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Direct Solid-phase Spectrophotometric Determination of Tartrazine in Soft Drinks Using β-Cyclodextrin Polymer as Support

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ABSTRACT

A sensitive and new solid-phase spectrophotometry for the determination of tartrazine in soft drinks has been developed. In this method, *β*-cyclodextrin epichlorohydrin polymer (*β*-CDP) of 60-80 mesh, which has less background absorbance in ultraviolet and visible region compared to other ion-exchanger resins, was used to support direct adsorption of tartrazine from aqueous solutions to form a solid complex (tartrazine-*β*-CDP complex). The effects of pH, temperature, time and ionic strength on the *β*-CDP including tartrazine were determined. The optimum conditions were obtained (pH, 1.0; temperature, 20°C; time, 40 min). The method was applied for the direct solid phase spectrophotometric determination of tartrazine in soft drink samples at the maximum absorption wavelength of 435 nm with satisfactory results.

Key words: Tartrazine, solid-phase spectrophotometry, *β*-cyclodextrin epichlorohydrin polymer (*β*-CDP)

INTRODUCTION

Tartrazine, one of the best known and the most commonly used food additives, is used in many medications as well as in foods. It is a synthetically yellow azo dye added mainly to colored carbonated drinks, fruit squash, soups, ice cream, sweet, chewing gum, jam, jelly and many convenience foods. It can be used with brilliant blue to produce various green shades. In addition, tartrazine can also be found in the shells of medicinal capsules. Researches have shown that tartrazine can be linked to asthma, certain rashes, hyperactivity (particularly in children) and migraine. In fact, tartrazine is the second most common cause of migraines in younger people. In Norway and Austria, tartrazine has been banned to be used in foods. In the United States, manufacturers are required to indicate on the label if a product contains tartrazine. Therefore, it is very important to determine the contents of tartrazine in foods. Some analytical methods have been reported including spectrophotometry[1-6], liquid chromatography[7-13], capillary chromatography[14], ion chromatography[8], flow injection analysis[15,16], voltammetry[17-20] and LC-MS[21]. Among these methods, spectrophotometry often suffers from poor sensitivity and interference from some anions, although they are widely used for the determination of tartrazine due to its excellent detection limits. On the other hand, chromatography suffers from time-consuming procedures and complicated instrumentation, whereas voltammetry is subjected to severe interference from other variable. So far there has been no report on the determination of tartrazine by direct solid-phase spectrophotometric determination of tartrazine in foods.

In the present work, *β*-cyclodextrin epichlorohydrin polymer (*β*-CDP) was used as a solid adsorbent and support to directly separate tartrazine from aqueous samples. A novel solid phase spectrophotometric method for the determination of trace amounts of tartrazine in foods has been developed. The method showed several important advantages including higher sensitivity than those of conventional spectrophotometries, low interference level, the use of conventional instrumentation and the simultaneous preconcentration and color development. The proposed method seemed to be a useful technique for the determination of trace amounts of constituents. An outline of direct solid phase spectrophotometry was given and trace amounts of tartrazine in some soft drink samples were determined.

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MATERIALS AND METHODS

I. *Reagents and Apparatus*

(I) *Apparatus*

A model Hitachi UV-2000 spectrophotometer (Hitachi Corporation, Japan) matched with 5-mm quartz cell (5×10 mm, 1.5 mL) was used for all spectrophotometric measurements. At the bottom of the quartz cell, a small hole was made by an emery wheel before use, in order to release the solution that existed in the colored polymer when the colored polymer was packed into the cell (Figure 1).

All pH-values of solutions were measured using a digital pH-meter, model pHs-29A (Tianjin Shengbang Scientific Instruments Company, Tianjin, China). A thermostatic rotatory shaker model Peking HZS-HA (Beijing Analytical Instrument Company, Beijing, China) was used for the adsorption procedure.

(II) *Reagents*

All reagents used were of analytical reagent grade or better without any additional purification. Milli-Q water (Millipore Company, Bedford, Mass, USA) was used throughout the experiment. A standard stock solution of 1.0 mg/mL tartrazine (Sigma, USA) was prepared by dissolving 0.2501 g of tartrazine in 250 mL of Milli-Q water. Working solution of 200 μg/mL tartrazine was prepared by appropriate dilution of the stock solution. β-CDP was synthesized as described above[22]. The detailed procedure was as follows: 40 g of β-CD, 10 g of soluble starch and 100 mL of 20% sodium hydroxide were added into a beaker. The mixture was vigorously stirred at 50-60°C for an appropriate period until the reactants were dissolved. A total of 60 mL of epichlorohydrin was added dropwise into this solution, and β-CDP was formed in 30 min. After washing with Milli-Q water 5-6 times, the polymer was dried at 100°C, ground and sieved into 20-40, 40-60, 60-80 and 80-100 mesh fractions, and then stored at room temperature (20°C) in a desiccator before use. Soft drink samples were purchased from local markets in Tianjin, China.

