Pitfalls in the chromatographic determination of the alkenone U_{37}^k index for paleotemperature estimation ☆

Joan Villanueva, Joan O. Grimalt*

Department of Environmental Chemistry (CID–CSIC), Jordi Girona 18, 08034 Barcelona, Catalonia, Spain

Abstract

Co-elution and irreversible adsorption are major error sources in the gas chromatographic determination of paleotemperatures from the analysis of C_{37} di- and triunsaturated ketones. The former are related to the interference of methyl hexatriaconta-(7E,14E,21E)-trienoate with heptatriaconta-(15E,22E)-dien-2-one. These compounds can be resolved using capillary columns with a thin coating (ca. 0.1 μm) of 100% dimethylsiloxane. Irreversible adsorption concerns both the di- and triunsaturated species and has significant effects (deviations of temperature measurements higher than 0.5°C) when the absolute total amounts of ketones introduced into the system are smaller than 10 ng. The higher adsorption of the $C_{37:3}$ species gives rise to deviations of the U_{37}^k measurements towards higher temperatures that become more important the lower is the proportion of this species in the mixture. This adsorption effect also increases the dispersion of the results as a higher standard deviation is observed the lower is the total amount of ketones and the proportion of the triunsaturated species.

1. Introduction

A recent major achievement in organic geochemistry has been the development of a method for the measurement of sea surface temperatures (SST) in the past oceans. The method is based on the gas chromatographic determination of the composition of C_{37}–C_{39} ketones in sedimentary sections. The relationship of this composition to temperature is based on biological and geochemical findings. First, cultures of the precursor algae, some coccolithophoridae species and particularly *Emiliania huxleyi*, show a well defined relationship between the proportion of di- and triunsaturated ketones and growth temperature conditions [1]. Second, the latitudinal spatial distribution of these ketones in the sediments underlying the world oceans shows a good correlation with the average water column temperatures [2,3].

Other properties have contributed to the general interest in these alkenones for paleotemperature measurement. Thus, they are specifically produced by a few coccolithophoridae algae and one species, *E. huxleyi*, is the principal precursor in the oceans [4], being widespread over the whole planet. Further, these ketones are major lipid compounds in the oceanic sediments and their distribution features related to temperature are not modified by diagenesis,

---

* Corresponding author.
☆ Presented at the *23rd Annual Meeting of the Spanish Chromatographic Society, Peñiscola, 19–21 October 1994.*

even in the case of strong organic matter oxidation effects [5]. These properties show that the sedimentary prymnesiophyte alkenone signal is probably related to the temperature of the corresponding overlying waters since it is unlikely to be modified by contributions from other biosynthesizers or post-depositional processes. The use of this method for SST determination during the Pleistocene is increasing, as it constitutes an interesting tool for the elucidation of a parameter of high climatic interest (defining the boundary conditions between atmosphere and hydrosphere), which can only be approached by complex and uncertain methods.

However, the relationship between C_{37}–C_{39} ketone composition and temperature is related to small changes in the relative distribution of these lipids and requires accurate and precise measurements of their concentration for reliable estimates. These requirements are particularly important in sediment core studies in which the temperature changes to be identified range within a few degrees Celsius. Since gas chromatography is widely used for these analyses, the development of methods for temperature prediction poses stringent requirements in terms of co-elution and irreversible adsorption and also dispersion of the area measurement. These aspects have to be mastered prior to general use. In this study, we determined the analytical constraints for the gas chromatographic determination of the C_{37} alkenones in sedimentary stratigraphic studies. Further, we found that if these relatively restricted analytical requirements are not fulfilled, major errors may be introduced as consequence of the use of the alkenone paleothermometer.

2. Theory

2.1. The U_{37}^k index

The experimental relationship between temperature and the distribution of di- and triunsaturated ketones synthesized by some coccolithophoridae is expressed by means of empirical equations that correlate the relative composi-

tion of the C_{37} homologues, those present in higher abundance, and temperature measurements. The relative composition of C_{37} ketones is expressed by means of two indices:

$$U_{37}^k = (C_{37:2} - C_{37:4})/(C_{37:2} + C_{37:3} + C_{37:4}) \quad (1)$$

where $C_{37:2}$, $C_{37:3}$ and $C_{37:4}$ are the concentrations of heptatriaconta-(15E,22E)-dien-2-one, heptatriaconta-(8E,15E,22E)-trien-2-one and heptatriaconta-(8E,15E,22E,29E)-tetraen-2-one, respectively. Eq. 1 is generally simplified to

$$U_{37}^k = C_{37:2}/(C_{37:2} + C_{37:3}) \quad (2)$$

Eq. 2 does not consider the concentrations of the tetraunsaturated homologue because this species is a minor component that is encountered only in oceanic sediments of mid- or high latitude.

