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Extraction of Trace Tetracycline by Phase-Separated Sublation

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As a pretreatment technology, the phase-separated sublation was utilized for the selective extraction and determination of trace tetracycline (TC) in medical wastewater. The results indicated that tetracycline turned into a binary complex (Cu-TC) in the presence of copper(II), which can be separated by phase-separated sublation easily. The extracted binary complex was determined by spectrophotometry. Batch pretreatment tests were carried out, such as the effects of solution pH, reagents dosage, ionic strength, reaction time, floatation time and nitrogen flow rate. The maximum adsorption was 381 nm, phase-separation reagent was 3 mol L^{-1} sodium chloride, sodium hydroxide solution was acidity regulator and *n*-propyl alcohol was solvent. Linear regression equation was A = 1.611 × 10^5C-0.011, lower limit of detection (LOD) was 8.1 × 10^{-7} mol L^{-1}.

Keywords: Tetracycline, Phase-separated sublation, Spectrophotometry, Copper.

INTRODUCTION

Tetracycline (TC) is a broad spectrum antibiotic synthesized by biological method, which is widely used in the prevention and treatment of livestock and poultry, aquatic plant and animal disease[1]. In natural water, the residual tetracycline drug is trace level. Moreover, the residue amount of tetracycline drug in aquaculture sediments and pharmaceutical wastewater may be higher[2]. The solubility of tetracycline in water is good, the decision procedure of trace tetracycline using phase-separated sublation is that tetracycline must be transformed into hydrophobic substance,we can obtain this hydrophobic substance *via* coordination reaction or association in the presence of collector.

The determining methods of antibiotics in the existing literatures include liquid chromatography mass spectrometry[3-5], Molecular imprinted extraction[6-8], immunochemistry[9], liquid phase extraction[10,11]. In this study, we will study the establishment of binary complex of tetracycline and copper, which was applied in the separation and enrichment of trace tetracycline in medical wastewater using phase-separated sublation. This is a novel method with regard to determination of trace tetracycline residues in environment.

The method has the high enrichment multiple, using non-toxic or low toxic solvents and easy to operation automated. Moreover, it can treat a large number of sample continuously and 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 extraction, secondary pollution was befallen 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 convenient post-processing. We will take advantage of this technology conjunction with the spectrophotometric measurement to detect tetracycline.

The basic principle of the technique is, first, we adjust ionic strength of test system with inorganic salt, the inorganic anions and cations ionized form the inorganic salt combine with a large number of water molecules. The hydrophile organic solvent that relys on hydrogen bonding to combine water molecule becomes hydrophobically because of no free water molecules, 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 in the function of surface tension. We can obtain the content of measuring substance by determining the absorbance of organic phase.

EXPERIMENTAL

Stock solution of tetracycline 1 × 10^{-3} mol L^{-1}: Accurately weighed 0.1202 g of tetracycline, dissolved with deionized water. 1 × 10^{-3} mol L^{-1} of Cu(II): accurately weighed 0.3990 g of anhydrous cupric sulfate, dissolved with deionized water

to 250 mL. Sodium chloride, pH 6-11 buffer solution: 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) 0.01 mol L^{-1} sodium hydroxide, tetrahytrofuran, *n*-propyl alcohol, *n*-butyl alcohol, $C_{19}H_{42}BrN$, $C_{12}H_{25}NaO_3S$, $C_{21}H_{38}ClN{\cdot}H_2O$, HCl, NaOH, H_2SO_4.

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-separated sublation equipment (selfmade, shown in Fig. 1).

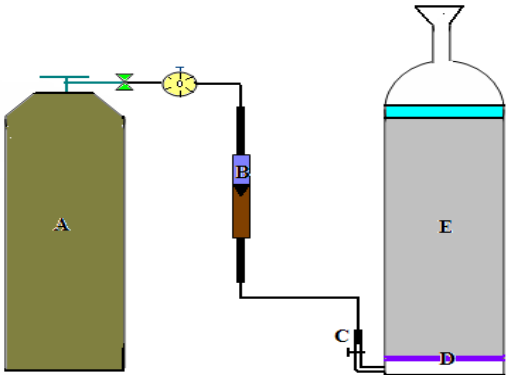

Fig. 1. Apparatus for phase-separated sublation (a) nitrogen cylinder (b) rotor flow meter; (c) impervious glass cock; (d) glass filter plate (e) flotation column