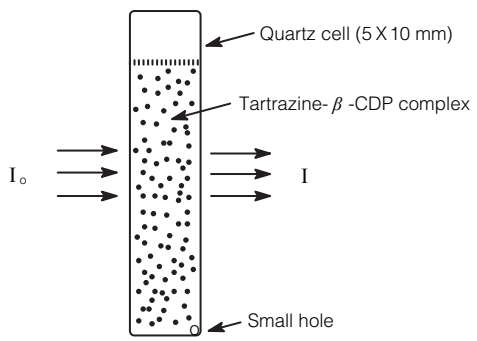

Figure 1. The quartz cell used and packing manner of tartrazine-β-CDP complex.

II. *Procedure*

An aliquot of 10-20 mL of soft drink sample was added to a 50-mL stoppered conical flask. Then, 2.5 mL of 1.0 mol/L hydrochloride acid solution and 0.5 g of β-CDP (60-80 mesh) were added. The mixture was kept for about 5 min and then made up to 25 mL. After the mixture was shaken mechanically at a rotation rate of 40 rpm for 40 min at room temperature, tartrazine-β-CDP complex was transferred into a 5-mm quartz cell and absorbance intensity was measured with a wavelength of 435 nm.

RESULTS AND DISCUSSION

I. *Absorption Spectra of Tartrazine*

The absorption spectra of tartrazine in β-CDP phase and in solution are shown in Figure 2. It can be seen that the maximum absorbance of tartrazine in β-CDP phase appeared at 435 and 399 nm in solution, respectively, and that the absorbance intensity in β-CDP phase is 8.3 times than that in solution. Maximal absorbance wavelength

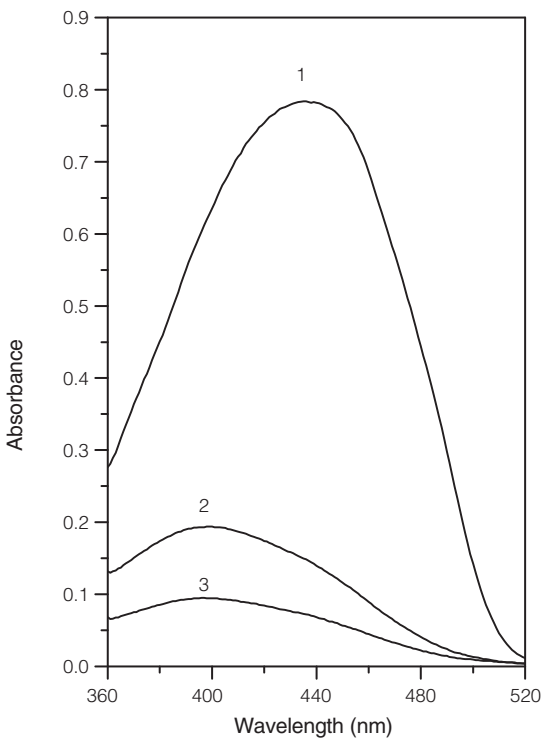

Figure 2. Absorption spectra of tartrazine in solution and β-CDP solid-phase. 1, Tartrazine in β-CDP solid-phase; 2, tartrazine in solution in the presence of β-CD; 3, tartrazine in solution in the absence of β-CD. The condition is pH=1, β-CDP (60-80 mesh): 0.5 g, tartrazine (200 μg/mL) 1 mL, shaking time: 40 min, room temperature, total volume: 25 mL.

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of tartrazine in *β*-CDP phase is shifted to a longer wavelength by 36 nm.

II. *Conditions of Tartrazine Included β-CDP*

(I) *Effect of the Particle Size on β-CDP Including Tartrazine*

Effect of the particle size on *β*-CDP including tartrazine is shown in Figure 3. It can be seen that the inclusive quantities of tartrazine in different mesh *β*-CDP were not identical. When the polymer was smaller particle size, inclusion quantity (*Q*) was the larger. It was desirable that tartrazine in the solution was adsorbed by the polymer as completely as possible. In addition the separation of the polymer from the equilibrated solution and its packing into a sample cell should be simple. For these reason the 60-80 mesh polymer was used for analysis.

