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Extraction of Sodium Copper Chlorophyll in Food Samples Based on Cloud Point Extraction with a Nonionic Surfactant

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Abstract: A new method was developed for the determination of sodium copper chlorophyll (SCC) by cloud point extraction preconcentration and spectrophotometry, for which Triton X-114 was selected as a nonionic surfactant. Several factors affecting the extraction efficiency of SCC and its subsequent determination, including the pH of the sample solution, salt and surfactant concentrations, and equilibration temperature and time, were studied and optimized. The extraction efficiency approached 99.4%. The calibration graph under the optimum conditions was linear in the concentration range of 3–220 mg/L with correlation coefficients > 0.9997 ($n = 8$). The limit of detection for the analytes was 0.6 mg/L ($S/N = 3$). The proposed method is inexpensive, simple, and accurate for the extraction and determination of SCC in food samples.

Keywords: Cloud point extraction; Sodium copper chlorophyll; Triton X-114

1 Introduction

Sodium copper chlorophyll (SCC) is a natural edible pigment approved by the United Nations Food and Agriculture Organization and the Chinese Standards Committee for food usage. It has been widely used in the food industry because of its inherent bright-green color, thermal stability, and special sterilizing and deodorizing properties.

Several methods have been developed for the determination of SCC, including spectrophotometry, fluorescence spectroscopy, high-performance liquid chromatography (HPLC), and capillary electrophoresis[1-4]. However, some of these methods, such as HPLC and capillary electrophoresis, are time-consuming and use very expensive apparatus. Spectrophotometry remains the most common method used for SCC determination because of its simplicity, precision, and accuracy. It is also the state-standard method in China for the determination of SCC in food[5]. However, the method is difficult to apply for the analysis of complex matrix samples without any pretreatment. SCC is usually present in food samples at low levels; thus, a preconcentration step, such as polyamide adsorption or solvent extraction[6], prior to spectrophotometry, is necessary to achieve appropriate levels of sensitivity. These preconcentration steps are complex and tedious, and cause organic solvent pollution and pigment decomposition. Therefore, development of a simple, efficient, and accurate technique for the analysis of SCC in foodstuffs is important.

Separation and preconcentration based on cloud point extraction (CPE) is becoming increasingly popular in analytical chemistry[7-9]. Most nonionic surfactants in aqueous solutions form micelles and the solution turns turbid when heated to the “cloud point temperature”. The micellar solution separates into a surfactant-rich phase and a diluted aqueous phase at temperatures above the cloud point temperature, during which the surfactant concentration is close to the critical micellar concentration (CMC). The small volume of the surfactant-rich phase obtained by this method permits the design of simple, inexpensive, highly efficient, and rapid extraction schemes that are also less harmful to the environment than extractions using organic solvents. CPE has been widely used to separate and pre-concentrate organic compounds and metal ions[10-14].

The present study adopted CPE for the extraction of SCC from food samples using a nonionic surfactant, Triton X-114. The effects of the sample solution pH, concentrations of salt and surfactant, equilibration temperature, and time on the extraction of SCC were also investigated.

2 Materials and Methods

2.1 Materials and Apparatus

Nonionic surfactant Triton X-114 was purchased from Sigma-Aldrich (St. Louis, MO, USA) and prepared in deionized water. SCC of 99% purity was purchased from Jianglai Biotechnology Co., Ltd. (Shanghai, China). Analytical-grade sodium chloride (NaCl) and hydrochloric acid (HCl) were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (China). All other chemicals used were of analytical grade.

A UV-2460 ultraviolet-visible spectrophotometer (Shimadzu, Japan) was used to record absorption spectra and absorbance measurements using 1 cm glass cells. A PHS-3C digital pH meter (Shanghai Dapu Instrument Company) was used for pH measurement. A thermostat bath (Henan Zhongliang Scientific Instrument Co., Ltd.) maintained at the desired temperature was used for the CPE experiments.

