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Clean-up procedures for the unbiased estimation of C_{37} alkenone sea surface temperatures and terrigenous *n*-alkane inputs in paleoceanography

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Abstract

Sample preparation procedures for the quick determination of C_{25}–C_{33} *n*-alkanes and the U^{k}_{37} index in small sediment sample amounts (ca. 1 g dry weight) have been developed. Alkaline hydrolysis and silica column fractionation are the two clean-up steps considered. The former eliminates the interferences from the sedimentary wax esters which dominate the lipid composition in high latitude Atlantic Ocean sediments. The latter has been designed to collect both lipid groups (C_{25}–C_{33} *n*-alkanes and C_{37} alkenones) in one single fraction avoiding the interferences from highly polar compounds which affect, in particular, the quantitative determination of the *n*-alkanes. The whole procedure has been shown not to affect the U^{k}_{37} measurements, even at low C_{37} ketone concentrations such as 10 ng which is the minimum threshold of total C_{37} alkenones in the gas chromatographic system for avoidance of adsorption-related interferences [1]. As shown with real case examples the usefulness of these clean up methods is highly dependent on the amounts of interfering lipid compounds present in the sediment extracts and their application may not be necessary for the open sea sediments present in most low latitude areas.

Keywords: Clean-up methods; Sea surface temperature; Alkenones; Alkanes

1. Introduction

The development of a method for the estimation of sea surface paleotemperatures based on the determination of the sedimentary composition of C_{37} unsaturated alkenones [2] has encouraged the widespread application of this technique. The method is based on the measurement of the concentrations of heptatriaconta-(15E,22E)-dien-2-one ($C_{37:2}$) and heptatriaconta-(8E,15E,22E)-trien-2-one ($C_{37:3}$) to determine a relative composition index [U^{k}_{37} = $C_{37:2}/(C_{37:2}+C_{37:3})$] that is calibrated against temperature [3–5].

The results have also prompted the investigation of other gas chromatography-amenable molecules as sources of paleoceanographic information, i.e., *n*-alkanes [6]. Thus, the demand for higher number of lipid determinations at higher temporal core resolution has increased considerably. Unfortunately, very limited attention has been devoted to the analytical criteria required to avoid erroneous U^{k}_{37} determinations and measurements of lipid abundances in the procedures developed.

In a previous paper [1], we considered several gas chromatographic pitfalls that may invalidate the

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reliability of the U^k_{37} determinations. One major source of error is the coelution of the C_{37} di- and triunsaturated ketones with other compounds. Adequate stationary phase selection, and hence polarity, of the capillary column may considerably reduce the importance of these interferences [1] (i.e., Fig. 1A). However, when these ketones are not dominant in the sedimentary lipid extracts sample clean-up is required (i.e., Fig. 2A).

Alkaline hydrolysis and column chromatography are the current methods of choice in these situations [7,8]. In paleooceanography, these procedures have to be scaled down (i.e., to 1 g of sediment) to minimize sample amount, analysis time and cost. This aspect is particularly important in view of the large number of samples to be analyzed in these studies (i.e., 50–500 per sediment core). However, possible biases in the U^k_{37} measurements resulting from these clean up applications have to be taken into account. The present paper is devoted to the development of some of these scaled down procedures for the analysis of n-alkanes and C_{37} ketones which do not introduce deviations in the U^k_{37} determinations. In all cases, the procedures aim towards the collection of these two types of lipids in one single fraction allowing their joint gas chromatographic analysis. This is feasible due to the lack of coeluting peaks between these two groups of compounds and involves a substantial reduction in terms of time and cost.

2. Experimental

2.1. Reagents and standards

Residue analysis dichloromethane, n-hexane, methanol and toluene were obtained from Merck (Darmstadt, Germany). Potassium hydroxide pellets pro analysi were also obtained from Merck and cleaned (three times) by extraction with n-hexane in an ultrasonic bath. Deionized water was purified with a Milli-Q system (Millipore). Neutral silica gel (Kieselgel 40, 70–230 mesh, Merck) was cleaned by extraction with dichloromethane in a Soxhlet apparatus for 36 h and, after solvent evaporation, stored deactivated. Alkenone standards, heptatriaconta-15E,22E-dien-2-one and heptatriaconta-8E,15E,22E-trien-2-one, were kindly provided by Prof. James

