetivo de remover y prevenir la aparición de coloides insolubles de fluoruro de calcio formados tras el tratamiento con ácido fluorhídrico. El primer tratamiento consistió en la remoción del gel ya formado mediante el procesamiento del residuo orgánico con ácido clorhídrico y posteriores lavados con una solución de pirofosfato de sodio. El segundo tratamiento incluyó un proceso de defloculación con pirofosfato de sodio previo a la primera etapa con ácido fluorhídrico, adaptado de protocolos previamente publicados, con el propósito de evitar la formación de coloides. El tercer tratamiento consistió en incrementar los lavados de neutralización entre las etapas ácidas, modificado de metodologías previas, realizando el tratamiento final con ácido clorhídrico en baño térmico. Los resultados obtenidos indican que la estrategia más efectiva fue permitir la formación de los coloides insolubles y posteriormente tratar el residuo con ácido clorhídrico en baño térmico. Por el contrario, la etapa intermedia de defloculación, comúnmente recomendada para este tipo de problemas, no resultó adecuada debido a la baja recuperación de residuo orgánico y a la persistencia en la formación de coloides.

Palabras clave. Palinología. Técnicas de procesamiento. Fluoruro de calcio.

INTRODUCTION

Palynology involves the study of microscopic organic-walled particles, both modern and fossil, that are resistant to aggressive chemical treatments (Hyde & Williams, 1944). These particles, called palynomorphs, encompass a broad range of terrestrial and marine representatives with plant

or animal affinities, including pollen grains, spores, dinoflagellate cysts, chitinozoa, fungal spores, microforaminiferal linings, prasinophytes, rotifers, scolecodonts, acritarchs, as well as arthropod and crustaceans organs and eggs, among others (Traverse, 2007; Riding, 2021). Palynomorphs exhibit a wide range of sizes, generally between 5 to 500 µm, and

occur in the geological record from Precambrian rocks to Quaternary sediments (Traverse, 2007). In addition, palynology also encompasses the study of phytoclasts, microscopic organic particles including plant cuticles, wood fragments, tracheids, and amorphous organic material (Tyson, 1995). Due to their small size, widespread distribution, abundance, and high preservation potential, palynomorphs and phytoclasts are commonly preserved in rocks of various ages and depositional environments (Faegri & Iversen, 1989). Consequently, these microfossils have broad application across numerous disciplines, providing valuable information of paleoenvironmental, paleoclimatic and paleoecological conditions, as well as biostratigraphic frameworks (Traverse, 1994).

Palynological studies require processing techniques that enable the extraction and concentration of palynomorphs from sedimentary matrices. This process involves the digestion of the mineral fraction of sediments or rocks to isolate the organic residue (Faegri & Iversen, 1989; Traverse, 2007). Traditional palynological techniques generally rely on digestion with strong mineral acids, mainly hydrochloric acid (HCl) and hydrofluoric acid (HF), to remove carbonate and siliceous components, respectively (Traverse, 2007). However, numerous methodological variations have been developed because protocols that are effective for certain sample types may not be suitable for others, and laboratory procedures may vary depending on reagent availability and analytical requirements.

Several authors have proposed methodologies to address the removal of dispersed clay particles, which may lead to the formation of insoluble calcium fluoride (CaF_2) colloids during the acid treatment steps involved in palynological preparations (Bates et al., 1978; Heusser & Stock, 1984; Batten, 1999; Riding & Kyffin-Hughes, 2004, 2006, 2010; O'Keefe & Eble, 2012; Moss, 2013; Pound et al., 2021, among others). These clay particles can form insoluble aggregates around both organic and inorganic particles, encapsulating palynomorphs and severely hindering their observation and analysis (Bates et al., 1978). A comprehensive review of the occurrence of calcium fluoride (CaF_2), commonly referred to as silica gel in palynological preparations, and methodologies for dispersed clay removal was provided by Riding (2021).

The present study evaluates three alternative approaches modified from previously published methodologies for samples in which traditional and non-traditional protocols may not effectively prevent colloid formation or facilitate its removal.