2.2 CPE Experiment

Briefly, a stock solution of 0.5 g/L SCC was prepared by dissolving 0.5 g of SCC in a volumetric flask containing 1 000 mL of water, from which diluted solutions of SCC were prepared. Each CPE experiment was conducted using a 25 mL

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tube containing different concentrations of Triton X-114, SCC, and NaCl heated in a constant-temperature bath for several minutes. The Triton X-114 concentrations were 1%, 1.5%, 2%, 3%, 4% and 5% (V/V), and the NaCl concentrations were 0.05, 0.08, 0.1, 0.15 and 0.2 g/mL, respectively. After complete phase separation, the tube was removed from the temperature bath and cooled for 2 min. The aqueous phase was removed by decantation, and the surfactant-rich phase was diluted in a volumetric flask containing 5 mL of water. The absorption spectrum of the SCC solution showed that maximum absorbance occurs at $\lambda_{\max}$ = 632 nm and the presence of surfactant has no significant effect on the $\lambda_{\max}$. Thus, the analyte was monitored at 632 nm. A blank solution was also subjected to the same procedure and each experiment was replicated three times.

2.3 Sample Preparation

The coating of sweet samples and fruit juice were dissolved in deionized water. All the samples were filtered using a 0.45 μm pore size membrane filter to remove suspended particulate matter and stored in the dark. The solution was treated under the recommended procedure.

3 Results and Discussion

3.1 Effect of pH

The effect of pH, which was adjusted with 0.1 mol/L HCl, on the extraction efficiency of SCC was studied over the pH range 3.0–10.0 (Fig. 1). The results indicated that the extraction efficiency of SCC is relatively high over the pH range 3.0–5.0. The ionic form of an analyte does not interact as strongly with the micellar aggregate as its neutral form; thus, a smaller amount of analyte is extracted[15]. These results indicate that pH should be adjusted to ensure that the analyte is present in its neutral form prior to CPE. The pH was thus controlled to 4.0 in the following experiments for easy adjustment.

3.2 Effect of Triton X-114 Concentration

Triton X-114 was chosen as the CPE medium because of its low cloud point temperature and high density, both of which facilitate phase separation. Successful extraction is achieved by maximizing the enrichment factor through minimizing the phase volume ratio, and the surfactant concentration is the main parameter affecting the phase ratio[16].

The effect of Triton X-114 concentration (1.0%–4.0%, V/V) on the performance of the extraction system was studied (Fig. 2). SCC absorbance increased upon increasing surfactant concentration from 1.0% to 2.0%. Low Triton X-114 concentrations were inadequate for the process. When a large surfactant concentration was used, the surfactant-rich phase obtained after CPE was too sticky, which makes subsequent handling more difficult[17]. An optimum surfactant concentration of 2.0% (V/V) was chosen for further studies based on the extraction efficiency of SCC.

3.3 Effect of NaCl Concentration

The effect of electrolytes on the cloud point temperature using nonionic surfactant solutions has been investigated[18]. The micelle size and aggregation number increase with increasing salt concentration, but the CMC remains constant. Available electrolytes can also change the cloud point temperature of nonionic surfactants.

To study the influence of electrolytes, different NaCl concentrations ranging from 0.05 g/mL to 0.2 g/mL were investigated (Fig. 3). The extraction efficiency of SCC increased upon increasing NaCl concentration from 0.05 g/mL to 0.1 g/mL; however, no significant difference was observed above 0.1 g/mL. A NaCl concentration of 0.1 g/mL was thus chosen for further studies.

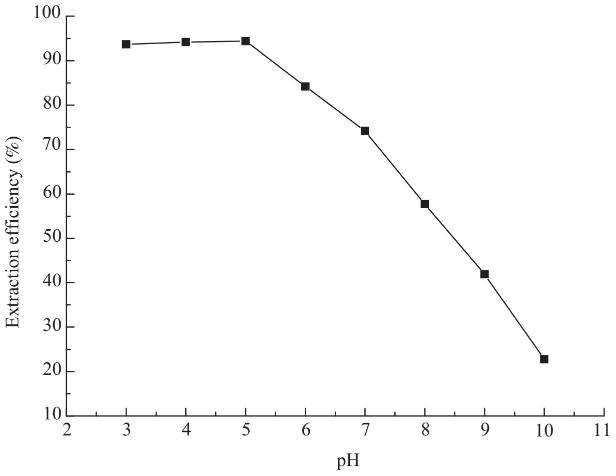

Fig. 1 The effect of pH on the extraction efficiency of 100 mg/L of SCC after CPE

Extraction condition: 2% Triton X-114, 0.1 g/mL NaCl; temperature: 40 °C; equilibrium time: 15 min.