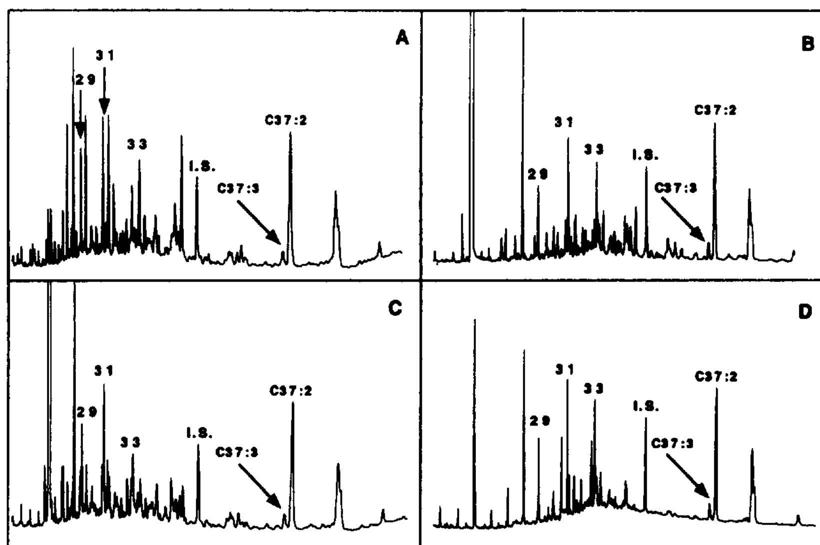

Fig. 1. Gas chromatograms of the sediment extract from a South China Sea low latitude sediment of glacial periods (Sample A, 8.30°N, 112.19°E). (A) Total extract. (B) After fractionation with a silica column. (C) Neutrals after alkaline hydrolysis. (D) Cleaned up extract after alkaline hydrolysis and column chromatography fractionation. Numbers refer to n-alkane chain length. $C_{37:2}$: heptatriaconta-(15E,22E)-dien-2-one. $C_{37:3}$: heptatriaconta-(8E,15E,22E)-trien-2-one. I.S.: internal standard.

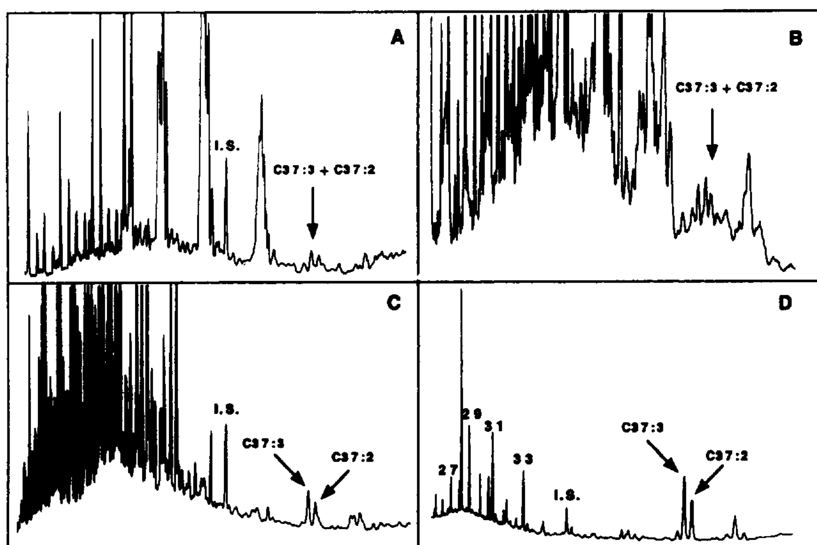

Fig. 2. Gas chromatograms of the sediment extract from a high latitude North Atlantic sediment (Sample B, 52.03°N, 21.06°W). (A) Total extract. (B) After fractionation with a silica column. (C) Neutrals after alkaline hydrolysis. (D) Cleaned up extract after alkaline hydrolysis and column chromatography fractionation. Numbers refer to *n*-alkane chain length. $C_{37:2}$: heptatriaconta-(15E,22E)-dien-2-one. $C_{37:3}$: heptatriaconta-(8E,15E,22E)-trien-2-one. I.S.: internal standard.

Maxwell (Environmental and Analytical Unit, School of Chemistry, University of Bristol, UK).