MATERIALS AND METHODS

Studied material

The studied material comprises 53 sediment samples collected from a Holocene section located in Puerto Visser, Área Natural Protegida de Rocas Coloradas, Golfo de San Jorge, Chubut, Argentina ($45^\circ 26' 37'' \text{ S}$; $67^\circ 09' 24'' \text{ W}$). Grain size analysis was performed at the laboratory of CADIC-CONICET (Centro Austral de Investigaciones Científicas) using a Malvern 2000 laser particle-size analyzer. The sedimentary composition of all samples is dominated by silt, with subordinate proportions of clay and sand. The samples were processed following the laboratory protocol described by Louwye et al. (2004). Carbonates and silicates were removed using cold hydrochloric acid (19 % HCl) and cold hydrofluoric acid (40 % HF), respectively. To preserve non-pollen palynomorphs (NPPs) and dinoflagellate cysts, no acetolysis, oxidation or heavy-liquid treatments were applied. Prior to acid treatment, two *Lycopodium clavatum* tablets (Batch No. 3862, containing 9666 spores) were added to every sample as an exotic marker (Stockmarr, 1971). In this study, the tablets were not used to quantify the concentration of the original Holocene palynomorph assemblages but rather to assess the efficiency of the different sample preparation procedures.

Organic residues were filtered through a 10 μm nylon mesh to concentrate palynomorphs, subsequently dehydrated in alcohol, and mounted on microscope slides using UV-curable acrylate as a mounting medium following the protocol proposed by Noetinger et al. (2017). The palynological slides and residual material are housed at the Centro Austral de Investigaciones Científicas (CADIC-CONICET), Ushuaia, Tierra del Fuego, Argentina.

The initial sample mass was approximately 15 grams; however, due to the scarce recovery of organic residue during the process, sample weights were progressively increased, ultimately ranging from 25 to 40 grams. The amount of sample processed should be adjusted according

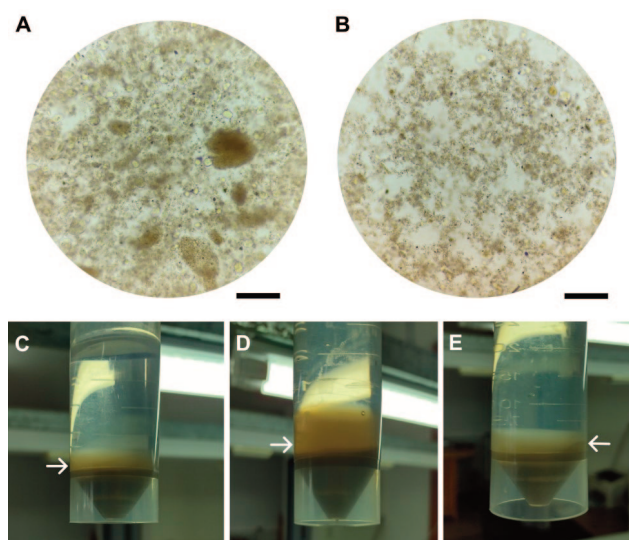


Figure 1. Microscopic view at 40x magnification showing calcium fluoride colloids that hinder microscopic palynomorph observation. A, Dense colloid distribution. B, Sparse colloid distribution. Scales = 20 µm (A, B). C–E, Photographs of three samples in tubes showing different concentrations of the jellified residue (white arrows).

to lithology to obtain sufficient organic residue for subsequent palynological treatments. Following the recommendations of Volkheimer and Melendi (1976), a minimum of 15 grams should be processed for shale and coal samples, 30 grams for siltstone and marl, and 40–50 grams for limestone and sandstone. After the first HF treatment of the first batch, all samples except one developed a jellified consistency, later identified as calcium fluoride colloids, which significantly hindered microscopic palynomorph observation (Fig. 1A–E). To overcome this issue, modified procedures incorporating combinations of previously published methodologies were implemented. Consequently, the three protocols were applied to successive stratigraphic intervals rather than to replicate samples. Therefore, the comparisons presented here should be regarded as documenting the outcomes of the methodological adjustments rather than as a controlled experimental evaluation of their relative performance.

