OPTIMIZATION AND DETERMINATION OF TARTRAZINE BY GREEN TWEEN-20 CLOUD POINT EXTRACTION FOR FOOD SAMPLES

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Abstract

A simple and low-cost cloud point extraction (CPE) method was developed for the determination of tartrazine in food samples by spectrophotometry detection at a wavelength 427.5 nm. The CPE was performed by utilizing Tween-20 and sodium carbonate (Na₂CO₃) as extractant and separation accelerator, respectively. Factors that influenced CPE such as surfactant and salt concentrations, pH and temperature were optimized in the context of extracting tartrazine from aqueous media. Under an optimal condition, the proposed CPE was applied for the determination of tartrazine in sweets and concentrated syrup juice, which represented food samples. A CPE-UV-Vis method showed linear calibration within the range of 1-12 mg L⁻¹ of tartrazine with a regression coefficient was 0.9957. The limit of detection (LOD) and limit of quantification (LOQ) of the method were 0.88 mg L⁻¹ and 2.96 mg L⁻¹, respectively. The relative standard deviation (RSD) was found to be < 3.00 %. The amount of tartrazine found in food samples was 1.22-6.12 mg L⁻¹. The results showed that the proposed CPE method was applicable for the determination of tartrazine content in the food samples was permitted according to the guidelines from the European Food Safety Authority (EFSA).

Keywords: cloud point extraction, Tween-20, sodium carbonate, tartrazine, food

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Introduction

Tartrazine or E102 is an azo dye that primarily used as a food colouring and food additive. It is a synthetic dye which has anionic and hydrophilic characteristics. An orange-yellow colour of tartrazine has been commonly used in cosmetic, fabric and food industries as an alternative synthetic dye to beta-carotene as well as a biological stain because it is cheap. Unfortunately, this synthetic dye has been reported to cause adverse effects. Tartrazine has potential to cause food intolerance, allergy reactions and other side effects such as hyperactivity, attention deficit hyperactivity disorder (ADHD) and Carpal Tunnel Syndrome (CTS) (Chaudary et al. 2003; Siddiquee & Shaarani 2017).

Cloud point extraction (CPE) method is a green alternative technique for liquid-liquid extraction (LLE) method, analyte pre-concentration and sample clean-up. The CPE method was selected in this study due to its efficiency and versatility. Instead of the existing chromatographic methods, CPE offers a simple, more economical, greener and eco-friendly method as it employs minimal use of toxic

substances. Besides that, routine analysis of the tartrazine in quality control analysis can be done as it does not require sophisticated instrumentation.

CPE is a suitable alternative separation and pre-concentration technique. It separates the hydrophobicrich phase in a nonpolar microenvironment from the aqueous supernatant when exceeding certain critical thermodynamic state known as the cloud point (Mondal et al. 2018). Usually, above a certain temperature known as cloud point temperature (CPT), a single phase non-ionic surfactant micelle aqueous solution separates into a dilute aqueous phase and a surfactant-rich phase together with any analyte solubilized in the hydrophobic core of the micelles (Purkait et al. 2005).

Ultraviolet-visible (UV-Vis) spectrophotometer is still the most attractive and popular method that has been applied in different fields of chemical analysis, especially in quality control because the technique is simple with high in speed, precision and accuracy. The combination of spectrophotometric detection with CPE was first proposed by Watanabe & Tanaka (1978) and Goto et al. (1977) who studied the extraction for the pre-concentration of manganese ion and zinc ion in water samples. In recent years, several reports have been published for removal of various heavy metals and dyes by CPE method coupling with spectrophotometric detection, indicating this method is still reliable (Bişgin et al. 2018; Guo et al.2017; Wang et al.2016)

In this study, determination of the tartrazine in food samples using a combined CPE with UV-Vis spectrophotometry analysis was carried out. CPE was prepared using non-ionic surfactant Tween-20 as an extractant and sodium carbonate (Na_2CO_3) as separation phase accelerator. This study was divided into two objectives: (1) To optimize the effect of the operating parameters including the concentrations of surfactant and salt, pH and equilibration temperature, in order to get the highest recoveries of tartrazine in an aqueous medium prior to real food samples. (2) To evaluate the effectiveness of the proposed CPE and concurrently determining the quantity of tartrazine trace in food samples. To the best of our knowledge, this is the first study that utilized the Tween-20 in CPE for determination of trace tartrazine dye in food samples.

