
An optimized thermal extraction system for preparation of water from fluid inclusions in speleothems

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ABSTRACT

The fluid inclusions present in speleothems (secondary mineral deposits formed in caves) are a relict sample of the parent seepage water from which the speleothem was deposited and determination of their composition can solve the palaeotemperature equation for the precipitation of speleothem carbonate. We have extracted fluid inclusions using a newly-designed thermal vacuum extraction method in stalagmites and stalactites from Nerja Cave, Southern Spain. Optimal conditions were found to involve heating samples crushed to 0.8-2mm for 3 hours at 300-400°C. Waters extracted from modern aragonitic speleothem samples produced results on the Meteoric Water Line defined by modern infiltration and other waters associated with the cave and hence demonstrate an absence of fractionation effects. This successfully demonstrates the utility of the method.

KEYWORDS | Fluid inclusions. Speleothems. Paleoclimate. Stable isotopes.

INTRODUCTION

Calcium carbonate deposits from caves, speleothems such as stalagmites, stalactites or flowstones, provide information on the nature of past climates and environments. Speleothems have become increasingly important as paleoenvironmental proxies, largely through advances in analytical instrumentation. Thus high-resolution TIMS Th/U dating has improved chronological control, whilst

the analysis of oxygen and carbon isotopes (Holmgren et al., 1999; Jiménez de Cisneros et al., 2003; Drysdale et al., 2004; Tămaş et al., 2005) and trace elements (Fairchild et al., 2000, 2006) has provided information on temperature, precipitation and vegetation cover. The speleothems may provide direct information on the isotopic composition of rainfall and, therefore, potentially identify the source of moisture for a given area. The oxygen isotope ratios of speleothem calcite ($\delta^{18}\text{O}$) and hydrogen isotope ratios of

speleothem fluid inclusion ($\delta^2\text{H}$) are water-isotope tracers, which enable the calculation of the isotopic composition of paleogroundwater and paleoprecipitation (Caballero et al., 1996; McDermott et al., 2006).

The growth of speleothems in cave systems occurs via the precipitation of calcium carbonate (either as calcite or aragonite) by dripwaters onto the cave floor. Speleothems generally contain fluid inclusions of varying sizes, from as small as 20 μm to 1 mm in diameter (Genty et al., 2002; Zhang, 2008). The inclusions are trapped samples of cave dripwater originating from local precipitation (Harmon et al., 1979; Yonge et al., 1985). Total amounts of inclusions that can be trapped normally range from 0.05 to 1.0 wt.% of the speleothem (Schwarcz et al., 1976; Schwarcz and Yonge, 1983). Climate-related data on cave paleowaters can be gained from the isotopic analysis of these fluid inclusions (McGarry et al., 2004) if the speleothems were formed under isotopic equilibrium condition. The oxygen isotopic composition of calcium carbonate ($\delta^{18}\text{O}$), formed in isotopic equilibrium with a water of fixed composition ($\delta^{18}\text{O}$), decreases with increasing temperature. However, it is now recognized that changes in the water composition form the main agent for change in $\delta^{18}\text{O}$ over time, and so one current research focus is the technically challenging extraction of coeval water from fluid inclusions to determine the $\delta^{18}\text{O}$ composition directly, or via the meteoric water line, from $\delta^2\text{H}$ (Matthews et al., 2000; Dennis et al., 2001; McGarry et al., 2004).

The growth of speleothems in caves is related to climatic controls such as temperature, precipitation and vegetation cover, and speleothem archives have significant potential for reconstructing past climates (Fairchild et al., 2006; McDermott, 2004; McDermott et al., 2006).

The $\delta^{18}\text{O}$ of fluid inclusions is generally viewed as a potentially unreliable indicator of the isotopic composition of the original water because fluid inclusions may undergo post-depositional oxygen isotope exchange with carbonate minerals (Schwarcz et al., 1976; Rozanski and Dulinski, 1987). The situation with hydrogen should be less ambiguous because the trapped water should not undergo post-depositional hydrogen isotope exchange. The analysis of the isotopes of hydrogen D/H as a proxy for $\delta^{18}\text{O}$ of water has been studied by several authors (e.g., Schwarcz et al., 1976; Harmon et al., 1979; Yonge, 1982; Schwarcz and Yonge, 1983; Rozanski and Dulinski, 1987; Goede et al., 1990; Matthews et al., 2000; Genty et al., 2002; McGarry et al., 2004) and is based on the well-known correlation of these isotopic systems for meteoric water. Worldwide, this is represented by the global Meteoric Water Line (MWL) relationship: $\delta^2\text{H} = 8 \delta^{18}\text{O} + 10$. In the present Eastern Mediterranean area, the deuterium excess parameter (d_{excess} : defined as the difference $\delta^2\text{H} - 8 \delta^{18}\text{O}$)

is higher than 10 because evaporation processes at the seasurface occur in low-humidity air masses (Andreo et al., 2004). The oxygen-hydrogen isotope relationship is better expressed by the Mediterranean Meteoric Water Line (MMWL) $\delta^2\text{H} = 8 \delta^{18}\text{O} + 22$ (Gat, 1980; Gat and Carmi, 1987).

