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RESEARCH ARTICLE

An improved method for fluorescence analysis of dissolved organic matter in cave drip water

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Abstract An improved synchronous fluorimetric method for the determination of dissolved organic matter in cave drip water, by adding ascorbic acid, is described. The method is based on the redox reaction between ascorbic acid and the electron-withdrawing constituents in dissolved organic matter. The results show that adding ascorbic acid can quench the minor peaks, at 200–300 nm, but does not affect the intensity of the main peaks at 300–500 nm. In addition, adding ascorbic acid can maintain relatively high and constant fluorescence intensity over a wide pH range (9–4).

Keywords drip water, synchronous fluorescence spectroscopy, ascorbic acid, pH

1 Introduction

Although present as in a trace amount, speleothem organic matter (OM) is a sensitive recorder of the overlying ecosystem (McGarry and Baker, 2000; Xie et al., 2003; Ban et al., 2008; Blyth et al., 2008; Gázquez et al., 2012). Luminescence spectrophotometry is widely applied to characterize speleothem OM due to its non-destructive and convenient manipulation features (e.g., Baker et al., 1996; Baker and Genty, 1999; McGarry and Baker, 2000; van Beynen et al., 2001; Baker, 2005; Hartland et al., 2010). During the fluorescence analysis of cave drip water using conventional methods, the complex nature of dissolved organic matter (DOM) in drip water allows for the influence of minor components on major components. In addition, fluorescence intensity and wavelength are affected by pH (Miano et al., 1988; Senesi et al., 1991a, b; McGarry and Baker, 2000; Patel-Sorrentino et al., 2002). Here we describe an improved method for fluorimetric determination of DOM in drip water that is accomplished by adding ascorbic acid (AA; vitamin C), with the aim of eliminating the influence of minor components on major components, and of maintaining a relatively high fluorescence intensity over a wide range of pH.

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2 Materials and methods

Drip water samples were collected from the Heshang Cave (30°27′N, 110°25′E, 294 m above sea level). For a detailed description of the cave, see Hu et al. (2008a, b). The drip water was collected at the former growth site of the HS-4 stalagmite, which was collected in 2001. During each field trip, the drip water was collected using a glass bottle that had been cleaned sequentially using diluted HCl, non-fluorescent detergent, and ultrapure water. Once transported to the lab, the water samples were kept frozen. The samples were collected during September 2012 to June 2013.

After warming to room temperature, an aliquot (10 mL) of drip water was transferred to a 25 mL test tube and a measured amount of AA was added. The solution was thoroughly mixed and allowed to stand for 1 hr. Fluorescence analysis was conducted with a Perkin-Elmer LS-55 Luminescence Spectrophotometer using a 150 W Xe lamp. The synchronous fluorescence intensity (SFI) was measured using a 1 cm quartz cell with wavelength scanning from 200 nm to 500 nm in steps of 45 nm.

3 Results and discussion

For synchronous fluorescence analysis, it is important to choose the optimal wavelength difference ($\Delta\lambda$) between excitation and emission. Using the samples (adding 0.01 g AA) collected in March 2013, we compared $\Delta\lambda$ values ranging from 1 to 150 nm at intervals of 10 nm. The results

show that when $\Delta\lambda$ was below 40 nm or above 50 nm, the SFI was lower or the peak shape was poor (Fig. 1). After comparing $\Delta\lambda$ from 40 to 50 in steps of 1 nm, we finally set the $\Delta\lambda$ value at 45 nm.