Reagents and chemicals

A commercial non-ionic surfactant Tween-20 was purchased from R&M chemicals (Selangor, Malaysia). Tartrazine was supplied by Sisco Research Laboratories Pvt. Ltd. (Chennai, India). A hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). Sodium carbonate (Na₂CO₃) was supplied by Sigma Aldrich (St. Louis, MO, USA). Fresh working standard solutions were prepared by diluting a stock solution and kept stable during the day of experiments. Food samples such as sweets and concentrated syrup juice were randomly purchased from local market. Milli-Q deionized water (Millipore, Bedford, MA) with 18.2 M Ω cm⁻¹ resistivities was used throughout the experiment.

Methods

Instrumentation

The ultraviolet-visible (UV-Vis) spectrophotometer (Model T60 UV-Vis Jenway spectrophotometer 6715, United Kingdom) with 1 cm glass cells was used to identify absorption spectra and absorbance measurements. The centrifuge machine (Model Nuve NF 800 centrifuge, Behsa, Iran) was employed to facilitate and accelerate the phase separation process. The pH meter (Model OH700, Switzerland) with a combined glass electrode was utilised to measure the pH values. The thermo-stated water bath (Model Memmert MNE 29L-1 Water Bath, Germany) was used to maintain the temperature in cloud point extraction (CPE) experiment.

General cloud point extraction (CPE) preparation

The cloud point extraction (CPE) method was adopted from Nambiar et al. (2017) with slight modifications. An aliquot of 10 mL standard solution of 10 mg L^{-1} tartrazine (analyte) was mixed with 1.5 mol L^{-1} Na₂CO₃ (salt) and subsequently transferred into a 20 mL of the centrifuge tube. Then, 4 %

(v/v) Tween-20 (extractant) was added into the solution. The solution was immersed in a thermostated water bath at 30 °C for 15 min prior to the phase separation and cooled to room temperature. During the phase separation, the tartrazine in the solution was extracted into the surfactant-rich phase and after the separation time was completed, a satisfactory increased of viscosity could be observed. Then, the aqueous and surfactant phase were individually removed using a syringe to minimize the risk of cross-contamination of the tartrazine analyte. The volume and concentration of the collected surfactant-rich phase were measured and isolated prior to an analysing step was carried out. The recovery of tartrazine in the surfactant-rich phase was measured using UV-Vis spectrophotometer at a wavelength of 427.5 nm. The data presented was the average of triplicate measurements.

Optimization of CPE parameters study

The effect of surfactant and salt concentrations, pH and equilibration temperature were optimized and evaluated. The effect of surfactant concentration was measured in 1-8 % (v/v) of Tween-20. The effect of salt concentration was varied from 0.5-2.5 mol L⁻¹. The effect of pH on the CPE method was evaluated at pH 4, 7 and 10. While the effect of equilibration temperature was studied at 20-90 °C. The details of the experiment are summarized in Table 1.

Experiment	Manipulated variable	Constant variable	
Effect of surfactant	Volume of Tween-20 (%, v/v)	Tartrazine (mg L ⁻¹)	10
concentration	1, 2, 4, 6, and 8.	$Na_2CO_3 \pmod{L^{-1}}$	1.50
		Temperature (°C)	30
		Incubation time (min)	20
		pH	10
Effect of salt	Concentration of Na ₂ CO ₃ (mol L ⁻¹)	Tartrazine (mg L ⁻¹)	10
concentration	0.50, 1.00, 1.50, 2.00, and 2.50.	Surfactant(%,v/v)	4
		Temperature (°C)	30
		Incubation time (min)	20
		pH	10
Effect of pH	pH	Tartrazine (mg L ⁻¹)	10
	4,7, and 10	Surfactant(%, v/v)	4
		$Na_2CO_3 \pmod{L^{-1}}$	1.50
		Temperature (°C)	30
		Incubation time (min)	20
Effect of temperature	Temperature (°C):	Tartrazine (mg L ⁻¹)	10
	20, 30, 50, 70, and 90	Surfactant(%, v/v)	4
		$Na_2CO_3 \pmod{L^{-1}}$	1.50
		Incubation time (min)	20
		pН	10