Speleothem $\delta^{18}\text{O}$ records the combined signatures of rainfall $\delta^{18}\text{O}$ and cave temperature, provided the calcite is precipitated in oxygen isotopic equilibrium (Hendy and Wilson, 1968). Methods for separating the two effects, such as the analysis of fluid inclusions that represent the original dripwater, are complicated and not routinely performed (Treble et al., 2005; Verheyden et al., 2008). Thus, many speleothem records are interpreted as combined records of rainfall and temperature (Bar-Matthews et al., 1999; McDermott et al., 2001), while some are recognised as being clearly dominated by rainfall (Wang et al., 2001). The $\delta^{18}\text{O}$ signal in the stalactite is primarily a response to two factors: 1) isotopic fractionation in the meteoric system and 2) isotopic fractionation in the cave system. Fractionation in the meteoric system governs the $\delta^{18}\text{O}$ value of rainwater. The fractionation in the cave system is less complicated. Yonge et al. (1985) showed that the isotope signal in the dripwater is almost constant throughout the year, even though the isotope signal in the rainwater is fluctuating. Changes in the cave temperature result in greater fractionation between the isotopic ratio in the dripwater and the calcite at lower temperatures (O'Neil et al., 1969; Friedman and O'Neil, 1977).

From dripwater and from the water in fluid inclusions, oxygen isotope ratios and deuterium/hydrogen (D/H) ratios can be determined. Measurements of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ from both dripwaters and fluid inclusions (Schwarcz et al., 1976; Yonge et al., 1985) showed that isotope ratios of these waters fell on the meteoric water line (MWL). Because of the possibility of oxygen isotopic exchange between the water in the inclusion and the carbonate host, the hydrogen isotopic composition of the fluid is used to calculate the original oxygen isotopic values for paleothermometry (Craig, 1961). Dennis et al. (2001) suggest that earlier problems with the analysis of fluid inclusions $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values were caused by incomplete recovery of extracted water. Fractionation problems appear particularly persistent for thermal decrepitation extraction which commonly fractionates $\delta^2\text{H}$ more than 20‰ (Yonge, 1982; McGarry et al., 2004). In recent years, advances in continuous-flow mass spectrometry have provided new techniques for the analysis of sub-microliter amounts of water. Vonhof et al. (2006) describe the first application of this continuous-flow pyrolysis technique to speleothem fluid inclusion analysis. Continuous-flow mass spectrometry for D/H analyses of inclusion water in speleothems was recently also successfully used by Siklosy et al. (2007). Verheyden et al. (2008) have observed the possible contamination of

the recovered inclusion water with hydration water of lime, responsible for the recovery of water with very negative $\delta^2\text{H}$ values.

Of pivotal importance in the present study is the application of a technique to analyze the stable isotope composition of fossil dripwater trapped as fluid inclusions in speleothem. We extracted water from fluid inclusions using the method of vacuum thermal extraction in speleothems from Nerja Cave, southern Spain, which grew during the Middle Pleistocene. To perform this extraction a high-vacuum system has been designed based on modifications of other methods proposed by different authors.

LOCATION AND DESCRIPTION OF MATERIAL STUDIED

Nerja Cave is situated in the easternmost part of Málaga province, in southern Spain (Fig. 1). The local climate is dry Mediterranean, with a mean annual temperature of 20°C. This area is characterized by vegetation dominated by grasses using the C_3 photosynthetic pathway. This cave is an excellent fossil record of its own history and also of the paleoclimatic and neoseismotectonic evolution of the site where the cave is located. The beginning of the karstification period which gave rise to its origin dates back more than 800,000 years and is probably even from the Lower Pleistocene or Upper Pliocene. Throughout the history of the cave different phases of active growth responsible for the formation of large volumes of speleothems are detected (Durán et al., 1993). All samples analysed were deposited in isotopic equilibrium according to the criteria of Hendy (1971). Analyses of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ and the relationship between these from two randomly selected growth layers showed that material was precipitated under isotopic equilibrium and is thus suitable for paleoclimate study (Jiménez de Cisneros et al., 2003).

