Asian Journal of Chemistry; Vol. 25, No. 15 (2013), 8657-8659

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14899

Determination of Trace Tetracycline Using Phase-Separation Sublation and Ultraviolet Detection

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(*Received*: 3 December 2012; *Accepted*: 26 August 2013) AJC-14020

A novel analytical method was proposed to determine trace tetracycline (TC) by phase-separation sublation using Mg(II) as a ligand. When pH is 7.0, tetracycline and Mg(II) can combine to a stable complex (Mg-TC). Under certain conditions, the complex can be separated from a solution by phase-separation sublation, because it has better hydrophobicity, this procedure estimated by HPLC was good. The optimum phase-separation floatation was performed. 3.5 mol L^{-1} sodium chloride as ion intensity regulator, caustic soda solution as acidity regulator and tetrahydrofuran as extraction solvent. The detection wavelength is 380 nm, linear regression equation is $A = 1.912 \times 10^{5}C + 0.011$, linear range is from 1.7×10^{-7} to 1.02×10^{-5} mol L^{-1}, floating rate is 89 %, enrichment multiple is 26.7. The recoveries of tetracycline in water was 96.1 to 98.2 %.

Key Words: Tetracycline, Phase-separation sublation, Ultraviolet detection.

INTRODUCTION

The main source of tetracycline (TC) in environment is pharmacy wastewater and animal egesta. When tetracycline antibiotics get into human body through food chain, it can lead to a variety of adverse reactions, Gastrointestinal reaction, such as double infection, On the growth of bone, teeth, liver damage, vitamin A deficiency, renal toxicity *etc*. At the present time, the handling techniques of tetracycline residual is immature and little[1]. The residual substance of tetracycline which enter into the environment *via* various channels is toxic and difficult to biodegradation. This is a serious threat for the natural ecological balance system and human health[2].

In recent years, several techniques, such as high performance liquid chromatography (HPLC)[3-5], enzyme linked immunosorbent assay (ELISA)[6] and gas chromatography with tandem mass (GC-MS)[7], have been developed for the determination of tetracycline. The formation of binary complex of tetracycline with rare earth elements have been reported[8].

In this work, a new method for analysis of trace tetracycline by phase-separation sublation cooperated with an ultraviolet detector is investigated, which is simple and quick. Moreover, it is satisfactory to determine trace tetracycline in water by this method. The technique of phase-separation sublation is a new type of sample pre-treatment technology which integrated two-water phase extraction with gas solvent flotation. The basic principle is, first, adjust ionic strength of test system with inorganic salt. The inorganic anions and cations ionized form the inorganic salt will combine with a large number of water molecules in the hydration process. The hydrophile organic solvent that relys on hydrogen bonding to water molecules and entering the water system becomes hydrophobically because no free water molecules can be combined, the system is separated quantitatively into the two-phase. Second, under the function of trapping agent, the hydrophobic compounds attached to the bubbles were floated into organic phase because of surface tension. We can get the content of measuring substance by determining the absorbance of organic phase. This technique has the high multiple of enrichment, a large number of treatment sample, using non-toxic or low toxic solvents and easy to automation. It can be used to detect trace component. At the same time, the method will overcome the following shortcomings: A great deal of organic solvent was used, the multiple of enrichment was low (2 to 3 times) in the aqueous two-phase extraction, secondary pollution was be decrease in the gas solvent floatation by using toxic organic solvents (benzene, toluene, *etc*.). This kind of method drew the characteristics with simple equipment, high sensitivity, fast measurement and simple and convenient post-processing. In this paper, this technology conjunction with the spectrophotometric measurement will be used in the detection of oxytetracycline. The results estimated with high-performance liquid chromatography is good. The Schematic diagram of phase-separation sublation was shown in Fig. 1.