Table 1. Manipulated and constant variables used in each optimization experiment of cloud point astrontion (CPE) mathed

Determination of extraction efficiency of tartrazine based CPE method

The extraction efficiency (%) of tartrazine based CPE system was computed as Equation (1):

$$Extraction efficiency (\%) = \frac{C_s V_s}{C_o V_o} \times 100$$
⁽¹⁾

Where C_s represents the tartrazine concentration in the surfactant-rich phase of volume (V_s), C_o represents the initial concentration of tartrazine, and V_o is the initial of the volume of tartrazine. Each analysis was carried out in triplicate.

Preparation of food samples

Sample preparation was conducted by adopting a simple method from El-Shahawi et al. (2013). Two

food samples, sweets and soft drink were purchased from the local market and used without prior treatment. The preparation of sweet sample was carried out by mixing it with 20 mL of deionized water and shaken well for 5 min until all particles were fully homogenized. After that, the mixture was filtered through a nylon filter paper (0.45 μ m x 47 mm) to prevent any residue leaves (retentate). Lastly, the filtrate was diluted with 50 mL of deionized water. The soft drink sample was prepared by diluting 10 mL of sample with 50 mL of deionized water after manual degassing process was completely carried out.

Result and Discussion

Optimization of the CPE method

Effect of surfactant concentration. The effect of surfactant concentration was investigated to identify the optimum volume of Tween-20 used that produced a maximum extraction efficiency of tartrazine in this CPE method. Tween-20 also known as polysorbate 20 was chosen in this CPE study due to its non-ionic surfactant type, which is stable and relatively nontoxicity. The effect of Tween-20 volume on the extraction efficiency of tartrazine in the CPE is shown in Figure 1.



Figure 1. Effect of Tween-20 surfactant concentration. Condition: tartrazine concentration: 10 mg L⁻¹, pH: 10, Na₂CO₃ concentration: 1.5 mol L⁻¹, temperature: 30 °C, incubation time: 20 min.

At the initial stage, the graph shows the lowest extraction efficiency because of the incomplete separation process. This could happen due to the insufficient micelle formation to entrap tartrazine molecule into the hydrophobic side of the surfactant, thus making the extraction inefficient. Later, the extraction efficiency of tartrazine increased as the Tween-20 volume increased by 4 % (v/v). This phenomenon occurred due to the increased number of surfactant-aggregate/micelles complexes as the volume surfactant increased (Wang et al. 2014). However, the trend of extraction efficiency is slowly decreased when the surfactant volume exceeded after 4 % (v/v). This phenomenon happened related to the phase-volume ratio. When the volume of surfactant-rich phase increased more than the desired amount, it decreased the phase-volume ratio. The decreased of the phase-volume ratio is affected by the increased viscosity and sticky of the surfactant-rich phase.

Thus, an excess volume of surfactant used would reduce the extraction efficiency. A successful CPE with maximum extraction efficiency could be obtained through optimizing the phase volume ratio (Noorashikin et al. 2017; Pourreza et al. 2011). From this study, 4 % (v/v) of Tween-20 has been selected for further parameters study. A less amount of surfactant used is preferred as it would be more economical. Figure 2 shows the proposed interaction between Tween-20 surfactant and tartrazine, which exhibit dipole-dipole interaction.



Figure 2. The interaction between Tween-20 (surfactant) molecule and tartrazine (analyte) molecule.

Effect of salt concentration. Salt plays a main role in the cloud separation with a combination of surfactant as it accelerates the separation. The effect of salt is due to the behaviour of some electrolyte properties of the salt solution, which act by increasing the dehydration of surfactant-rich phase (Khammas et al. 2014). In addition, the purpose of salt addition in CPE method is to ease a surfactant-rich and aqueous-rich in phase separation because it has ability to change the aqueous phase density, lower the CPT, cause a salting-out phenomenon and decrease the surfactant-rich phase volume, thus increase the analyte transfer from this aqueous phase to surfactant-rich phase (Heydari & Hosseini 2016; Heydari et al. 2015).