The dominant mineral in most stalactites and stalagmites at Nerja is aragonite, although some speleothems are calcite-

aragonite mixes. The aragonite is metastable and may revert with time to calcite. Thin sections were studied to investigate the nature of speleothem fluid inclusions. Petrographic analysis of speleothems studied, reveals the presence of fluid inclusions (Fig. 2). Microscopic inspection of thick slices of speleothems show the fluid inclusions to be trapped in spherical or elongated, rounded cavities which are isolated from one another. Most of the cavities are completely filled with water. Variations in the inclusion density define laminae, as first noted by Kendall and Broughton (1978, 1993). The dominant texture is a calcite/aragonite in a palisade (Folk and Assereto, 1976) or columnar crystals (Kendall and Broughton, 1993; González et al., 1992, 1993a, b). The calcite displays mainly microcrystalline fabrics and subordinately columnar ones (*sensu* Frisia et al., 2000). As a whole, it presents a very compact internal structure, with little porosity; two kinds of fabrics can be distinguished: dark compact crystals, that are translucent, and appear dark on polished sections because the incident light is absorbed, and a white porous calcite/aragonite that appears white because the light is reflected by the pores and the inclusions. Consequently, we are likely to find more fluid inclusions in white porous parts of the samples. Yonge (1982) found little correlation between milkiness and water content, but more recent works seem to confirm this relationship (Dennis et al., 1996, 1998). From the stalactites and stalagmites of Middle Pleistocene (190,000 to 160,000 years ago), a total of 36 samples were selected for extraction of the inclusion water. For the dating of the samples the uranium-series disequilibrium method of Ivanovich and Harmon (1992) was used.

ANALYTICAL METHOD

Petrographic and thermal analyses

The mineralogical composition of the samples was determined by X-ray diffraction, using a Panalytical



FIGURE 1 | Map of study area.

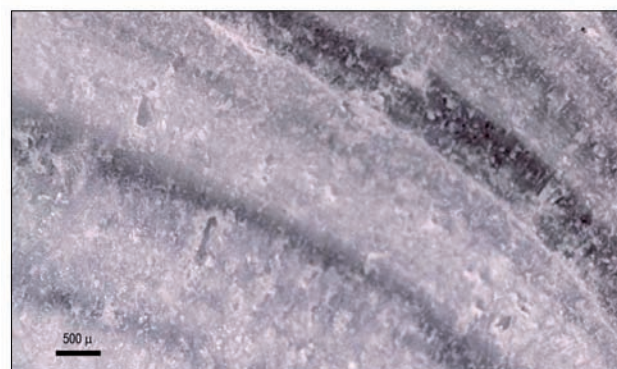


FIGURE 2 | Reflected light image of a speleothem surface with typical fluid inclusions found.

X'PERT pro diffractometer. Results from the XRD analysis show that the studied material consists of aragonite. Speleothem mineralogy and fluid inclusions are interpreted to be primary, and no signs of secondary alteration, such as corrosion features and recrystallization were found, which leads us to conclude that fluid inclusion isotope composition is undisturbed since the time of formation.

Thermal analysis comprises a group of techniques in which a physical property of a substance is measured as a function of temperature, while the substance is subjected to a controlled temperature programme. In differential thermal analysis, the temperature difference that develops between a sample and an inert reference material is measured, when both are subjected to identical heat treatments. DSC (Differential Scanning Calorimetry) is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned. The main application of DSC is in studying phase transitions, such as melting, glass transitions, or exothermic decompositions. These transitions involve energy changes or heat capacity changes that can be detected by DSC with great sensitivity. The result of a DSC experiment is a curve of heat flux versus against temperature or versus against time.

Differential thermal analysis (Fig. 3) made on the samples selected prior to the process of extraction of the

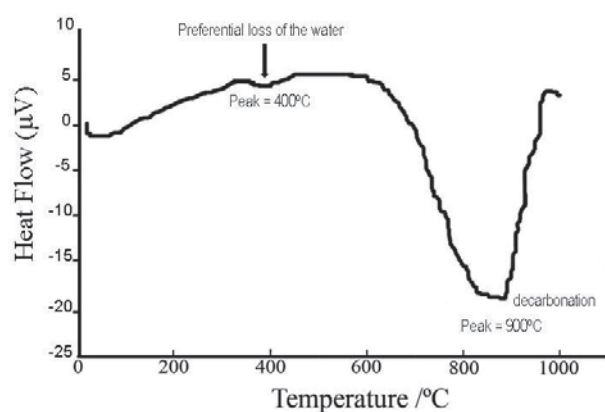


FIGURE 3 | Thermogravimetric analysis of an aragonitic sample. The sample is heated at a controlled rate and a plot of heat flow versus temperature is produced. In this graph it is possible to observe the changes of the sample in function of the increase of temperature. In the 300-400°C interval there is a first loss of mass that corresponds to the water that occupies the inclusions in the materials.