Experimental procedure: 5 mL of 1 × 10^{-4} mol L^{-1} tetracycline was transferred into a 50 mL beaker, 6 mL of 1 × 10^{-4} mol L^{-1} Cu(II) and 0.2 mL of 0.01 mol L^{-1} NaOH solution were added in turn, left for 5 min. The mixture solution was transferred to the floatation pool and diluted to 200 mL volume with 3 mol L^{-1} of NaCl, 5 mL of *n*-propyl alcohol was added to the floating pool. Detection was finished on an ultraviolet detector setting at 381 nm. The experiment of phase-separated sublation was in the conditions of 10 min of floating time and 20 mL min^{-1} of flow rate.

RESULTS AND DISCUSSION

Absorption spectrum: The standard solution of tetracycline and Cu-tetracycline were scaned successively in the wavelength range of 200-500 nm by spetrophotometer, the test conditions were that pH was 7 and the concentration of tetracycline and Cu(II) was 1 × 10^{-4} mol L^{-1}. The reference solution was deionized water when the standard solutions of Cu(II) and tetracycline were scaned. Besides, the reference solution was a reagent blank when the standard solution of Cu-TC was scaned. The absorption spectrum is shown in Fig. 2. It is find that the strong interaction has happened between tetracycline and Cu(II) by comparing the adsorption curve of Cu-TC. The absorption maximum of binary complex was 381 nm.

Effect of acidity: Adjusting the acidity of experimental system in two ways of 0.01 mol/L of sodium hydroxide co-operated with hydrochloric acid (corrected with acidometer) and buffer solution, we investigated the forming process and flotation effect of Co-TC with the change in acidity. The results were showed in Fig. 3.

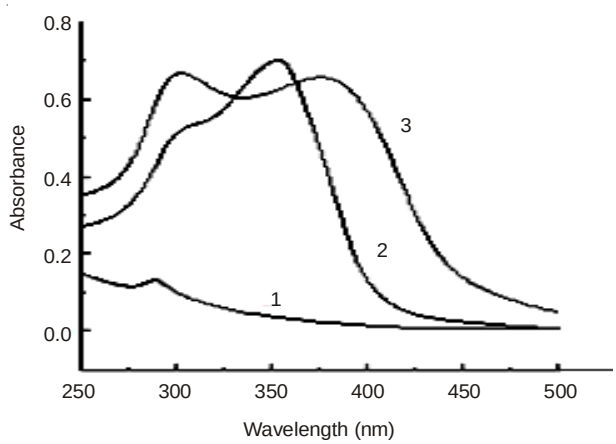

Fig. 2. Absorption spectra (1) Cu(II); (2) TC; (3) Cu-TC

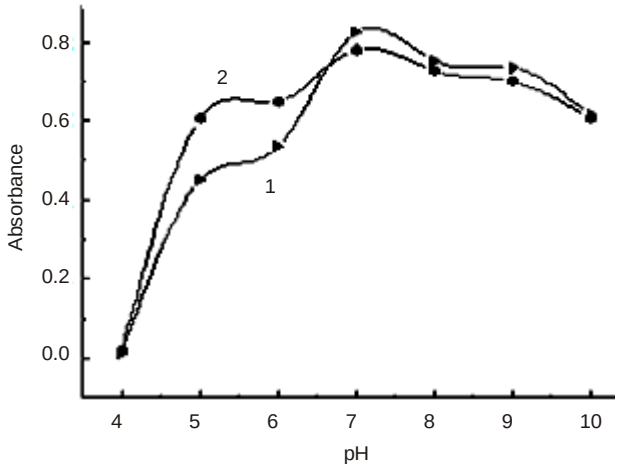

Fig. 3. Select of acidity adjusting method; (1) adjusting acidity with sodium hydroxide; (2) adjusting acidity with butter solution

Obviously, either or both of sodium hydroxide cooperated with hydrochloric acid and buffer solution, the maximum absorption peak of mixed solution of tetracycline, Cu^{2+} appeared at pH of 7. Fuethermore, the floating effect was better using sodium hydroxide cooperated with hydrochloric acid. So 0.2 mL of 0.01 mol L^{-1} sodium hydroxide was used in the experiment.