(II) *Effects of Temperature and Time on β-CDP Including Tartrazine*

Effects of the temperature and time on *β*-CDP including tartrazine are shown in Figure 4. It can be seen that in the inclusion reaction of tartrazine, the absorbance intensities decrease gradually as temperature increase, i.e. *Q* decreases gradually as temperature increases, where *Q* is maximal at room temperature which was used for further analysis. In addition, the higher temperature is the more easily to reach the inclusive equilibrium and the shorter the inclusive time is, it accords with general adsorption reaction rules. In order to extract the tartrazine on *β*-CDP adsorbent, shaking was adopted instead of stirring in the present work. Under such conditions, no destruction of the polymer particles occurred, but often observed when stirred very rapidly. The shaking time required for attaining the adsorption equilibrium depended on the system. The results showed that 10 min reaches the inclusive equilibrium at 60°C, 25 min at 40-50°C, 30 min at 30°C and 40 min at 20°C, respectively. Based on these results, 40 min (20°C) was chosen as shaking time for further experiment.

(III) *Effect of pH on β-CDP Including Tartrazine*

Tartrazine molecule contains benzene ring group

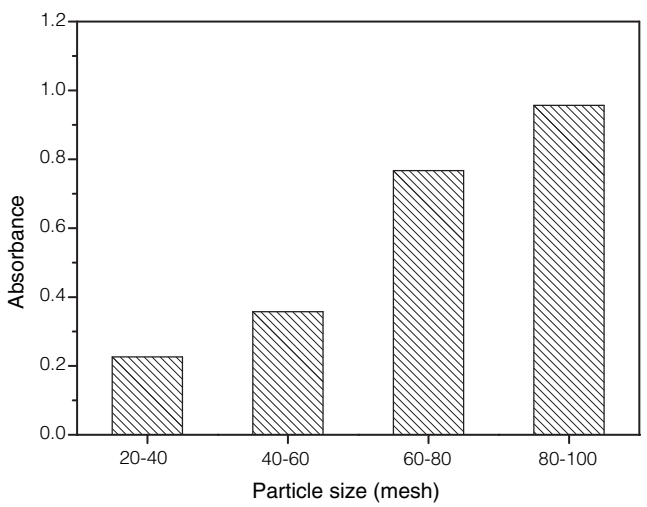


Figure 3. Effect of the particle size on *β*-CDP including tartrazine. The condition is pH=1, *β*-CDP: 0.5 g, tartrazine (200 μg/mL):1 mL, shaking time: 40 min, room temperature, total volume: 25 mL.

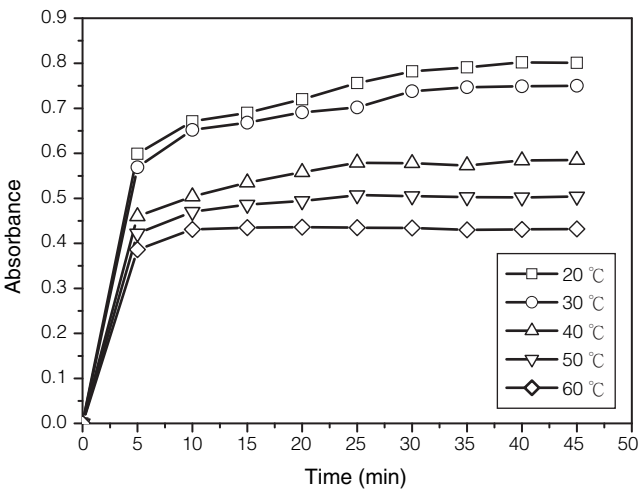


Figure 4. Effect of temperature and time on *β*-CDP including tartrazine. The condition is pH=1, *β*-CDP (60-80 mesh): 0.5 g, tartrazine (200 μg/mL): 1 mL, total volume: 25 mL.