fluid inclusion water allows the detection of the endothermic or exothermic processes in the samples indicative of the temperature at which these changes take place. In our case, there is an initial loss of mass in the temperature interval between 300°C and 400°C, indicating that at these temperatures there is a preferential loss of the water that occupies the inclusions of these materials. Decarbonation appears at a great endothermic peak of 900-1000°C. These results have been used to select the optimal working temperatures for extracting the water from the inclusions, thus avoiding the isotopic fractionation observed by other authors in this process (Wilburn and Sharp, 1993).

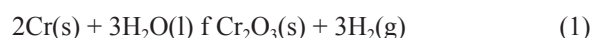
Description of the method of extraction of fluid inclusions

Samples of from 3.5 to 5g of aragonite were sampled from speleothems selected which were previously shown to have been deposited in oxygen isotopic equilibrium with their seepage waters. The isotopic variations of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ show no significant relation and the corresponding linear regression between both parameters was established giving very low correlation coefficients (Jiménez de Cisneros et al., 2003). According to Hendy's criteria (1971), this provides good evidence of equilibrium deposition of this speleothem with the groundwater.

We used the method of heating the crushed sample that permits the recovery of inclusion water. Thermal decrepitation methods were first systematically investigated by Yonge (1982) and involve heating speleothems from 400°C to 850 - 900°C to yield 0.5 - 2µl of water. At these temperatures the carbonate undergoes decomposition and the water has to be cryogenically separated from the much larger amount of CO_2 . Yonge (1982), Goede et al. (1990) and Matthews et al. (2000) observed a 20-30% depletion in the hydrogen isotope composition of the extracted modern inclusion waters relative to contemporary cave waters. This fractionation is not observed in the fluid inclusions analysed by Genty et al. (2002), or using the vacuum crushing method of Dennis et al. (2001), and therefore is ascribed to the analytical extraction procedure. The cause of this fractionation has not been determined, though suggested possibilities include hydride formation or calcite-bound water (Verheyden et al., 2008). Matthews et al. (2000) determined a depletion of 30% for Soreq cave speleothems and McGarry et al. (2004) give an identical value. Earlier studies of vacuum crushing also encountered isotopic depletions due to inefficient recovery of water. Dennis et al. (1998, 2001) were able to improve the method by crushing the sample in a specially-designed high vacuum cell followed by heating the crushed sample at 150°C for 1 hour to totally desorb the water from the calcite surfaces. Vonhof et al. (2006) describes the first application of continuous-flow pyrolysis furnace (Thermofinnigan

TC-EA) connected to a crusher and cold trap unit. Their fluid inclusion δD values were well in agreement with the isotopic composition of dripwaters coming from the same cave as the studied speleothems.

Our vacuum line is illustrated schematically in figure 4. It is made of glass with a high-vacuum greaseless stopcock (valves Young) and Pirani vacuum gauge (10^{-3} mbar). We extracted inclusion water using a recovery system modified by Matthews et al. (2000), Dennis et al. (2001) and McGarry et al. (2004). The sample was crushed to 0.8 - 2mm fragments and heated in a vacuum of 10^{-3} mbar. Following crushing and heating the released water is cryo-distilled into a cold trap and held at liquid nitrogen temperature, for a period of up to 10 minutes in a static vacuum. After the initial capture, the trap temperature is raised to -120°C and any CO_2 and other non-condensable gases are pumped to waste. During this process $\approx 3 - 5\mu\text{l}$ of water were extracted. The inclusion water was transferred to crimp-top vials with rubber/PTFE crimp caps which were fully sealed with a crimping tool. Stable hydrogen ($\delta^2\text{H}$) isotopes in water samples were measured using a continuous-flow GV Instruments mass spectrometer attached to an Elemental elemental analyzer with a liquid autosampler (EuroVector). The on-line Cr reduction method used has been described by Morrison et al. (2001). Water samples ($0.7\mu\text{l}$) are injected into an injector port heated at 150°C . The injector is connected to a reaction tube filled with Cr and heated at 1080°C . The reduction of the water occurred into the reaction tube can be described by the eq, (1)



The memory effect was evaluated and minimized analysing 2 sample replicates.