Effect of the concentration of sodium chloride: The phase-seperated ability of sodium chloride was studied. The result showed that it could not completely uncouple between salt-water phase and organic phase when the concentration of sodium chloride was less than 3 mol L^{-1}. So 3 mol L^{-1} of sodium chloride was used in the research.

Effect of reagents dosage: According to the experimental method, 5 mL of 1 × 10^{-4} mol L^{-1} tetracycline was used. We have investigated the absorbance trend of organic phase after flotation with the change in dosage of Cu^{2+}. The experimental results were showed in Fig. 4.

By observing the absorbance curve of Cu-TC, it is observed that the absorbance of organic phase after floatation gradually increased with the addition of Cu^{2+}. Further more the absorbance of organic phase after floatation reached the

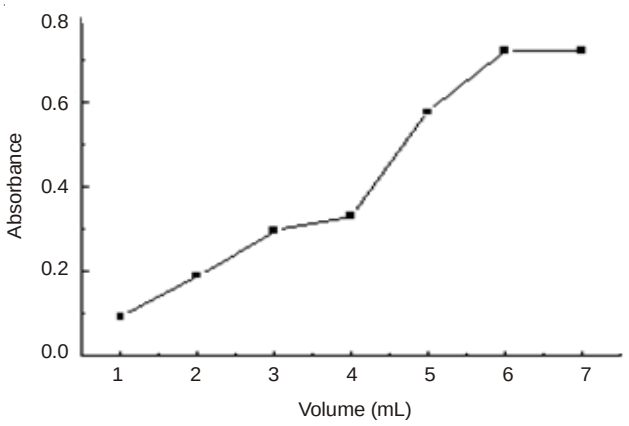

Fig. 4. Effect of added dosage change of Cu(II) to organic-phase absorbance

maximum when the dosage of Cu^{2+} was 6 mL. So 6 mL of 1×10^{-4} mol L^{-1} Cu^{2+} was added.

We also studied the effect to floatation to use different of organic solvent, the result suggested that *n*-propyl alcohol was better.

Effect of reaction time, floating time, nitrogen flow and coexisting substances: Effect of reaction time, floating time, nitrogen flow and coexisting substances on phase-separated sublation was investigated. The conclusion indicated that the organic-phase absorbance rose gradually with the extension of time for phase-separated sublation and accretion of flow rate, the particular case was showed in Fig. 5. 5 min of recation time, 20 mL min^{-1} of flow rate and 10 min of floatation time were chosen.

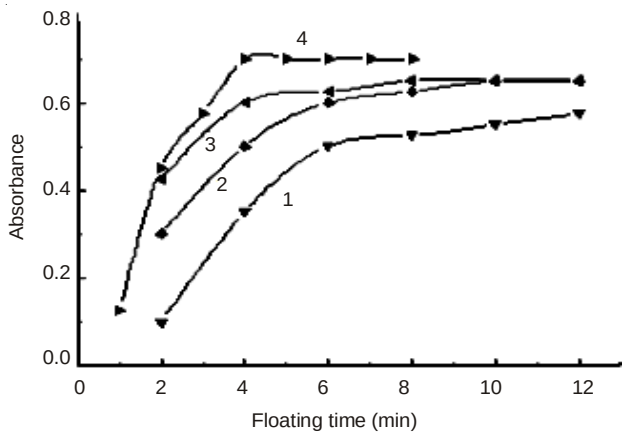

Fig. 5. Effect of floatation time and flow rate; (1-3) flow: 10 mL/min, 20 mL/min, 30 mL/min; (4) reaction time

5 mL of 1×10^{-4} mol L^{-1} TC was used in the interference experiment. Effect of several metal ions, non-metallic ions and organic compounds in test system on phase-separated sublation was studied. When the relative error was less than ± 5 %, the permissible amount of coexisting substances (to multiply) was showed in Table-1.

The interference experiment showed that the selectivity of test method for tetracycline was better when the metal ions was masked by sodium tartrate.