Implemented palynological techniques

Three different treatments were applied during sample processing. The first was designed to remove calcium fluoride colloids that had already formed in an initial set of samples. The second aimed to prevent colloid formation

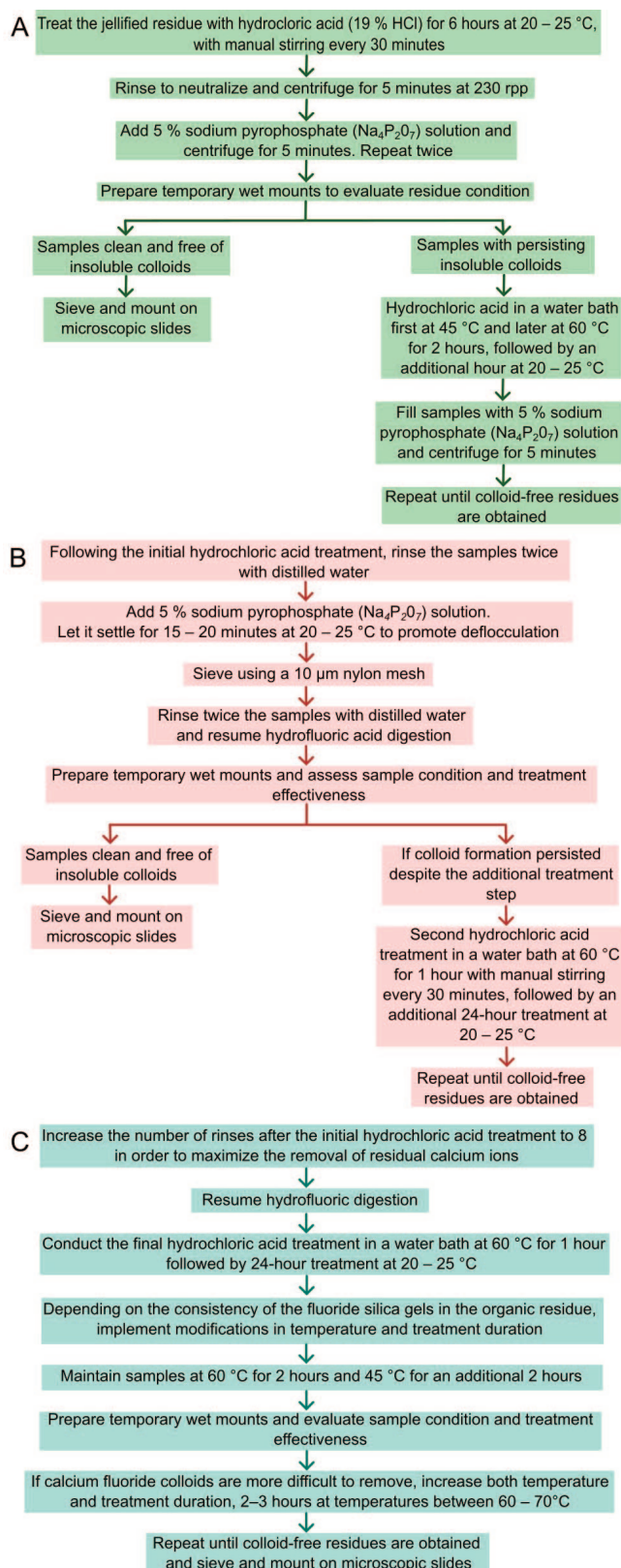
through a sample deflocculation and disaggregation procedure using sodium pyrophosphate, modified from the methods proposed by Bates et al. (1978) and Batten (1999). The third treatment involved increasing number of rinsing steps between the initial hydrochloric acid and hydrofluoric acid steps, modified from Batten (1999), while the second hydrochloric acid treatment was carried in a water-bath following modifications to the protocol of Louwye et al. (2004).

Sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$), also known as tetrasodium pyrophosphate, is a highly soluble, non-hazardous inorganic salt commonly used as a dispersing and peptizing agent (Bates et al., 1978). In palynology studies, it is widely employed to disperse clay particles, promote deflocculation, and improve the separation of organic material from fine-grained sediments, such as clay or shale. Its effectiveness is associated with its ability to bind cations, particularly calcium ions, thereby reducing electrostatic attraction among particles and preventing aggregation (Bates et al., 1978).

First treatment (Fig. 2A). The first treatment consisted of processing the jellified residue with hydrochloric acid (19 % HCl) for 6 hours at 20–25 °C, with manual stirring every 30 minutes. Following treatment, the samples were neutralized and centrifuged for 5 minutes. Subsequently, a 5 % sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) solution was added as deflocculant and centrifuged for 5 minutes. This step was repeated twice.

Following treatment, temporary wet mounts were prepared to evaluate residue condition. Samples that appeared clean and free of insoluble colloids were separated and subsequently filtered and mounted. Some samples required repeated treatments; therefore, hydrochloric acid was subsequently performed in a water bath, first at 45 °C and later at 60 °C for 2 hours, followed by an additional hour at 20–25 °C. After treatment, samples were again filled with a 5 % sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) solution and centrifuged for 5 minutes. This procedure was repeated until colloid-free residues were obtained.

Second treatment (Fig. 2B). Due to the low amount of recovered residue from the first method, the quantity of processed material was increased. This treatment was designed to prevent calcium fluoride colloid formation. Therefore, following the initial hydrochloric acid treatment, an intermediate sieving step using a 10 µm nylon mesh was



done following modified procedures based on Bates et al. (1978) and Batten (1999).

Prior to sieving, 5 % sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) solution was added and allowed to settle for 15–20 minutes at 20–25 °C to promote deflocculation. Heating was intentionally avoided to prevent damage to delicate paly-nomorphs. However, only a limited amount of material was retained on the 10 µm mesh, while the sieved clay fraction displayed pronounced expansive behavior (Fig. 3A–C).

Following this step, samples were rinsed twice with distilled water and hydrofluoric acid digestion was resumed. In some samples, calcium fluoride colloids did not develop even organic residue recovery remained extremely low. In others, colloid formation persisted despite the additional treatment step. Consequently, the second hydrochloric acid treatment was conducted in a water bath at 60 °C for 1 hour with manual stirring every 30 minutes, followed by an additional 24-hour treatment at 20–25 °C.

Temporary wet mounts were prepared after each treatment cycle to assess sample condition and determine treatment effectiveness.

Third treatment (Fig. 2C). For this procedure, the amount of processed sediment ranged from 25 to 31 grams. In this treatment, the rinsing step performed after the initial hydrochloric acid treatment was modified from the procedure described by Batten (1999), increasing the number of rinses from 4 to 8 in order to maximize the removal of residual calcium ions. Additionally, the final hydrochloric acid treatment described by Louwey et al. (2004) was conducted in a water bath at 60 °C for 1 hour, followed by 24-hour treatment at 20–25 °C.

Depending on the consistency of the fluoride precipitates present within the organic residue, modifications in temperature and treatment duration were implemented. In some cases, samples were maintained at 60 °C for 2 hours and 45 °C for an additional 2 hours. In samples where calcium fluoride colloids were more difficult to remove,

Figure 2. Flow charts illustrating the followed steps of the conducted methodologies. A, First treatment: treating the jellified residue. B, Second treatment: intermediate sieving step, modified from Bates et al. (1978) and Batten (1999). C, Third treatment: modified from Batten (1999) and Louwey et al. (2004).

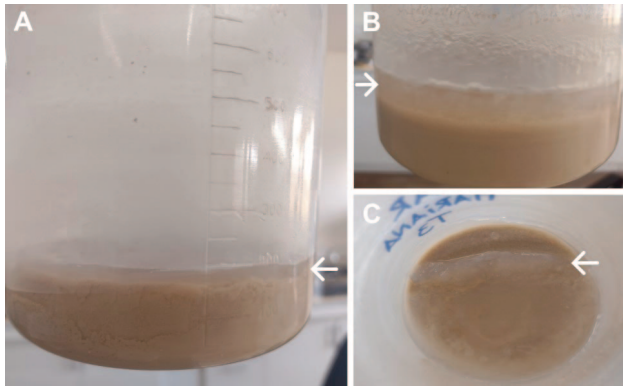


Figure 3. Photograph of the sieved clay fraction with expansive behavior after deflocculation and sieving (second treatment). The white arrows in A, B and C point to a jelly-like suspension formed upon hydration in different samples.

both temperature and treatment duration were increased, ranging from 2 to 3 hours at temperatures between 60 and 70 °C.