Oxygen isotopes were determined by the $\text{H}_2\text{O-CO}_2$ equilibration method described by Epstein and Mayeda (1953). For the equilibration process, a multiflow device was used. The CO_2 was injected into each vial with the

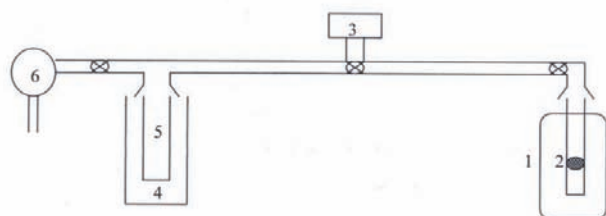


FIGURE 4 | Fluid inclusion extraction line (high vacuum system): 1) Oven/furnace; 2) Sample; 3) Ultra-Torr connectors; 4) Cold trap at liquid nitrogen temperature; 5) Sample tube; 6) Vacuum Pump.

sample of water. The equilibration occurred at a constant temperature of 40°C for a duration of 420 minutes. The oxygen isotope ratios of the equilibrated CO_2 were measured using a GV Instruments dual-inlet mass spectrometer. Two replicates of each sample were analyzed.

International reference materials (SMOW, GISP, SLAP) and two laboratory standards (BLM and LAN) were used throughout the isotopic water analyses to ensure high quality results. The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ results are given with respect to VSMOW with a precision greater than 0.5‰ and 0.05‰, respectively. The data were normalized according to the method described by Nelson (2000) and Nelson and Dettman (2001).

RESULTS AND DISCUSSION

Under favourable conditions, the $\delta^{18}\text{O}$ signal of calcite can be transformed into absolute temperature. This can be achieved by measuring the isotopic composition in fluid inclusions in the speleothem (Harmon et al., 1979; Gascoyne et al., 1981; Goede et al., 1986; Yonge et al., 1981). Fluid inclusions, representing fossil seepage water, are formed in speleothems when small volumes of dripwater become trapped within the precipitating calcite (Rowe et al., 1998).

In order to carry out the routine of work of extraction of the water present in the fluid inclusions of carbonates numerous tests have been made to optimize the experimental design. A total of 36 new samples of speleothem aragonite, weighing ~ 3.5 to 5g each, were crushed. The results obtained to assess the optimal conditions of the extraction process of waters in carbonates are shown in Table 1. The varied parameters were time and temperature, using the initial and final sample weights to obtain the extracted percentage of water. The temperature was varied between 300°C , 400°C and 500°C in accordance with the data collected in the DSC diagrams which indicate that at these temperatures the preferential loss of the water that occupies the fluid inclusions takes place, and the time was varied between a minimum of 2h and a maximum of 4h. Figure 5 illustrates that the optimal conditions are obtained by heating the crushed sample in high vacuum at $300\text{-}400^{\circ}\text{C}$ for 3 hours. The water is released by thermal decomposition of the speleothem sample in a quartz tube attached to a vacuum line. The inclusion water was transferred to a completely sealed 2mL crimp top vials with rubber.

In this study, the isotopic composition of the recovered water in samples submitted to the extraction process defined could be compared with the published results of two years' isotopic study of rain and dripwaters in this cave (Caballero et al., 1996). The paleoclimatic significance of

TABLE 1 | Experimental results to establish the optimal conditions of the extraction process of waters in aragonitic samples