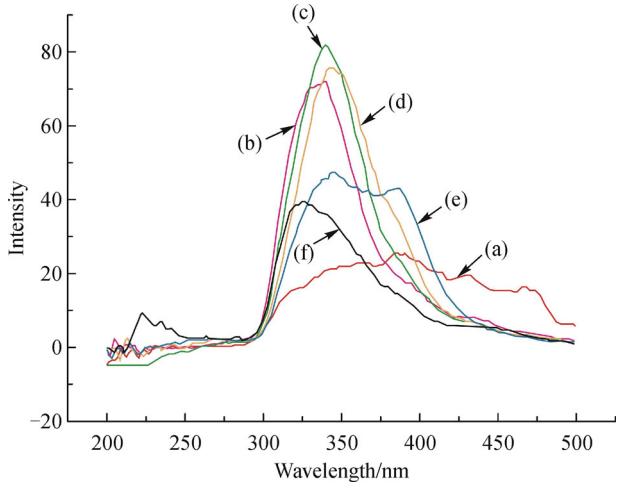

Fig. 1 Synchronous fluorescence spectra from 10 mL drip water (collected in March 2013) adding 0.01 g AA at different $\Delta\lambda$: (a) 20 nm, (b) 40 nm, (c) 45 nm, (d) 50 nm, (e) 60 nm, and (f) 90 nm.

Using the wavelength difference of 45 nm, we assessed the influence of adding AA to the drip water. Before adding AA, the synchronous fluorescence spectrum of the water collected in March 2013 showed some minor peaks between 200 nm and 300 nm (Fig. 2(a)). After adding 0.01 g AA to 10 mL drip water, these minor peaks between 200 nm and 300 nm disappeared (Fig. 2(b)). As a comparison, adding AA to pure water did not show any obvious spectral peak in the scanning range (Fig. 2(c)). The results reveal that AA can quench the fluorescence effect from some compounds and thereby eliminate their influence on the major peaks during the fluorescence analysis of DOM in cave drip water.

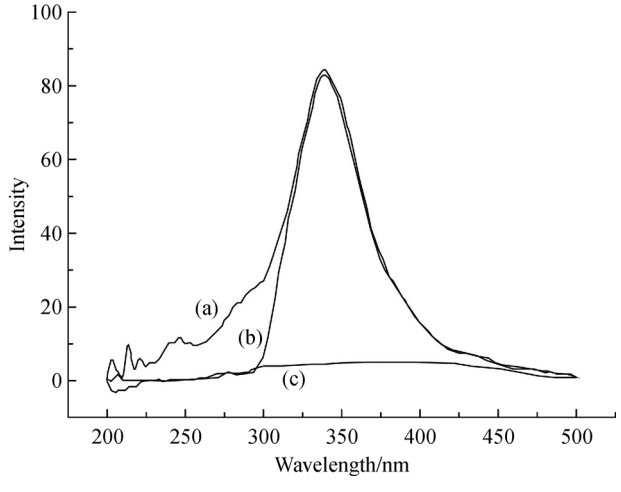

Fig. 2 Synchronous fluorescence spectra of drip water (collected on March 2013). (a) Without adding AA. (b) With 0.01 g AA in 10 mL drip water. (c) With 0.01 g AA in 10 mL pure water.

Fluorophores in DOM can be separated into two groups, one with electron withdrawing substituents and the other with electron donating substituents (Senesi et al., 1991b). Peaks at 200–300 nm in aquatic solutions probably originate from protein-like fluorophores, whereas fulvic-like fluorophores normally have maximal fluorescence intensity at 300–400 nm excitation wavelengths (Hudson et al., 2007). Consequently, the disappearance of minor peaks at 200–300 nm after adding AA probably results from the reaction between AA and protein-like fluorophores. AA is a strong reducing agent (Yang et al., 1997; Arya et al., 2000). In contrast, our results suggest that the reactivity between AA and fulvic/humic acids is quite weak, so adding AA does not affect the SFI in drip water.

AA is a weak acid (pKa = 4.10 at 25 °C). Adding AA to drip water can change its pH. A number of studies have shown that fluorescence intensity increases with pH, probably due to changes in the fluorescence characteristics of the acidic functional groups and changes in the conformation of the organic compounds (Coble, 1996; Mobed et al., 1996; McGarry and Baker, 2000). Our results show that SFI values reach high and near constant values when the concentration of AA is between 0.1 and 1.0 mg/mL (Fig. 3). When the AA concentration was higher than 1.0 mg/mL, SFI decreased sharply, corresponding to a decrease in pH to below 4. The study clearly shows that adding AA in the proper amount can maintain relatively high fluorescence intensity over a wide pH range (pH 9–4).