The temperature never exceeded 70 °C in order to prevent damage and disaggregation of delicate palynomorphs, since elevated temperatures may adversely affect the preservation of fragile structures, particularly the relatively thin phragma of *Protoperidinium* and *Alexandrium* cysts, leading to their disintegration (Matsuoka & Fukuyo, 2000).

RESULTS

A total of 53 samples were processed using the three successive preparation protocols. The amount of sediment processed varied among the implemented methodologies according to lithology and the organic residue recovered, ranging approximately from 15 to 40 grams (Tabs. 1–3). The treatments applied, including the number of samples, sediment mass processed, number of replicates, and number of *Lycopodium clavatum* spores recovered in the first, second, and third treatment, are summarized in Tables 1, 2 and 3, respectively. The initial protocol (Tab. 1) resulted in low recovery of organic residue, leading to subsequent modifications of the procedures (Tabs. 2, 3) that improved the recovery of sufficient organic residue for palynological analysis in the processed intervals.

First treatment: HCl + 5 % sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) solution (Tab. 1)

A total of 13 samples were processed, with sediment amounts ranging from 15.17 to 25.83 grams (M1–M13). Each sample was treated with HCl for 6 hours at 20 – 25 °C and $\text{Na}_4\text{P}_2\text{O}_7$, except for sample 3 (M3), in which no colloid formation was observed during processing (Tab. 1). The

TABLE 1. Summary of the results obtained from the first treatment consisting in HCl + 5 % sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) solution.

Sample number	Amount processed (gr)	Type of treatment	Number of replicates	<i>Lycopodium clavatum</i> added	<i>Lycopodium clavatum</i> counted
M 1	15.21	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	2	19332	958
M 2	15.17	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	2	19332	1445
M 3	15.25	no colloids formed	-	19332	729
M 4	25.19	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	4	19332	333
M 5	25.17	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	5	19332	698
M 6	25.59	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	5	19332	671
M 7	25.23	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	5	19332	1024
M 8	25.82	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	2	19332	1037
M 9	25.25	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	2	19332	607
M 10	25.33	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	3	19332	883
M 11	25.14	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	2	19332	436
M 12	25.83	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	2	19332	737
M 13	25.62	HCl + $\text{Na}_4\text{P}_2\text{O}_7$	2	19332	143

number of replicates varied between two and five per sample, depending on the effectiveness of colloid removal (Tab. 1). The number of *Lycopodium clavatum* spores recovered after processing ranged from 143 to 1445 (Tab. 1), and this variability could reflect differences in sample processing efficiency.

Second treatment: intermediate deflocculation and disaggregation using a 5 % sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) solution. Modified from Bates et al. (1978) and Batten (1999) (Tab. 2)

For the second treatment, 10 samples were processed (M14–M23), with sediment masses ranging from 30.15 to 40.69 grams (Tab. 2). Half of the samples (M14–M18) were treated with $\text{Na}_4\text{P}_2\text{O}_7$ (deflocculation step) followed by sieving, prior to HF digestion (Tab. 2). This procedure resulted effective in preventing colloid formation; however, only a small amount of organic residue was recovered. The remaining samples (M19–M23) required additional treatments with HCl at 60 °C, because colloids still formed despite the deflocculation step (Tab. 2). The number of *Lycopodium clavatum* spores recovered ranged from 48 to 1069 (Tab. 2).