Figure 3. Effect of Na₂CO₃ salt concentration. Condition: tartrazine concentration: 10 mg L⁻¹, Tween-20 concentration: 4 % (v/v), pH 10, temperature: 30°C, incubation time: 20 min.

Figure 3 shows the extraction efficiency of tartrazine gradually rises up from an initial concentration of salt (0.10 mol L⁻¹) to 1.50 mol L⁻¹. Then, the extraction efficiency is slowly decreased after 1.50 mol L⁻¹. This situation could be explained as a low concentration of salt is inadequate to increase the cloud point temperature (CPT) as well as obtain a complete separation phase. Besides, as the

concentration of salt exceeded the optimum amount, the salt would become saturated and reduced the extraction efficiency. Moreover, the salting-out interaction occurs and induced inter-attraction between surfactant-aggregate complexes itself, consequently caused the precipitation of salt and surfactant molecules.

 Na_2CO_3 salt was selected in this CPE study because the salting-out effect has been reported to be more favourable for divalent salt (Asman & Azlie 2018). The influence of salting-out effect is due to the dehydration of the polyoxyethylene chain by cations and high water molecule self-association by anions (Purkait et al. 2006). Besides, Na^+ is considered as a kosmotropic ion that exhibits strong interaction with water molecule than water itself, thus has ability forming a phase separation by breaking the water-water hydrogen bonds (Asman et al. 2018; Zain et al. 2014). Similar observations were reported on Na_2CO_3 salt in CPE for determination of carmine and Sudan dyes in food samples, respectively (Heydari et al. 2014; Liu et al. 2007). In this study, the maximum extraction efficiency of tartrazine was found at 1.50 mol L⁻¹ of Na_2CO_3 salt concentration. Thus, 1.50 mol L⁻¹ was selected for further parameter study as the optimum salt concentration in this study.

Effect of pH. The effect of pH on extraction efficiencies of tartrazine was examined in three different pH media, which were pH 4 (acidic medium), pH 7 (neutral medium) and pH 10 (alkaline medium). As illustrated in Figure 4, the extraction efficiency of tartrazine showed an increasing trend from acidic to alkaline media, which indicated the extraction efficiency of tartrazine was more favourable and optimum at pH 10. The reason for this behaviour is related to the pK_a value of tartrazine, which is 9.43 and favoured an alkaline condition (Zhao 2013; Pérez-Urquiza & Beltrán 2001). At a lower pH less than the pK_a value, the tartrazine is protonated and its ionic characteristics increased, resulted in less solubilization of tartrazine in the hydrophobic micelles and less extraction of tartrazine. At higher pH above the pK_a value, the tartrazine is deprotonated and behaved like a hydrophobic molecule, which solubilized easily in the micelles and resulted in higher extraction of tartrazine (Purkait et al. 2005). Thus, the alkaline medium (pH 10) provides the optimum extraction efficiency of tartrazine.



Figure 4. Effect of pH. Condition: tartrazine concentration: 10 mg L⁻¹, Tween-20 concentration: 4 % (v/v), Na₂CO₃ concentration: 1.5 mol L⁻¹, temperature: 30°C, incubation time: 20 min.

Effect of equilibration temperature. The effect of temperature (Figure 5) shows the extraction efficiency of tartrazine is rapid increases from 20 °C to 30 °C. At the initial temperature, no measurable mobilities were observed because no reverse micelles existed to stabilize the charges due to unreached critical micelle concentration (CMC) (Michor & Berg 2015). However, the extraction efficiency of tartrazine showed satisfactorily highest at 30 °C. At this temperature, the clouding phenomenon happened and reached the CMC.