With the optimized experimental conditions, we selected two water samples, collected on March 2013 and September 2012, respectively, to check the reproducibility of our method. Using the same procedure for both water samples resulted in quite a low standard error (< 0.36%; Fig. 4).

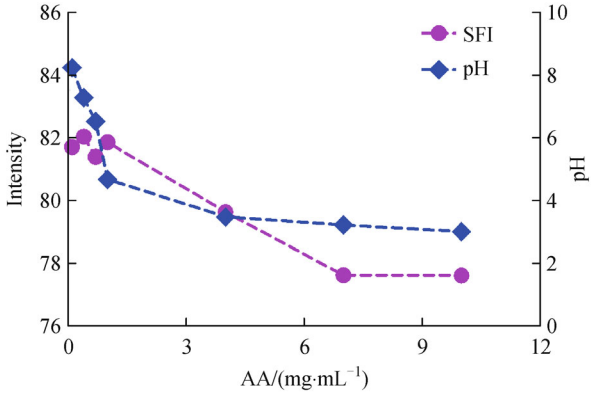

Fig. 3 Variation in SFI and pH of drip water (collected in March 2013) with different AA concentration.

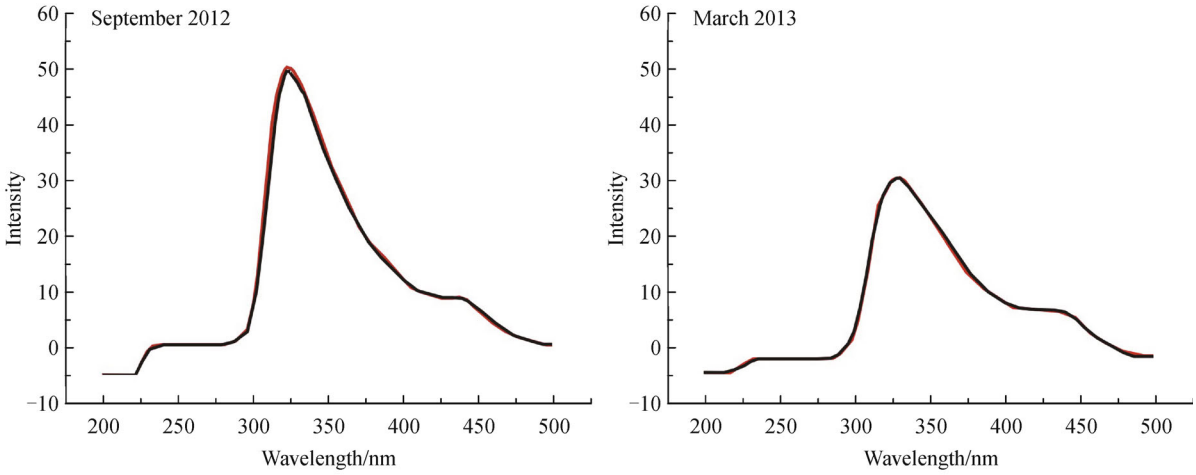

Fig. 4 Reproducibility test by way of analysis of two drip water samples collected in September 2012 and March 2013.

4 Conclusions

In this study, we considered the influence of AA on the fluorescence intensity and peak shape during analysis of DOM in cave drip water. Using water samples collected from the Heshang Cave for a case study, the results show that adding AA can quench the minor peaks, at 200–300 nm, but does not affect the intensity of the main peaks at 300–500 nm. This phenomenon probably results from the reductive reaction between AA and the protein-like substances in the drip water. Thus our improved method for fluorescence analysis on drip water has the advantage of eliminating the influence of the protein-like compounds on the predominant fluorophors at 300–500 nm. In addition, adding AA can maintain relatively high fluorescence intensity over a wide pH range (pH 9–4).