Third treatment: increased rinsing + HCl in water bath. Modified from Batten (1999) and Louwye et al. (2004) (Tab. 3)

Under the third approach, 30 samples (M24–M53) were processed, with sediment amounts ranging from 25 to 31.23 grams (Tab. 3). All samples underwent additionally rinsing steps before HF digestion, and the final HCl treatment was performed in a water bath. The number of replicates (additional HCl in water bath) varied among 12 samples, ranging from 1 to 5, whereas 18 samples required no further treatment because the initial procedure successfully prevented colloid formation (Tab. 3). The number of *Lycopodium clavatum* spores recovered ranged from 25 to 917 (Tab. 3).

DISCUSSION

Although the three treatments successfully removed or prevented the formation of calcium fluoride colloids, their observed effectiveness varied among samples. Because the protocols were progressively modified during palynological processing, the three methodologies were applied sequentially to different sets of samples from the Holocene sedimentary section at Puerto Visser rather than to replicate subsamples of the same sediment. Procedures that proved

TABLE 2. Summary of the results obtained from the second treatment consisting in intermediate deflocculation and disaggregation using a 5 % sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) solution; modified from Bates et al. (1978) and Batten (1999).

Sample number	Amount processed (gr)	Type of treatment	<i>Lycopodium clavatum</i> added	<i>Lycopodium clavatum</i> counted
M 14	30.16	$\text{Na}_4\text{P}_2\text{O}_7$ + sieving	19332	695
M 15	30.15	$\text{Na}_4\text{P}_2\text{O}_7$ + sieving	19332	144
M 16	33.19	$\text{Na}_4\text{P}_2\text{O}_7$ + sieving	19332	1069
M 17	31.13	$\text{Na}_4\text{P}_2\text{O}_7$ + sieving	19332	504
M 18	33.13	$\text{Na}_4\text{P}_2\text{O}_7$ + sieving	19332	557
M 19	39.27	$\text{Na}_4\text{P}_2\text{O}_7$ + sieving + HCl 60 °C	19332	572
M 20	40.69	$\text{Na}_4\text{P}_2\text{O}_7$ + sieving + HCl 60 °C	19332	128
M 21	40.05	$\text{Na}_4\text{P}_2\text{O}_7$ + sieving + HCl 60 °C	19332	368
M 22	40.16	$\text{Na}_4\text{P}_2\text{O}_7$ + sieving + HCl 60 °C	19332	48
M 23	39.68	$\text{Na}_4\text{P}_2\text{O}_7$ + sieving + HCl 60 °C	19332	410

TABLE 3. Summary of the results obtained from the third treatment consisting in increased rinsing + HCl in water bath; modified from Batten (1999) and Louwye et al. (2004).

Sample number	Amount processed (gr)	Type of treatment	Number of replicates (HCl step)	<i>Lycopodium clavatum</i> added	<i>Lycopodium clavatum</i> counted
M 24	25.86	Increased rinse + HCl in water bath	2	19332	839
M 25	25.46	Increased rinse + HCl in water bath	2	19332	917
M 26	25.63	Increased rinse + HCl in water bath	3	19332	395
M 27	25.35	Increased rinse + HCl in water bath	2	19332	480
M 28	25.78	Increased rinse + HCl in water bath	5	19332	48
M 29	25.91	Increased rinse + HCl in water bath	5	19332	555
M 30	25.45	Increased rinse + HCl in water bath	-	19332	361
M 31	25.50	Increased rinse + HCl in water bath	3	19332	535
M 32	25.14	Increased rinse + HCl in water bath	3	19332	318
M 33	25.33	Increased rinse + HCl in water bath	3	19332	25
M 34	25.16	Increased rinse + HCl in water bath	-	19332	331
M 35	26.00	Increased rinse + HCl in water bath	-	19332	742
M 36	25.33	Increased rinse + HCl in water bath	-	19332	429
M 37	25.11	Increased rinse + HCl in water bath	-	19332	405
M 38	25.00	Increased rinse + HCl in water bath	-	19332	288
M 39	25.32	Increased rinse + HCl in water bath	-	19332	83
M 40	25.33	Increased rinse + HCl in water bath	-	19332	425
M 41	25.03	Increased rinse + HCl in water bath	-	19332	77
M 42	25.85	Increased rinse + HCl in water bath	-	19332	138
M 43	25.56	Increased rinse + HCl in water bath	-	19332	242
M 44	25.17	Increased rinse + HCl in water bath	-	19332	188
M 45	25.07	Increased rinse + HCl in water bath	1	19332	124
M 46	25.28	Increased rinse + HCl in water bath	1	19332	114
M 47	26.31	Increased rinse + HCl in water bath	1	19332	582
M 48	31.02	Increased rinse + HCl in water bath	-	19332	60
M 49	31.23	Increased rinse + HCl in water bath	-	19332	125
M 50	30.25	Increased rinse + HCl in water bath	-	19332	107
M 51	30.35	Increased rinse + HCl in water bath	-	19332	336
M 52	30.23	Increased rinse + HCl in water bath	-	19332	205
M 53	31.08	Increased rinse + HCl in water bath	-	19332	43