Sample	T (°C)	t (hours)	W _f -W _i	% H ₂ O	Sample	T (°C)	t (hours)	W _f -W _i	% H ₂ O
EX1/NE	200	2	0.0227	0.0045	EX33/NE	350	3	0.0809	0.0162
EX2/NE	200	2	0.0217	0.0043	EX34/NE	350	4	0.0838	0.0167
EX3/NE	200	2	0.0266	0.0053	EX35/NE	350	4	0.0793	0.0159
EX4/NE	200	3	0.0274	0.0055	EX36/NE	350	4	0.0819	0.0164
EX5/NE	200	3	0.0269	0.0054	EX37/NE	400	2	0.0737	0.0147
EX6/NE	200	3	0.0281	0.0056	EX38/NE	400	2	0.0718	0.0143
EX7/NE	200	4	0.0301	0.0060	EX39/NE	400	2	0.0775	0.0155
EX8/NE	200	4	0.0379	0.0076	EX40/NE	400	3	0.1155	0.0231
EX9/NE	200	4	0.0278	0.0056	EX41/NE	400	3	0.1740	0.0348
EX10/NE	250	2	0.0148	0.0030	EX42/NE	400	3	0.1118	0.0223
EX11/NE	250	2	0.0267	0.0053	EX43/NE	400	4	0.1050	0.0209
EX12/NE	250	2	0.0285	0.0057	EX44/NE	400	4	0.1076	0.0215
EX13/NE	250	3	0.0274	0.0055	EX45/NE	400	4	0.1039	0.0208
EX14/NE	250	3	0.0297	0.0059	EX46/NE	450	2	0.0895	0.0179
EX15/NE	250	3	0.0335	0.0067	EX47/NE	450	2	0.0919	0.0184
EX16/NE	250	4	0.0312	0.0062	EX48/NE	450	2	0.0940	0.0188
EX17/NE	250	4	0.0304	0.0061	EX49/NE	450	3	0.1314	0.0263
EX18/NE	250	4	0.0316	0.0063	EX50/NE	450	3	0.1265	0.0253
EX19/NE	300	2	0.0228	0.0046	EX51/NE	450	3	0.1222	0.0244
EX20/NE	300	2	0.0254	0.0051	EX52/NE	450	4	0.1238	0.0247
EX21/NE	300	2	0.0313	0.0063	EX53/NE	450	4	0.1304	0.0260
EX22/NE	300	3	0.0352	0.0070	EX54/NE	450	4	0.1212	0.0242
EX23/NE	300	3	0.0300	0.0060	EX55/NE	500	2	0.1088	0.0217
EX24/NE	300	3	0.0355	0.0071	EX56/NE	500	2	0.1089	0.0218
EX25/NE	300	4	0.0303	0.0061	EX57/NE	500	2	0.1082	0.0216
EX26/NE	300	4	0.0293	0.0059	EX58/NE	500	3	0.1203	0.0240
EX27/NE	300	4	0.0314	0.0063	EX59/NE	500	3	0.1208	0.0241
EX28/NE	350	2	0.0413	0.0083	EX60/NE	500	3	0.1192	0.0238
EX29/NE	350	2	0.0494	0.0099	EX61/NE	500	4	0.1131	0.0226
EX30/NE	350	2	0.0550	0.0110	EX62/NE	500	4	0.1234	0.0246
EX31/NE	350	3	0.0820	0.0164	EX63/NE	500	4	0.1250	0.0250
EX32/NE	350	3	0.0851	0.0170					

the full isotopic record of the samples will be discussed in a future publication. Here we present only the isotopic measurements on fluid inclusions, and discuss their implications from a methodologic point of view.

The liberated inclusion water was analyzed for $\delta^2\text{H}$ and $\delta^{18}\text{O}$. The results of oxygen and hydrogen isotopic composition of the recovered water are presented in Table 2 and are plotted in Figure 6. The oxygen isotope composition ($\delta^{18}\text{O}_{\text{VSMOW}}$) for recovered water, has a mean value of -7.1‰ ranging from -7.5 to -6.2‰ . $\delta^2\text{H}$ values vary between -52.3 and -44.4‰ (VSMOW) and the mean -49.97‰ (VSMOW). For some local rainshowers and from the dripwaters in the cave the average value is -37.2‰ for $\delta^2\text{H}$ (VSMOW). $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the recovered water are plotted on the Global Meteoric Water Line (GMWL, Figure 6). In this figure the isotopic data obtained in the fluid inclusions along with those of the rainwater of the zone are presented and drip waters of the Cave of Nerja (Caballero et al., 1996). It is possible to state

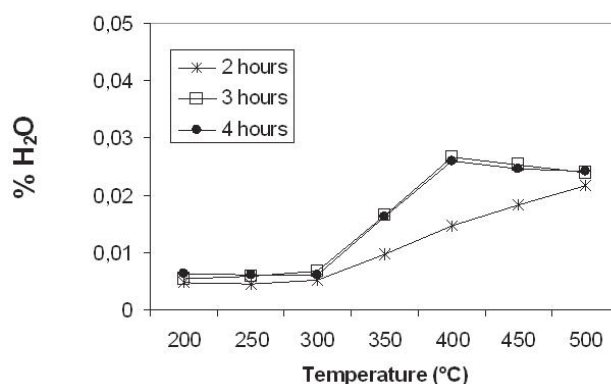


FIGURE 5 | Representation of experimental values obtained in the test to optimize the method. Between 200-300°C at 2, 3 and 4 hours only 24% of total water is recovered. At 400°C with 3-4 hours heating, practically 100% of water is recovered.