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References

Arya S P, Mahajan M, Jain P (2000). Non-spectrophotometric methods for the determination of Vitamin C. Anal Chim Acta, 417(1): 1–14

Baker A (2005). Thermal fluorescence quenching properties of dissolved organic matter. Water Res, 39(18): 4405–4412

Baker A, Barnes W L, Smart P L (1996). Speleothem luminescence intensity and spectral characteristics: signal calibration and a record of palaeovegetation change. Chem Geol, 130(1–2): 65–76

Baker A, Genty D (1999). Fluorescence wavelength and intensity variations of cave waters. J Hydrol (Amst), 217(1–2): 19–34

Ban F, Pan G, Zhu J, Cai B, Tan M (2008). Temporal and spatial variations in the discharge and dissolved organic carbon of drip waters in Beijing Shihua Cave, China. Hydrol Processes, 22(18): 3749–3758

Blyth A J, Baker A, Collins M J, Penkman K E H, Gilmour M A, Moss J S, Genty D, Drysdale R N (2008). Molecular organic matter in speleothems and its potential as an environmental proxy. Quat Sci Rev, 27(9–10): 905–921

Coble P G (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Mar Chem, 51(4): 325–346

Gázquez F, Calaforra J M, Rull F, Forti P, García-Casco A (2012). Organic matter of fossil origin in the amberine speleothems from El Soplao Cave (Cantabria, Northern Spain). International Journal of Speleology, 41(1): 113–123

Hartland A, Fairchild I J, Lead J R, Baker A (2010). Fluorescent properties of organic carbon in cave dripwaters: effects of filtration, temperature and pH. Sci Total Environ, 408(23): 5940–5950

Hu C, Henderson G M, Huang J, Chen Z, Johnson K R (2008a). Report of a three-year monitoring programme at Heshang Cave, Central China. International Journal of Speleology, 37(3): 143–151

Hu C, Henderson G M, Huang J, Xie S, Sun Y, Johnson K R (2008b). Quantification of Holocene Asian monsoon rainfall from spatially separated cave records. Earth Planet Sci Lett, 266(3–4): 221–232

Hudson N, Baker A, Reynolds D (2007). Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters—A review. River Res Appl, 23(6): 631–649

McGarry S F, Baker A (2000). Organic acid fluorescence: applications to speleothem palaeoenvironmental reconstruction. Quat Sci Rev, 19 (11): 1087–1101

Miano T M, Martin J P, Sposito G (1988). Fluorescence spectroscopy of humic substances. Soil Sci Soc Am J, 52(4): 1016–1019

Mobed J, Hemmingsen S L, Autry J L, McGown L B (1996). Fluorescence characterisation of IHSS humic substances: total luminescence spectra with absorbence correction. Environ Sci Technol, 30(10): 3061–3065

Patel-Sorrentino N, Mounier S, Benaim J Y (2002). Excitation-emission fluorescence matrix to study pH influence on organic matter

fluorescence in the Amazon basin rivers. Water Res, 36(10): 2571–2581

Senesi N, Miano T M, Provenzano M R (1991a). Fluorescence spectroscopy as a means of distinguishing fulvic and humic acids from dissolved and sedimentary aquatic sources and terrestrial sources. Humic Substances in the Aquatic and Terrestrial Environment, 33: 63–73

Senesi N, Miano T M, Provenzano M R, Brunetti G (1991b). Characterisation, differentiation and classification of humic substances by fluorescence spectroscopy. Soil Sci, 152(4): 259–271

van Beynen P, Bourbonniere R, Ford D, Schwarcz H (2001). Causes of colour and fluorescence in speleothems. Chem Geol, 175(3–4): 319–341

Xie S, Yi Y, Huang J, Hu C, Cai Y, Collins M, Baker A (2003). Lipid distribution in a subtropical southern China stalagmite as a record of soil ecosystem response to palaeoclimate change. Quat Res, 60(3): 340–347

Yang J, Tong C, Jie N, Zhang G, Ren X, Hu J (1997). Fluorescent reaction between ascorbic acid and DAN and its analytical application. Talanta, 44(5): 855–858