effective for some samples did not consistently yield comparable results in others (Tabs. 1–3), therefore, the observed differences should not be interpreted as controlled comparison of methodological performance. Instead, they document the sequential adjustments implemented during sample processing to improve organic residue recovery throughout the sedimentary sequence. These results suggest that no single treatment can be considered universally more effective than the others and that treatment performance may depend on sample-specific characteristics.

These variable responses during palynological processing highlight the importance of considering sediment properties and composition when designing palynological protocols. Colloid formation is likely related to the mineralogical composition of the sediment, particularly fine-grained components such as clay. Nevertheless, the overall preservation of both the recovered Holocene palynomorphs and the *Lycopodium clavatum* spores added as exotic markers ranged from moderate to good, suggesting that the modified procedures did not significantly affect specimen integrity. As highlighted by Riding (2021), the routine use of exotic marker particles provides a reliable means of assessing the effectiveness of palynological processing. Successful recovery of the added *Lycopodium* spores indicates that the preparation procedure has been effective, whereas the absence of both exotic markers and indigenous palynomorphs suggests that palynomorphs have been lost or destroyed during processing. Conversely, the recovery of exotic markers in the absence of indigenous palynomorphs confirms that the sample is genuinely barren, rather than reflecting a failure of the preparation procedure.

Palynofacies analysis revealed abundant phytoclast content in all samples, which may explain the relatively low recovery of *Lycopodium clavatum* spores observed throughout the treatments (Tabs. 1–3).

Van der Kaars (1991) proposed the use of sodium pyrophosphate ($\text{Na}_2\text{P}_2\text{O}_7$) as a processing method for removing clay from siliciclastic Quaternary sediments. Subsequently, Moss et al. (2005, 2016) employed this reagent as a deflocculating agent, sieving the samples through 180 and 8 μm meshes prior to hydrochloric acid treatment. Moss (2013) further described the use of 10 % tetrasodium pyrophosphate in heated beakers at 100 °C

for 40 minutes, followed by sieving through 200 and 8 μm meshes before resuming conventional processing procedures.

Similarly, Bates et al. (1978) and Batten (1999) proposed the use of sodium pyrophosphate ($\text{Na}_2\text{P}_2\text{O}_7$) in a water bath for 10 to 20 minutes before and after the hydrofluoric acid (HF) treatment. These authors recommended repeating the procedure 3 to 5 times to ensure complete clay removal from the supernatant. In addition, Batten (1999) described resuspending the organic residue in $\text{Na}_2\text{P}_2\text{O}_7$ and subsequently sieving it through a 5 μm nylon mesh after the completion of the conventional procedure.

For the Puerto Visser samples, colloid formation was observed following hydrofluoric acid treatment, indicating that insoluble colloids were produced by reactions between fluoride ions derived from hydrofluoric acid and cations present in the solution, most likely associated with silt and clay fractions from the sediment. Consequently, the second treatment incorporated a deflocculation stage prior to HF digestion, following a procedure modified from those described by Bates et al. (1978) and Batten (1999). Samples were allowed to settle in sodium pyrophosphate solution for approximately 20 minutes before sieving through a 10 μm mesh to remove clay particles.