2.2. Sample preparation and extraction

Prior to extraction, the sediment samples were freeze-dried and manually ground for homogenization. After addition of an internal standard of *n*-hexatriacontane, 2 to 3 g of sediment were extracted three times in an ultrasonic bath (20 min) with 3×10 ml of dichloromethane. The extracts were collected together and evaporated to dryness under a nitrogen stream.

2.3. Alkaline hydrolysis

The evaporated extracts were dissolved with 3 ml of 6% potassium hydroxide in methanol and stored overnight. Non acidic compounds were recovered by extraction with *n*-hexane (3×3 ml). The resulting extracts were cleaned with water (1 ml) to eliminate possible KOH traces. This last step was skipped when the samples were to be treated by column chromatography.

2.4. Column chromatography

The columns (30×0.5 cm I.D.) were packed with 2 g of silica (dead volume ca. 2.5 ml). The adsorbent was suspended in the same solvent mixture to be used for compound elution. Different mixtures of solvents were added for evaluation of different chromatographic conditions (5:5, 7:3, 8:2 dichloromethane–*n*-hexane). The collected solvents were evaporated under a nitrogen stream and redissolved with 20 μl of toluene.

2.5. Gas chromatographic analysis

Prior to instrumental analysis, the extracts were derivatized overnight with bis-trimethylsilyl-trifluoroacetamide (BSTFA) at room temperature. These extracts were analyzed in a Varian Model 3400 equipped with a septum programmable injector (SPI), a flame ionization detector and a CPSIL-5 CB (Chrompack, Middelburg, Netherlands) capillary column (50 m×0.32 mm I.D.) coated with 100% dimethylsyloxane (film thickness 0.25 μm). Hydrogen was the carrier gas (50 cm/s). The oven temperature was programmed from 90 to 200°C at

20°C/min, then to 305°C at 15°C/min, holding time 20 min, and to 320°C at 15°C/min, final time hold 19 min. Injector temperatures were programmed from 90°C (holding time 1 min) to 300°C at 200°C/min (final time hold 10 min). Then, the injector was cooled to 90°C with a gas stream.

3. Results and discussion

The maximum temperature changes between the Last Glacial Maximum and the present interglacial in most open sea surface oceanic waters (excluding the North Atlantic) were in the range of 1–3°C [9]. This conclusion is also supported by the temperature data calculated from the U^{k}_{37} index [10–12]. These maximum ranges of variation require temperature proxies with maximum error tolerances of 0.5°C to provide the paleotemperature measurements with a quantitative meaning which may be useful for paleoclimatic models.

The relationship between U^{k}_{37} index and temperature has been established by means of top core sediment analyses and correlation with surface waters [5,13] or *Emiliania huxleyi* cultures under temperature controlled conditions [3,4]. There is general agreement on the slope and range of application of the resulting straight lines [3–5]. According to these results the maximum U^{k}_{37} deviation to fulfil the 0.5°C requirement is 0.0165 [1,3].

3.1. Column chromatography

Elution of silica columns with solvent series of increasing polarity allows the sequenced withdrawal of the major lipid groups, i.e., *n*-alkanes, aromatic hydrocarbons, esters, ketones, aldehydes and alcohols [7,14–16], from the sediment extracts. This approach is, in principle, useful for the separation of *n*-alkanes and C_{37} ketones from other major sedimentary lipids. As indicated above, in the present study, the column chromatography procedure was aimed at isolating these two compound classes in one single fraction. Since *n*-alkanes are poorly retained in silica columns, the main aspect to be addressed in the fractionation procedure is the separation of ketones in the presence of large amounts of alcohols.

This objective has been addressed from the study of the separation of small amounts of C_{37} alkenone standards (200 ng) from larger amounts of cholestanol (4000 ng) and octadecanol (4000 ng). The results of the elution behaviour of this mixture under different mixtures of dichloromethane and *n*-hexane are summarized in Table 1. Recoveries higher than 100% are explained by partial evaporation of solvent prior to the gas chromatographic analysis (samples were redissolved with only 20 μl of toluene).

Each of the values shown in Table 1 corresponds to the average of three replicates. The standard deviations range between 1–2 and 3–7% of the mean for the ketones and polar compounds (*n*-octadecan-1-ol and cholestan-3β-ol), respectively. The absolute error in the U^{k}_{37} index due to this alkenone variability is smaller than 0.003, which corresponds to absolute temperature errors below 0.08°C [1,3].