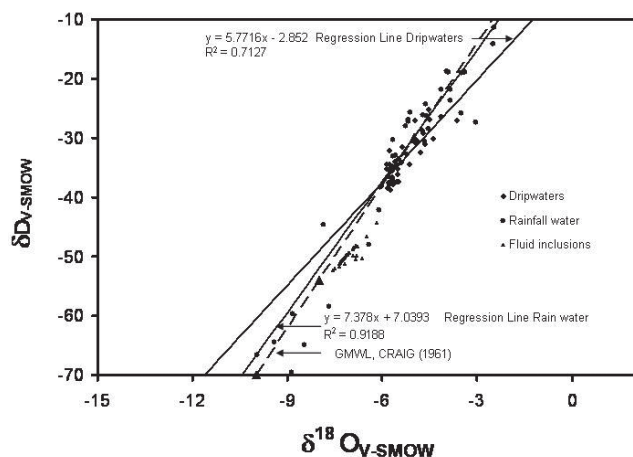


FIGURE 6 | Oxygen and hydrogen isotopic composition of the recovered water from fluid inclusions. The isotopic data of the rainwater and dripwaters of the Cave of Nerja are presented. In addition, it's plotted the regression lines, equations and correlation coefficients from the D/H data of dripwaters and rainfall water.

that the results obtained from $\delta^2\text{H}$ and $\delta^{18}\text{O}$ are reasonably homogenous and that these values of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of the fluid inclusions are aligned on the line of meteoric waters and hence that they are waters of meteoric origin, and that do not display mixture with another type of water. They present values similar and close to those shown by Caballero et al. (1996) for dripwaters in the cave although somewhat more negative. On the other hand, this similarity between the isotopic values of the fluid inclusions and those of seepage waters are also stated by Genty et al. (2002) in their study on fossil waters from two stalagmites in the Caves Villars and Clamouse (France).

TABLE 2 | Deuterium and oxygen content of the analysed fluid inclusions of aragonitic samples from Nerja Cave

Recovered water from fluid inclusion	$\delta^{18}\text{O}_{\text{SMOW}} \text{‰}$	$\delta\text{D}_{\text{SMOW}} \text{‰}$	Recovered water from fluid inclusion	$\delta^{18}\text{O}_{\text{SMOW}} \text{‰}$	$\delta\text{D}_{\text{SMOW}} \text{‰}$
1	-7.33	-50.91	19	-7.10	-49.62
2	-7.26	-50.55	20	-7.05	-49.31
3	-6.20	-44.38	21	-6.85	-50.46
4	-7.50	-51.93	22	-7.31	-50.83
5	-7.57	-52.32	23	-7.12	-49.72
6	-7.24	-50.42	24	-6.90	-48.46
7	-7.34	-51.01	25	-7.27	-50.56
8	-7.37	-51.72	26	-6.94	-49.82
9	-6.49	-46.67	27	-7.19	-50.11
10	-6.78	-48.31	28	-7.38	-51.19
11	-7.18	-51.22	29	-7.28	-50.66
12	-6.83	-49.78	30	-6.63	-50.38
13	-7.07	-49.41	31	-7.28	-50.62
14	-6.92	-48.56	32	-7.18	-50.09
15	-7.22	-50.32	33	-7.34	-50.96
16	-7.34	-50.98	34	-6.85	-48.14
17	-7.15	-49.91	35	-6.95	-48.71
18	-7.21	-50.21	36	-7.34	-50.96

TABLE 3 | Data $\delta^{18}\text{O}$ of fluid inclusions experimentally obtained using extraction system and the theoretical $\delta^{18}\text{O}$ values which are simply calculated from the D/H data using the relationship Craig (1961): $\delta\text{D} = 8\delta^{18}\text{O} + 10$ "Global Meteoric Water Line". The $\delta^{18}\text{O}$ results are given with respect to VSMOW with a precision greater than $\pm 0.05\text{‰}$.

Sample	$\text{‰} \delta^{18}\text{O}_{\text{exp}}$	$\text{‰} \delta^{18}\text{O}_{\text{theor}}$	Sample	$\text{‰} \delta^{18}\text{O}_{\text{exp}}$	$\text{‰} \delta^{18}\text{O}_{\text{theor}}$
1	-7.33	-7.61	19	-7.10	-7.45
2	-7.26	-7.57	20	-7.05	-7.41
3	-6.20	-6.80	21	-6.85	-7.56
4	-7.50	-7.74	22	-7.31	-7.60
5	-7.57	-7.79	23	-7.12	-7.47
6	-7.24	-7.55	24	-6.90	-7.31
7	-7.34	-7.63	25	-7.27	-7.57
8	-7.37	-7.72	26	-6.94	-7.48
9	-6.49	-7.08	27	-7.19	-7.51
10	-6.78	-7.29	28	-7.38	-7.65
11	-7.18	-7.65	29	-7.28	-7.58
12	-6.83	-7.47	30	-6.63	-7.55
13	-7.07	-7.43	31	-7.28	-7.58
14	-6.92	-7.32	32	-7.18	-7.51
15	-7.22	-7.54	33	-7.34	-7.62
16	-7.34	-7.62	34	-6.85	-7.27
17	-7.15	-7.49	35	-6.95	-7.34
18	-7.21	-7.53	36	-7.34	-7.62