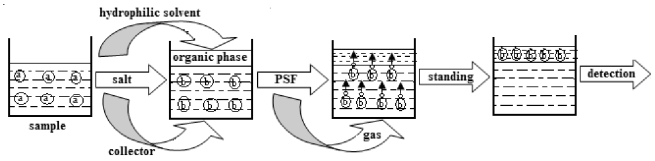

Fig. 1. Principium diagram of phase-separation sublation (a) tetracycline; (b) Mg-TC; ( ) bubble

EXPERIMENTAL

All reagents were of analytical reagent grade. 1×10^{-4} mol L^{-1} of tetracycline, 1×10^{-4} mol L^{-1} of a standard Mg(II) solution, 0.01 mol L^{-1} sodium hydroxide, tetrahydrofuran, HAc-NaAc (pH = 4-6), *tris*-HCl (pH = 7), K_2HPO_4-KH_2PO_4 (pH = 8), $Na_2B_4O_7$ (pH = 9), Na_2CO_3-Na_2HCO_3 (pH = 10).

UV-265 ultraviolet-obviously spectrophotometer (Shimadzu Corporation); high performance liquid chromatograph (Aglient Technology 1200 series); 722 grating spectrophotomete (Sichuan Ninth Instrument Factory); 320-S acidometer (Meteler-Toledo Instrument Ltd); electronic analytical balance (Switzerland Meteler Company); phase-separation sublation equipment (home made, Fig. 2).

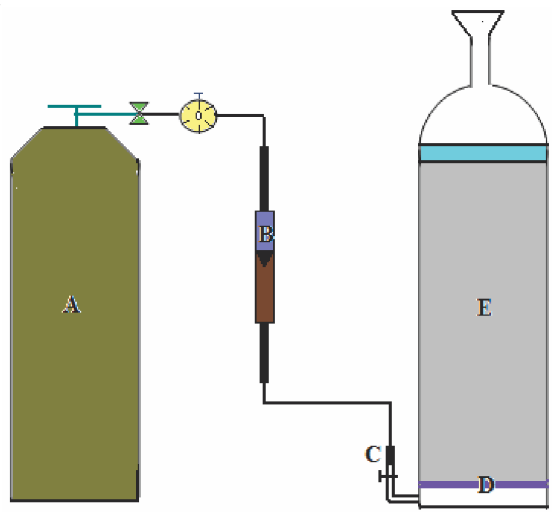

Fig. 2. Apparatus for hydrophilic solvent floatation. (A) Nitrogen cylinder (B) Rotor flow meter (C) Impervious glass cock (D) Glass filter plate (E) Flotation column

Experimental procedure: 5 mL of 1×10^{-4} mol L^{-1} tetracycline, 6 mL of 1×10^{-4} mol L^{-1} Mg(II) and 0.2 mL of 0.01 mol L^{-1} NaOH solution were added to a 50 mL beaker, after standing for 5 min, the mixture solution was transferred into the floatation pool and diluted to 150 mL volume calibration line with 3.5 mol L^{-1} of NaCl, 5 mL of tetrahydrofuran was added. Detection of organic-phase and aqueous-phase was performed on an ultraviolet detector setting at 380 nm after phase-separartion sublation in the conditions of 10 min of floatation time and 20 mL min^{-1} of gas flow rate.

RESULTS AND DISCUSSION

Absorption spectrum: The spectrum is shown in Fig. 3. From Fig. 3, we can find that the strong interaction between tetracycline and Mg(II) has happened by observing the shift of absorption maximum of tetracycline solution. The absorption maximum of tenary complex was 380 nm.

Effect of acidity: To investigate the effect of acidity on the formation of binary complex and phase-separation sublation, the test conditions was in the light of procedures. Fig. 4 show that the optimal acidity of binary complex formation and phase-separation sublation was adjusted with NaOH/HCl and buffer solution. The results was at pH = 7.0. 0.2 mL of 0.01 mol L^{-1} NaOH solution was added in the experiment.