Heating was intentionally avoided during this stage to minimize thermal exposure and prevent potential damage to delicate palynomorphs. Because only a small amount of residue was retained on the mesh, this step was conducted only once. Despite these modifications, insoluble colloids still developed in approximately half of the processed samples, requiring additional treatment with hydrochloric acid (19 % HCl) for 6 hours.

Several authors have indicated that deflocculation is generally more effective after hydrofluoric acid digestion (Traverse, 2007; Riding, 2021 and references therein). However, for the Puerto Visser samples, implementation of post-HF deflocculation was not feasible because colloid formation had already significantly compromised residue quality.

The first and second treatments were not pursued further because of the limited recovery of organic residue and the prolonged processing time involved. In contrast, the third treatment yielded the highest quantity of organic

residue and proved to be the most operationally efficient.

For the Puerto Visser samples, the results indicate that the most effective processing strategy involved allowing colloidal particles to form after HF treatment and subsequently removing them through additional hydrochloric acid (HCl) treatments at controlled temperature below 70 °C to avoid damage to delicate palynomorphs. Although the present study focused on evaluating previously published sample preparation methodologies designed to overcome and prevent colloid formation, future research incorporating detailed mineralogical characterization of the sediments could improve prediction of sample behavior during processing and facilitate the selection of the most appropriate treatment for each sediment type. A better understanding of sediment mineralogy may also contribute to identifying potential relationships between sediment composition and colloid formation. Furthermore, future studies applying the three palynological methodologies to the same sediment sample would enable a more robust and direct comparison of their relative effectiveness on organic residue recovery.

Nevertheless, alternative non-standard processing techniques have increasingly been proposed, particularly hydrofluoric-acid-free methods for extracting palynomorphs from sediments and sedimentary rocks (Riding & Kyffin-Hughes, 2004, 2006, 2010; O'Keefe & Eble, 2012). These methodologies commonly employ sodium hexametaphosphate ($\text{NaPO}_3)_6$, hydrogen peroxide (H_2O_2), and related reagents to promote sample disaggregation or deflocculation (Riding & Kyffin-Hughes, 2010). However, these non-hazardous approaches could not be evaluated in the present study because the necessary reagents were not available at the laboratory.

CONCLUSIONS

Three modified processing protocols were implemented to remove and/or prevent the formation of calcium fluoride colloids.

The first treatment, applied after colloid formation had already occurred, involved hydrochloric acid treatment initially at room temperature and subsequently in a water bath at 45 °C and 60 °C. Following each acid step, residues

were rinsed and centrifuged three times in a sodium pyrophosphate solution.

The second treatment incorporated an intermediate clay deflocculation stage following the initial hydrochloric acid digestion, modified from the procedures described by Bates et al. (1978) and Batten (1999). Although the first application proved successful, subsequent attempts resulted in renewed calcium fluoride colloids formation. Consequently, the final hydrochloric acid treatment described by Louwye et al. (2004) was modified and performed in a water bath at 60 °C for 1 hour, followed by an additional 24-hour treatment at 20–25 °C.

The third treatment involved increasing the number of rinsing steps between the initial hydrochloric acid and hydrofluoric acid treatments following modifications of Batten (1999), while maintaining the modified hydrochloric acid water bath treatment described above. Although increasing the number of rinses initially improved processing efficiency, several samples still required additional hydrochloric acid treatments at elevated temperatures.

Overall, for the Puerto Visser samples, the most effective strategy consisted of allowing calcium fluoride colloids to form and subsequently removing them through controlled hydrochloric acid treatments conducted in water baths. In this particular case, intermediate deflocculation procedures commonly recommended in the literature were not viable because residue recovery remained extremely low and colloid formation persisted despite treatment.

The modified protocol proposed here may provide a useful alternative when conventional palynological processing methods fail to produce satisfactory results, particularly in cases where no standardized approach is available for the simultaneous removal of finely disseminated clay particles and calcium fluoride colloids formed during sample processing.

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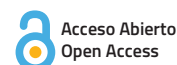
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