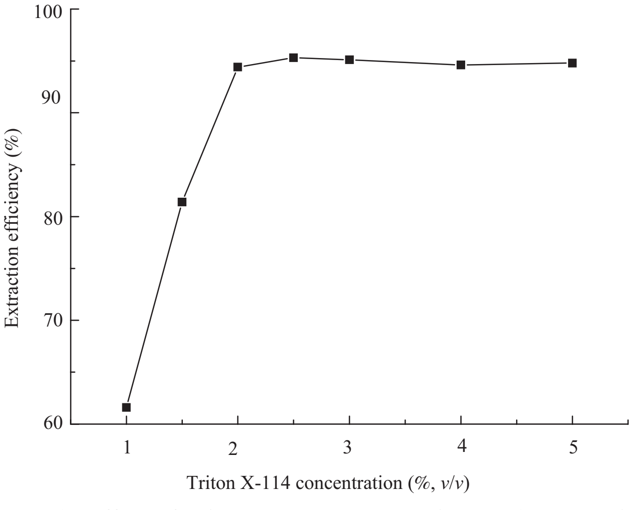

Fig. 2 The effect of Triton X-114 concentration on the extraction efficiency of 100 mg/L of SCC after CPE

Extraction condition: pH 4.0, NaCl concentration 0.1 g/mL; temperature: 40 °C; equilibrium time: 15 min.

3.4 Effect of Equilibrium Temperature

Triton X-114 can form normal micelles in aqueous solution. Its cloud point temperature in pure water is about 25 °C. To obtain a more favorable preconcentration factor, CPE must be performed at temperatures higher than the cloud point temperature[19].

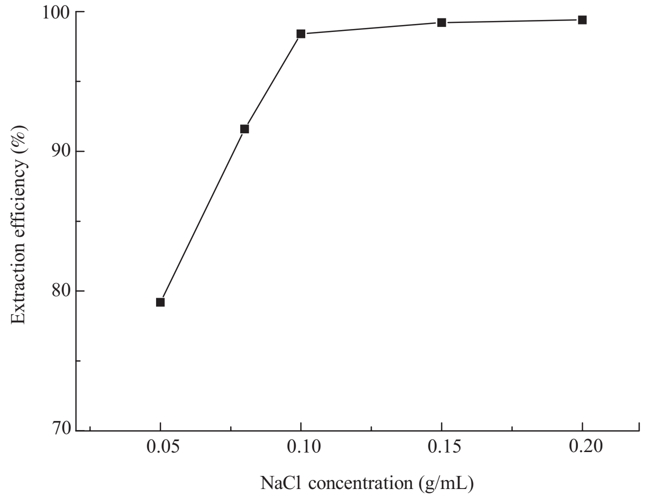

Fig. 3 The effect of NaCl concentration on the extraction efficiency of 100 mg/L of SCC after CPE
Extraction condition: 2% Triton X-114, pH 4.0; temperature: 50 °C; equilibrium time: 15 min.

The effect of temperature on the extraction efficiency was studied in the temperature range 30–80 °C (Fig. 4). The volume of the surfactant-rich phase decreases with increasing equilibration temperature because hydrogen bonds are disrupted and dehydration occurs. Consequently, the amount of water in the surfactant-rich phase also decreases. The extraction efficiency was found to maximize at temperatures above 50 °C; thus, 50 °C was selected as the equilibrium temperature in this study to achieve complete extraction.

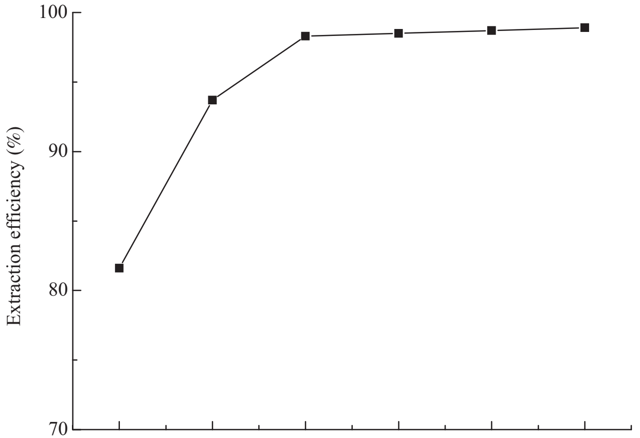

Fig. 4 The effect of equilibrium temperature on the extraction efficiency of 100 mg/L of SCC after CPE
Extraction condition: 2% Triton X-114, pH 4.0, NaCl 0.1 g/mL; equilibrium time: 15 min.