Elution with a mixture of dichloromethane–*n*-hexane (5:5) separates completely the ketones from the alcohols. However, the C_{37} ketones elute over relatively large volumes (92% recovery after an elution/dead volume ratio of 5.6). Fractionation between the unsaturated C_{37} ketone species is observed in these conditions which results in major changes in U^{k}_{37} index when the eluates of fractions I–IV (Table 1) are analyzed separately. Mixtures of dichloromethane–*n*-hexane (7:3) do not allow complete separation of C_{37} alkenones and *n*-octadecanol. Furthermore, these conditions also involve some spreading of the C_{37} alkenones among fractions I–IV, giving rise to U^{k}_{37} calculation errors.

Complete separation from the alcohols is obtained with dichloromethane–*n*-hexane (8:2). The alkenones elute in the first 8 ml (elution/dead volume ratio 3:2) while the alcohols only appear after addition of 12 ml of solvent. No discriminating fractionation between the $C_{37:2}$ and $C_{37:3}$ ketones is observed after elution of 8 ml of this solvent mixture which avoids errors in the measurement of the U^{k}_{37} index.

3.2. Recoveries and influence of the analytical procedures on U^{k}_{37} measurements

Performance tests on gas chromatographic columns have shown that the U^{k}_{37} measurements can be affected by irreversible adsorption of alkenones to

Table 1
Recovery tests of $C_{37:2}$ alkenones, *n*-octadecanol and cholestanol in a silica column eluted with three different dichloromethane–*n*-hexane mixtures (5:5, 7:3, 8:2)

Solvent (DCM:Hex)	Fraction	Volume (ml)	Recovery			U^k_{37}	Errors	
			C_{37} Alkenones (%)	*n*-Octadecanol (%)	Cholestanol (%)		U^k_{37}	Temperature (°C)
(5:5)	I	8	7.8	0	0	0.922[a]	0.177[b]	5.3[c]
	II	2	43	0	0	0.823	0.078	2.3
	III	2	34	0	0	0.653	−0.092	−2.8
	IV	2	7.3	0	0	0.420	−0.325	−9.8
(7:3)	I	8	94	0	0	0.739	−0.006	−0.20
	II	2	5.4	0	0	0.755	0.010	0.30
	III	2	4.5	1.5	0	0.734	−0.010	−0.33
	IV	2	0	40	0.3			
(8:2)	I	8	109	0	0	0.745	−0.003	−0.11
	II	2	0	0	0			
	III	2	0	10	0			
	IV	2	0	70	5			

Four fractions obtained after successive addition of 8, 2, 2 and 2 ml have been collected for each test. Complete separation of alkenones from the alcohols is achieved using a volume of 8 ml of dichloromethane–*n*-hexane (8:2). The U^k_{37} index of the C_{37} alkenone standard is 0.7415.

[a] Calculated from the C_{37} alkenones eluting in each fraction.

[b] The spread of C_{37} alkenones among various fractions leads to significant U^k_{37} errors. This spread is avoided in the conditions developed in this study [8 ml of dichloromethane–methanol (8:2)].

[c] Calculated from U^k_{37} with the equation of Prahl and Wakeham [3].

the capillary column [1]. This effect is significant at low alkenone concentrations and defines a minimum concentration threshold for reliable U^k_{37} measurements [1].

Partial irreversible adsorption may also occur in preparative column chromatography. Other methods of fractionation, i.e., alkaline hydrolysis, may eventually introduce biases in this index due to the partial recovery of trace amounts of these ketones or to coelution with lipids which have not been separated with this method. Thus, both the above described fractionation method and alkaline hydrolysis have been tested for possible U^k_{37} biases in the analysis of small amounts of C_{37} ketones (Table 2). The range of ketones considered (20 to 200 ng) is representative of the amounts currently encountered in high resolution studies (e.g., 500 *y*) involving the analysis of ca. 1 g of sediment in sites such as the central Atlantic Ocean or the South China Sea.

In cases of silica column chromatography with elution with dichloromethane–*n*-hexane (8:2), alkaline hydrolysis and the combination of both methods, the errors observed are always smaller than 0.012 (equivalent to 0.4°C; Table 2) showing that neither column chromatography nor saponification are significant sources of error.

On the other hand, in cases of column chromatography, alkaline hydrolysis and their combined application, the recoveries for *n*-alkanes and C_{37} ketones are in the range of 90–110% (Table 2), which also supports the suitability of these procedures for quantitative studies.