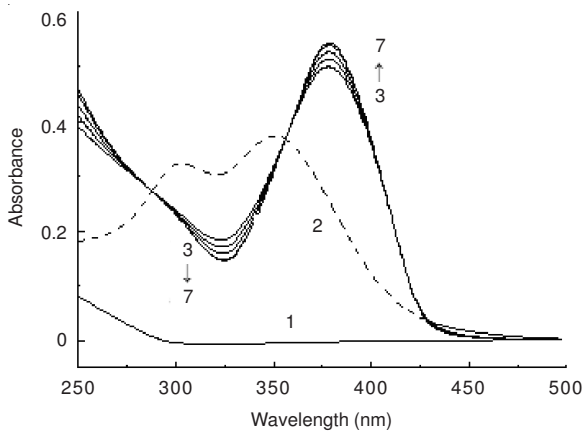

Fig. 3. Absorption spectra of tetracycline in the absence and presence of Mg(II). 1. Mg(II); 2. tetracycline; 3-7.Different concentrations of Mg(II)

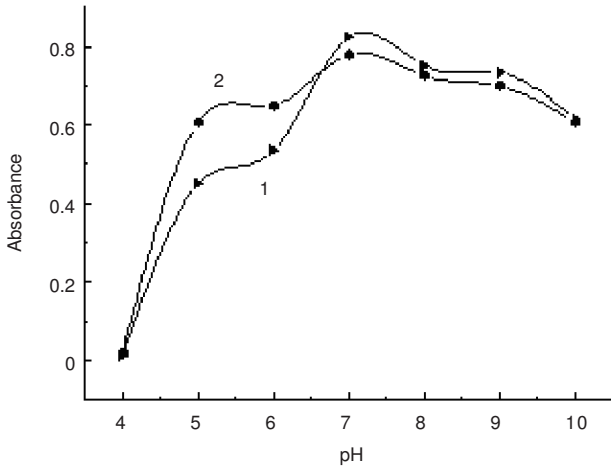

Fig. 4. Effect of acidity. 1. NaOH/HCl; 2. buffer solution

Effect of reagents dosage and ionic strength: The experimental outcome indicated that 3.5 mol L^{-1} NaCl as phase-seperation salt, 6 mL Mg(II) as complexant and 5 mL tetrahydrofuran as solvent was feasible, moreover, the various ionic strength made no impression on floating result.

Effect of reaction time, floatation time and nitrogen flow rate: Effect of reaction time, floatation time and nitrogen flow rate on phase-separation sublation was investigated. The organic-phase absorbance rose gradually with the extension of time for phase-separation sublation and accretion of flow rate and reaction time, the particular case was showed in Fig. 5. 20 mL min^{-1} of flow rate and 5 min of floatation time were chosen by integrated analysis.

Reaction mechanism: Studies showed that it is coordination reaction between tetracycline and Mg(II). The maximum possibility of coordination compound forming process is shown in Fig. 6.

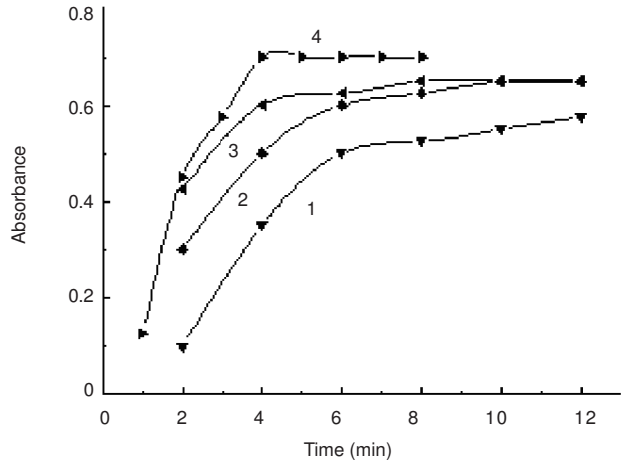

Fig. 5. Effect of reaction time,floatation time and nitrogen flow rate. 1. draft rate 10 mL/min; 2. draft rate 20 mL/min;3. draft rate 30 mL/min; 4. reaction time