2.2. Temperature-U_{37}^k equations

Diverse empirical equations relating temperature measurements and the U_{37}^k index have been obtained. All equations have a linear form and have been generated by least-squares fitting to experimental data sets. These data were obtained from either *Emiliania huxleyi* cultures under temperature-controlled conditions [1,6], water column measurements [1,7] (the U_{37}^k in the filtered particles is related to average temperatures in the sampling site) or top core sediment analyses [3,8] (the U_{37}^k analyses are related to average temperatures in the overlying water column). The regression coefficients obtained in each case do not differ significantly. The equation most widely used is that reported by Prahl and Wakeham [1] and we shall consider this for simplicity:

$$U_{37}^k = 0.033T + 0.043 \quad (3)$$

where T is temperature in °C.

2.3. Paleoclimatic requirements for U_{37}^k

The transformation of Eq. 3 gives

$$T = 30.3U_{37}^k - 1.30 \quad (4)$$

and

$$\Delta T = 30.3 \Delta U_{37}^{k} \qquad (5)$$

Temperature predictions are useful for paleo-climatic models when the maximum error tolerance is 0.5°C. According to Eq. 5, this condition involves a maximum error of 0.0165 for the U_{37}^{k} determinations. Therefore, the summed systematic and random errors of the chromatographic determination of $C_{37:2}$, $C_{37:3}$ and $C_{37:4}$ must not exceed this threshold.

2.4. Chromatographic precision

A maximum error of 0.0165 in the U_{37}^{k} measurements requires relative peak quantification errors of the order of 1%. To achieve this precision, replicate injections and determination of the resulting standard deviation are needed. Taking into account the large number of U_{37}^{k} determinations required in each study, *ca.* 50–500 samples per sediment core, a feasible number of replicates is three. In these conditions, Student's *t*-test can be used to determine the maximum standard deviation that can be accepted in the chromatographic system to give errors below the 0.0165 threshold:

$$s = 0.0165 \cdot 3^{-1/2} / t \qquad (6)$$

where *s* is the standard deviation of the U_{37}^{k} replicate measurements and *t* is the tabulated statistic for two degrees of freedom. According to the tabulated *t* values for 99, 97.5 and 95% confidence levels, the maximum standard deviation for the calculated U_{37}^{k} must be 0.0041, 0.0066 and 0.0098, respectively.

3. Experimental

3.1. Materials

Residue-analysis grade isooctane was obtained from Merck (Darmstadt, Germany). The alkenone standards, heptatriaconta-(15*E*,22*E*)-dien-2-one and heptatriaconta-(8*E*,15*E*,22*E*)-trien-2-one, were kindly provided by Professor J.R. Maxwell (Organic Geochemistry Group, University of Bristol) [9].

3.2. Capillary columns

Two columns were used for the determination of alkenones: (a) DB-5 (J&W Scientific, Folsom, CA, USA): 5% phenyl–95% methylsiloxane, 25 m × 0.25 mm I.D., 0.25 μ film thickness; and (b) CPSIL-5CB (Chrompack, Middelburg, Netherlands): 100% dimethylsiloxane, 50 m × 0.32 mm I.D., 0.12 μm film thickness.

3.3. Gas chromatographic equipment

Two chromatograms were used for the analyses: (A) Carlo Erba Model HRGC5300 equipped with a flame ionization detector and on-column injection; carrier gas, hydrogen (50 cm/s); oven temperature, programmed from 80 to 200°C at 20°C/min, then to 295°C at 6°C/min, holding the final temperature for 20 min; detector temperature, 320°C, injection (solvent, isooctane) either in the splitless mode (300°C, split valve close for 55 s, hot needle technique) or on-column (70°C); and (B) Varian Model 3400 equipped with a septum programmable injector (SPI) and a flame ionization detector; carrier gas, oven and detector temperatures as for (A); injection temperature, programmed from 80°C (held for 1 min) to 300°C at 200°C/min, holding the final temperature for 15 min; the injector was cooled to 80°C with a gas stream.