In order to fully interpret the data in $\delta^2\text{H}$ vs. $\delta^{18}\text{O}$ it is first necessary to consider the $\delta^2\text{H}$ vs. $\delta^{18}\text{O}$ relationships of present-day cave waters. These are presented in Figure 6 which plots the annual isotopic analyses of cave water, together with the published results of two years of dripwaters in this cave. Cave and rain water average isotopic values are seen to follow the MWL relationship but the cave waters are displaced to lighter values relative to rain water. A value of $\delta^{18}\text{O}$ of water from fluid inclusions obtained permits the calculation of the temperature in the study area during the deposition of the materials studied. To do this, and because we know the value of $\delta^{18}\text{O}_{\text{carbonate}}$ and $\delta^{18}\text{O}_{\text{water}}$ we only have to place these values in the equation of paleotemperatures after Grossman and Ku (1986). When inserting the value $\delta^{18}\text{O}_{\text{water}} = -7.1\text{‰}$ obtained from fluid inclusions and $\delta^{18}\text{O}_{\text{carbonate}} = -4.5\text{‰}$ mean value of Middle Pleistocene speleothems (Jiménez de Cisneros et al., 2003), the calculated temperatures are between 10 and 13°C; these temperatures are somewhat lower than those obtained in previous works in the area whose calculations were based on a value of $\delta^{18}\text{O}_{\text{water}} = -5.2\text{‰}$ (Caballero et al., 1996). However, these results agree with the observed changes in temperature in the study area during the formation of the speleothems: somewhat colder than current values and containing values more negative fossil water reflects the ambient temperature in the area.

Moreover, from the experimentally obtained values of $\delta^2\text{H}$ we have deduced the theoretical $\delta^{18}\text{O}$ value of water from fluid inclusions and compared it with those obtained

experimentally following the vacuum extraction process. The results, shown in Table 3, verify the reliability of the method of extraction, since experimental values and theoretical values (obtained from $\delta^2\text{H}_{\text{exp}}$) are close enough to ensure that there has been no isotopic exchange between water and carbonate.

The results obtained in this work show that the method developed for the extraction of fluid inclusion waters in carbonate is effective. The use of this method on speleothems for the purpose of obtaining paleoclimatic data should enable highly reliable paleotemperature information to be obtained. The direct application of the method developed on speleothems from the south of the Iberian Peninsula will allow the isotopic composition of paleowaters (fluid inclusions), and consequently climatic variations in this area of the Mediterranean, to be established.

CONCLUSIONS

The $\delta^{18}\text{O}/\delta^2\text{H}$ analyses of fluid inclusion waters in fossil carbonate speleothems can provide significant data on paleowaters. The fluid inclusions represented the parent drip that deposited the speleothems. The only way to obtain an absolute measurement of past climate is to measure the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ content of the dripwaters feeding the speleothem that is trapped in the fluid inclusions. This technique could be of great value in reconstructing past continental climates.

Assuming that the fluid inclusions are isotopically representative of the parent seepage waters, we can calculate the temperatures of speleothem formation from this data. A consequence of this is that the temperature of speleothems is possibly comparable to that presently observed in the cave.

The method of thermal vacuum extraction presented here is capable of recovering the water from fluid inclusion in speleothems. From the experimentally-obtained values of $\delta^2\text{H}$ we have deduced the theoretical value of $\delta^{18}\text{O}$ of water from fluid inclusions and compared with those obtained experimentally following the vacuum extraction process. The results confirm the reliability of the method of extraction because theoretically the data obtained from the $\delta^2\text{H}$ values are very similar to those provided experimentally through the extraction process, by which we can ensure that there has been no isotopic exchange between water and carbonate.

The results for the hydrogen and oxygen isotope compositions of this water indicate that their isotopic compositions follow the meteoric water line (MWL), as expected for meteoric waters that do not show evidence

of mixing with other types of water. This technique could, in principle, be of great value in reconstructing past continental climates.

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