3.5 Effect of Equilibrium Time

The shortest incubation time must be employed for completion of extraction and efficient phase separation. The effect of equilibrium times ranging from 5 to 30 min on the extraction efficiency was investigated (Fig. 5). The extraction efficiency of SCC increased upon increasing equilibrium time from 5 min to 10 min, but did not change from 10 min to 30 min. Thus, an equilibrium time of 10 min was chosen for further studies.

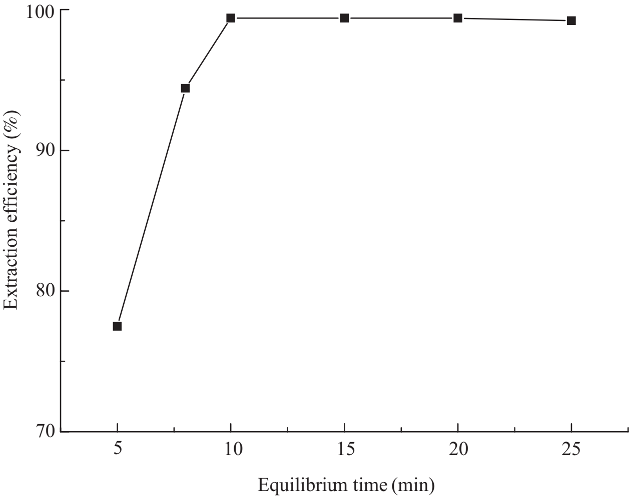

Fig. 5 The effect of equilibrium time on the extraction efficiency of 100 mg/L of SCC after CPE
Extraction condition: 2% Triton X-114, pH 4.0, NaCl 0.1 g/mL; temperature: 50 °C.

3.6 Characteristics of the Method

The calibration curve was linear in the range of 3–220 mg/L SCC under optimized conditions. The equation of the line obtained was $A = 0.0016\,C + 0.0045$ with a regression coefficient (R) of 0.9997 ($n = 8$), where A is the absorbance and C is the concentration of SCC (in mg/L). The limit of detection (LOD) based on three-times the standard deviation of the blank ($S/N = 3$) was 0.6 mg/L, and the relative standard deviation (RSD) values for 50 and 100 mg/L of SCC were 2.15% and 1.46%, respectively.

3.7 Sample Analysis

To verify the accuracy of the proposed method, the SCC contents in certified reference materials of sweet and fruit juice were determined by the proposed method (Table 1). In addition, the recovery experiments with different amounts of SCC showed that recoveries of 98.8%–102.0% are very reasonable for analysis.

Table 1 Determination of sodium copper chlorophyll (mg/L) in food samples ($n = 3$)

Sample	Certified value	Added (mg/L)	Found (mg/L)	Recovery (%)
Spiked solution		50	49.8 ± 0.6	99.6
		100	99.1 ± 0.4	99.1
Sweet	15.6 ± 0.3	0	16.2 ± 0.6	103.8
		50	67.2 ± 0.8	102.0
		100	115.0 ± 1.1	98.8
Fruit juice	45.2 ± 0.5	0	45.9 ± 0.6	101.5
		50	96.2 ± 0.7	100.6
		100	145.7 ± 1.2	99.8

4 Conclusions

The results show the practicability of the proposed method for the extraction and determination of SCC in food samples.

The optimal extraction variables were studied based on the cloud point extraction technique. Under optimal conditions, i.e., 2% Triton X-114 (*V*/*V*), pH 4.0, NaCl concentration of 0.1 g/mL, and cloud point extraction for 10 min at equilibrium temperature of 50 °C, the extraction efficiency of SCC was maximized (99.4%).

The proposed method is simple, inexpensive, efficient, and accurate for the extraction of SCC. Furthermore, CPE could be employed as a new and effective approach for the rapid extraction and preconcentration of SCC from various real samples without affecting spectrometric analysis.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

[1] MORTENSEN A, GEPPEL A. HPLC-MS analysis of the green food colorant sodium copper chlorophyllin[J]. Innovative Food Science and Emerging Technologies, 2007, 8(3): 419-425.