4. Results and discussion

4.1. Co-elution

One obvious potential cause of systematic errors in U_{37}^{k} measurements is co-elution of the $C_{37:2}$, $C_{37:3}$ and $C_{37:4}$ ketones with other compounds present in the solvent extracts. Fortunately, the high molecular mass of these ketones (*m/z* 526–530) involves high-temperature elution (*ca.* 300°C) in a chromatographic zone where few marine sedimentary lipids are encountered. Possible problems related to thermal ketone decomposition were not observed on comparing the analyses performed at 300°C with lower final temperature programmes. Elution at a final

temperature of 300 or 310°C is recommended in order to have sharp peaks for easier quantification. Obviously, this aspect is particularly important when the ketones are in low concentration.

In practice, the co-elution problems concern the C_{36} di- and triunsaturated methyl alkenoates. Other compounds that could also elute in the same temperature range, e.g. some glycerides, have not been found to be significant in this respect. The abundance of C_{36} di- and triunsaturated methyl alkenoates relative to ketone concentrations increases with the latitude of the sampling station (colder temperatures).

The high efficiency and adequate selectivity needed to avoid co-elution of these alkenoates with the alkenones are achieved by using capillary columns of small film thickness (ca. 0.1 μm) coated with 100% dimethylsiloxane (Fig. 1). Other columns widely used for this type of analysis, such as 5% phenyl–95% methylsiloxane columns [1,6,8,10,11], do not allow a correct separation between methyl hexatriaconta-(7E,14E,21E)-trienoate and heptatriaconta-(15E,22E)-dien-2-one. The poor resolution with this stationary phase is observed even with columns of small film thickness (Fig. 1).

4.2. Irreversible adsorption

In paleoceanography, the requirements for larger numbers of determinations and higher temporal core resolution encourages the scaling down of the methods towards the use of smaller amounts of sediment samples. On these grounds, the reliability of the gas chromatographic U_{37}^k determination with decreasing amounts of ketones was tested using $C_{37:2}$ and $C_{37:3}$ standard solutions that were repeatedly diluted and injected in triplicate. This test of column performance was carried out with on-column injection to minimize the possible disturbances associated with the introduction of the analytes outside the column.

According to the results, the dispersion between replicates increases considerably as the amounts of ketones introduced into the system are decreased (Fig. 2). Small amounts of ketones
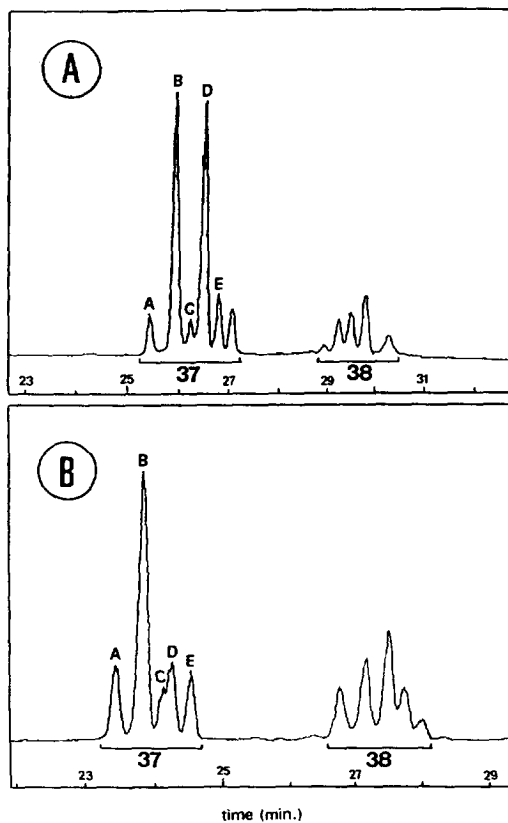

Fig. 1. Representative gas chromatograms of sedimentary samples containing mixtures of long-chain ketones and alkenoates on analysis with (A) CPSIL-5CB and (B) DB-5 columns. Peaks: A = heptatriaconta-(8E,15E,22E,29E)-tetraen-2-one; B = heptatriaconta-(8E,15E,22E)-trien-2-one; C = methyl hexatriaconta-(7E,14E,21E)-trienoate; D = heptatriaconta-(15E,22E)-dien-2-one; E = methyl hexatriaconta-(14E,21E)-dienoate; 37 and 38 refer to the eluting peak clusters of the heptatriacontane and octatriacontane homologues.

also lead to a decrease in accuracy. Deviations of the average values towards U_{37}^k indices corresponding to warmer temperatures are observed in the analysis of more diluted standards.