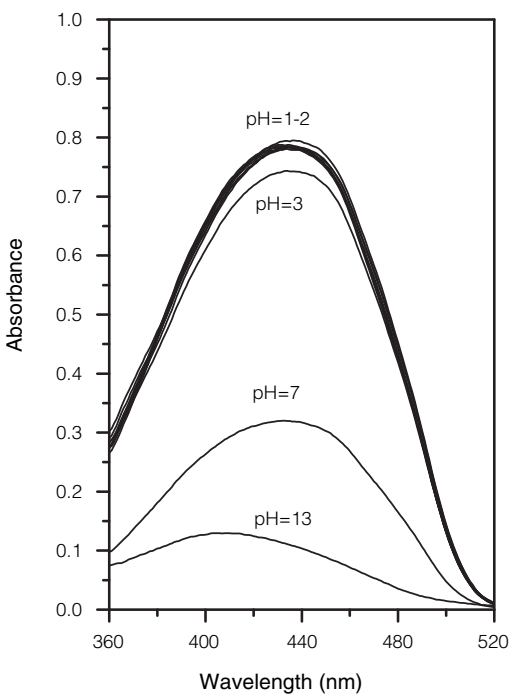
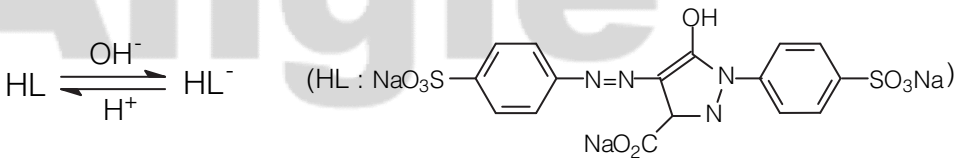


Figure 5. Effect of pH on *β*-CDP including tartrazine. The condition is *β*-CDP (60-80 mesh): 0.5 g, tartrazine (200 μg/mL): 1 mL, shaking time: 40 min, room temperature, total volume: 25 mL.



Scheme 1. Types of tartrazine molecules in different pH conditions.

whose size and geometry are suitable for the hydrophobic cavity of β-CDP. The types of tartrazine molecule existed in solution are changeable as pH-values changes of solution because of ionization (Scheme 1).

In acidic and neutral conditions, tartrazine is very stable and exists with neutral molecule. On the contrary, the stability of tartrazine decreases in basic medium, because hydroxyl group takes place ionization to form the related phenyl salt, i.e. the solution color lightens and even appears to red. Maximum absorbance shifts to shorter wavelength region (428 nm). As *β*-CDP cavity is hydrophobic, the neutral molecules of tartrazine in acidic and neutral medium are easily included by *β*-CDP than the related salt of tartrazine in basic medium. Weak polar and no-polar compounds are easily adsorbed to enter the *β*-CDP cavities and form the inclusion complexes in general conditions. The absorption spectra of *β*-CDP including tartrazine in different pH ranges are shown in Figure 5. The results showed that absorbance intensities maximum appeared in pH values 1-2 and decreased with the increasing pH values. Based on Figure 5, pH 1.0 was selected for further experiments.

(IV) *Effect of Ionic Strength on β-CDP Including Tartrazine*

Effect of ionic strength on the formation of *β*-CDP including tartrazine complex was determined and the result is shown in Figure 6. It can be seen that there was little increase in absorbance when the concentration of sodium chloride changes from 0.01 to 0.1 mol/L. Furthermore, there was slight decrease in absorbance when the concentration of sodium chloride is over 0.5 mol/L. In conclusion, the ionic strength has little effect on the inclusion of tartrazine in *β*-CDP. Therefore, the ionic strength is not specially considered, but controlled at 0.1 mol/L in further research

(V) *Calibration, Precisions and Detection Limit of Tartrazine*

The calibration curve of tartrazine was constructed in the concentration range of 0.04-4.8 μg/mL under the optimum conditions. The calibration curve of tartrazine with good linearity is expressed by the equation: A= 0.0143 + 0.0078·W (R = 0.9995, P < 0.0001), where A denotes the absorbance intensity of tartrazine, and W represents the mass of tartrazine (μg) in sample solutions. The precision of the proposed method for five replicate determinations at 30 μg of tartrazine was 2.2% of relative standard deviation (RSD) according to the IUPAC method[23]. The detection limit of the method for tartrazine was 0.04 ppm, which was calculated as the concentration corresponding to a three-fold standard deviation obtained by five subsequently repeated measurements of a reagent blank.