Method sensitivity: A series of different volume of 1×10^{-4} mol L^{-1} tetracycline was applied to the phase-separated sublation. The adsorbance of floating organic phase was determined successively, the working curve of adsorbance and concentration of tetracycline was described, the least square method was used to calculated the data.

TABLE-1
EFFECT OF COEXISTENCE SUSTANCES

Coexistence	Added (10^{-3} mol/L)	Relative error (%)	Coexistence	Added (10^{-3} mol/L)	Relative error (%)
Zn^{2+}	1	1.0	Erythromycin	5	1.1
Fe^{3+}	5	1.1	L-Histidine	50	0.9
Ni^{2+}	5	0.7	L-Leudine	50	1.3
Mg^{2+}	3	0.6	L-Cystine	40	0.7
Ca^{2+}	5	0.9	Glycine	30	0.8
Ag^{+}	1	2.0	Penicillin	10	0.6
Co^{2+}	5	1.9	Gentamicin	8	0.7
Chloramphenicol	2	0.3	Glucose	10	1.0
CO_3^{2-}	4	2.0	Starch	70	0.5

Linear regression equation: $A = 1.611 \times 10^5 C - 0.011$

Linear range: 8.7×10^{-7}-2×10^{-5} mol L^{-1}

Molar absorption rate: 1.611×10^5 L mol^{-1} cm^{-1}

Detection limit: 8.1×10^{-7} mol L^{-1}

Floatation rate: 79.2 %

Enrichment multipules: 26.7

Composition of coordination compound: The outmost energy level group of Cu(II) is $3d^9 4s^0 4p^0$. When the reaction has taken place between Cu(II) and tetracycline, the four equal energy of hybridized orbital of dsp^2 should be formed because of electronic transition. So we could get a binary complex of Cu-TC. Applied with mole ratio method and equimolar continuous variations method,we could obtain the complex ratio was 1:1, the detemining wavelength is 381 nm.

According to the Chen Guozhen rule, the formation of complex was showed in Fig. 6.

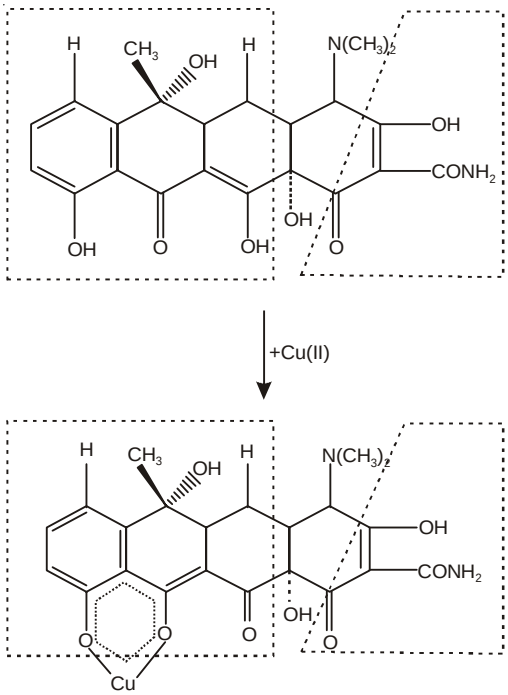

Fig. 6. Reaction mechanism of binary complex

Application test: According to the test procedure, the three samples taken from hospital were determined after vacuum filtration. The results were shown in Table-2.

TABLE-2
DETERMINATION RESULTS OF
TETRACYCLINE (n = 5, 10^{-5} mol/L)

Sample	Found	Added	Total found	Recovery (%)	RSD (%)
1	0.97	1.00	1.87	94.9	1.3
2	1.10	1.00	2.12	100.9	1.6
3	1.21	1.00	1.91	86.4	2.0

Conclusion

In this research, we set up a new sample pretreatment technology of phase-separated sublation,it was used for selective extraction and determination of trace tetracycline (TC) in hospital wastewater. The results showed that tetracycline can be transformed into binary complex (Cu-TC) in the presence of copper(II), which can be separated by phase-separated sublation easily. The extracted binary complex was determinated by spectrophotometry.This technique can be applied in the field of analysis and assessment of trace component in the food and medicine.

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