The clouding phenomenon could be observed when the CPE system reached a cloudy phase at a welldefined temperature, known as CPT. During the CPT, a single phase micellar solution transferred to surfactant-rich and aqueous phases known as micelles formation or CMC (Mahajan, 2011). Micelles formation happened due to the hydrophobic effect, which the surfactant hydrocarbon chain tends to minimize water contact, thus leads to the formation of two phases (Goddard & Ananthapadmanabhan 1994). Simultaneously, this phenomenon delivered the analyte to dissolve into the micelles along with the formation of two phases separation (Melnyk et al. 2015). From Figure 5, the clouding phenomenon has clearly formed at 30 °C and produced the highest extraction efficiency of tartrazine. At this optimum temperature, the surfactant added was satisfactorily exceeding the CMC obtained. Also, the CPE system was sufficiently heated exceed the CPT, thus leading the tartrazine to dissolve into the micelles and forming two phases.



Figure 5. Effect of temperature. Condition: tartrazine concentration: 10 mg L^{-1} , Tween-20 concentration: 4 % (v/v), Na₂CO₃ concentration: 1.5 mol L^{-1} , pH:10, incubation time: 20 min.

Afterward, the extraction efficiency of tartrazine was rapidly decreased after 30 °C and above because the CMC changed with temperature (Michor & Berg 2015). When the micelle concentration increased after the CMC, the mobility rises, before falling off at higher temperatures due to screening caused by intermicellar charging. Besides, the maximum tartrazine particle mobility decreased because of the dehydration of the ethylene oxide of the surfactant that reduced the CMC. As temperature increased higher, the maximum amount of analyte particle mobility would be decreased and reduced the micelle formation (Heydari & Hosseini 2016).

Originally, the CPT of Tween-20 was approximately at 70-77 °C. However, the CPT in this study declined to 30 °C due to the secondary effect of salt known as salting-out (Kulichenko et al. 2009). The presence of sodium carbonate (Na₂CO₃) reduced the CPT in a ratio dependent on electrolyte concentration (Pourreza & Zareian 2009).

Validation and application of the CPE method to real samples. In order to validate the reliability of the proposed CPE, spiking and non-spiking standard tartrazine on real food samples were used. Spiked tartrazine in food samples was 10 mg L⁻¹. The linear calibration graph was in the range of 1-12 mg L⁻¹ with a correlation of determination (R^2) was 0.9957. The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated using S/N*3 and S/N*10, where S/N is the signal to noise ratio. The LOD and LOQ values were 0.88 mg L⁻¹ and 2.96 mg L⁻¹, respectively.

The amounts of tartrazine in the food samples are in the range of 1.22-6.12 mg L⁻¹ with relative standard deviation (RSD) is < 3 % as shown in Table 2. This result showed that the tartrazine content in both samples followed the regulation of the European Food Safety Authority (EFSA) (2009), which the maximum limit of tartrazine content in the sweet (confectionary product) is 300 mg L⁻¹ and the soft drink (non-alcoholic drink) is 100 mg L⁻¹. This CPE demonstrated good reliability for the determination of tartrazine in food samples. Therefore, the possibility for determination of other analogues dyes based in food samples using developed CPE could be considered.

Table 2. Determination of tartrazine in food samples using CPE				
Food Samples	Tartrazine added (mg L ⁻¹)	Tartrazine in real sample + standard tartrazine spiked (mg L ⁻¹)	$\begin{array}{c} \mbox{Tartrazine found} \\ \mbox{in the real sample} \\ \mbox{(mg } L^{\text{-1}}) \end{array}$	RSD (%), n =3
Sweets				
	-	11.22	1.22	1.44
	10	11.51	1.51	0.12
Soft drink	-	1.27	1.99	2.52
	10	5.61	6.12	0.74

Conclusion

A simple and cost-effective CPE method was developed for the determination of tartrazine in food samples prior to its spectrophotometric detection. The optimum CPE condition was successfully achieved by studying the concentrations of surfactant and salt, pH and temperature. The tartrazine content in food samples conformed the European Food Safety Authority (EFSA). The proposed CPE has shown a good performance to determine the tartrazine in food samples, thus the possibility of tracing the amount of other dyes in food samples could be considered in the application in food safety and quality analysis.

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