3.3. Samples

The usefulness of these methods can be evaluated from the analysis of two representative samples of the sediments usually encountered in the open ocean. Sample A (8.30°N, 112.19°E) is a low latitude sediment of glacial periods from the South China Sea in which wax esters are in small proportion and the C_{37} alkenones are the predominant lipids. Sample B (52.03°N, 21.06°W) is characteristic of the high latitude North Atlantic sediments that contains high concentrations of wax esters and other lipids together with a small relative proportion of C_{37} alkenones.

Table 2
Recoveries and U^k_{37} indices corresponding to the analysis of diverse C_{37} alkenone amounts by the alkaline hydrolysis and column chromatography procedures developed in this study

Procedure	C_{37} alkenone amounts (ng)	Recoveries (%)		U^k_{37}	Error	
		C_{36} *n*-alkane	C_{37} alkenones		U^k_{37}	Temperature (°C)
Alkaline hydrolysis	60	90	113	0.733	−0.012	−0.4
	20	92	110	0.736	−0.009	−0.3
Column chromatography	20	93	105	0.754	0.009	0.2
	60	95	109	0.737	−0.008	−0.3
	200	96	110	0.741	−0.004	−0.1
Alkaline hydrolysis and Column chromatography	60	108	96	0.746	0.001	0
	20	110	91	0.758	0.013	0.4

The U^k_{37} index of the alkenone standard is 0.7415.

The chromatograms corresponding to the solvent extracts of these two samples are shown in Fig. 1A and Fig. 2A. Comparison of these two figures clearly illustrates the impossibility of the determination of the *n*-alkane concentrations and the U^k_{37} index in the second case.

The results obtained from column chromatography, alkaline hydrolysis and/or the combination of both methods are illustrated in Figs. 1B–D and Figs. 2B–D). The corresponding U^k_{37} determinations are summarized in Table 3. In the first case, the differences between the indices measured with each procedure are smaller than 0.015 which corresponds to 0.4°C. There no substantial improvement in the U^k_{37} measurements as consequence of the clean up procedures. Nevertheless, even in this case, with samples being apparently free from interferences, the use of the hydrolysis step is recommended to eliminate wax esters that may accumulate in the capillary column shortening its live time.

Conversely, major differences are observed in sample B as consequence of the application of each clean up procedure (Figs. 2B–D). The differences may account up to 0.046 which corresponds to 1.4°C. In this second case, only column chromatography is not sufficient for the elimination of the interferences with the C_{37} alkenones. For this purpose, the most efficient clean up procedure is alkaline hydrolysis. Thus, the difference in U^k_{37} measurements between this step and alkaline hydrolysis combined with column chromatography is 0.001 (0.1°C). Column chromatography is useful for the elimination of interferences from the *n*-alkanes (Fig. 2C,D). However, this procedure is only efficient after

Table 3
C_{37} alkenone concentrations (ng/g) and U^k_{37} index obtained from the application of the alkaline hydrolysis and column chromatography procedures to South China Sea (A) and North Atlantic Ocean (B) sediments

Sample	Method	C_{37} alkenones	U^k_{37}	Temperature
A	Extraction	490	0.905	26.1
	Column chromatography	470	0.904	26.1
	Alkaline hydrolysis	520	0.920	26.5
	Alkaline hydrolysis and column chromatography	470	0.903	26.1
B	Extraction	51	0.446	12.2
	Column chromatography	47	0.444	12.1
	Alkaline hydrolysis	38	0.403	10.9
	Alkaline hydrolysis and column chromatography	59	0.400	10.8

alkaline hydrolysis of the extracts. Single column chromatography following the above described conditions is not sufficient to obtain quantifiable gas chromatographic traces of the n-alkane distributions (Fig. 2B).

4. Conclusions

The combination of alkaline hydrolysis and column chromatography affords the analysis of n-alkanes and C_{37} alkenone U^{k}_{37} index in open ocean sediments even when these compounds are minor constituents of the sedimentary lipid extract. The column chromatography procedure developed in this study allows the joint analysis of these two paleooceanographic proxies in the same lipid fractions representing a considerable advantage in terms of analysis time and cost. The clean up procedures developed do not modify the U^{k}_{37} measurements, even at very low C_{37} ketone concentrations (20 ng). Nevertheless, as shown in the real case examples, the need for these cleanup procedures is highly dependent on the amounts of interfering compounds in the sediments to be studied.

Acknowledgments

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