Curve fitting of the equation

$$U_{37}^k = (C_{37:2} - A_2)/(C_{37:2} - A_2 + C_{37:3} - A_3)$$

$$(7)$$

to the average replicate values obtained in these tests gave values of 0.1 and 0.25 for A_2 and A_3, respectively. This equation is typical of partial
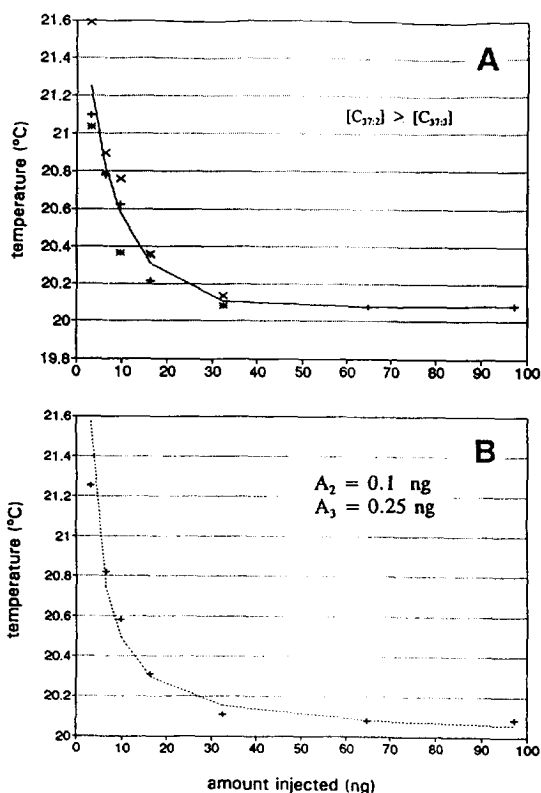
Fig. 2. Changes in the U_{37}^k index with decreasing concentration of total alkenones. (A) Average index of the experimental determinations, where ×, + and * correspond to replicates of the same mixture; (B) curve-fitting line as described in Eq. 7, where + = average points of the replicate determinations shown in (A), the dotted line is the curve-fitting line to these average points and A_2 A_3 are the curve-fitting adsorption constants defined in Eq. 7.

irreversible adsorption of both ketones in the capillary column. The A_2 and A_3 values show that the triunsaturated species is more adsorbed, leading to U_{37}^k deviations towards warmer temperatures. These curve-fitted values, and the curves displayed in Fig. 2, also define a threshold concentration by which the analyses involving total alkenone amounts below that limit are not reliable. In the chromatographic system used for this test, alkenone amounts below 10 ng gave rise to deviations of more than 0.5°C in the predictions.

As these threshold concentrations are related to adsorption effects, the observed values depend on the performance of the column used in

the analyses. The trend described in Fig. 2 was obtained with a new CPSIL-5CB column. In columns that have lost some of their initial performance (e.g., after 200 injections), the effects of loss of precision and accuracy increase considerably. This is illustrated in Fig. 3, where the U_{37}^k values obtained after SPI injection in one new and one old column are compared for a series of two standard mixtures diluted at various ratios. The two mixtures correspond to representative cases of warm and cool temperatures and low and high proportions of $C_{37:3}$. Note that the term "old" column refers to a column used for multi-purpose analysis, e.g., analyses of all sorts of lipids in addition to ketones. If the column is only used for C_{37}–C_{39} ketone analysis, this decrease in performance is not observed.

According to Eq. 7, higher average U_{37}^k deviations in the measurement of mixtures having lower $C_{37:3}$ concentrations should be expected. Thus, the trend towards higher U_{37}^k indices (higher temperature) at smaller ketone amounts is observed in all cases shown in Fig. 3, even when the dominant ketone is the triunsaturated
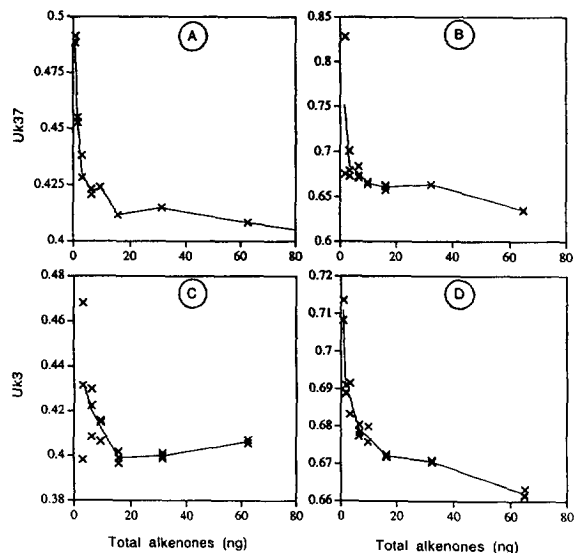

Fig. 3. U_{37}^k indices corresponding to two series of standard mixtures of $C_{37:2}$ and $C_{37:3}$ alkenones diluted in various ratios. (A, B) Injection into a new column; (C, D) injection into an old column (e.g., that use for multi-purpose analysis after 200 injections). The two mixtures represent commonly encountered values of cold (A, C) and warm (B, D) waters.