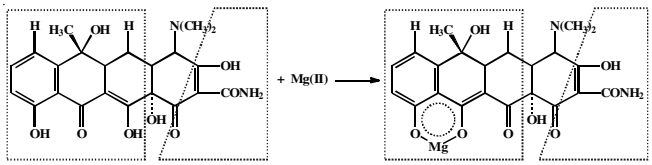

Fig. 6. Coordination compound forming process of tetracycline and Mg(II)

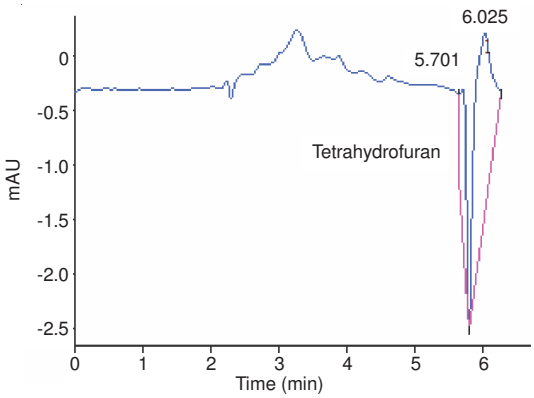

Fig. 7. Chromatogram of tetrahydrofuran

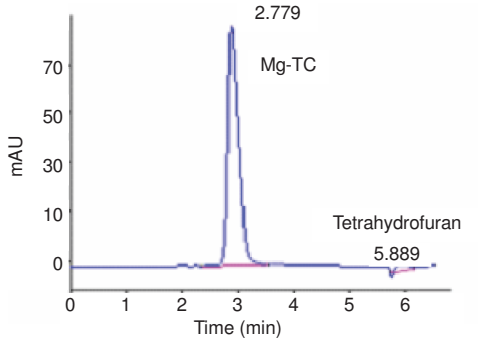

Fig. 8. Total chromatogram of organic phase

Liner range and limit of detection: The detection of tetracycline was determined and it was 1.1×10^{-7} mol L^{-1}. The liner range of analytical method was calculated and it was 1.7×10^{-7}-1.02×10^{-5} mol L^{-1}.

Floating rate and enrichment multiples: The experiment indicated that the floating rate was 89.0 % and the enrichment multipules was 26.7.

Evaluation of flotation: The standard solution of tetracycline and floated organic phase were used for chromatography analysis in the option conditions: Diamonsil C_{18} column; acetonitrile-water flowing phase and flow rate 0.8 mL min^{-1}; VWD1A detector; wavelength 350 nm, the results are showed in Figs. 7 and 8. Comparing Fig. 7 with Fig. 8, it is observed that the complex of Mg(II)-TC (2.5×10^{-6} mol L^{-1}) had been floated efficiently into tetrahydrofuran. Calculated with chromatography standard curve method, the floating rate was 89.2 %. The chromatography analysis experiment showed that the complex Mg-TC has a good solubility in tetrahydrofuran, at the same time; it showed that the complex has a good hydrophobicity, because the good hydrophobicity was the key to effective flotation of the complex.

Sample analysis: Take three sorts of waste-water of manufacturer and made it up to test solution. The content of tetracycline in waste-water was determined using the experimental procedure, the results were shown in Table-1.

TABLE-1
DETERMINATION RESULTS OF TC IN MEDICINE (n = 5, 10^{-6} mol L^{-1})

Tablets	Found	Added	Total found	Recovery (%)	RSD (%)
1	0.51	1.00	1.46	97.0	0.89
2	0.49	1.00	1.45	97.2	1.22
3	0.89	1.00	1.81	98.2	0.98

ACKNOWLEDGEMENTS

This research was financially supported by Henan Provincial Natural Science Foundation of China (No. 2009c610002).

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