[2] BOHN T, WALCZYK T. Determination of chlorophyll in plant samples by liquid chromatography using zinc-phthalocyanine as an internal standard[J]. Journal of Chromatography A, 2004, 1024(1-2): 123-128.

[3] BUSCHMANN C, LICHTENTHALER H K. Contribution of chlorophyll fluorescence to the reflectance of leaves in stressed plants as determined with the VIRAF-spectrometer[J]. Zeitschrift für Naturforschung C, 1999, 54(9): 849-855.

[4] DEL GIOVINE L, FABIETTI F. Copper chlorophyll in olive oils: identification and determination by LIF capillary electrophoresis[J]. Food Control, 2005, 16(3): 267-272.

[5] GB/T 23749-2009. Determination of sodium copper chlorophyllin in foods-spectrophotometric method[S]. Beijing: China Standards Press, 2009.

[6] GARCÍA A L, NICOLÁS N. Establishing errors in the spectrophotometric determination of chlorophylls using different solvents[J]. Journal of Plant Physiology, 1998, 152(4): 392-398.

[7] YANG X, JIA Z, YANG X, et al. Cloud point extraction-flame atomic absorption spectrometry for pre-concentration and determination of trace amounts of silver ions in water samples[J]. Saudi Journal of Biological Sciences, 2017, 24(3): 589-594.

[8] MUHAMMAD B, TASNEEM G K, HASSAN I A, et al. Application of conventional and modified cloud point extraction for simultaneous enrichment of cadmium, lead and copper in lake water and fish muscles[J]. Journal of Industrial and Engineering Chemistry, 2016, 40: 137-144.

[9] NONG C, NIU Z, LI P, et al. Dual-cloud point extraction coupled to high performance liquid chromatography for simultaneous determination of trace sulfonamide antimicrobials in urine and water samples[J]. Journal of Chromatography B, 2017, 1051: 9-16.

[10] GIEBUŁTOWICZ J, KOJRO G, PIOTROWSKI R, et al. Cloud-point extraction is compatible with liquid chromatography coupled to electrospray ionization mass spectrometry for the determination of antazoline in human plasma[J]. Journal of Pharmaceutical and Biomedical Analysis, 2016, 128: 294-301.

[11] GLEMBIN P, KERNER M, SMIRNOVA I. Cloud point extraction of microalgae cultures[J]. Separation and Purification Technology, 2013, 103: 21-27.

[12] EL-ABBASSI A, KIAI H, RAITI J, et al. Cloud point extraction of phenolic compounds from pretreated olive mill wastewater[J]. Journal of Environmental Chemical Engineering, 2014, 2(3): 1480-1486.

[13] SILVA W P N, DO NASCIMENTO A E G, DE ALENCAR MOURA M C P, et al. Study of phenol removal by cloud point extraction: A process optimization using experimental design[J]. Separation and Purification Technology, 2015, 152: 133-139.

[14] SAMADDAR P, SEN K. Cloud point extraction: A sustainable method of elemental preconcentration and speciation[J]. Journal of Industrial and Engineering Chemistry, 2014, 20(4): 1209-1219.

[15] SUN C, XIE Y, TIAN Q, et al. Separation of glycyrrhizic acid and liquiritin from licorice root by aqueous nonionic surfactant mediated extraction[J]. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2007, 305(1): 42-47.

[16] KIMCHUWANIT W, OSUWAN S, SCAMEHORN J F, et al. Use of a micellar-rich coacervate phase to extract trichloroethylene from water[J]. Separation Science and Technology, 2000, 35(13): 1991-2002.

[17] QIN X Y, MENG J, LI X Y, et al. Determination of venlafaxine in human plasma by high-performance liquid chromatography using cloud-point extraction and spectrofluorimetric detection[J]. Journal of Chromatography B, 2008, 872(1-2): 38-42.

[18] PURKAIT M K, BANERJEE S, MEWARA S, et al. Cloud point extraction of toxic eosin dye using Triton X-100 as nonionic surfactant[J]. Water Research, 2005, 39(16): 3885-3890.

[19] SUN C, XIE Y, TIAN Q, et al. Cloud point extraction of glycyrrhizic acid from licorice root[J]. Separation Science and Technology, 2007, 42(14): 3259-3270.