Table 1
Standard deviations resulting from replicate analyses of two alkenone mixtures using new and old CPSIL-5CB columns

Cold temperature, $U_{37}^k = 0.405$			Warm temperature, $U_{37}^k = 0.665$		
Total alkenones (ng)	Standard deviation (ng)		Total alkenones (ng)	Standard deviation (ng)	
	New column	Old column		New column	Old column
0.78	0.0015	n.d.	1.62	0.0010	0.029
1.56	0.00097	n.d.	3.24	0.0041	0.0088
3.13	0.0031	0.0029	6.49	0.0013	0.0042
6.26	0.0011	0.0088	9.73	0.0019	0.0021
9.39	0.00	0.0042	16	0.00027	0.0010
15	0.00	0.0021	32	0.00024	0.00080
31	0.00	0.0010	65	0.00082	
63	0.00	0.00080			
83	0.00				

species. Further, the decrease in accuracy associated with the use of an old column is considerably higher when the measured U_{37}^k corresponds to ratios with a lower abundance of the $C_{37:3}$ species. Accordingly, the curves displayed in Fig. 3 are fully consistent with the constant values resulting from the curve fitting of Eq. 7. In some cases, these average U_{37}^k deviations in mixtures with a low proportion of $C_{37:3}$ are even observed at high ketone abundances.

The modifications in the measured U_{37}^k not only concern decreases in accuracy. As shown in Table 1, the standard deviation of the measurements is considerably higher when the old column is used. Further, the differences are more important for the warm (maximum standard deviation 0.08 vs. 0.0045 ng for the old and new columns, respectively) than for the cold temperatures (0.03 vs. 0.007 ng, respectively). This difference corresponds to the higher analytical dispersion for the mixtures containing lower concentrations of the species more affected by irreversible adsorption.

5. Conclusions

Two types of distortion effects were encountered in the study of the analytical constraints for the gas chromatographic determination of the composition of C_{37} di- and triunsaturated ketones for SST measurement. One is more specific to deviations at low temperature and the other is particularly significant at high temperature. The former involves the co-elution of methyl hexatriaconta-(7E,14E,21E)-trienoate with heptatriaconta-(15E,22E)-dien-2-one and can be resolved by using capillary columns with a thin coating (ca. 0.1 μm) of 100% dimethylsiloxane. The second concerns the irreversible adsorption of these ketones, particularly of the $C_{37:3}$ species. However, both error sources may be significant (error >0.5°C) for all measurements.

Under the practical operating conditions selected for automatic analysis (average measurement of three replicate injections per sample), the adsorption effects in the capillary column define a sedimentary C_{37} alkenone threshold of 100 ng/g as the lowest concentration limit for reliable paleotemperature determination.

References

[1] F.G. Prahl and S.G. Wakeham, Nature, 320 (1987) 367.

[2] S.C. Brassell, G. Eglinton, I.T. Marlowe, U. Pflaumann and M. Sarnthein, Nature, 320 (1986) 129.

[3] A. Rosell, Ph.D. Thesis, University of Bristol, 1994.

[4] M.H. Conte and G. Eglinton, Deep-Sea Res., 40 (1993) 1935.

[5] F.G. Prahl, G.J. de Lange, M. Lyle M. and M.A. Sparrow, Nature, 341 (1989) 434.

[6] F.G. Prahl, L.A. Muehlhausen and D.L. Zahnle, Geochim. Cosmochim. Acta, 52 (1988) 2303.

[7] E.L. Sikes and J.K. Volkman, Geochim. Cosmochim. Acta, 57 (1993) 1883.

[8] E.L. Sikes, J.W. Farrington and L.D. Keigwin, Earth Planet. Sci. Lett., 104 (1991) 36.

[9] J.A. Rechka and J.R. Maxwell, Org. Geochem., 13 (1988) 727.

[10] J. Klok, J.M. Baas, H.C. Cox, J.W. de Leeuw, W.I.C. Rijpstra and P.A. Schenck, Org. Geochem., 6 (1984) 265.

[11] J.P. Jasper and R.B. Gagosian, Paleoceanography, 4 (1989) 603.