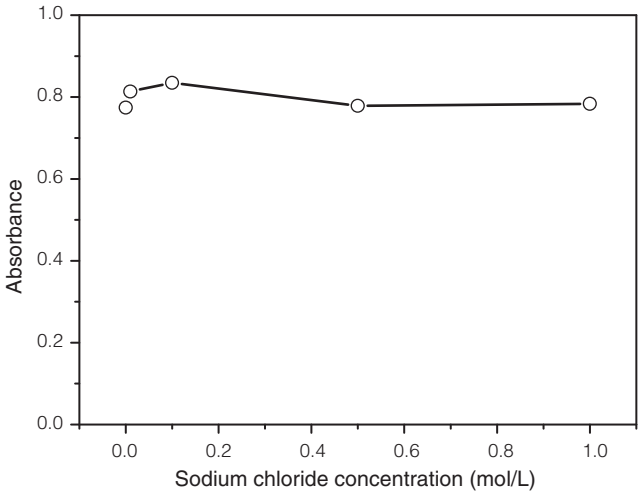


Figure 6. Effect of ionic strength on *β*-CDP including tartrazine. The condition is pH=1, *β*-CDP (60-80 mesh): 0.5 g, tartrazine (200 μg/mL): 1 mL, shaking time: 40 min, room temperature, total volume: 25 mL.

(VI) Effect of Foreign Ions and Antioxidants

The tolerance limits of foreign ions, antioxidants and oxidants frequently found in foods were determined as the maximum added quantities of foreign ions with 40 μg of tartrazine. The limits resulted in less than 5% RSD in the absorbance intensity of tartrazine (Table 1). Most common ions including Zn^{2+}, Cu^{2+}, Cr^{3+}, Ni^{2+}, Fe^{3+}, Co^{2+} and Mn^{2+} were tolerated in relatively high concentration or did not interfere. Tartrazine was stable to antioxidants and oxidants, which embodied the stability of artificial synthetic pigment.

III. *Determination of Samples*

The proposed method was applied to the determination of tartrazine in soft drink samples. The results are given in Table 2. The tartrazine contents in four different samples were between 21 and 67 μg/mL. In order to

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Table 1. Effect of foreign ions, antioxidants and oxidants on the determination of tartrazine (tartrazine: 40 μg)

Foreign ions	Mass ratio of ions to tartrazine	Relative error (%)	Foreign ions	Mass ratio of ions to tartrazine	Relative error (%)
Zn^{2+}	250	0	Fe^{3+}	250	2.0
Al^{3+}	200	3.2	Co^{2+}	250	0.9
Cu^{2+}	250	-4.5	Mn^{2+}	250	3.6
Mg^{2+}	250	2.7	Pb^{2+}	250	1.8
K^{+}	250	5.4	Sn^{2+}	250	0.6
Cr^{2+}	250	3.4	Vc	1500	4.6
Ca^{2+}	250	1.8	I^{-}	12500	3.4
Ni^{2+}	250	4.5	$S_2O_3^{2-}$	395	4.0
Fe^{2+}	250	-4.5	H_2O_2	12500	2.3

Table 2. The contents of tartrazine in soft drink samples and recoveries

Sample	Tartrazine (μg/mL)		Added (μg/mL)	Found (μg/mL)	Recovery (%)
	This method	HPLC			
Smart apple	31 ± 0.7[a]	34 ± 0.3	200	195	97.5
Farmer apple	21 ± 0.4	23 ± 0.3	200	192	96.1
Gatorade lemon	67 ± 1.5	70 ± 1.4	200	193	96.2
Marinda apple	39 ± 0.8	42 ± 0.7	200	192	96.1

[a] Average ± relative standard deviation for five determinations.

further check the validation of the proposed method, tartrazine contents in the samples were determined simultaneously by HPLC[10,12] (Table 2). As can be seen, the results obtained by the proposed method are in good agreement with those obtained by HPLC.

CONCLUSSIONS

A sensitive and selective solid-phase spectrophotometry for the determination of trace amounts of tartrazine was developed, in which the separation, concentration and determination of tartrazine took place simultaneously. Tartrazine molecules were included by *β*-CDP to form a solid supramolecular complex, i.e. tartrazine-β-CDP complex. The stability and absorbance intensity of tartrazine were then prolonged comparing with those in solution. It is easy to pack tartrazine-β-CDP complex into a 5-mm quartz cell (5 × 10 mm, 1.5 mL) by the use of a pipette. The method is convenient for the determination of 0.04-4.8 μg/mL of tartrazine. In addition, the polymer used can be regenerated by 1.0 mol/L sodium hydroxide with a 96.9% regenerated yield. Solid-phase spectrophotometry seems to be more useful than traditional liquid-phase spectrophotometry.

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